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The Effect of Alhagi maurorum Medik on Mitochondrial Function

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https://doi.org/10.34737/w85z1

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The Effect of *Alhagi maurorum* Medik on Mitochondrial Function

Juwairiya Zulfiqar Butt

A thesis submitted in partial fulfilment of the requirements of the University of Westminster for the degree of Doctor of Philosophy

September 2023

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Abstract

Mitochondrial bioenergetics are crucial for the optimal activity of the cell and are emerging therapeutic targets for the treatment of several metabolic syndromes and cancer. *Alhagi maurorum* plant is traditionally used in several clinical conditions due to its potential anti-inflammatory, hepatoprotective and gastrointestinal effects.

In this study changes in cell viability, reactive oxygen species (ROS) levels, mitochondrial membrane potential (MMP), oxidative phosphorylation (OXPHOS), calcium homeostasis and mitochondrial morphology in MCF-7 and MCF-10-A (control) cells after treatment with *Alhagi maurorum* for 24hr and its main bioactive compound hordenine for 6hr, 24hr, and 48hr are observed. Results indicate that cells treated with *Alhagi maurorum* have significantly reduced cell viability, ROS levels, MMP, and intracellular calcium concentrations at higher concentrations that suggest cytotoxic effects, while moderately reduced effects at 100 µg/ml, indicating possible stimulation of several other cellular processes such as cell death, antioxidant systems, and immune responses. Furthermore, *Alhagi maurorum* extract modulated various parameters of mitochondrial respiration too including, basal respiration, ATP production, proton leak, spare respiratory capacity, maximal respiration, and non-mitochondrial respiration. In the cells treated with hordenine, the key bioactive compound of *Alhagi maurorum,* results indicate that hordenine has no significant effect on MCF-7 and MCF-10-A after 24hr and 48hr of exposure, including cell viability, ROS levels, MMP, intracellular calcium concentrations and mitochondrial respiration. However, in MCF-10-A after 6hr of the treatment ROS levels are significantly reduced at concentrations 0.1 µM, 10 µM, 500 µM, and 1000 µM. Furthermore, no significant changes to mitochondrial morphology are observed at lower concentrations of *Alhagi maurorum* and hordenine tested in MCF-7 and MCF-10-A, though only small, rounded networks of mitochondria, increased in number in case of hordenine suggest possible occurrence of the process of fission.

In conclusion, my work shows that *Alhagi maurorum* extract modulates mitochondrial function, and effects are concentration-dependent while one of the main bioactive compounds of hordenine phenethylamine alkaloid, has more limited effects. Characterisation of the extract through HPTLC and LC-MS analysis showed presence of more than one compound in the extract.

List of Conference Posters

Hordenine Regulates Redox state and Mitochondrial Functions in Malignant and Non-malignant Breast Cell Lines in Concentration Dependent Manner. The 21st International Congress of International Society for Ethnopharmacology (Ethnopharmacology 2022) taking place at China Medical University, Taichung, Taiwan, May 28-31, 2022.

Hordenine: Redox State and Mitochondrial Functions in Malignant and Non-Malignant Breast Cell Lines. Poster presented at 70th International Congress and Annual Meetings of the Society for Medicinal Plant and Natural Product Research (GA), Thessaloniki, Greece, August 29, 2022.

Acknowledgements

I would like to express my sincere gratitude to my mentor Professor Jimmy David Bell. I am grateful for his encouragement and wisdom throughout my PhD journey. I also extend my gratitude to my director of studies Dr Anthony Booker and second supervisor Professor Michael Heinrich (University College London, London, United Kingdom) for their constant support and guidance throughout my PhD. An enormous thank you to Dr Rhys Mould for his valuable time teaching me in the lab and sharing his research experience with me. I am also very grateful for the assistance and expertise of Dr Meliz Sahuri-Arisoylu in the beginning of my PhD program. I would like to thank Francesca Scotti (University College London, London, United Kingdom) for the LC-MS experiments.

I would like to acknowledge the support of Professor Zaheer-Ud-Din (Government College University, Lahore, Pakistan) for the collection of plant sample from Pakistan for this research.

I am extremely thankful to the team members Professor Louise Thomas, Steve Woodley and Ifigeneia Kalampouka for being there for any additional support and assistance.

Most importantly, I would like to thank my parents and my husband for their financial support to full fill my passion of higher education from abroad and my children for their love and understanding throughout this journey.

Declaration of Contributors

I declare that all the material contained in this thesis is my own work. Any assistance is detailed below:

LC-MS Analysis

Francesca Scotti performed LC-MS experiments of plant *Alhagi maurorum* and data is analysed by the author.

List of Abbreviations

ATP	Adenosine triphosphate
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ANOVA	Analysis of variation
ANT	Adenosine nucleotide translocator
ADP	Adenosine diphosphate
APAF1	Apoptotic protease activating factor
AIF	Apoptosis-inducing factor
Bad	Bcl-2 associated death promoter.
Bak	Bcl-2 homologous antagonist killer
Bax	Bcl-2 associated protein X
Bcl	Cell lymphoma
Bcl-2	B-cell lymphoma
BSA	Bovine serum albumin
Ca^{+2}	Calcium ion
CoA	Coenzyme A
Cyt c	Cytochrome c
CSCs	Cancer stem cells
DCF	2',7' –dichlorofluorescin
DCFDA	2',7' –dichlorofluorescin diacetate
DNA	Deoxyribonucleic acid
DAMPs	Damage-associated molecular patterns
DMEM	Dulbecco's modified Eagle's medium
DI	Deionized
DMEM	Dulbecco's modified Eagle's medium
DNA	Deoxyribonucleic acid
Drp1	Dynamin-related protein
ETC	Electron transport chain
FAD/FADH	Flavin adenine dinucleotide
FADD	Fas-associated protein with death domain

FasL	Fas ligand
FBS	Foetal bovine serum
FCCP	Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone
GIT	Gastrointestinal tract
IMM	Inner mitochondrial membrane
HL60	Human acute myeloid leukemia cells
HPTLC	High Performance Thin Layer Chromatography
MAPK	Mitogen activated protein kinase
MAVS	Mitochondrial antiviral-signalling protein
MCU	Mitochondrial calcium uniporter
MEME	Modified Essential Media Eagle
miRNA	Micro ribonucleic acid
MOMP	Mitochondrial outer membrane permeabilization
M.W	Molecular weight
mPTP	Mitochondrial permeability transition pore
mtDNA	Mitochondrial DNA
MitoTAM	Mitochondrially targeted tamoxifen
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MMP	Mitochondrial membrane potential
MS	Mass Spectrometry
NAD/NADH	Nicotinamide adenine dinucleotide
NADP/NADPH	Nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NO	Nitric oxide
NOX	NADPH oxidase
Nrf2	Nuclear factor (erythroid-derived 2)-like 2
Na+	Sodium ion
NMR	Nuclear Magnetic Resonance
OCR	Oxygen consumption rate
OMM	Outer mitochondrial membrane
OXPHOS	Oxidative phosphorylation

PAMPs	Pathogen-associated molecular patterns
PBS	Phosphate buffered saline
PCD	Programmed cell death
PI3K	Phosphoinositide 3-kinase
P53	Upregulated modulator of apoptosis
PRL	Prolactin
PRRs	Pattern-recognition receptors
RIPA	Radioimmunoprecipitation assay
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPMI	Roswell Park memorial institute medium
RIPK3	Receptor-interacting serine/threonine protein kinase 3
Smac/DIABLO	Second mitochondria derived activator of caspases
TCA	Tricarboxylic acid cycle/Kreb's cycle/citric acid cycle
TMRE	Tetramethyl rhodamine ethyl ester
TNF-α	Tumour necrosis factor-α
TP53	Tumour protein 53, commonly referred to as p53
tRNA	Transfer RNA
UTI	Urinary tract infections
UV	Ultraviolet
VDAC	Voltage dependant anion channel

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Statement of Problem

Mitochondrial dysfunction has been reported in several acute and chronic diseases, for example fatigue, obesity, diabetes, hypertension, and cancer. Therefore, regulating mitochondrial functions may offer new therapeutic opportunities. *Alhagi maurorum* Medik plant has been reported to have potential anti-inflammatory and anti-cancer activities however, it has not been assessed in reference to possible impact on mitochondrial functions.

Aims and Objectives

Aim: To assess the impact of *Alhagi maurorum* Medik and its bioactive compounds on mitochondrial function.

Objectives:

1. To determine if cell viability is affected by *Alhagi maurorum* extract and its bioactive compound hordenine.
2. To assess the effect of *Alhagi maurorum* extract and its main bioactive compound on mitochondrial functions, including reactive oxygen species (ROS) production, mitochondrial membrane potential (MMP), intracellular Ca^{+2} concentrations, and mitochondrial respiration.
3. To determine the effect of *Alhagi maurorum* extract and hordenine on mitochondrial morphology.
4. To identify the principal bioactive compounds associated with the mitochondrial effects induced by *Alhagi maurorum* extract.

Hypothesis

I hypothesize that *Alhagi maurorum* extract and selective secondary metabolite hordenine can regulate mitochondrial functions, including mitochondrial membrane potential (MMP), adenosine triphosphate (ATP) production, reactive oxygen species (ROS) levels, intracellular Ca^{+2} concentrations, oxidative phosphorylation (OXPHOS) and mitochondrial morphology.

Chapter 1

Introduction

1.1 Mitochondria- Impact Life

Mitochondria are critical semi-autonomous organelles essential for the optimal activity of the cell. Mitochondria meet the functional needs of the cell because their size, structure and number vary considerably, according to their physiological role (Liu et al., 2019; Calvo and Mootha, 2010).

The mitochondrion is commonly known for its classically appreciated role as the powerhouse of the cell, but now after intensive research it is also considered as master regulator of the cellular system. It serves as a biosynthetic hub for the synthesis of macromolecules essential for nucleotides, fatty acids, cholesterol, amino acids, glucose, heme, and iron-sulphur proteins by taking part in critical bioenergetic circuitries such as the tricarboxylic acid (TCA) cycle (Martinez-Reyes and Chandel, 2020) and oxidative phosphorylation (OXPHOS) (Ait-Aissa et al., 2019; Allen et al., 2011). It also serves as a waste management hub, degrading misfolded mitochondrial proteins intrinsically through the system of proteases in their compartments, for example the ubiquitin proteasome system (UPS) which is a part of mitochondrial quality control system and removes dysfunctional mitochondria involved in critical cellular processes of cell death most importantly mitophagy to maintain healthy cellular environment (Xiao and Loscalzo, 2019; Spinelli and Haigis, 2018;Wang and Youle, 2009). The mitochondria are also important to the cell signalling circuitry**,** either serving as a physical platform where protein-protein signalling interactions occur or regulating production and levels of different signalling molecules such as reactive oxygen species (ROS) and Calcium (Ca^{2+}), implicated in several other important cellular processes including initiation of immunogenic responses, growth, and differentiation. However, here focus will be mainly on the first three main aspects of mitochondrial functions because they are interrelated, crucial for mitochondrial health and significant therapeutic targets (Wang and Youle, 2009; Liu et al., 2019; Gibellini et al., 2015; Antico et al., 2012)

Mitochondria, being sensitive organelles, rapidly detect and respond to any changes in their cellular environment caused by endogenous or exogenous stimuli such as nutrients (selenium), co-factors, enzymes, toxins, alcohol and even frequently prescribed drugs as

seen in the case of carnitine, which is an essential co-factor required for the transport of long chain fatty acids into the mitochondrion for β-oxidation and depletion of Co-enzyme Q10 (CoQ10), essential for the transport of high energy electrons across the electron transport chain (ETC). At present, mitochondrial damage is assessed indirectly through several clinical markers for example, altered blood levels of lactate, pyruvate, and organic acid in urine (Yun and Finkel, 2014).

Since discovery, mitochondria continued to be a subject of research in various disciplines of biological and medical sciences, now their emerging role as a pharmacological target is a subject of research in the field of modern medicine (Ernster, and Schatz, 1981; Pagliarini and Rutter, 2013) (For an overview of mitochondrial research see Figure 1).

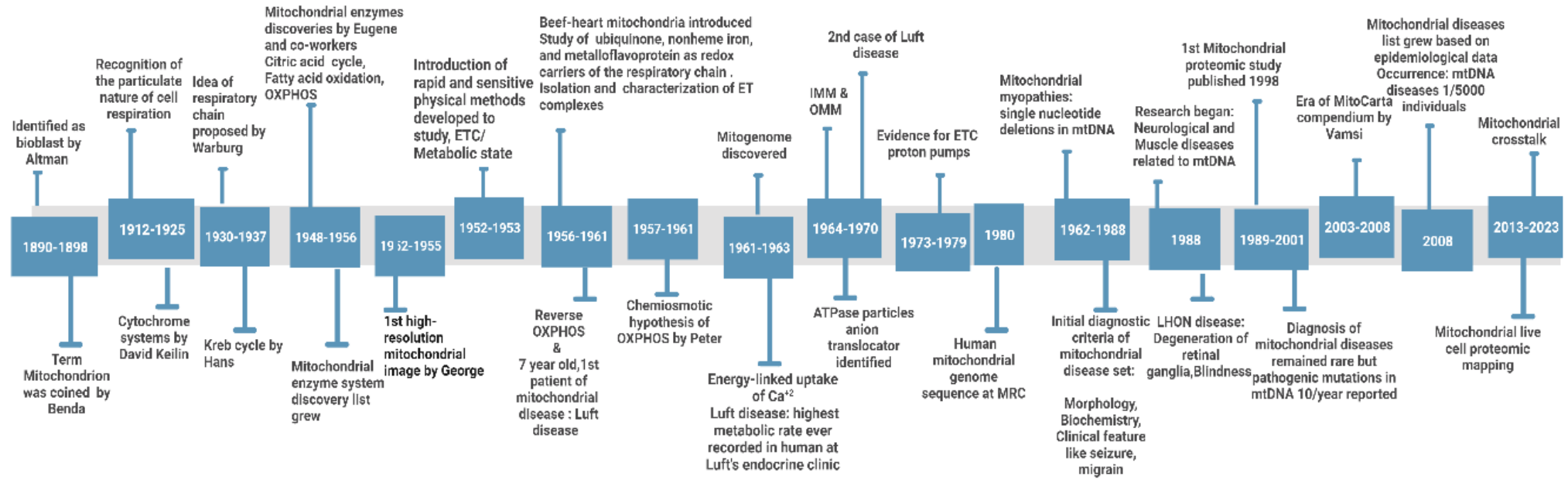


Figure 1: Mitochondrial research overview: Mitochondria are interesting organelles for researchers of various discipline (Created with BioRender).

1.2 Mitochondrial Morphology

Mitochondria are compartmentalized intracellular organelles normally occupying an area between 0.75 and 3 μm^2 , round to oval in shape and range in size from 0.5 to 10 μm, found in the cytoplasm of the eukaryotic cells (Veltri et al., 1990; Piomboni et al., 2012). The outer mitochondrial membrane (OMM) is porous for most of the molecules such as metabolites and products of cellular respiration and faces cytosol. Proteins residing on OMM play significant role in mitochondrial remodelling, particularly mitochondrial anti-viral signalling proteins (MAVS), mitochondrial fusion proteins (MFN1, MFN2), anti-apoptotic proteins and mitochondrial voltage-dependent anion channels (VDACs) also termed as porins (De Stefani et al, 2012). The inner mitochondrial membrane (IMM) protrudes into the mitochondrial matrix and forms invaginations called cristae that hold the main protein complexes of the main energy-generating system, the ETC. In addition to this, mitochondrial fusion protein optic atrophy 1 (OPA1) also resides in the inner mitochondrial membrane (Rastogi et al., 2019). The organelle's central mass is a gel like matrix which contains mitochondrial DNA (mtDNA), also called mitogenome.

Common geometric features that describe morphology of mitochondrion includes shape, size, position, and dynamics measured at nanometre scale to the micron scale and all the above-mentioned geometric features are interrelated and collectively define mitochondrial morphology. Remodelling of mitochondrial architecture in response to the cell's metabolic requirements results in either punctuated mitochondria (<1 μm in length) or interlocked connected networks of mitochondria (>3 μm in length) (Collins et al, 2021; Dowling et al, 2021). Different mitochondrial morphological states have been linked to various physiological as well as pathophysiological states. Since, fragmented mitochondria are observed under stressed and different states of cellular death where, elongated morphology has been linked to the efficient production of ATP though, full impact of mitochondrial morphology on the mitochondrial matrix and OXPHOS still needs to be fully explored **(**Figure 2).

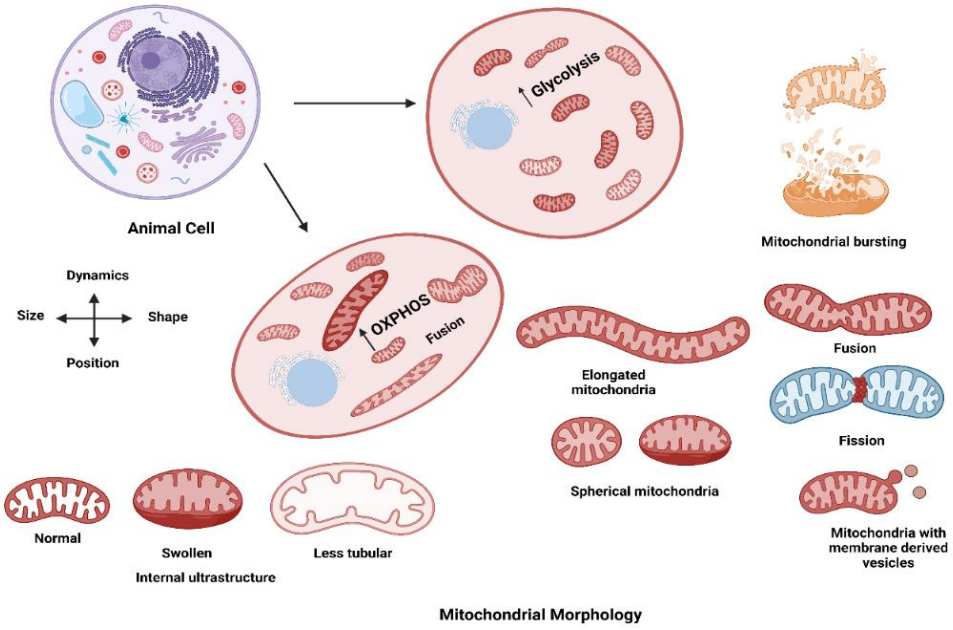


Figure 2: Mitochondrial morphological remodeling: Different mitochondrial shapes indicate dynamic nature of mitochondria under physiological and pathological conditions. Fibroblasts (Differentiated cells) possess elongated mitochondria with increased OXPHOS. Fragmented mitochondrial network with higher glycolytic rate and sensitive to death (Created with BioRender).

Although the dynamic nature of mitochondria was observed over 100 years ago, the main proteins involved in the maintenance of mitochondrial morphology have only been identified in the past few decades and lack full mechanistic information (Lewis and Lewis, 1914; Garcia et al., 2019; Lotz et al., 2014). Also, the mitochondrial proteome is found to be dynamically regulated as observed in the case of human cardiac cells that contain fewer mitochondria as compared to muscle cells, so relatively low number of proteins too. This indicates the impact of mitochondrial density on the mitochondrial functions depending upon the metabolic requirements of the cells even in two highly metabolically active cells (for more information about mitochondrial morphology and cell type, see Table 1) (Martinez-Reyes and Chandel, 2020; Ait-Aissa et al., 2019; Xiao and Loscalzo, 2019; Liu et al., 2019; Gibellini et al., 2015;). Similarly, higher concentration of mitochondria are also found in the active growth cones of the developing neurons further supporting the relationship of mitochondrial density and mitochondrial remodelling based on metabolic requirements of the organs (Zhao et al., 2012; Mils, 2015; Benard et al., 2007).

Table 1: Mitochondrial morphology and structural diversity

Cell type	Mitochondrion size, shape, position
Fibroblasts	Long filaments (1–10 μm in length, diameter of ~ 700 nm)
Hepatocytes	Uniform spheres or ovoids in shape
Vascular smooth muscle	Ovoid or rod-shaped organelles
Endothelium	Tubular mitochondrial network
Skeletal muscle	Ovoid structures: Have two populations (one positioned close to the sarcolemma that are round and smaller while other embedded among the myofibrils)
Pancreatic acinar cells	Three different regional groups, functionally unconnected. 1. Peripheral basolateral region near plasma membrane 2. Around the nucleus 3. In the periphery of the granular region separating the granules from the basolateral area
Cardiac myocytes	Three distinct populations, 1. Perinuclear (more rounded & densely packed) 2. Subsarcolemmal 3. Interfibrillar
Cultured vascular smooth cells	Long filamentous entities, loops, and networks and recurring structural classes such as small spheres, swollen spheres, straight rods, twisted rods, branched rods, and loops
Native vascular smooth cells	Solitary spheres and rods of various sizes

Furthermore, a new role of mitochondrial morphology in the maintenance of cellular homeostasis has been shown to influence mitochondrial bioenergetic status (Navaratnarajah et al., 2021). Rapid but temporary morphological adaptations of mitochondria in response to metabolic flux are generally known to be mediated by the processes of fission and fusion [for detailed process of fission and fusion possibly involved in mitochondrial remodelling, see Figure 3 (Table 2)]. Fission and fusion processes are the most studied processes though the impact of some undefined processes that are also influencing mitochondrial morphology such as branching/de-branching and extension/retraction is still unknown. This is an emerging area of research and as mitochondrial morphological changes are observed in complex and rare diseases there is intense need for more studies to explore the connection between mitochondrial morphology and mitochondrial bioenergetics to design highly effective drugs to target mitochondrial morphology (Galloway, Lee and Yoon, 2012; Navaratnarajah et al.,

2021). Interestingly, mitochondrial morphological remodelling also has a significant impact on the initiation of immune cell metabolism in response to pathogens a process still needs understanding but critical for new emerging field of immunometabolism (O'Neill et al, 2016; Angajala et al, 2018; Rambold and Pearce, 2018).

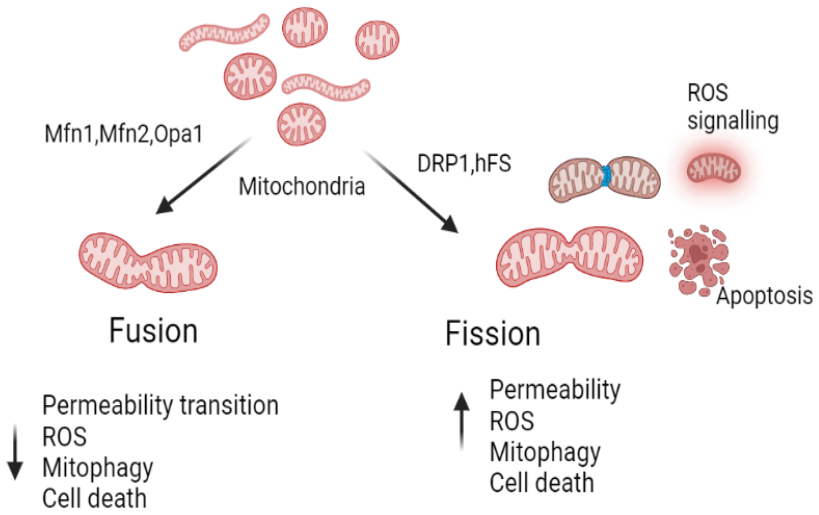


Figure 3: Schematic of process of fission and fusion. Fusion (Increased oxidative capacity), Fission (Decreased oxidative capacity). MFN1 and MFN2 mediate fusion of the OMM which is followed by fusion of IMM mediated by OPA1 and results in elongated mitochondria with increased ATP production and OXPHOS rate. Various cycles of fission and fusion maintain mitochondrial health and help to eliminate dysfunctional mitochondria or repair any damaged mitochondria through a process called mitophagy controlled by Pink1 and Parkin molecules. Apoptotic cells further release messengers as signals for phagocytosis in the form of metabolites (inflammatory, ApoEVs (apoptotic extracellular vesicles), PS (Phosphatidylserine) molecules) (Rastogi, et al., 2019) (Created with BioRender).

Table 2: Role of pro- and anti-apoptotic proteins in morphological remodelling

Protein	Protein characteristics	References
Bcl-2 (Apoptosis regulator)	Increases mitochondrial size Increases mitochondrial complexity	Kowaltowski et al., 2002
Bax/Bak (Proapoptotic)	Increases fragmented mitochondria Increases apoptosis induction	Desagher and Martinou, 2000
Bid (Proapoptotic)	Increases apoptosis induction Increases mitochondrial cristae fusion Increases lipid translocase activity	Scorrano, Ashiya, et al., 2002 :

1.3 Mitochondrial Cellular Crosstalk

As mitochondria are considered critical organelles due to their role in cellular metabolism, they are also important in deciding the fate of the cell (Sun et al., 2015; Wiedemann and Pfanner, 2017). Recent studies about the functional roles of mitochondria suggest that mitochondrial activities and involvement of these organelles in multiple processes are tightly controlled by nuclear DNA through anterograde regulation of gene expression (Arnold, et al., 2006; Audano et al., 2020). More than 1000 mitochondrial proteins are encoded by nuclear genes and are synthesized as precursors in cytosol and are transported into mitochondria through five main transport pathways such as the carrier, β-barrel and the pre-sequence pathways. Nuclear encoded mitochondrial proteins are transported into the mitochondrial matrix through the TOM/TIM complex located on OMM and IMM, respectively (Wiedemann and Pfanner, 2017; Suhm, et al., 2018; Picard, Wallace and Burelle, 2016). Mitochondrial protein import is a strictly controlled process and largely dependent upon mitochondrial energetics, though full molecular mechanism of transport of proteins is not known. Many scientific studies suggest mitochondria undergo intracellular stress and trigger specific nuclear gene transcription patterns through retrograde communication pathways (Chang, Shtessel and Lee, 2015; Braymer and Lill, 2017). This mito-cytosol-nuclear crosstalk is essential for the maintenance of cellular homeostasis (Xia, et al., 2019) (Figure 4).

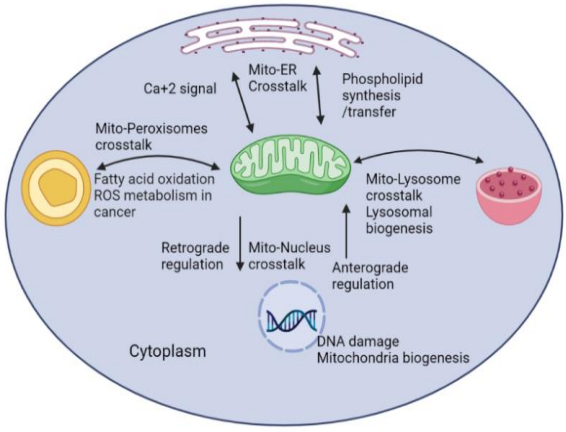


Figure 4: Mitochondrial crosstalk with other organelles: Mitochondria endoplasmic reticulum (ER) crosstalk for Ca^{2+}homeostasis. Mitochondria-lysosome crosstalk for lysosomal biogenesis. Mitochon-

drila peroxisomes crosstalk for fatty acid oxidation and ROS metabolism. Mito nuclear crosstalk, antero-grade regulation and retrograde regulation for transcription proteins, mitochondrial biogenesis (Created with BioRender).

Mitochondria and lysosomes communicate for fine tuning of cell's functionality as both organelles take part in the metabolism and participate in the events of catabolism as well as anabolism (Scorrano et al., 2019). Lysosomes, which are membrane bound organelles in cells, are involved in cellular processes of breaking down of large molecules of carbohydrates, lipids, proteins and prevent infections by digesting pathogens due to the presence of hydrolytic enzymes. However, dysregulation of any of the mitochondrial activity affects the functions of lysosomes and results in many pathologies such as Parkinsonism disease and Huntington's disease (Demers-Lamarche et al., 2016; Baixauli et al., 2015). Mitochondria-lysosome crosstalk is thought to be mediated by AMPK and mTORC1 pathways (Mullins and Bonifacino, 2001).

Peroxisome, another dynamic organelle of the cell, coordinates with mitochondria in processes like redox regulation, inflammatory and immune signalling (Fransen, Lismont and Walton, 2017). Similarly, endoplasmic reticulum (ER) and mitochondria form microdomains, known as mitochondria-associated ER membranes, and are necessary for Ca^{2+} signaling, and mitochondrial dynamics. So, various studies on mitochondrial association with other organelles in the cell have shown these intracellular talks are essential to maintain cellular and mitochondrial homeostasis (Veeresh et al., 2019).

1.4 Mitochondrial Disorders

Mitochondrial disorders are multiple medical disorders produced due to defects in mitochondrial function (Garcia-Berumen et al., 2019). Symptoms due to mitochondrial defects range from mild to life threatening, such as fatigue, weakness, developmental abnormalities, diabetes, cognitive disabilities, liver failure, metabolic disorders, and cancer (Singh et al., 2015; Gorman et al., 2015; Wallace, 2010). Mitochondrial DNA contains 37 genes out of which 13 genes code for the components of respiratory chain complexes (Ruhoy and Saneto, 2014; McFarland et al., 2010; Wallace, 2010). Although, maternal inheritance of mtDNA mutations (deletions, duplications), is reported primarily, but most of the mitochondrial disorders are sporadic in nature and show different clinical presentations with similar phenotype that suggest this area needs to be further explored. However, lactic acidosis (metabolic acidosis) and Leigh's disease (neurological disorders) are some

examples of primary mitochondrial disorders (Lopez, 1998; Chinnery, 2021; Seifert et al., 2015; Marra et al., 2021; Taylor and Turnbull, 2005).

Secondary mitochondrial disorders occur during lifetime and are caused by alterations in some of the major steps of mitochondrial metabolism. Among several causes altering normal metabolism, the most commonly reported are the changes in mitochondrial bioenergetics or defects in substrate utilization, for example, PDH complex (Pyruvate dehydrogenase) which connects glycolysis to the TCA cycle and any alteration in the activity affects pyruvate metabolism and ultimately glycolysis, gluconeogenesis and the citric acid cycle as seen in diabetes, obesity, and even various types of cancers (Currie et al., 2013, Tennant et al., 2010; DeFronzo and Tripathy, 2009; Du, et al., 2013; DeFronzo and Tripathy, 2009; Du, et al., 2013; Kerr, 2013). Similarly, excess of fructose triggers apoptosis, uric acid and contribute to cardiovascular diseases (Longo et al., 2006; DiDonato, 1997; Stanley, 2004; Imbe et al., 2018; Ohara et al., 2018; Du, et al., 2013; Feinman and Fine, 2013; Calvo et al., 2014; Gnaiger, 2014) and fumarase deficiency causes mitochondrial encephalomyopathy (De Vivo, 1996; Luft et al., 1962; Liang, Ahmad and Sue, 2014). Furthermore, defects in OXPHOS coupling results in some of the abnormalities of the respiratory chain affecting ATP production and causes several neurodegenerative diseases and nonthyroidal hypermetabolism commonly known as Luft disease (Sivitz and Yorek, 2010). Neurodegenerative disorders like Parkinson and Huntington's disease are some examples of mitochondrial disorders due to defects in multi protein complexes of the ETC (Mimaki, et al., 2012; Fullerton et al., 2020; Fernndez-Vizarra and Zeviani, 2015). These defects are typically due to mitochondrial DNA mutations (Mayr et al., 2015; Vyas, Zaganjor and Haigis, 2016). Any defect in even one complex of electron transport chain ETC can alter the functions of other complexes for example, complex-III which is a master regulator of mitochondrial respiratory chain which can halt the assembly of complex I and biogenesis of Complex IV (cytochrome *c* oxidase) (Signes and Fernandez-Vizarra, 2018; Letts et al., 2019). Also, deficiency of Coenzyme Q_{10} (CoQ_{10}) an antioxidant and essential electron carrier of the mitochondrial respiratory chain has been linked to nephrotic conditions (Sacconi, et al., 2010; Shiau et al., 2022) and defects of Complex V are linked to fatal neonatal mitochondrial encephalopathy (Signes and Fernandez-Vizarra, 2018; Letts et al., 2019; Karolinska Institutet, 2021).

1.5 Mitochondrial Functions Evolving Therapeutic Targets

Cancer is a global health concern and one of the leading causes of death. It is a multifaceted disease caused by genetic, epigenetic, proteomic, transcriptomic, and metabolic changes (Hanahan and Weinberg, 2011; Alkhazraji et al., 2019; Cuyas et al., 2018). Nearly 10 million deaths are reported in 2020 according to the World Health Organization (WHO) report published in 2022, and among several benign and chronic types the most common types are, breast, lung, colon, rectum, and prostate cancers that are mainly caused by smoking, high body mass index (BMI), alcohol, poor lifestyle and certain infections in low- and middle-income countries such as hepatitis (Spivak et al., 2021; Schockel et al., 2015).

Breast cancer is the most common cancer particularly in women contributing 12.5% of the total number of newly diagnosed cancers in 2020 (Sung et al., 2021; Franze et al., 2020; Guo et al., 2019). The incredible inter- and intra- heterogeneity of cancer is attributed to mutated genes, their altered behaviors, and defective cellular pathways of respiration and metabolism (Rogalinska, 2016; Kozakiewicz, et al., 2021; Taanman, 1999; Vyas, Zaganjor and Haigis, 2016; Perciavalle et al., 2012; Gaude and Frezza, 2014; Stroud et al., 2016; Warburg, Wind and Negelein, 1927; Reichert and Neupert, 2004; Wallace, 2012). Based on various scientific studies published to date, it has become much clearer now that cancer cells show variable bioenergetics states, as some cancers rely on aerobic glycolysis while others on OXPHOS, and even metabolic coupling occurs in some aggressive cancers and cause chemoresistance. Otto Warburg has emphasized the occurrence of metabolic reprogramming in cancer cells since 1920 - the Warburg Effect. Though metabolic events are not always oncogenic drivers, it is more likely there is also some role in the activation of other associated cellular pathways that induce oncogenesis. For example, mitochondrial protein p32 is known to maintain high levels of OXPHOS, promote tumorigenesis in breast, thyroid, pancreas, skin, ovarian, prostate and colon cancers, by either acting as a metabolic switch between aerobic glycolysis and OXPHOS or activating oncogenic signalling pathways by stimulating Akt and mTORC proteins and promoting proliferation. The full impact of p32 on mitochondrial activity is still unknown, but it has also been shown to regulate Ca^{2+} concentrations in the mitochondrial matrix, which is coupled with the process of OXPHOS. P32 mitochondrial protein could be a potential therapeutic target considering the antiapoptotic role and as a positive modulator of Akt/mTORC signalling pathway. Based on current evidence, p32 is found to be primarily located in mitochondrial matrix, but it is also a multicompartment protein and found on plasma membrane and nucleus so, therefore its functional role needs further study as on nucleus p32 protein may interact with tumor

suppressor protein p53, altering its function, and also increases nuclear localization in cancer cells and on plasma membrane, facilitating cell migration (Yenugonda et al., 2017; Sotgia et al., 2011; Egusquiza-Alvarez, et al., 2021). Similarly, high levels of mitochondrial STAT3 and mitochondrial transcription factor A are also significant to sustain mitochondrial respiration in the process of KRAS induced lung cancer (Sotgia et al., 2011; Pfanner, Warscheid and Wiedemann, 2019). The translocases of the inner membrane (TIM) complexes are among several other protein transport machineries also linked to electron transport transmembrane potential. Based on mitochondrial proteomic studies, multiple protein import pathways could be potential therapeutic targets as they relate to bioenergetics and mitochondrial morphology (Schmidt, Pfanner, and Meisinger, 2010).

Moreover, each cell experiences about 1.5×10^5 oxidative insults per day and mitochondria are very sensitive to internal and external stressors and release several signalling molecules to maintain cell homeostasis. ROS produced in the cell as byproducts of metabolism and mitochondria are the main site of ROS production. Several species of ROS are produced in the cell, however, the most reported are superoxide ($O2-\bullet$), hydroxyl radical ($OH^{\bullet}$), nitric oxide (NO), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). ROS regulate various activities governed by kinase pathways such as such as mitogen activated protein kinases (MAPKs), Akt to promote cell growth (Yang, Anzo and Cohen, 2005; He et al., 2012; Latimer and Veal, 2016). Cell have naturally conserved mechanisms to balance ROS production, such as its ROS scavenging system through activation of the transcription factor FOXO3a, which induces expression of manganese superoxide dismutase (MnSOD),the chief ROS scavenging enzyme in mitochondria (Brand, 2016; Perillo et al., 2020; Sanz, 2016; Scialo, Fernandez-Ayala and Sanz, 2017b; Barcena, Mayoral and Quiros, 2018). It is very important to understand the role of levels of ROS, as a certain level of ROS is required to maintain normal cellular function. "Mitohormesis" is a cellular protective response where lower levels of ROS are induced to initiate diverse cytosolic and nuclear responses to improve life's expectancy through regulation of metabolism and immune system. Modulation of levels of ROS is very significant in designing ROS therapies used to either prevent or delay many chronic diseases (Ristow and Schmeisser, 2014; Palmeira et al., 2019; Lingappan, 2018; Holmstrom and Finkel, 2014).

The Mitochondrial membrane potential (MMP) is one of the most significant indicators of mitochondrial health and any prolonged fluctuations in MMP may cause bioenergetic stress. MMP, along with proton gradient (ΔpH), develops the transmembrane

potential of hydrogen ions which are used to synthesize ATP (Zorova et al., 2018). Modulation of OMM is not sufficient for the release of cytochrome *c* protein from the cristae, however, depolarization causes structural remodeling of the inner membrane which leads to the release of cytochrome *c* from cristae to the intermembrane space (Gottlieb et al., 2003; Sanz, 2016; Xie et al., 2020) (Figure 5).

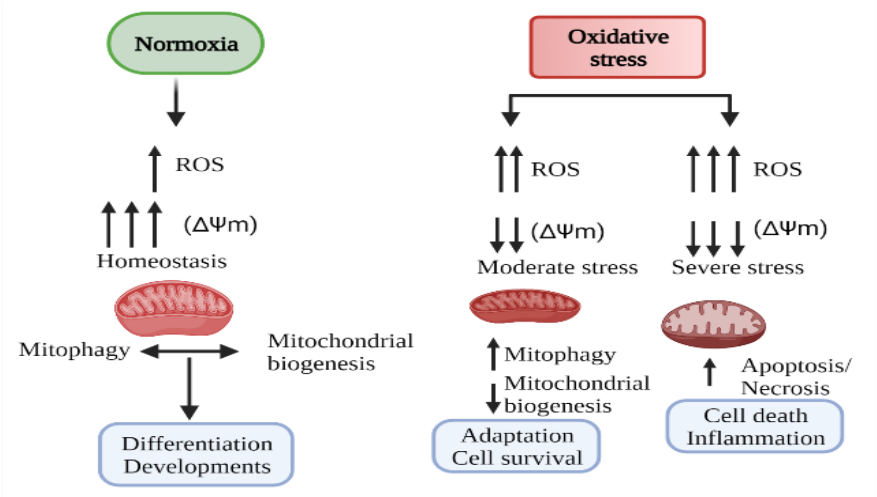


Figure 5: Illustration of ROS and MMP. Normoxia: is considered normal physiological state where slight increase in ROS levels promotes differentiation and development whereas under conditions of Oxidative stress with increase in ROS levels above celluar threshold limit leads to cell death due to depolarisation of mitochondrial membrane (Created with BioRender).

Impaired OXPHOS is another signal of mitochondrial dysfunction which is critical to produce ATP inside mitochondria. Mitochondria are the major source of ATP in the cell due to the presence of ETC on the IMM. Nicotinamide adenine dinucleotide (NAD) coenzyme is another indicator of mitochondrial health. It is synthesized from the simple amino acid building blocks aspartic acid and tryptophan and exists in two forms; an oxidized (NAD^+) and a reduced form (NADH) (Blacker et al., 2014; Yang et al., 2007; Srivastava, 2016; Williamson, Lund and Krebs, 1967; Wang et al., 2013). NAD^+/NADH ratio has significant effect on the redox state of the cell by controlling key enzymes such as glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and pyruvate dehydrogenase (PDH) (Zhang, Wang and Mo, 2018; Stein and Imai, 2012; Pittelli et al., 2011;Pumiputavon et al., 2017). The main role of NAD^+ in redox regulation is the reduction to NADH, as part of beta oxidation, glycolysis, and the citric acid cycle (Hopp, Gruter and Hottiger, 2019;). Emerging

roles of NAD^+ as an extracellular signalling molecule is also a significant discovery, although full impact on health and disease is still not clear, but it has been known to enhance glycolysis (Srivastava, 2016; Williamson, Lund and Kreb, 1967). Boosting NAD^+ through dietary supplementation improves range of physiological and pathological conditions (Xie, et al., 2020; Kotnik et al., 2017; Kuriyama and Maeda, 2019).

Intracellular Ca^{2+} concentration is another indicator of mitochondrial health. Though the ER is one of the main storage sites of Ca^{2+}, other organelles such as mitochondria, Golgi apparatus, lysosomes and peroxisomes also participate in Ca^{2+} signalling. How much Ca^{2+} homeostasis is crucial for the cellular system is yet to be explored, however Ca^{2+} overload results in oxidative stress and several pathological conditions, for example cardimyopathies (Shimizu et al., 2015; Marchi and Pinton, 2014; Carafoli, 2003; Peitzsch et al., 2017; Arnold et al., 2020).

There is exceptional potential of mitochondria as a therapeutic target for several diseases and various types of cancers and a recent focus of research is to target these intriguing organelles, however due to some discouraging findings that indicates variability of tumours for example multiform of glioblastoma (Reviewed by Hanif) and inter and intra tumor heterogeneity there is a need for more intensive research (Hanif et al., 2017). Even the same tumors differ in type of mutations from individual patients indicating tumors are unlikely to be treated by targeting a single pathway, gene or signalling molecule (Fisher, Pusztai and Swanton, 2013). So, a current trend is to design anti-cancer drugs that can specifically destabilise mitochondria named as “Mitocans”, which will be explained later in detail (Section 1.6).

Moreover, the body’s immune system is trained enough to recognize cancer cells but not cancer stem cells (CSCs) that are reproduced as normal, sustain harmful effects, cause relapse after treatment and result in failure of many immunotherapies (The Ludwig Center, 2021; De Francesco, Sotgia, and Lisanti, 2018; Cui, Wen and Huang, 2017; Kalyana-raman et al., 2018; Bobrovnikova-Marjon and Hurov, 2014; Falkowska et al., 2015).

1.6 Mitocans

After several years of research, now there is unprecedented focus on the discovery of more efficient drug candidates and invariant, clinically relevant therapeutic targets, due

to drug resistance, side effects of existing therapies, disease relapse and the differences in the patterns of even the same disease in different individuals. Developing mitocans is one such attempt in progress to develop anticancer agents that can specifically target certain genes, cellular pathways of complex diseases, including cancers, because aberrant mitochondrial metabolism in cancer also makes them more attractive therapeutic targets (Ralph et al., 2006; Neuzil et al., 2013; Spivak et al., 2021). Mitocans produce effects by destabilizing and altering the functions of mitochondria, such as ETC blockers (Figure 6) (Ralph et al., 2006; Rohlena et al., 2011; Neuzil et al., 2007; Dong et al., 2007; Chen et al., 2009; Guzzo et al., 2014). Scientists are expecting these mitocans will have fewer side effects on normal healthy cells. As the process of tumorigenesis and development are regulated by mitochondria, they could also offer a general approach for the effective treatment because of their functional role in most of the diseases like cancer, inflammation and aging (Baggetto and Testa-Parussini, 1990; Bernal et al., 1983; Ferrarini et al., 2021; Dong et al., 2011; Rohlenova et al., 2017; Neuzil et al., 2013; Ashkenazi et al., 2017; Ghoneum and Said, 2019; Du et al., 2019; Dong and Neuzil, 2019). Some of the main classes of mitocans are hexokinase inhibitors, ETC inhibitors, BcL-2 family of proteins inhibitors, inhibitors of VDAC/ANT channels, and lipophilic cations which target inner membrane, the citric acid cycle and mtDNA (Figure 6). Some examples of anticancer drugs showing effects through mitochondria are already in clinical trials and are listed in Table 3.

Table 3: Anti-cancer drugs and their mechanism of action on mitochondrial function.

Mitochondrial function	Mechanism of action/Tumor type	Clinical trial	References
ETC	Papuamine inhibits ATP production /lung	NA	Min et al., 2020
	Metformin inhibits Complex I/ Colon,Ovary, Breast, Prostate	CT	Wheaton et al., 2014
	Tamoxifen inhibits Complex I/ Breast	FA	Daurio et al., 2016
	Mito-Tam inhibits Complex I/ Breast	CT	Bryant et al., 2017
	α-TOS inhibits Complex II/ Breast	PC	Dong et al., 2008
	MitoVES inhibits Complex II/ Breast	PC	Dong et al., 2011
	VLX600 inhibits Complex IV/ Colon	PC	Zhang et al., 2014
	Tigecycline inhibits Complex I Inhibits Complex IV/ Leukemia	FA	Skrtic, et al., 2011
	Gamitrinib inhibits ATPase activity/ Prostate	PC	Chae et al., 2012
TCA Cycle	AGI-5198 inhibits IDHs activity/ Glioblastoma	CT	Golub et al., 2019
	Dichloroacetate inhibits IDHs activity/ Brain	CT	Dunbar et al., 2014
Glycolysis & OXPHOS	2-deoxyglucose (2-DG) competitor for binding hexokinase /Lung, Prostate,Ovary,Breast	CT	Xu, et al., 2018
	Metformin/2DG inhibits ATP production/ Lung, pancreas	CT	Cheng et al., 2012
	ABT737/2DG inhibits OXPHOS/ Ovary	NA	Xu et al., 2018
Signalling pathways	Venetoclax Bcl-x_L inhibitor/ Leukemia, Lymphoma	FA	Aghvami et al., 2018
	Navitoclax Bcl-Xl/Bcl2 inhibitor/ Breast, Lung Prostate, Colon	CT	Cui, Wen, and Huang, 2017
	ECPU-0001 Bcl2 inhibitor/ Lung	PC	Mongre et al., 2019
	Gossypol LDHA inhibitor, NADH competitor/ Breast, Brain, Prostate	CT	Missiroli et al.,2020

Abbreviations: ETC (electron transport chain); TCA Cycle (tricarboxylic acid cycle); OXPHOS (Oxidative phosphorylation); IDH (isocitrate dehydrogenase); ATP (adenosine triphosphate); LDHA (lactate dehydrogenase); NADH (nicotinamide adenine dinucleotide) (Dong et al., 2020). Abbreviation: CT(Clinial trial), PC(Preclinical),FA (FDA Approved)

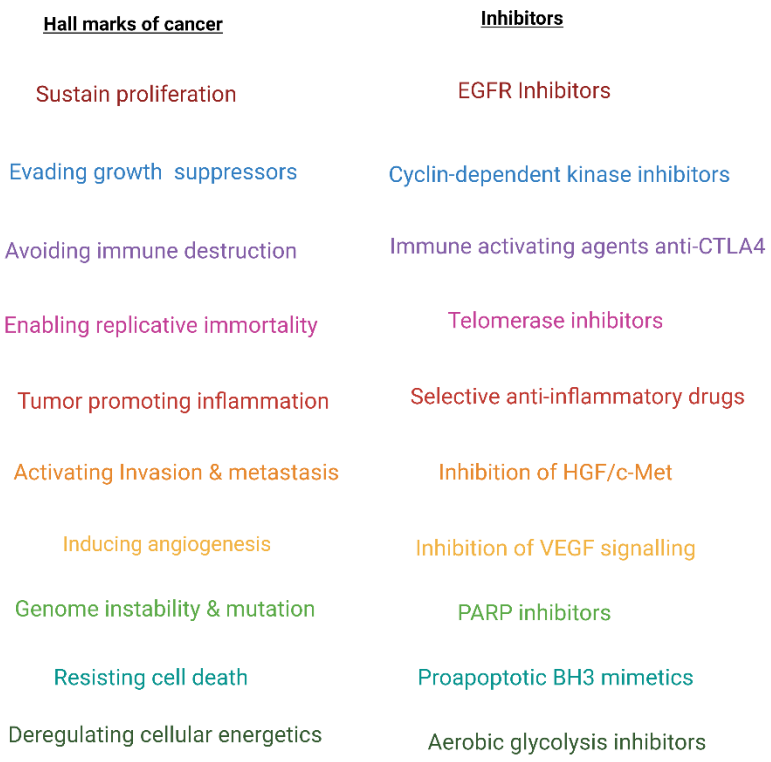


Figure 6: Hallmarks of cancer and their inhibitors: Original hallmarks (self-sufficiency in growth signals, insensitivity to anti-growth signals, tissue invasion and metastasis, limitless replicative potential, sustained angiogenesis (blood vessel growth) and evasion of apoptosis), enabling factors (Inflammation and instability genes), emerging hallmarks (Reprogramming metabolism and immune evasion) (Created with Biorender).

1.7 Mitochondria and Phytotherapy

The tradition and knowledge of drug development from natural sources, particularly plants based, is centuries old, for example morphine (*Papaver somniferum* L.) and digoxin (*Digitalis purpurea* L./ (Wachtel-Galor and Benzie, 2011). Plants, either used for medicinal purposes or as functional foods, have provided several classes of bioactive compounds (micro molecules and macro molecules), including phenolics, alkaloids, flavonoids, which are rich in valuable bioactivities, such as antioxidant, anti-inflammatory, anticancer, antibacterial, or antiviral activities (Koo, Song and Bae, 2018; Rahman et al., 2011; Shati et al., 2020). However, the exact pathways through which phyto-active compounds produce their effects are still not clear, due to the diverse nature of most of these bioactive compounds. How-

ever, some compounds show characteristic and distinctive mechanisms, such as the sulforaphane effect through cancer stem cell self-renewal pathway (Liu, Min and Bao, 2009), curcumin targeting a plethora of signalling pathways in pancreatic cancer (Glienke et al., 2009; Schutte et al., 1998), capsaicin homovanillic acid derivative targeting Trx-ASK1 signalling and mitochondrial transport chain during apoptosis (Pramanik and Srivastava, 2012), epigallocatechin-3-gallate stimulating mitochondrial membrane depolarization (Qanungo et al., 2005; Urra et al., 2013; Li et al., 2017), resveratrol inhibiting proliferation through hedgehog signalling pathway (Mo et al., 2011), polymethoxylated flavones inducing Ca^{+2} mediated apoptosis, magnolol inhibiting EGFR/PI3K/Akt signalling pathway (Lee, Park and Roh, 2019), lectins targeting programmed cell death pathways (Fu et al., 2011), ascorbic acid affects bone cancer, chlorbenzoyl berbamine (natural derivative of berbamine) modulating NF-κB and JNK signalling pathways (Yun et al., 2016;Zhu et al., 2020; Wang et al., 2015; Plitzko, Kaweesa and Loesgen, 2017). Considering the above evidence and the association of mitochondria with different diseases and signalling pathways, mitochondria prove to be a promising model for testing of biological activities of new bioactive compounds (Table 4) (Yu et al., 2022; Liu et al., 2019; Sinha et al.,2019; Fernandes et al., 2017; Arauna et al., 2019; Cho et al., 2019).

Table 4: Natural Compounds Affecting Mitochondrial Functions

Compounds	Cell line	Mechanism	Reference
Lupeol (*Bombax ceiba* L.*)*	Renal cell carcinoma SK-RC-45 cells	Altered mitochondrial dynamics Increased fission Increased apoptosis	Sinha et al., 2019 Zhang et al., 2015 Zhang et al., 2017
Fucoidan (Marine brown algae)	MCF-7, MDA-MB-231, HeLa	Increased DNA fragmentation Decreased MMP Increased apoptosis	Zhang et al., 2011 Ayman, May, Mohammad, 2021
Ganoleuconin (*Ganoderma leucocontextum T.*)	Liver cancer cell line Huh7.5	Decreased ATP production Increased apoptosis Decreased MMP	Sinha et al., 2019 He et al., 2016
Betulinic acid (*Betula pendula R.*)	HeLa cells	Increased ROS, Decreased MMP	Xu, et al., 2017
Genipin (*Gardenia jasminoides* J.)	Lipid bilayer membranes	Decreased uncoupling protein 2 (UCP2)	Kreiter, et al., 2019
Alternol (*Taxus baccata* L.)	PC-3 prostate cancer cells	Decreased mitochondrial respiration, Decreased isocitric acid, fumaric acid, malic acid	Li et al., 2019 Liu et al., 2007 Zhang et al., 2018 Chan, et al., 2015
Dihydromyricetin (*Ampelopsis grossedentata/ Nekemias grossed-entata* (Hand.-Mazz.) J.Wen & Z.)	HepG2 cells	Increased Bax and Bad Decreased apoptosis	Zhang, et al., 2017
Cathachunine (*Catharanthus roseus* L.)	HL60 cells	Increased ROS mediated intrinsic apoptosis Decreased MMP	Wang et al., 2016
Dentatin (*Clausena excavate* B.)	HepG2 cells	Increased cytoplasmic cytochrome *c* Increased Bax Decreased Bcl-2	Gong et al., 2017
Plumbagin (*Plumbago zeylanica* L.)	MG63	Increased ROS Altered protein levels of Bcl-2, Bax, Bcl-xL, Bak	Chao et al., 2017
Aellinane (*Euphorbia aellenii* R.)	Ovarian cancer cell line	Increased apoptosis via mitochondrial pathway	Nabatchian et al., 2017

1.8 Scope of Complementary Medicine in Pakistan

Pakistan has a rich tradition of using medicinal plants for the treatment of various health related issues including cancer and rheumatism (Kanwal and Sherazi, 2017; Welz, Emberger-Klein and Menrad, 2018). Along with advanced medical treatments, traditional medicine is still widely practiced in Pakistan as complementary and alternative medicine (CAM), mainly due to limited health care facilities and poverty. Ayurvedic and homeopathic system of medicines have been accepted as a part of national health care system of Pakistan.

Pakistan holds a great diversity of flora due to its distinct geographical position and diverse climatic conditions. More than 6,000 vascular plant species and 5,600 species having 22 families and 150 genera are described in the flora of Pakistan (Stewart, 1972; Ali and Qaiser, 1986; Mallon, 1991). Over 1,000 species have been evaluated for possible phytochemicals and around 350-400 species are traded and used by the manufacturers of Unani and Homeopathic medicines. There is very high demand of exploring the medicinal plants of Pakistan as there are around 40,000-50,000 practitioners of Greco-Arabic medicine, Ayurvedic and folk medicine locally known as Tabibs and Vaids in addition to unregistered practitioners of rural areas utilize more than 200 plants in the preparation of their medicinal recipes. Though there are some challenges that need consideration, such as over exploitation of resources due to unsustainable use of medicinal plants by local people and herbal medicine manufacturers, scientists (pharmacologist/biochemist/botanist) and pharmaceutical companies, who process large quantities of medicinal plants to obtain small quantities of biologically active compounds (Shinwari and Shah, 2007; Shinwari and Shinwari, 2010; Ekor, 2014).

1.9 *Alhagi maurorum* Medik: Multipurpose Herb

Alhagi maurorum M. (Fabaceae) is a multipurpose herb commonly used in the Greco-Roman and the Unani system of medicines (Measer, 2019; Xu et al., 2021). Folklore claims about the use of Alhagi species are reported in various countries such as Egypt (gastrointestinal track - GIT, liver and UTI disorders), India (laxative, diuretic, asthma, rheumatism, fever), Iran (kidney stones, laxative, cardiac pains, diuretic), China (rheumatism, cancer), Palestine (urinary tract disorders), Turkey (tonic), Jordan (kidney stones), Afghanistan (diarrhea, jaundice, skin

wounds, appetizer), Pakistan (eyesight improvement, antirheumatic, stomachache), Qatar (migraine, rheumatism, jaundice), other South Asian countries (hemorrhoids, GIT), Saudi Arabia (analgesic, antioxidant, diuretic, cough, angina, antipyretic, anti-tussive, dysenteries, gastritis), Uzbekistan (eczema) (reviewed by Tavassoli et al., 2020). However, it is mainly explored due to its antioxidant potential and nutritive value (Sarkar et al., 2020; Davinelli et al., 2013). *Alhagi maurorum* is a rich source of biologically active compounds, including phenolics and alkaloids (Samejo et al., 2012; Loizzo et al., 2014; The Plant List, 2010; EOL, Encyclopedia of the life, 2014). Almost all parts of *Alhagi maurorum* plant are used in traditional system of medicine. Its phytochemistry and its use in Greco-Roman and Unani system of medicine make it a promising medicinal plant. It is marketed under the name of "Toranjabin" (Atta and Abo, 2004; Measer., 2019; Ara et al., 2020; Aslam et al., 2016). Ardekani reported in his study about temperament of this plant in Iranian traditional medicinal system as hot and dry and suggested it is due to the presence of its constituent alkaloidal amines (Parvinroo et al., 2014; Ardekani, Rahimi, and Mohammad, 2010; Atta et al., 2010). Furthermore, previously published research studies have ascribed therapeutic properties of anti-rheumatism and nutritive value to alkaloids present in plant extract, for example phenethylamine (tyramine, hordenine, β- phenethylamine (β-PEA), N-methyl mescaline (Irsfeld, Spadafore and Prub, 2013:Rafehi et al., 2021), isoquinoline alkaloid (salsolidine) (Tundis et al., 2012) and pyrrole (pyrrolizidine, pyrrolezanthine) alkaloids (Guan et al., 2020; Thu et al., 2020). Some pharmacological evaluation of different extracts prepared in different solvents have already been reported as cytotoxic effects by Sulaiman using leaves and flowers extract on the human leukaemia cell line (IC_{50} 16.0-22.0 μg/ml) (Asghari et al., 2016; Sulaiman et al., 2013). Its gastroprotective effects in rabbit using aqueous ethanolic extract and aqueous extract, anti-inflammatory effect, antipyretic effect using ethanolic extract of root, hepatoprotective effect using ethanolic extract of aerial parts in albino rats, and antifungal effect using methanolic extract have already been investigated (Sheweita et al., 2016; Neamah, 2012; Alqasoumi et al., 2008; Ara et al., 2020; Aslam et al., 2016; Ahmad et al., 2010).

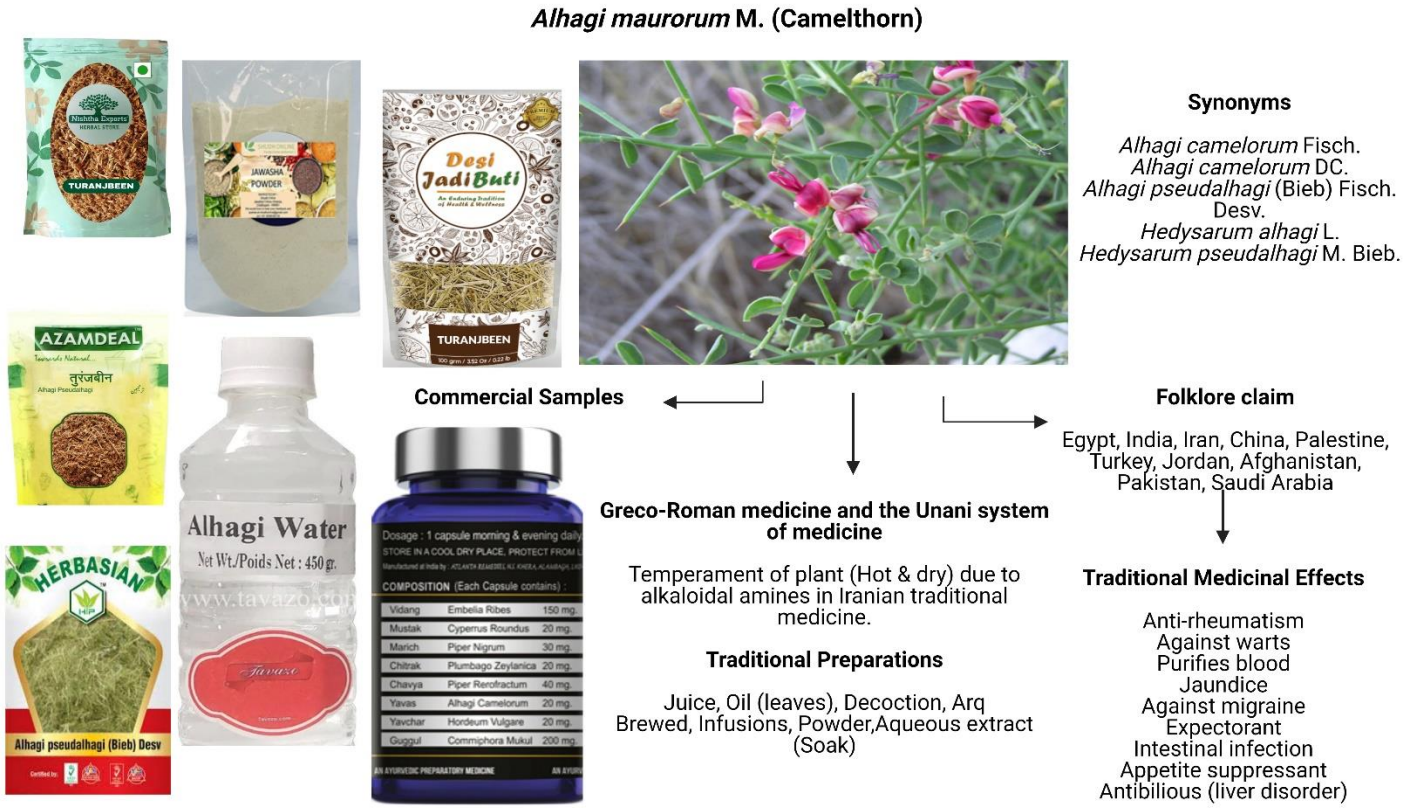


Figure 7: *Alhagi maurorum's* commercially available samples, folklore claims in different regions of the world and different form of traditional preparations used to achieve traditional medicinal effects in different traditional system of medicine mainly Unani and Greco- Roman (Alchetron, 2018; Asghari and Tewari, 2007) (Created with Biorender).

In addition to pharmacological evaluation, chemical investigation using fresh stems of *Alhagi maurorum* by Ghosal and Srivastava showed the presence of mixture of alkaloids such as tyramine derivative, isoquinoline and pyrrole alkaloids in addition to other chemical compounds (Ghosal and Srivastava, 1973; Asghari and Tewari, 2007) (Table 5). Most of the alkaloids are under investigation but very limited data is available about their pharmacological effects, pyrrolizidine alkaloids have been investigated for their anti-inflammatory effects (Wei, Ruan and Vrieling, 2021; Hoang et al., 2015; Kaltner, 2020; Kazemi, Fatemi, and Wink, 2018), salsolidine has a potential role in the treatment of Alzheimer's disease (Tundis et al., 2009), tyramine and it's derivatives acts as catecholamine releasing agent and shares pharmacological properties with hordenine but unable to cross the blood brain barrier and have highly variable pharmacokinetics in humans (Rafehi et al., 2019), N-methyl mescaline has been investigated as a pharmacological chaperone design for reducing risk factor of Parkinson's disease (dysfunction of glucocerebrosidase enzyme that result in mitochondrial dysfunction) to treat misfolded protein (Huang, 2014) and β- phenethylamine (β-PEA) acts as a CNS stimulant in humans (Irsfeld et al., 2013) and hordenine consumed as supplement.

Alhagi maurorum is considered as a cheaper and ever-available source for lupeol (a bioactive triterpenoid) which is also found in root bark. *Alhagi* honey contain sugar exudates polysaccharides (galactose, uronic acid) (Gonchaov, 2001), epigeal parts of plants contain catechins, proanthocyanidins (Islambekov et al., 1982) whereas whole plant extract reported to contain, octadecane, eicosane; di-, tri- and tetra-cosane, triterpene and derivatives (Ghosal and Srivastava, 1973; Ghosal and Srivastava, 2016), alhacidin and alhacin (Muhammad et al., 2015). There are some studies which have reported alhagifoline A, pseudalhagin, alhacin, alhacidin (Al-Snafi, 2015; Li et al., 2010), lupeol (Laghari et al., 2011) in addition to steroids, alkaloids from the aerial parts of this plant (Sultan et al., 2011; Xiuwei et al., 1996; Guijie et al., 2010 and Laghari et al., 2012b) and showed presence of twelve different compounds of new iso flavonolignan and flavanol, alhagitin and alhagidin from the root bark. Furthermore, some studies have been published about the contents of the seed oils of plant *Alhagi maurorum* and found presence of saturated and unsaturated fatty acids and some metals such as Cu, Al, Mn (Hashem and Alfarhan, 1993; Singh et al., 1999) (Table 5).

So far, quantitative data of the most of the compounds of Alhagi spp. is not available, though some studies have reported presence of 4.22 mg/g of phenolic acid (200 g root powder), 8.36 ± 1.33 of alkaloids The n-butanol fraction exhibited the highest total phenolic content (20.78 ± 0.63 μg gallic acid eq./mg), while the ethyl acetate fraction exhibited the highest total flavonoids (14.41 ± 0.03 μg quercetin eq./mg) and tannins (9.44 ± 0.36μg) (Mostafa et al., 2019)

Table 5: *Alhagi maurorum*: Pharmacological effects

Biological System	**Pharmacological Effects**	**Extract**	**References**
Gastrointestinal	Curing stomach, intestinal complaints, liver ailments (Hepatoprotective)	Ethanolic	Alqasoumi, 2007
	Anti-inflammatory (reduces the thickness of paw edema)	Aqueous	Neamah, 2012
	Anti-diarrheal (Increased contraction duodenal smooth muscles (low conc.), sedative effect (high conc.)	Methanolic	Gutierrez et al., 2007
	Anti-ulcerogenic effect (Decreased acid output)	Ethanolic	Shaker, Mahmoud, Mnaa, 2010
Systemic	Antioxidant effect (Decreased malondialdehyde levels) Antioxidant effect (scavenging free radicals)	Aqueous Alcoholic	Neamah, 2012 Awaad et al., 2011
	Anti-tumour (increase immune activity)	Extract	Laghari et al., 2012
Urinary tract	Enlargement of ureter to expel stones	Ethanolic	Marashdah, and Al-Hazimi, 2010.
	Diuretic, reduction of pH and crystalluria	Extract	Mirdeilami, et al., 2011
Antimicrobial	Antibacterial activity	Methanolic	Bonjar, 2004
	Antifungal activity	Ethanolic	Al-Askar, 2012
Blood	Decrease bilirubin levels, serum creatinine phosphate level and lipid per oxidation	Extract	Nabavizadeh and Nabavi, 2010 Khushbaktova et al., 1992
CNS	Antinociceptive (protection against writhing)	Ethanolic	Awaad et al., 2011
	Antinociceptive (traditional use)	Methanolic	Hudaib et al., 2008
Genome	Genotoxicity (causes DNA damage at a conc. Of 5µg/ml)	Extract	Etebari et al., 2012

1.10 Phenethylamine Alkaloid: Hordenine

There is now enough evidence available to support that alkaloids have had a significant role in medicine since ancient times and remained a source of medicines. For example, psilocin affecting perception, caffeine stimulating CNS, morphine and cocaine relieving pain (Avois et al., 2006), cephalosporin, and penicillin being used as antibiotics (Cushnie, and Lamb, 2014), quinine as antimalarial drug (Achan et al., 2011). Phenethylamine (PEA) is a natural monoamine alkaloid and trace amine marketed as dietary supplement mainly to increase cognitive functions and to reduce weight (Andersen and Fogh, 2001; Galitzky et al., 1988; Seneca, 2007; Kaltner et al., 2020). Hordenine is an organic compound synthesized from tyrosine (Figure 9) (for detailed steps of synthesis see Figure 10), also known as eremursine, N,N-dimethyltyramine, peyocactine, *p*-hydroxy-N,N-dimethylphenethylamine, 4-[2-(Dimethylamino) ethyl] phenol and occurs naturally in wide range of plants such as *Ariocarpus fissuratus* E., *Hordeum vulgare* L., (Ghosal and Srivastava et al., 1972; Schultes, 1937), *Panicum miliaceum* L. (Brady and Tyler, 1958), *Lophophora williamsii* L. (Rao, 1970), *Schinus terebinthifolia* R., *Citus aurantium* F. (Mahmoud et al., 2021; Smith, 1977; Weaton and Stewart, 1970; Kim, Lee et al., 2013; Reilly, 1981) and *Anahalonium fissuratum* E (Spath and Roder, 1922; Spath, 1919; Kapadia and Fayez, 1970; Lima et al., 2015). It has taken its name from *Hordeum vulgare* (Barley) member of grass family which is one of the major cereal grains cultivated 10,000 years ago (Guo et al., 2018; Roman, Betz and Hildreth, 2007; Xiong, Li, and Jin, 2014). *Tamarindus indica* L. is traditionally used as an antiasthmatic agent and is reported to be a promising anti-diabetic drug containing hordenine in its leaves, bark and even flowers (Saxena et al., 2022). So, possibly diuretic, psychostimulatory, and anti-obesity activities of *Tamarindus indica* are due to hordenine (Ghosh and Das, 2010). In addition to the above-mentioned plants, hordenine is also one of the interesting alkaloids reported from *Cannabis sativa* L. (Atakan, 2012). Hordenine is structurally related to ephedrine, a central nervous system stimulant, has adrenergic-like actions and releases neurotransmitters epinephrine (adrenaline) and norepinephrine (noradrenaline) to increase energy and focus. Hordenine is also a vital ingredient of nutritional supplements marketed with the claim as CNS stimulant, nootropic agents (increase energy level), it lifts the mood, promotes

weight loss, and increases athletic performance. Some examples of these supplements are synephrine, higenamine and hordenine, which are marketed as the best pre-workout stimulants, improve ability to use fat for fuel and promote mental arousal. Some examples of supplements available in the market are shown in Figure 8 (Efimova, et al., 2020; Lovett, Hoult, and Christen, 1994; Li et al., 2020).

Figure 8: Commercially available dietary supplements containing hordenine.

Dietary supplements are easily available in pharmacies, food stores and groceries without any prescription, so, they are used as complementary therapy globally (Kotnik et al., 2017; Kuriyama and Maeda, 2019; Anders and Schroeter, 2017; Li et al., 2017; Liu, Ye and Guo, 2020). However, like all other medicines, dietary supplements can also cause allergic reactions, adverse effects and interact with prescription medicines (Dwyer, Coates and Smith, 2018; Omar et al., 2019; NCCIHT, 2021) therefore these should also be consumed based on scientific evidence (Brady and Tyler, 1958; Webmed, 2022).

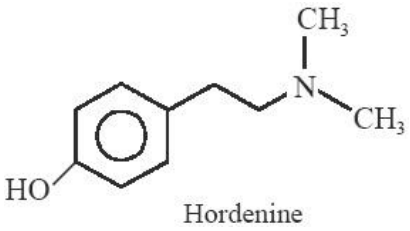


Figure 9: Structure of hordenine

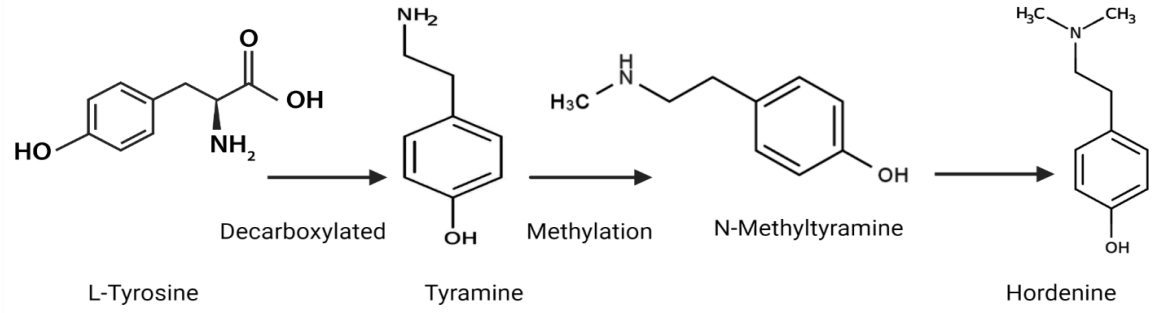


Figure 10: Biosynthesis of hordenine in three steps: Hordenine is produced in plants from tyramine in a stepwise methylation process (Leete and Marion, 1954; Stribney and Kirkwood, 1954; Thomas, 1963; Massicot and Marion, 1957; Brady and Tyler, 1958. In 1937 Raoul proposed based on the structural similarity of hordenine and tyrosine following series of three step reaction of biosynthesis of hordenine: (I) L-Tyrosine decarboxylated to tyramine (II) Methylation of tyramine to N-methyltyramine (III) Methylation of N-methyltyramine to hordenine (Raoul, 1937; Frank and Marion, 1956; Liu and Lovett, 1993a-1993b).

Additionally, not only the availability of hordenine from different sources but also the pharmacological features and physicochemical properties make it a potential candidate for research as it is a low molecular weight compound with amphoteric in nature, selectively deaminated by MAO-B, crosses the blood brain barrier, and has short half-life of 1-2hr (Barwell, 1989; Lebecque et al., 2018).

Pharmacokinetics studies of hordenine have shown bioavailability of 66.2% after oral administration in a rat model and in humans after one hour of beer consumption maximum plasma levels of 12.0-17.3 nM were achieved (Ma et al., 2015; Sommer et al., 2020). In 2020, Sobiech designed and utilized magnetic core-shell molecularly imprinted

sorbent to study pharmacokinetics of the hordenine present in human plasma after consuming dietary supplements and noted highest concentration of 16.4 ± 7.8 μg/l (65 ± 14 min), $t_{1/2}$ 54 ± 19 min and V_d (volume of distribution) 6000 ± 2600 (66 ± 24 l/kg) (Sobiech et al., 2020).

In vitro and *in vivo* studies of hordenine

In vitro: Hordenine and Cellular Processes

1) Mitogen Activated Kinase (MAPK) Signalling Pathway

As the prevalence of pituitary tumors in general population is very high and prolactinomas are the most recurrent pituitary tumors (Daly et al., 2009; Ciccarelli et al., 2005). To restore pituitary functions therapeutic aim is to lower excessive serum prolactin (PRL) levels and the size of the tumor. Dopamine under physiological conditions is responsible for inhibiting prolactin production. Dopamine D2 receptors are expressed on the membranes of lactotroph cells (Ben-Jonathan and Hnasko, 2001; Wong et al., 2015). Wang et al., (2020) investigated the effect of hordenine extracted from traditional Chinese herb *Hordeum vulgare* (Wang et al., 2014) on prolactinomas. Wang et al., (2020) investigated the antiprolactinoma effect of hordenine in a rat model and showed decreased level of the p38, ERK1/2, and JNK protein expression and the production of inflammatory cytokines TNF-α, IL-1β, and IL-6 possibly through MAPK signalling pathway. Hordenine significantly reduced the size of pituitary tumors in rats and restored serum prolactin levels, and he proposed hordenine inhibited the production of PRL by regulating MAPKs signalling (Lu, Wang and Zhan, 2019; Weichert et al., 2015; Hu et al., 2017). MAPK is a key signalling pathway involved in several physiological and pathological functions by activating three sequentially activated protein kinases (See Figure 11 for detailed process) (Zhan, 2019).

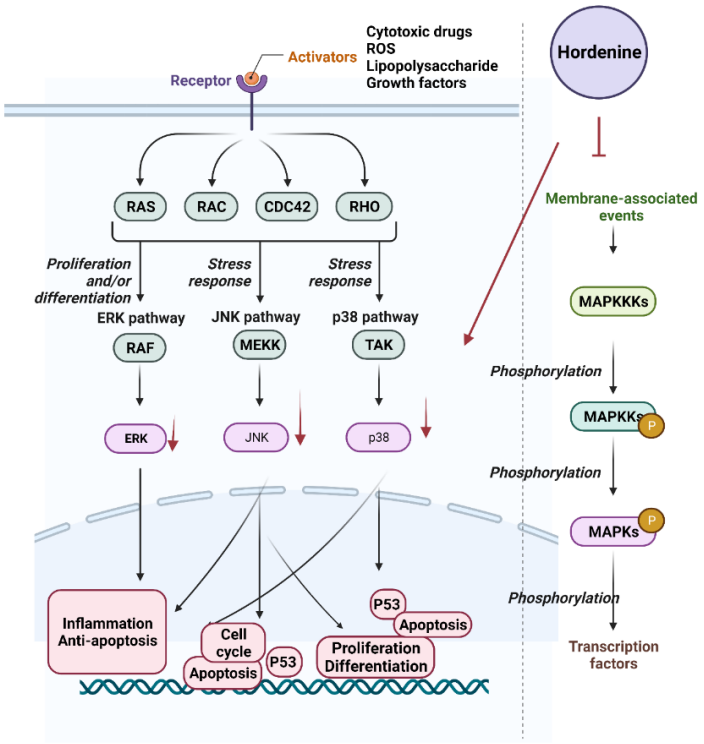


Figure 11: Hordenine modulation of MAPK pathway. Extracellular signal-regulate kinase (ERK1/2), Jun kinase (JNK), MAPK. Each MAPK pathway is initiated by diverse extracellular as well as intracellular stressors such as oxidative stress, ER stress, reductive stress, hormones, and growth factors. Stress response is a very broad term encompassing several molecular mechanisms that are initiated depending on the nature, strength, duration of the stress and cell types. Protein phosphorylation is the most rapid and effective response initiated against stress among many others. (Created with BioRender).

Wang et al., in 2014 further showed hordenine has inhibited PRL secretion through DRD2 agonist which further regulate cyclic adenosine monophosphate (cAMP)/PKA/CREB pathways in two cells; high expression of estrogen induced rat pituitary tumor MMQ cells and low expression of radiation induced rat pituitary tumor GH3 cell. However, this study also investigated total barley maiya alkaloids and proposed synergistic phenomena responsible for efficient way of reducing PRL levels (Figure 12) (Sussman et al., 2020; Gong, Tao, Wang, et al., 2021; Recouvreux et al., 2016; Yang et al., 2020; Gao et al., 2017).

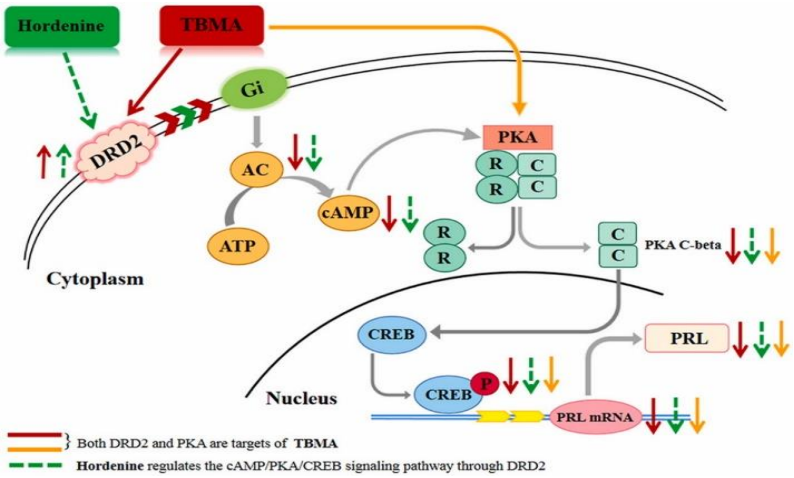


Figure 12: Hordenine and synergistic effect. cAMP Response Element Binding Protein (CREB), Dopamine D2 Receptor (DRD2), Mesenger RNA (mRNA), PKA (Protein Kinase Pathway), PRL (Prolactin), Protein Kinase A (PKA), Total Barley Maiya Alkaloid (TBMA) (Wang et al., 2014; Lu et al., 2019)

2) Pyruvate Dehydrogenase Kinase (PDK)

Protein kinases are important drug targets, governing many cellular pathways in various diseases, including cancers and metabolic disorders. Nuclear-encoded mitochondrial multienzyme complex the pyruvate dehydrogenase (PDH) complex provides primary link between glycolysis and TCA cycle. The enzymatic activity of PDH involves phosphorylation and dephosphorylation cycles, regulated by products and substrates of the reaction inside mitochondria (Gudi, et al., 1995; Xu et al., 2019). The phosphorylation of PDH results in inactivation of its enzymatic activity completely due to highly specific pyruvate PDK. Pyruvate Dehydrogenase Kinase 3 (PDK3) plays a pivotal role in metabolic switching during progression of cancer. Anwar, et al. (2020) investigated effects of hordenine in lung cancer cell lines and suggested, hordenine is a potential inhibitor of PDK3 gene that in chemoresistance drives glycolysis by forming a positive feedback loop with HSF1. So, targeting this mitonuclear communication may represent a novel approach to overcome chemoresistance (Figure 13) (Anwar et al., 2021; Xu et al., 2021).

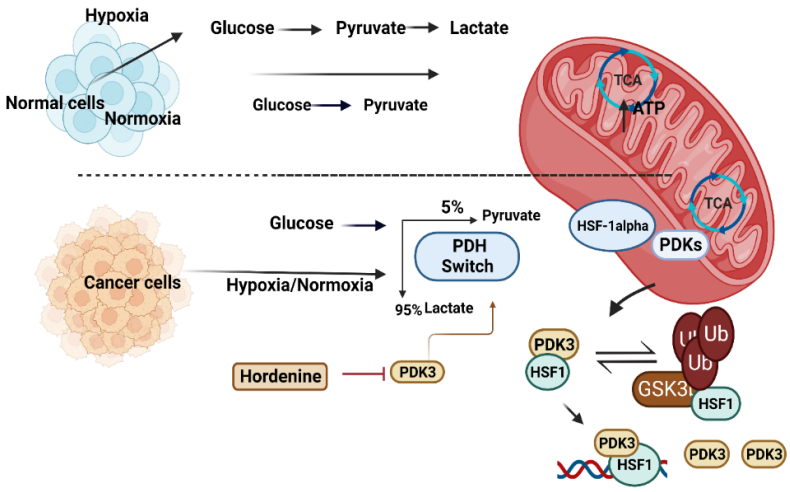


Figure 13: Hordenine binds and inhibits PDK3 gene and eventually activity of Pyruvate Dehydrogenase (PDH) is altered that offers metabolic switch between glycolysis and TCA cycle. Heat Shock Transcription Factor 1(HSF protein coding gene highly conserved and regulate transcriptional responses mainly non stress related such as metabolism and development), Glycogen Synthase Kinase-3 beta (GSK3b), Ubiquitin (Ub), Tricarboxylic Acid (TCA) (Anwar et al., 2021; Xu et al., 2021) (created with Biorender).

3) Nuclear Factor Erythroid 2–related Factor 2 (Nrf2)

The prevalence of diabetes and its associated complications is growing globally (Harding, et al., 2019). Diabetes is a disorder of glucose metabolism and may result in multiple organ failure. There is need for novel agents which either enhance or complement the actions of insulin (Wang et al., 2017). Shuhao et al., (2018) investigated the effect of hordenine alone and in combination with insulin. They observed significant effects in reduction of fasting and postprandial blood glucose levels. In addition to this, hordenine has shown significant renal protective activity (diabetic nephropathy) in combination with insulin. Authors suggested based on evidence the hordenine/insulin combination produced effects through inhibition of Nrf2 expression (Zhao et al., 2018; Su et al., 2018). Under unstressed conditions low levels of Nrf2 offer basal expression of its targeted genes while under stressed conditions cysteine modified KEAP1 (Kelch-like ECH-associated protein 1 (KEAP1) is unable to degrade Nrf2 and higher levels of antioxidant and pro-oxidant genes are produced (Figure 14).

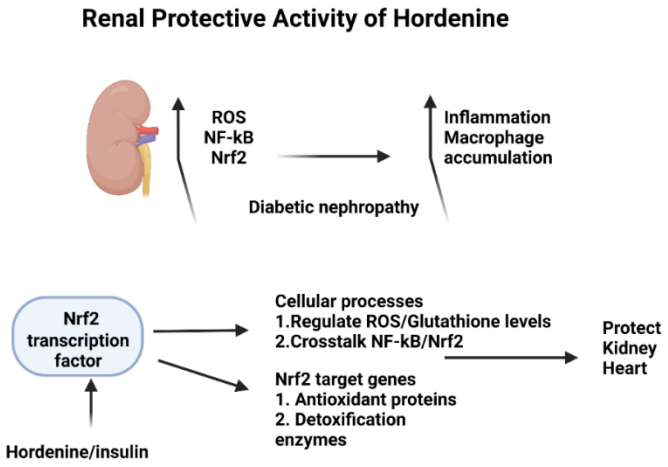


Figure 14: Renal protective activity of hordenine: Hordenine protects kidney through Nrf2 essential transcription factor and regulate multiple homeostatic functions such as inflammation, redox metabolism, and proteostasis. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) (Created with BioRender).

4) Cyclic Adenosine Monophosphate (cAMP)

Melanin protects human skin by absorbing free radicals and shielding from ultraviolet light. However, abnormal levels of melanin result in skin disorders. Many studies have shown cAMP-mediated pathways are the major signaling pathways involved in this process (Bos, 2006). cAMP is a second messenger used for intracellular signal transduction and is associated with the function of kinases in many biochemical processes such as sugar regulation and lipid metabolism. Activation of associated genes is linked to growth of cancer (Ali et al., 2016). Hordenine is reported to be produced as secondary metabolite during germination stage in barley so when germinated barley extract and ungerminated barley extracts were used in study, germinated barley extract exhibited potent inhibitory effect. Further studies confirmed hordenine involved in pigmenting activity through cAMP pathway and altered CREB (cAMP response element binding protein) phosphorylation as well as microphthalmia-associated transcription factor (MITF) associated genes expression (Bertolotto et al., 1998; Busca and Ballotti, 2000; Kim et al., 2013) (Detailed pathway see Figure 15).

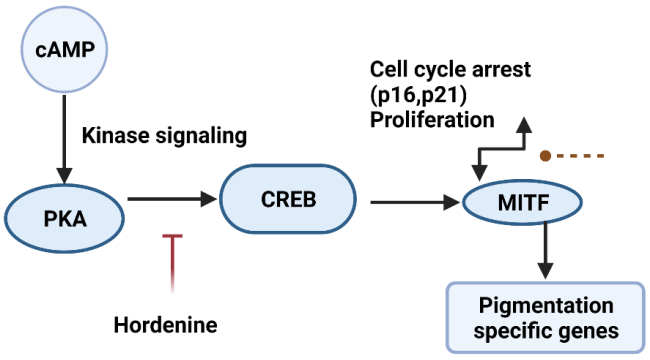


Figure 15: Hordenine and cAMP. Microphthalmia-associated transcription factor (MITF), CREB (cAMP response element binding protein) (created with Biorender).

5) Akt Signaling and Nuclear Factor κB (NF-κB)

Clinical syndromes of acute respiratory failure increase morbidity as well as mortality. If a patient survives an acute lung injury their quality of life remains affected. Prevalence of acute lung injury and acute respiratory distress is so common that there is need to find new drugs (Johnson and Matthay, 2010). In a recent study, the effect of hordenine is investigated in acute lung injury induced by lipopolysaccharide. Protein kinase B (Akt) involves in various important cellular processes such as cell proliferation, apoptosis, metabolism of glucose and a complex protein NF-κB which leads to cytokine production, survival of cell and even DNA transcription. Hordenine has shown effect on the activation of both inflammatory factors. It also has been shown to affect the MAPK signalling, which is associated with heat shock, pro-inflammatory cytokines. Zhang et al., (2021) recently reported the effectiveness of hordenine treatment of acute lung injury both *in vitro* and *in vivo*.

6) Dopaminergic Pathway

Dopaminergic pathways are involved in hedonic food intake and can lead to overconsumption. Dopamine D2 receptor is particularly involved in food reward and ultimately increased consumption (Hoch, Kreitz, et al., 2015; Baik, J.H., 2013). Sommer, Hubner, Kerdawy, et al., (2017) observed hordenine caused D2R-promoted G-protein activation (Shonberg, Lopez et al., 2014). It also has shown functional selectivity by antagonized D2-

mediated β-arrestin recruitment. So, this study has associated mood elevating effects of beer to hordenine a component of beer though its concentration in beer depends on malting process (Brauers, Steiner and Daldrup, 2013). Hordenine is identified as food-derived dopamine receptor agonist (Sommer et al., 2020).

7) Hordenine Activating Serotonin Receptor

Serotonin (5-hydroxytryptamine) neurotransmitter is involved in complex, multifaceted biological functions. In humans it has diverse effects on mood, anxiety, sleep, appetite, GIT motility, eating, temperature (Beattie and Smith, 2008; Rosen, 2009; Collet et al., 2008). In humans 95% of 5-HT receptors are present in GIT which promote transmittance of signals from the gut lumen to nerve cells and smooth muscles. So, many studies have reported it regulates digestive functions, bone mass and organ development too. Hordenine (antibiofilm) activates 5 HTR 4b in human colon epithelial cells which is associated with wound healing and increased cell motility (Yasi et al., 2019).

8) Hordenine Selectivity for Monoamine Oxidase (MAO)

Monoamine oxidases (MAO) catalyze oxidation of amines and are present in the outer membrane of mitochondria in various cell types. Neurotransmitters are inactivated by MAOs. Normal levels of MAOs have clinical significance as imbalance is linked to many diseases such as depression, attention deficit disorder, schizophrenia, migraines (Domino and Khanna, 1976; Meyer et al., 2006; Filic et al., 2005). In a study deamination of hordenine was conducted in rats which showed its high selectivity for MAO-B in liver (Barwell et al., 1989; Fagervall and Ross, 1986) and is not deaminated by intestinal MAO-A. So, dietary hordenine is unlikely to be deaminated by intestinal MAO-A more likely will be absorbed and affect the sympathetic nervous system.

9) Hordenine Antibiofilm Agent

Hordenine extracted from sprouting barley has shown quorum sensing inhibitory activity against *Pseudomonas aeruginosa* (food borne pathogen) at lower concentrations. It also has inhibited acyl-homoserine lactones levels which is an important intercellular signaling molecule

through which bacteria monitor density of their population. It is synthesized by members of Luxl family of proteins. Treatment with hordenine decreased the quorum sensing-related extracellular factors and genes (lasI, lasR, rhlI) (Zhou, et al., 2018; Zohary and Hop, 2000).

In vivo studies

Hordenine remained the focus of research over a century ago, Hefter in 1894, found hordenine induced CNS paralysis in frogs without affecting blood circulation at lower doses however, with increased doses caused hypertension and eventually induced death. Rietschel, (1937), proposed the cause of through stimulation of the heart muscle (Rietschel, 1937; Jensen et al., 2021). Schweitzer and Wright, in 1938, studied derivatives of hordenine and reported dimethylcarbamic ester of hordenine hydrochloride, (D.-C.-Hord. -HCI) and dimethylcarbamic ester of hordenine methiodide, (D.-C.-Hord. -MeI) have similar anticholinesterase activity, group of drugs used to neurodegenerative diseases for example, Alzhemier disease and Parkinson diseases (Kapadia and Fayez, 1970). Frank et al., observed respiratory distress, increased heart rate and flehman response in horses after administration of hordenine (Frank et al., 1990; Hart, 1983*).* Overall, these studies highlighted the significance of hordenine for the treatment of CNS disorders and called for further study.

Hordenine Supplements

Thermogenic supplements are used by adults to boost energy and control body fat without scientific evidence (Hoffman et al., 2009). Many natural compounds are added to these supplements, for example, caffeine in combination with ephedra produces thermogenic effects targeting lipolytic metabolic pathway in fat adipocytes and reduces depression with addition of phenethylamine (Bell et al., 2004; Pittler and Ernst, 2004; Hoffman et al., 2006; Dulloo et al., 1999).

In a recent study on Meltdown® (SUP), which contains caffeine (increases resting metabolic rate), yerba mate extract (a stored fat mobilizer and appetite suppressant), tetradecylthioacetic acid (boosts mitochondrial fatty acid oxidation), methyl synephrine (acts as a mild stimulant), phenylethylamine (promotes mood elevation and lipolysis), yohimbine (facilitates lipolysis), and hordenine, researchers measured parameters including oxygen consumption (VO2) and respiratory quotient (RQ), energy expenditure, heart rate, fat oxidation rate, and blood pressure, the investigators concluded that the supplement effectively increased

acute energy expenditure and had a strong inotropic effect on heart rate and systolic blood pressure. However, the study also suggested supplements possess cardiovascular risks therefore should be used with caution (Roberts et al., 2005; Heck and De Mejia, 2007; Ro et al., 2015).

In 2017 a recipe of nutraceutical capsule containing hordenine, caffeine and β-phenylethylamine was patented with the claims that it enhances mental clarity and improves stamina, and energy (Allison et al., 2018; Jason, Guillermo and John, 2018).

In the most recent work on hordenine, Xu et al., showed effects of hordenine through shingilipid pathway for the treatment of ulcerative colitis which is also involved in several signal transduction pathways of cell proliferation, cell migration as well as inflammation. Overview of hordenine biological activities and cellular pathways reported in recent publications, see Table 6 and Figure 16. So, based on available evidence, hordenine has some potential to be researched as a new drug lead.

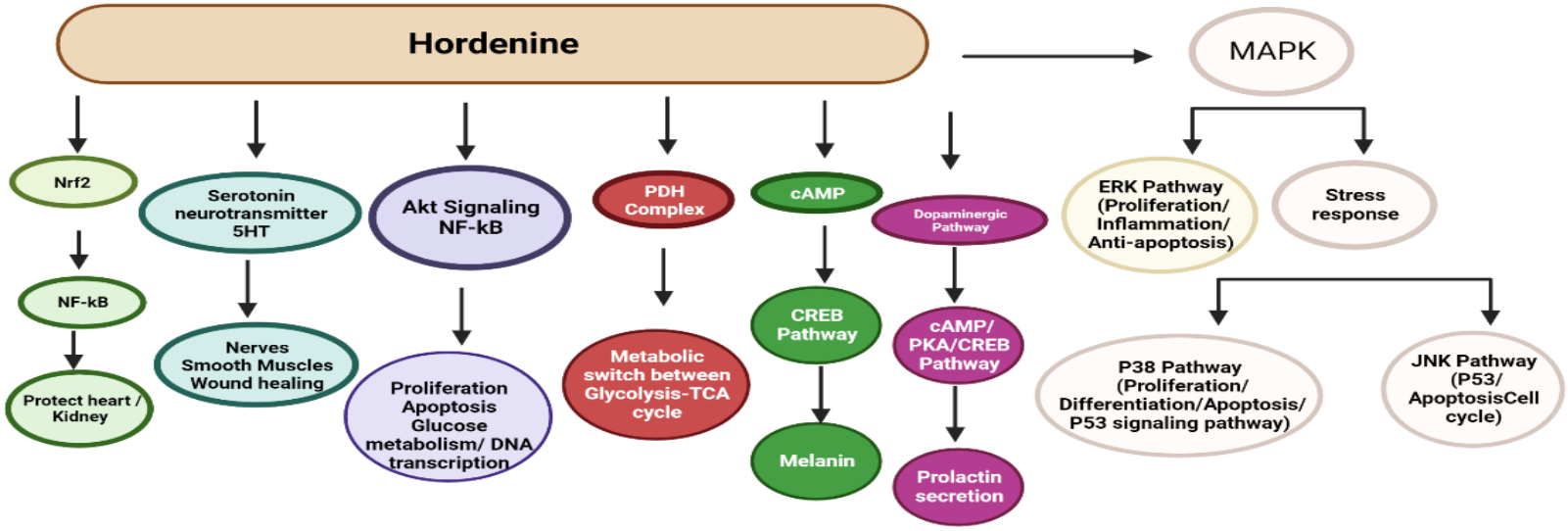


Figure 16: Hordenine and cellular pathways: Hordenine produce anti-prolactinoma effect in rat model through MAPK pathway supported by decline in Extracellular signal-regulated kinase (ERK1/2), Jun kinase (JNK), P38 protein expression level and the production of inflammatory cytokines TNF-α, IL-1β, and IL-6 and inhibited PRL secretion through DRD2 agonist which is regulated through cAMP /PKA/CREB pathways in two cell lines MMQ cells (high expression)-oestrogen-induced rat pituitary tumour cells and GH3 cells (low expression)- radiation-induced rat pituitary tumour cells: Hordenine is also potential inhibitor of PDK3 gene, an important drug target to block PDH complex a switch between glycolysis and citric acid cycle targeting this mitonuclear communication may represent a novel approach to overcome chemoresistance. Hordenine enhance or complement actions of insulin acitivating Nuclear Factor Erythroid 2–related Factor 2 (Nrf2), regulate melanin through cyclic Adenosine Monophosphate (cAMP):cAMP-mediated pathways that is CREB (cAMP response element binding protein) phosphorylation, treat acute lung injury through Protein kinase B (Akt) Signalling and Nuclear Factor κB (NF-κB) and heals wound through serotonin (5-hydroxytryptamine) (Created with Biorender).

Table 6: Biological Activities of Hordenine

Biological Activities	Concentration	References
In mouse colonic epithelial cells (MCECs). Effects on ulcerative colitis: reduced disease activity index (DAI),relieved colon oedema, lesions, inflammatory cells infiltration through sphingolipid pathway.	Dextran sodium sulphate (DSS) DSS+hordenine 50 mg/kg, DSS+hordenine 25 mg/kg, DSS+hordenine 12.5 mg/kg	Xu et al., 2023
PBCEC, CCK8 kit contain (lymphocytes (T cells, B cells, and NK cells), monocytes, and dendritic dendritic cells), human colon adenocarcinoma (Caco-2)	0.1 μM to 100 μM	Hahn et al., 2022
Hordenine protects against lipopolysaccharide-induced acute lung injury by inhibiting inflammation	12.5, 25, 50, 75–100 μg/ml	Zhang et al., 2021
Hordenine a potent inhibitor of PDK3 in human lung cancer cells (A549 and H1299)	5.4 μM	Anwar et al., 2020
Antiprolactinoma effect of hordenine by inhibiting MAPK signaling pathway activation in rats	152.8 ($p < 0.01$), 76.4 ($p < 0.01$), 38.2 mg/kg ($p < 0.05$)	Wang et al., 2020
Quorum sensing (QS) inhibitory activity	0.5 to 1.0 mg ml^{-1}	Zhou et al., 2018
Binds to and activates the dopamine D2 receptor	13 μM	Sommer et al., 2017
Anti-melanogenic effects of hordenine and its mechanism in human melanocytes	500 μM (Treated duration 5 days)	Kim et al., 2013
Hordenine exerts anti-melanogenic effects by inhibiting the cAMP signaling pathway	0-100 μM, (Treatment duration upto 5 days)	Kim et al., 2013
No measurable contraction of vasa deferentia in rat.	150 μM	Ghosh and Das, 2010
Significantly potentiated the tonic response to submaximal dose of nor adrenaline and inhibited those to Tyramine	25 μM	Barwell, 1989

Chapter 2

Materials and Methods

2.1 Plant Collection

Whole plant of *Alhagi maurorum* was collected from Pattoki, Pakistan. *Alhagi maurorum* is not very hardy in nature and grows in sunny season and alkaline soil. Around 10 kg of the plant was collected by farmers when it was in its flowering stage and were transported under the supervision of Assistant professor Shumaila Arshad, (The University of Lahore), Lahore, Pakistan, to the Botany department of the Government College University, Lahore, Pakistan (Figure 17). All samples used in this research were collected in a single lot from August – September 2020. *Alhagi maurorum* was washed three times with water to remove soil and any contaminant and then left to dry under shade which is a traditional method to preserve maximum phyto-constituents of the plants (Zhang et al., 2021; Gasecka et al., 2020; Roshanak, Rahimmalek and Goli, 2016).

Figure 17: Sampling area *Alhagi maurorum* (Map of Pakistan, 2022)

2.2 Plant Identification

Alhagi maurorum was authenticated for this research by Professor Zaheer-ud-Din (Botany department of Government College University (GCU), Lahore, Pakistan).

Figure 18: Sample of desiccated *Alhagi maurorum* used in this study.

Following organoleptic evaluation *Alhagi maurorum* was identified, briefly as thorny shrub, intricately branched, aerial parts were not very hard and greenish in colour. It generally grows 1 m tall, but roots penetrate up to 6 m (deep root system) in soil and were very hard along with rhizome. Greenish stems bear axillary spreading very sharp spines. Leaves are alternate, simple, ovate, entire, and elliptic. Petioles are short up to 2 mm long while stipules are 1 mm in length. Leaves are hairy and light green in colour. Flowers are pinkish to maroon having short pedicel. Flowers arise from axil and are not too many in number. Fruits are pod shaped and are reddish brown in colour. Authenticated voucher specimens were deposited in the Herbarium of GC University (**Herbarium code**: GC. Herb. Bot. 3332). Samples of the desiccated plant were transported to the University of Westminster, London, UK fully sealed contained in cardboard boxes by air using DHL service (Figure 18).

2.3 Plant Extraction

2.3.1 Pulverization

Dried soft stem parts were separated and pulverized to fine powder using a laboratory scale mini mill/commercial blender. Then the powder obtained after milling was passed through sieve of stainless steel of mesh number 70 (Aperture 125 µm) (Figure 19).

Figure 19: Mini Mill: Pulverization of *Alhagi maurorum*

2.3.2 Solvent Extraction

Different solvents were used in the extraction procedures based on some published studies (Chemat, Vian, Cravotto, 2012; Zhang, Lin & Ye, 2018; Gallo, 2022), including methanol, chloroform, ethanol, and water. 0.5gm of powdered sample was mixed with 5ml of each solvent in clean glass test tubes. All tubes were shaken and were allowed to stand for 30 minutes. The solutions obtained were then observed under visible light as well as UV light for the detection of their colour. Based on the colour results and published literature ethanol was selected as an appropriate solvent for the extraction. After selecting solvent different concentrations of ethanol (50%, 70%, 99.8%) were also tested for extraction. Plant material was macerated for 72hr and 70% ethanol was found to be more appropriate solvent for extraction because 99.8% ethanol extracted all types of organic compounds this includes polar and non-

polar compounds and even chlorophyl of the plant. So, based on these results 70% ethanol was chosen as the most effective solvent for extraction (Sultana, Anwar, and Ashraf, 2009; Cao et al., 2022; Urabee et al., 2021) (For the details of extracting solvent see Table 7).

Table 7: List of extraction solvents

Solvent	Percentage
Methanol (CH_3OH)	99.8% (Analytical grade) (Sigma, UK)
Chloroform ($CHCl_3$)	Anhydrous, ≥ 99%, (0.5-1.0% ethanol) as stabilizer (Sigma, UK). Analytical grade
Water	Distilled water
Ethanol (CH_3CH_2OH)	a. 99.8 % (Absolute ethanol) (Sigma, UK) b. 70% (70:30) c. 50% (50:50)

Fine powder of *Alhagi maurorum* was dissolved in 70% ethanol in a 1:10 ratio and solution was agitated three times a day. After 72hr of maceration, the macerant was removed and filtered through Whatmann filter paper #1 (medium fast) to remove residue/debri.

Then filtered extract was concentrated using rotary evaporator (Buchi Rotavapor R-215) under reduced pressure 100 mbar and at temperature not exceeding 40 °C. Concentrated extract was dried in incubator at 37 °C in petri dishes to increase the surface area. Dried extract was homogenized using pestle mortar and kept at -20 °C for experiments in tightly closed bottles. Extract was found to be soluble in 70% ethanol and thereafter extract was reconstituted in 70% ethanol for pharmacological testing.

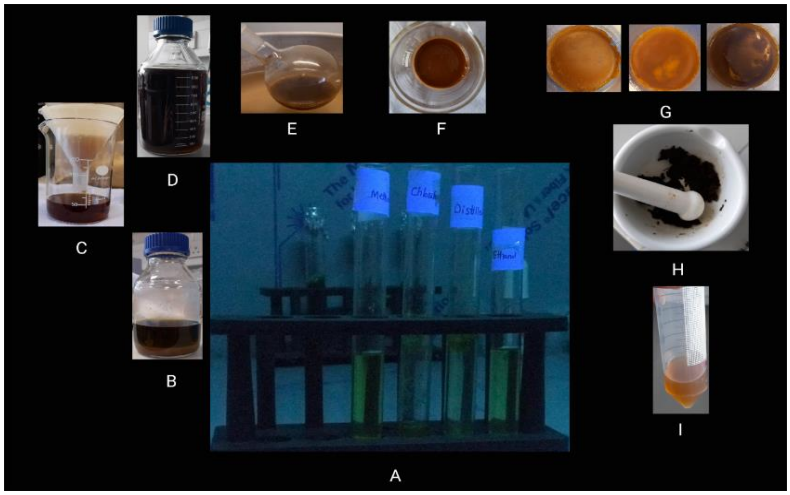


Figure 20: Schematics of extract preparation of *Alhagi maurorum* with 70% Ethanol Solvent A .Fluorescence analysis for the selection of suitable solvent B. Maceration of pulverized powder in 70% Ethanol for 72hr C. Filtration of macerated extract through Whatmann filter paper # 1 D. Filtered extract E. Solvent evaporation using rotary evaporator F. Concentrated extract G. Dried extract at 37°C H. Homogenization of extract using pestle and mortar I. Extract completely soluble in 70% ethanol which is used as solvent for cell culture experiments.

2.4 High Performance Thin Layer Chromatography analysis of *Alhagi maurorum* extract

High Performance Thin Layer Chromatography (HPTLC) (CAMAG, Muttenz, Switzerland) provided an excellent separation of components of the extracts (Varghese, 2013; Reich and Schibli, 2006). For HPTLC analysis chapters on HPTLC about herbal materials were used as a reference, published by The United States Pharmacopoeia (USP-NF 2015) and the European Pharmacopoeia (Ph. Eur. 2017).

After extracting the *Alhagi maurorum* (for details of extraction see Section 2.3.2) with ethanol 70%, characterization was performed with HPTLC. For quantitative analysis of constituents of dried extract of *Alhagi maurorum* dried extract 100 mg was reconstituted in 70% ethanol (1ml). The reconstituted sample was centrifuged at 3000 rpm for 5 minutes.

Reconstituted sample was filtered using 0.45 µm Nylon syringe filter. Various solvent combinations (mobile phase) were used to develop plates (for details of solvent see Table 13) and 2 µl sample was applied to HPTLC plate (Precoated Silica gel plates 60 F_{254} (Merck or equivalent), 10 x 10 cm) by Automatic TLC Sampler (ATS4) (CAMAG, Muttenz, Switzerland) using proper needle type on Silica coated plate band wise (8 mm) at a flow rate of 1.0 ml/min and column temperature was kept at 25°C. Plates were then dried for 20 minutes at 100°C. For the preparation of reference 100 mg hordenine powder was dissolved in 1ml of ethanol which was later used as a reference compound.

After successful sample application plate was moved to the Automatic Development Chamber (ADC2) (CAMAG, Muttenz, Switzerland) for the development of chromatogram saturated with preconditioning solvent and developing solvent under controlled conditions of humidity to final solvent migration distance of 70mm.

2.5 Liquid Chromatography-Mass Spectroscopic Analysis of *Alhagi maurorum* extract

To determine the exact molecular weight of the compounds in *Alhagi maurorum* extract Liquid Chromatography-Mass Spectroscopic (LC-MS) analysis was performed on Shimadzu LC-MS system in Chemistry Mass Spectrometry Facility, School of Pharmacy, University College London, and London, United Kingdom (Heyman and Meyer, 2012; Kamboj and Saluja, 2011). LC-MS spectrometer technique used combination of liquid chromatography (LC) and mass spectroscopy (MS) where LC allowed physical separation of analytes. Ions having strongest interaction with stationary phase left the column in the end and the time an analyte spends in the column is the characteristics of that analyte and is called retention time (RT) and response was captured in the form of peaks. It is one of the common methods used to identify compounds. Later separated ions in LC were driven into the mass spectrometer. A mass spectrometer (MS) an analytical instrument produces a beam of gas phase ions from analytes (sample) as a result sorting of mixture of ions based on m/z ratio when electrical or magnetic fields or both were applied. As a result, digital output signals in the form of peaks appeared and the m/z ratio and abundance or intensity of each ionic species detected was determined. 10 µl of *Alhagi maurorum* extract sample was introduced through direct infusion through syringe connected to the pump. Separation was achieved using mobile phase containing 0.2% formic

acid in water (A) and a mixture of ethanol and acetonitrile (1:1, v/v) used at the flow rate 0.3 ml/min. Shimadzu was used for data processing and reporting (Kowalczyk and Kwiatek, 2018; Hoffmann et al., 2014; Vassiliadis et al., 2019).

2.6 Cell Culture

All cell culture work was carried out aseptically to ensure that all cell lines were not infected with fungi, bacteria, or cross contaminated with other cell lines. Following are the processes, materials and equipment used in this research.

Chemicals

1. Hordenine, M.W (165.23). Analytical grade (HPLC (Assay > 97.5%); Impurities <3.0% water)
2. Foetal Bovine Serum -heat inactivated (Gibco®,Thermo Fisher Scientific UK)
3. Dulbecco's modified Eagle medium (DMEM)- liquid, high glucose with L-glutamine, D-glucose, sodium pyruvate (Gibco®, Thermo Fisher Scientific UK)
4. Phosphate Buffer Saline (PBS), pH 7.4 with potassium phosphate, sodium chloride and dibasic sodium phosphate (Gibco®, Fisher Scientific UK)
5. 0.25%TrypLE-EDTA liquid (Gibco®, Thermo Fisher Scientific UK)
6. Penicillin-Streptomycin (100X solution) (Gibco®, Thermo Fisher Scientific UK)
7. Dimethyl sulphoxide: DMSO (AnalaR BDH, UK)
8. Horse serum (Sigma, UK)
9. Glutamine (Sigma, UK)
10. Cholera toxin, 100 ng/ml of final volume (1 mg/ml stock) (Sigma, UK)
11. Insulin, 10 µg/ml of final volume (10 mg/ml stock) (Sigma, UK)
12. Extracellular growth factor, 20 ng/ml of final volume (100 µg/ml stock) (Sigma, UK)
13. Hydrocortisone, 0.5 mg/ml final volume, (1mg/ml stock) (Sigma, UK)

2.6.1 Cell Lines Used in Research

Two cell lines were used in this research MCF-7 and MCF-10-A. After defrosting, cells were seeded and incubated in humidified atmosphere, with in appropriate size T75-flasks, at 37°C, at 5% CO_2. Cells reaching 70% confluency were detached by using TrypLE-EDTA and were used for further analysis. Following a standard protocol, cells were seeded in 96 well plate for cell viability, ROS, MMP, intracellular Ca^{+2} assays and 24 well plate for Seahorse mitostress assay throughout the experiments.

1. Breast Cancer Cell Line (MCF-7)

MCF-7 is an adherent, human epithelial cancer cell line derived from a metastatic mammary adenocarcinoma cultured in Minimum Essential Media (MEM) (Sigma, UK) (Soule et al., 1990). MCF-7 cells cultured in Minimum Essential Media (MEM) (Sigma, UK) was also supplemented with 10% Fetal Bovine Serum (FBS), 1% l-glutamine and 1% penicillin/streptomycin.

MCF-7 is estrogen receptor-positive (ER+); progesterone receptor positive (PR+) and human epidermal growth factor receptor-2 negative (HER2-) breast cancer cell line. Low metastatic potential and presence of steroid receptors make MCF-7 cell line suitable *in vitro* model for several studies such as endocrine therapy, drug sensitivity (Horwitz, Costlow and McGuire, 1975). In addition to the above-mentioned features MCF-7 cells form domes, capable to grow in monolayers and are sensitive to cytokeratin (CK) structural proteins expressed by epithelial cells which help them to withstand mechanical stress (Camarillo et al., 2014).

2. Non-Cancerous Breast Cell Line (MCF-10-A)

MCF-10-A is an adherent, human epithelial non-cancerous breast cell line and found to exhibit immortality in low Ca^{2+} media (Soule et al., 1990). These were cultured in a mixture of Dulbecco's Modified Eagle's Media and Ham's F12 nutrient mix (DMEM: F12) (Life Sciences, UK), supplemented with 5% horse serum (Sigma, UK), 1% penicillin/streptomycin, 20 ng/ml epidermal growth factor (Sigma, UK), 0.5 mg/ml hydrocortisone (Sigma, UK), 100 ng/ml cholera toxin (Sigma, UK) and 10 µg/ml insulin (Sigma, UK).

MCF-10-A (human breast epithelial cell line) is a widely used *in vitro* non-cancerous breast epithelial cell model. MCF-10-A is estrogen receptor negative (ER-) and forms acinus-like

spheroids (3D model) a good feature to study the microenvironment of the cell. However, unlike MCF-7 cell line MCF-10-A lack anchorage-independent growth (Qu et al., 2015). Cells between passages 5 to 15 were used in this research and tested periodically for mycoplasma infection using the Plasmo Test Mycoplasma Detection Kit (Invivo Gen, UK).

2.6.2 Thawing Cell Lines from Liquid Nitrogen

Cell lines bought commercially and received in frozen form. So, cells must be thawed and cultured before use. Cells must be thawed rapidly and diluted slowly using warm growth medium to eliminate dimethyl sulfoxide, an organosulfur compound (DMSO) which is used as a cryoprotectant. DMSO also prevents water crystallization that would cause cell lysis during cryopreservation. Cells kept in liquid nitrogen were defrosted, transferred to T75 flask, and kept in incubator at appropriate temperature 37° C with 5% CO_2.

2.6.3 Subculture of Cell Lines (Cell Passaging/Cell Splitting)

Cells should be observed often to check their confluency as adherent cell lines continue to grow until the medium is depleted of nutrients. Once cell lines covered the surface area of the flask, they were sub-cultured after three days to prevent the culture dying as well as to increase the number of cells for different assays. Following aseptic procedure, media was aspirated off and discarded from flask. Cells were washed with PBS solution to remove dead cells from the flask and later detached following five to seven minutes incubation using TrypLE (10% of the total volume of media) which hydrolyses proteins that attach the cells to the bottom of the flask in case of adherent cells. After incubation detached cells transferred to 15ml falcon tube after adding fresh media and centrifuged at 1000G for about 5 minutes. Then supernatant was aspirated off and pellet is dispersed using fresh media. Then cells were transferred to new flasks labeled containing fresh media and kept in an incubator at 37°C, 5% CO_2.

2.6.4 Cell Seeding

Cells were seeded in consistent numbers for experiments. Old media from cells was aspirated and cells were washed using PBS buffer and were left in incubator after adding

TrypLE in incubator at 37°C, 5% CO_2. Once cells were detached after diluting with fresh media were transferred to falcon for centrifugation at 1000 G for 5 minutes. After centrifugation old media was aspirated off and pellet was resuspended in fresh media. For cell counting, cells (20 µl) were diluted with Trypan blue. Then cells were counted using Countess automated cell counter. A consistent number of cell concentrations were added to wells with the appropriate volume of media. Two different plates were used,

1) 100 µl total well volume for 96 well plates (25,000 cells per well)
2) 500 µl total well volume for Seahorse assay 24 well plate (30,000 cells per well)

Following equation is used to calculate the number of cells / well required for the experiments,

Seeding Density = Desired number of cells / Total number of cells X 1000

2.6.5 Freezing Cells for Storage in Liquid Nitrogen

Cells are frozen to be stored for future use, but the procedure should be carried out slowly to avoid osmotic shock which is caused by extracellular and intracellular ice formation and to minimize the effect of heat produced by DMSO when it is mixed to the water. Cells should be viable up to 90% at the time of freezing. Trypsinize cells using TrypLE followed by centrifugation step as explained previously. After the removal of supernatant from pellet add 90% of FBS and 10% DMSO and resuspend that are later stored into cryogenic vials and these vials are then stored into Liquid Nitrogen containers.

2.6.6 MTT Reduction Assay (Cell proliferation Assay)

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay is a colourimetric assay used to determine cell proliferation.). Principle of this assay is based on MTT (yellow tetrazole) reduction by mitochondrial reductase enzyme which is NADPH-dependent to Formazan (1-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan, Thiazolyl blue formazan) (purple) (Mosmann, 1983) (Figure 21).

For MTT Assay cultured cells were seeded in a 96-well plate at a density of 2.5 x 10^4 cells per well and left overnight to adhere. Cells were treated with different concentrations of AM extract/hordenine prepared in fresh media. Post treatment (AM extract for 24hr and hordenine for 6, 24, 48hr) cells were washed with PBS. Then fresh phenol red-free media (100 µL) was added in each well containing treated cells followed by addition of MTT solution (10 µl/well). Plates were covered in aluminum tinfoil to protect from light and incubated at 37°C, 5% CO_2, for 3hr. After incubation, media was aspirated, and resultant crystals were dissolved in 100 µl MTT solvent (DMSO) (Lysis buffer). Viability is quantified by ultraviolet (UV)-Vis spectrometry (SPECTROstar Nano, BMG Labtech, and Germany) with a microplate reader.

Equation,

Absorbance normalized = Absorbance (570nm) – Absorbance (690nm) (Background)

% Cell viability = Absorbance (Treated) / Absorbance (untreated control) X 100

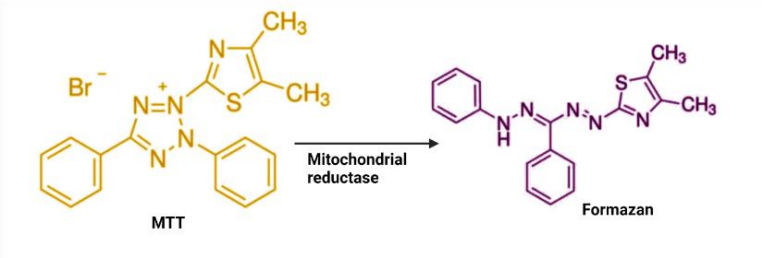


Figure 21: Cell Viability Assay: MTT assay for determination of Cell viability (Created with BioRender).

2.6.7 Detection and Quantification of Cellular ROS

Cellular ROS levels were investigated in this study using a 2',7'–dichlorofluorescein diacetate (DCFDA) assay (Abcam, UK). The fluorescence produced is due to presence of ROS which converts DCFDA into the highly fluorescent compound 2',7' –dichlorofluorescein (DCF) is detected and quantified using fluorescence spectroscopy (FLUOstar Optima, BMG Labtech, UK) (Excitation 485 nm, Emission 520 nm) (Henley et al., 2017; Keston and Brandt, 1965; LeBel et al., 1992). Cultured cells were seeded in a 96-well plate at a density of 2.5 x 10^4 cells per well and left overnight to adhere. Cells were treated with AM extract and hordenine prepared in fresh media. Post treatment, media was aspirated off and cells were washed with PBS (100 µl). Later the buffer was replaced with DCFDA stain prepared in 1X buffer. Treated plates were

incubated at 37°C for 45 minutes. DCFDA stain was removed and replaced with PBS (100 µl). Plates were covered in aluminum tin foil to avoid photobleaching effect. Fluorescence was measured using fluorescence plate reader using excitation/ emission filters (Table 8).

Table 8: Typical working concentrations of DCFDA

Preparation of reagents	
1 x Buffer	Sterilize ddH_2O using syringe filter. Mix 9 ml of sterile ddH_2O with 1ml of 10X buffer
DCFDA Stain	2.1 ml of 1X buffer and 2.1 µl of DCFDA stain (20 µM)

Equation,

% of ROS = As-Cb/C-Cb x 100

Where,

C= Fluorscence of control (Average of at least 3-5 wells)
Cb= Fluorscence of blank (Average of at least 3-5 wells)
As= Fluorscence of sample

2.6.8 Detection and Quantification of Mitochondrial Membrane Potential

TMRE (tetramethylrhodamine, ethyl ester) (Abcam, UK) is a cell based qualitative assay to assess the changes in membrane potential of active mitochondria. It is a red-orange dye, due to positive charge readily accumulates in mitochondria having relatively negative charge (Xu et al., 2021; Jaune et al., 2021). Cultured cells were seeded in a 96-well plate at a density of 2.5 x 10^4 cells per well and left overnight to adhere. Post treatment, cells were stained with TMRE prepared in fresh media (100 µl). Stained plates covered in aluminium foil were incubated at 37°C, 5% CO_2 environment for 30-35 minutes. The stain was aspirated off and cells were washed twice with PBS to remove background fluorescence caused by cell culture media and replace with PBS. Fluorescence was measured using fluorescence spectroscopy (FLUOstar Optima BMG Labtech, UK) and selecting appropriate filter set (excitation/emission (544 nm / 590 nm) (Table 9).

Table 9: Typical working concentrations of TMRE

Working Concentrations

Serial Dilution	1 µl of TMRE in 99 µl media (10 µM) 50 µl of 10 µM solution in 2.5ml (200 nm)

Equation,

% of MMP = As-Cb/C-Cb x 100

Where,

C= Fluorscence of control (Average of at least 3-5 wells)
Cb= Fluorscence of blank (Average of at least 3-5 wells)
As= Fluorscence of sample

2.6.9 Detection and Quantification of Intracellular Ca^{+2}

The acute intracellular Ca^{2+} levels are measured with the red fluorescent dye Rhod-2 (Abcam, UK). Basic principle involves the hydrolysis of the ester to Rhod-2 by cellular endogenous esterase which will bind to Ca^{2+} and once compound is in cells, localizes to mitochondria because ester itself is non-fluorescent and does not bind to Ca^{2+} directly. Chemical name of Rhod-2 is 1-[2-Amino-5-(3-dimethylamino-6-dimethylammonio-9-xanthenyl) phenoxy]-2-(2-amino-5-methylphenoxy) ethane-N,N,N',N'-tetraacetic acid, chloride (Dajindo, 2022)(Figure 22). Cultured cells were seeded in a 96-well plate at a density of 2.5 $x10^4$ cells per well and left overnight to adhere. Post treatment cells were stained with Rhod-2 prepared in fresh media (100 µl/well). Stained plates covered in aluminum foil and incubated at 37°C, 5% CO_2 environment for 40minutes.Then stain was aspirated from wells and cells were washed with PBS twice and replaced with PBS (100 µl/well). Fluorescence was measured using fluorescence spectroscopy (FLUOstar Optima, BMG Labtech, UK) and selecting appropriate filter set (excitation/emission (544 nm/590 nm) (Table 10).

Table 10: Typical working concentrations of Rhod-2

Working Concentration	
Dilute stock in media (1 µM-5 µM)	2.1 µl stock in 2.1 ml media (1 µM)

Equation,

% Of Intracellular Ca^{2+} = As-Cb/C-Cb X 100

Where,

C= Fluorscence of control (Average of at least 3-5 wells)
Cb= Fluorscence of blank (Average of at least 3-5 wells)
As= Fluorscence of sample

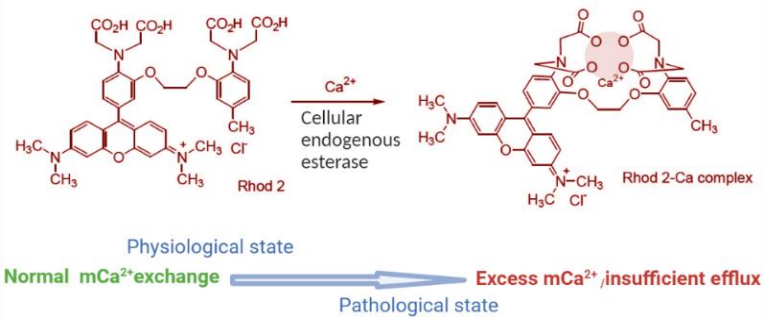


Figure 22: Acute Intracellular Ca^{2+} Assay. (Created with BioRender).

2.6.10 Seahorse Cell Mito Stress Assay

The mitochondrial respiration was measured using the Seahorse cell mitostress assay, the standard assay often used for assessing the mitochondrial respiration. The Agilent Seahorse XF delivers real time and direct measurements of mitochondrial metabolism with high specificity. Oxygen consumption rate (OCR) of plated cells was measured in real time by modulating ETC in cell well by adding modulators, oligomycin (Cheng et al., 2012; Kim et al., 2020), carbonyl cyanide-4 (trifluoromethoxy) phenylhydrazone (FCCP) (Reda et al., 2019), antimycin and rotenone (Sanchez-Alvarez et al., 2020) through built in injection ports on XF sensor cartridges (Figure 23) (Table 11).

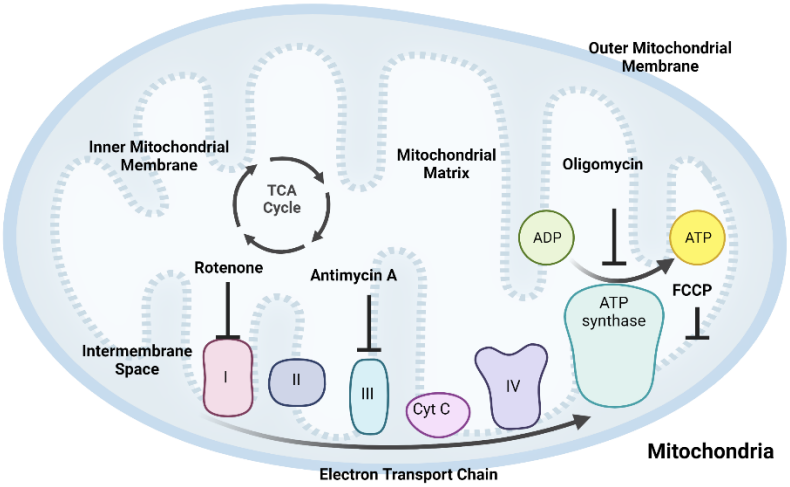


Figure 23: Mitochondria: Site of action of ETC modulators on large mitochondrial protein respiratory complexes located in inner mitochondrial membrane. Proton pumping complexes of ETC are mainly targeted in Mitostress assay. Oligomycin (Complex V/ATP Synthase inhibitor), FCCP (ETC uncoupling agent), Antimycin (Complex III/ ubiquinone-cytochrome c oxidoreductase inhibitor), Rotenone (Complex I/ NADH-ubiquinone oxidoreductase inhibitor) [(Agilent, 2021) (Image created with BioRender)].

On the first day cultured cells were seeded using XF24 cell culture microplates following two step seeding process. First, cells seeded to the desired final concentration in 100 µl of the growth medium without seeding in background correction wells (A1, B4, C3, D6). The seeded plate was incubated for 3hr to allow cells to adhere completely then 150 µl of growth medium was added to make the total volume in well 250 µl. Seeded plates were incubated overnight at 37°C, 5% CO_2. On the second day of treatment various concentrations of AM extract and hordenine were prepared in fresh media. Old media from wells was aspirated and fresh media containing treatments was added while keeping cell volume at this stage around 500 µl. Post treatment seeded plates were incubated at 37°C, 5% CO_2. 1 ml of XF Calibrant was added into each well of the Utility Plate and incubated overnight at 37°C in a non-CO_2 incubator. On the third day, the assay media was prepared by warming 49 ml of XF Base at 37°C and adding Sodium pyruvate (500 µl) and Glucose 200g/ml solution (500 µl). Treated cells were washed with assay media (500 µl) twice. After addition of assay media (500 µl) in each well including blanks, the plate was placed at 37°C in non-CO_2 incubator for 45 minutes. ETC modulators were prepared in the assay media as follows,

Table 11: Typical working concentrations of ETC modulators

Modulators	Stock	Final Conc.	Function	Effects on OCR
A. Oligomycin	5 mg/ml	1 µg/ml	ATP synthase inhibitor	Decrease
B. FCCP	10 mM	10 µM	Uncoupling agent	Increase
C. Antimycin + Rotenone	10 mM	10 µM	Complex III and I inhibitors	Decrease

Prepared modulators as described in Table 10 were loaded to ports A (55 µl), B (60 µl) and C (65 µl) of the utility plate. This calibration plate was loaded to Agilent Seahorse XF Analyzer and replaced after calibration step (15 minutes) with cell culture plate from non-CO_2 incubator (Kosgodage et al., 2018)

Table 12: Equations of key parameters of seahorse mito-stress assay (Agilent, 2021)

Parameter	Rate measurement equation
Non-Mitochondrial Respiration	Minimum rate measurement after rotenone/antimycin injection
Basal respiration	(Last rate measurement before injection of oligomycin) - (Non-mitochondrial respiration rate)
Maximal respiration	(Maximum rate measurement after FCCP injection) - (Non-mitochondrial respiration)
Proton (H+) leak	(Minimum rate measurement after oligomycin injection) – (Non-mitochondrial respiration)
ATP production	(Last rate measurement before injection of oligomycin) - (Minimum rate measurement after oligomycin injection)
Spare respiratory capacity	(Maximal Respiration) - (Basal respiration)

2.6.11 Morphological Analysis Using Fluorescent Microscopy

MCF-7 and MCF-10-A cells were seeded in 96 well plates and left overnight to adhere. After treating cells with *Alhagi maurorum* and hordenine they were observed under microscope. The Mito tracker deep red stain was used to observe the changes in mitochondrial morphology. MCF-7 and MCF-10-A cells were washed with PBS and replaced with phenol red free media

and stained with 100nM Mito tracker deep red dye for 35 minutes and were kept in incubator at 37 ºC, 5% CO_2. Cells were replaced with PBS and observed under microscope.

Statistical analysis

All statistical analysis was performed on Prism 5.04 (GraphPad) (San Diego, USA). For multiple comparisons and to find specific pair wise differences Ordinary One-way ANOVA test was applied, where mean of each group is compared to the mean of the control group was corrected by Dunnett's Multiple Comparison Test. Outlier test was applied to the data using Rout (robust regression and outlier removal) method based on false discovery rate which is recommended as it can find any number of outliers (definitive/likely) in the data. For some analysis one sample t test was also applied. Values are presented as mean ± standard error and p value =/<0.05 is considered statistically significant. Significant differences are indicated with asterisks (* if p< 0.05, **if p<0.01, *** if p<0.001, **** if p<0.0001).

Chapter 3

Results of *Alhagi maurorum* extract

3.1 Characterization of *Alhagi maurorum* with HPTLC

The high-performance thin layer chromatography (HPTLC) fingerprints of ethanolic extract of plant *Alhagi maurorum* showed the presence of a several phytochemicals as illustrated by different bands in (Figure 24). Various developing solvents were tried to develop chromatograms to find out the most suitable solvent. First, mobile phase used was composed of toluene, ethyl acetate and ethanol in the ratio of 70:30:5 reported for AM extract in literature (Chen et al., 2021; Hashim et al., 2016a-2017b) which separated the components of AM extract which is indicated by different bands in the chromatograms D (Figure 24). Then a mobile phase is composed of chloroform, methanol and con. Ammonia having ratios of 80:20:10 reported for hordenine was used for the AM extract and hordenine as a reference compound was also added (Ghosal and Srivastava, 1973; Varghese and Alka, 2013). Reference compound hordenine used was also dissolved in ethanol (100 mg/ml). A band appeared relative to the reference compound (hordenine) which indicated possible presence of hordenine in AM extract as shown in chromatogram B (Figure 24). Furthermore, one mobile phase composed of toluene, ethyl acetate, ethanol, and ammonia 25% was used which yielded less bands and indicated incomplete separation of constituents of AM extract as shown in chromatogram C (Figure 24). Mobile phase composed of ethyl acetate: methanol: ammonia 35% separated bioactive compounds of AM extract (Figure A) and then these developed plates were then dried. After drying plates were analysed at 254nm and 366nm using TLC Visualiser which were later derivatized and heated (CAMAG TLC Plate Heater) at 70 °C for 5 min. Finally, the cooled plates were analysed at different wavelengths of light (254nm, 366nm, white light (TLC Visualiser, CAMAG, Muttenz, Switzerland). A summary of the developing solvents is in Table 13 for reference. The peak tables were also generated and the RF values, peak height of unknown compounds were recorded (Hashim et al., 2016; Bian et al., 2022; Thammarat et al., 2021).

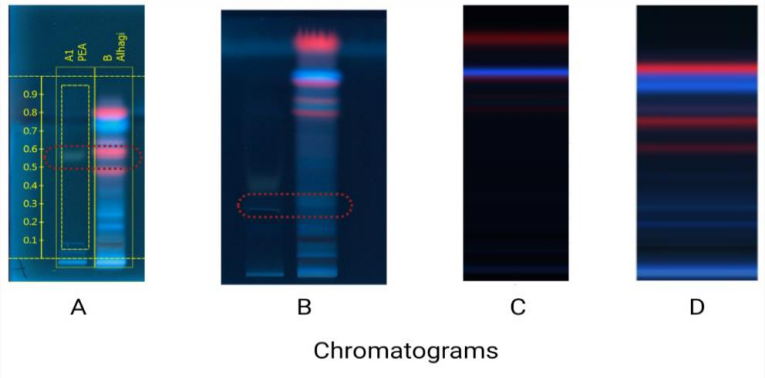


Figure 24: HPTLC Analysis: results view at wavelength 366nm. Separation of *Alhagi maurorum* extract in HPTLC analysis. Chromatograms for *Alhagi maurorum* obtained with different mobile phase. A. Ethyl acetate: Methanol: Ammonia 35% (Phenethylamine), B. Chloroform: Methanol: Conc. Ammonia (hordenine), C. Toluene: Methanol: Ammonia 25% (*Alhagi* extract) D. Toluene: Ethyl acetate: Ethanol (*Alhagi* extract).

Table 13: List of developing solvents for HPTLC analysis.

Sample	**Mobile Phase / Developing solvents**	**References**
Alhagi maurorum extract	Toluene: Ethyl acetate: Ethanol (70:30:5)	(Chen et al., 2021)
Alkaloids	Toluene: Ethyl acetate: Methanol: Ammonia 25% (30:30:15:1)	HPTLC Book
Phenethylamine	Ethyl acetate: Methanol: Ammonia 35% (85:10:5)	(www.camag.com, 2017)
Hordenine	Two solvent system: Solvent 1: Chloroform: Methanol: Conc. Ammonia (80:20:10) for hordenine detection Solvent 2: Chloroform: Methanol: Acetic Acid (75:15:10) beta phenethylamine	(Ghosal and Srivastava, 1973)

These sets of fingerprints of sample at 254 nm and 366 nm, and at white light are to derive values for migration distances (Rf) and corresponding intensities (AU). So, these

chromatograms hold two-dimensional information (Rf *vs.* AU). Fingerprints generated by HPTLC are richer source of information as various bands of different colours indicate different compounds (Islam et al., 2021). Previous literature has shown phenolic compounds exhibit different spot colouration when exposed to UV light specifically, flavonoids, show orange or yellow spots, flavones are dull brown in colour. Purple-red-orange indicates the presence of anthocyanins that are coloured water-soluble pigments. They appear as red, blue, and purple as different shades of blue colour were also detected which possibly indicate presence of different classes of phenolics. Simple phenolics (salicylic acids, gallic acids) dot fluoresces (UV light) and so, some dark absorbing blue spots were also observed. Dark and light blue indicate presence of coumaric, ferulic acid (Locher et al., 2017; Milojkovic et al., 2016). Violet blue indicates saponins and blue terpenoids. As different numbers of blue bands appeared when different developing solvents were used, therefore, more repeats are required to confirm these results. Bright reddish orange colour is also observed which indicates the presence of alkaloids. As it is very common to detect alkaloids because there are more than 10,000 alkaloids distributed in 300 different species of plants. Our results indicate presence of higher contents of flavonoids and alkaloids in 70% ethanol extract of *Alhagi maurorum.* Previously, reported literature indicated alkaloids are detected at Rf values 0.18-0.92 and AUC 2800-above 10,000 (Karthika and Paulsamy, 2015; Kulkarni et al., 2014) (Figure 25).

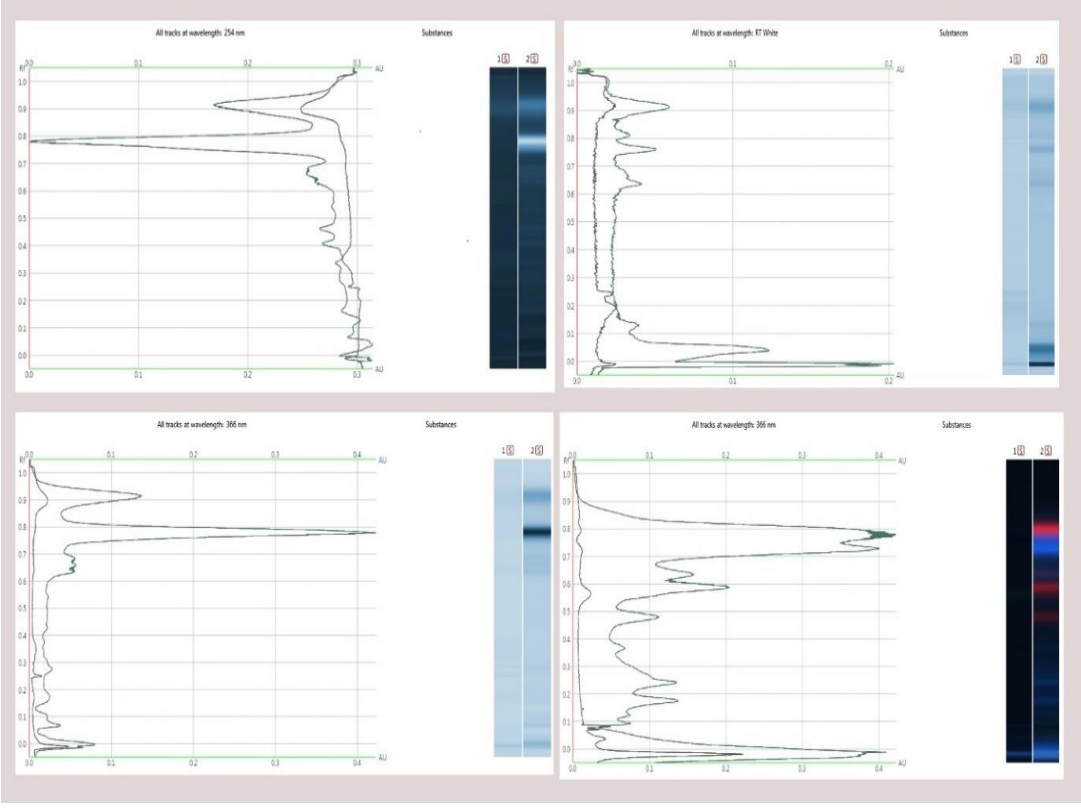


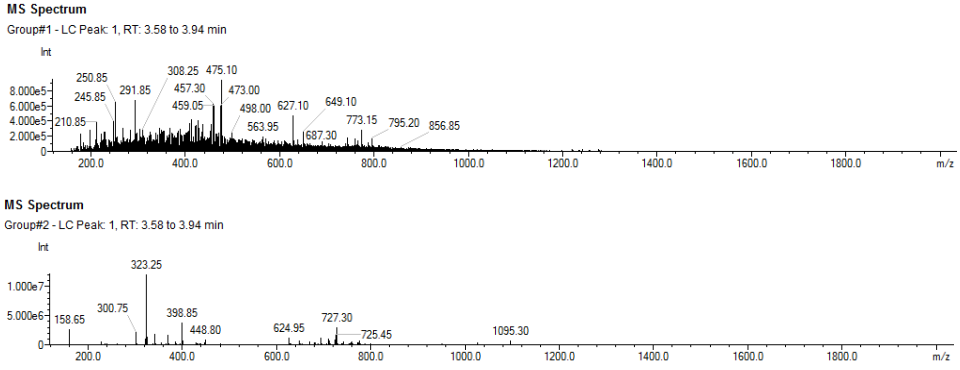
Figure 25: Detection of tracks of *Alhagi maurorum extract* and reference (hordenine) at different wavelengths (254 nm, RT White, 366 nm) in HPTLC analysis.

3.2 Characterization of *Alhagi maurorum* with Liquid Chromatography-Mass Spectroscopic

Liquid chromatography and mass spectroscopic analysis was performed. Data was acquired in the positive ionization mode (ESI+) and negative ionization mode (ESI-). Several compounds have been identified including phenolics and derivatives. Detection at 254 nm (Figure 26).

Screening of chemically diverse *Alhagi maurorum* for medicinal compound.

Peak 1[ES+/ES-]



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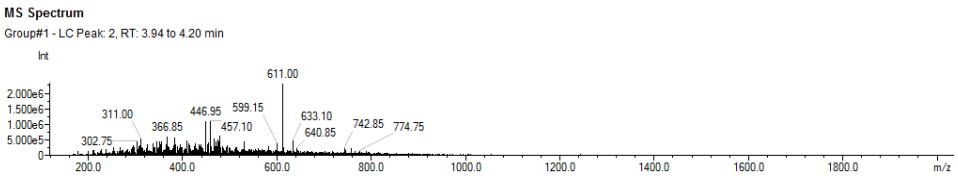
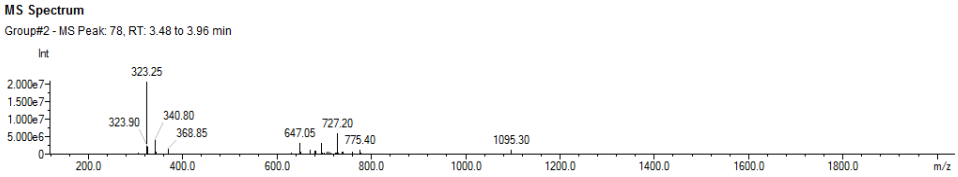
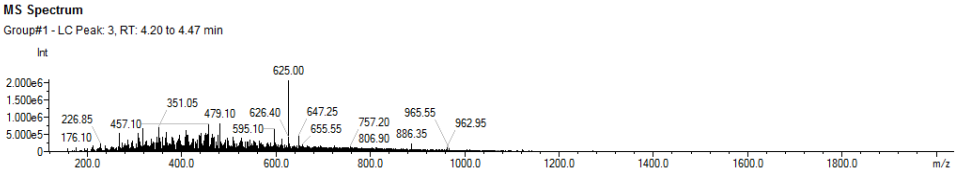
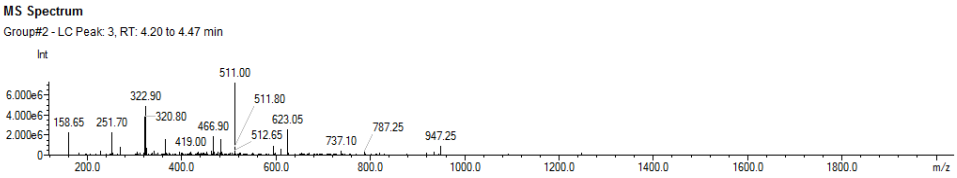




Peak 3





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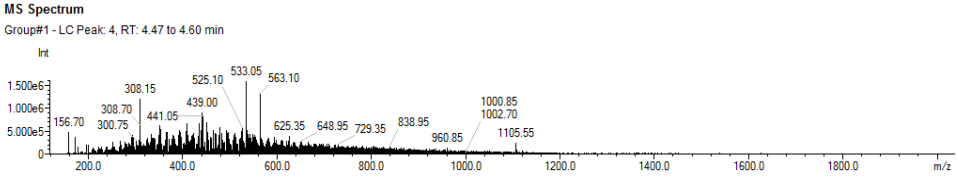

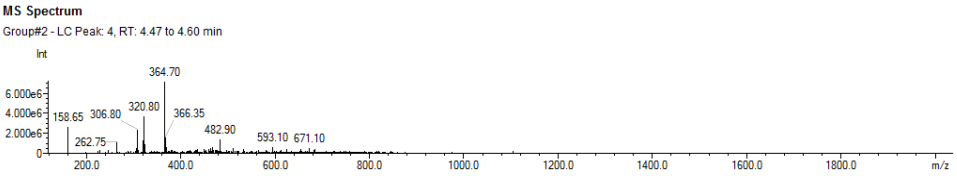

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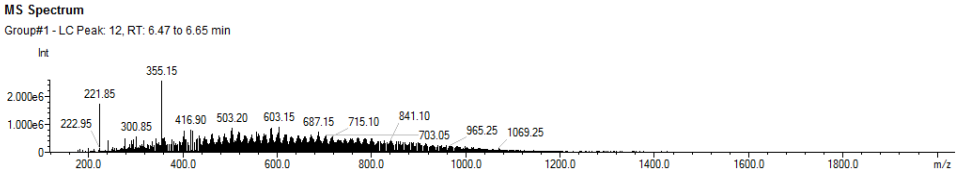

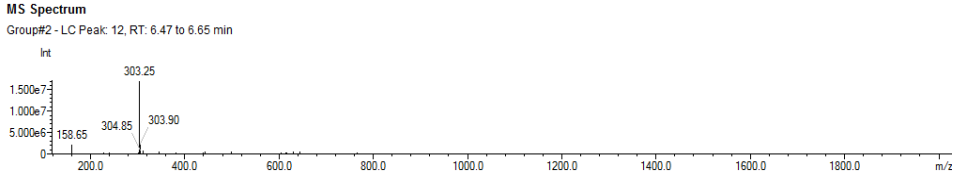

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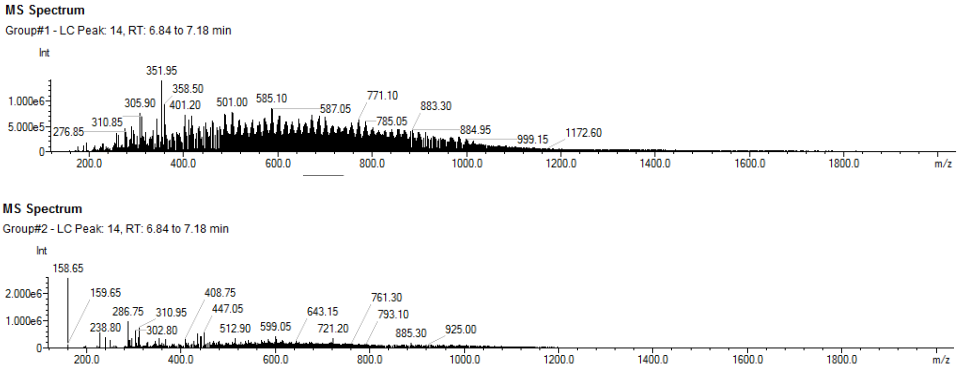

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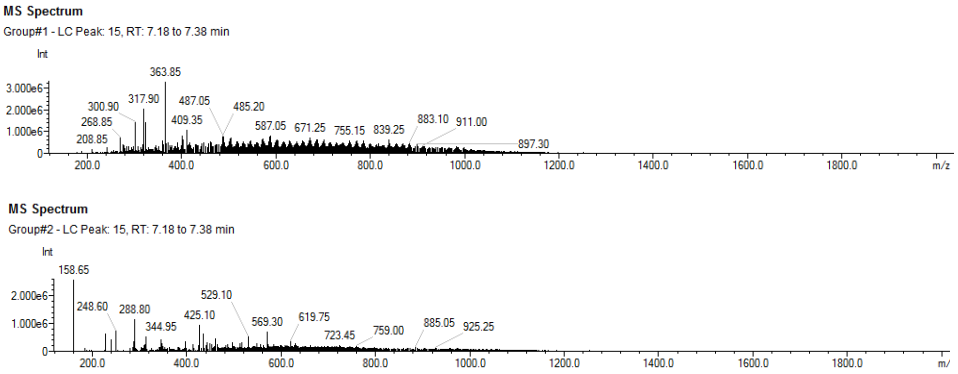
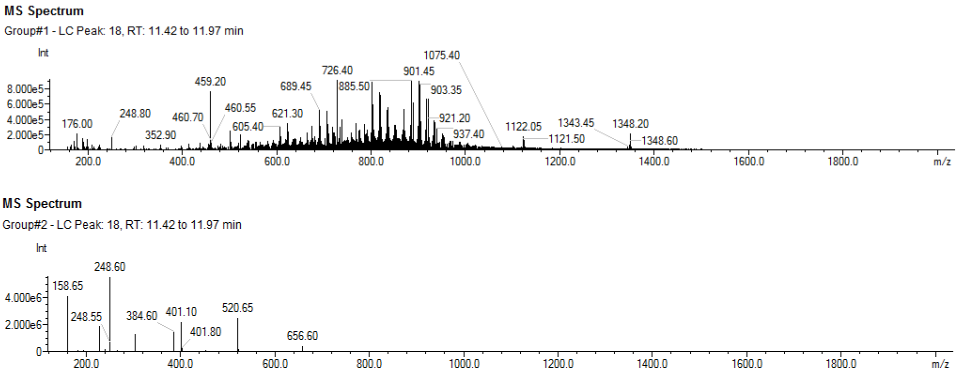
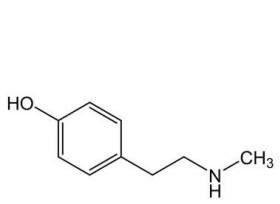


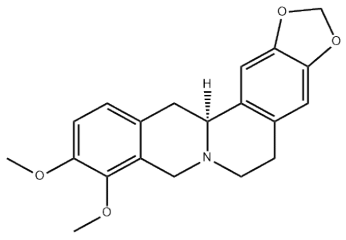
Peak 8



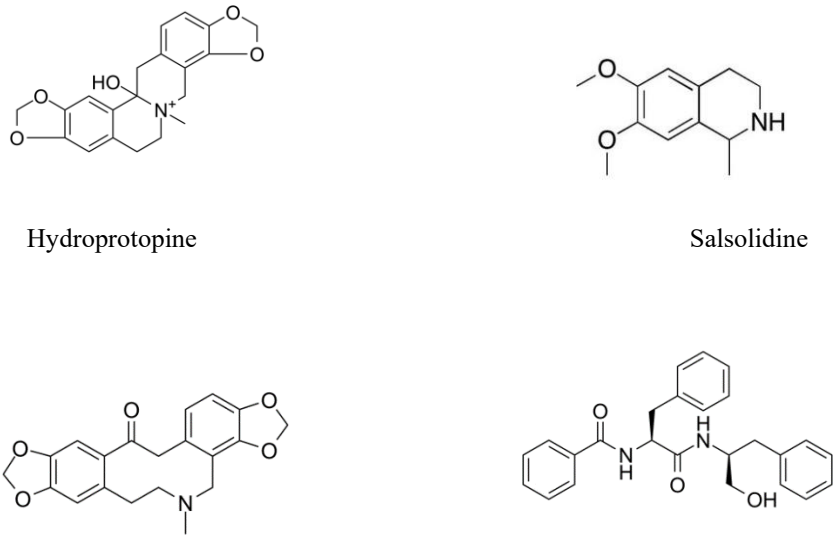
(A)



N-methyltyramine



Canadine



Hydroprotopine Salsolidine

Protopine Aurantinamide

(B)

Figure 26: (A) LC-MS Chromatograms of *Alhagi maurorum* extract. Detection is done at -254, 2+20. 155-2000mz.Sample infused through autosampler and the mass-to-charge ratio (*m/z*) 155-2000mz. (B) Chemical structures of some important compounds.

LC-ESI-MS method was used in this study, due to high efficiency in separation as well as sensitivity of alkaloids in the positive mode to identify potential bioactive compounds. Tentatively mainly alkaloids were identified at this stage by aligning the molecular weight based on proposed charge to mass ratio with PubChem, google scholar, dictionary of natural products and MassBank databases. Spectra was acquired in the mass range of 155-2,000. A group of alkaloids were detected, possibly phenethylamine hordenine (m/z 158.65) though tyramine (m/z 138)/ N-methyltyramine (151.2) was not detected on selected spectra range as reported previously but N-methyltyramine (151.2) is proposed based on peak observed (156.70). Furthermore, lysergic acid ergot derivatives, protopine, stylopine were also detected and possibly have fragmentation pattern as of hordenine (m/z 158.65). Isoquinoline alkaloids identified for example protopine, cryptopine, stylopine which have shown to have hepatoprotective activity and antiproliferative activity based on observed peaks in positive ionization mode. Five peaks reported in PubChem for a group of heterohexacyclic alkaloids and considering molecular weight closest compounds identified were protopine (m/z 353.4), hydroprotopine (m/z 354.38), stylopine (m/z 323.3) and cryptopine (m/z 368.85) based on reported peaks (m/z 354,275:171,149:288), and (m/z

370,369.8,369.9,369.7,370.1) respectively. (S)-N-Methylcanadine (m/z 354.4) benzylisoquinoline alkaloid, a quaternary ammonium ion that was S-canadine (m/z 339.4) bearing an N-methyl substituent. Canadine was reported to be immediate precursor of berberine and act as a calcium channel blocker. Aurantiamide (m/z 402.5), an isoindole alkaloid previously being reported from *Alhagi sparsifolia* was potentially detected which previously being reported to have an anti-neuroinflammatory activity and block activation of NF-kB, phosphorylation of JNK and p38 of MAPK pathway identified based on reported peaks (m/z 385,400, 402). Salsolidine (m/z 207.27), a tetrahydroisoquinoline alkaloid, known as a stereoselective competitive MAO A inhibitor identified. Caffeoyl-hexose-deoxyhexoside potentially also found based on the following $[M - H]^-$ at m/z 487.0 and fragment ion peak detected at m/z 308 (Li et al., 2018; Jianshe et al., 2015) as reported in literature (Table 14).

Some other classes of organic compounds of pharmacological significance were also tentatively identified as well such as diclofenac (NSAID), posaconazole (Antifungal), catechol etc.

Table 14: LC-MS Analysis: Tentative identification of alkaloids and other compounds in *Alhagi maurorum* extract

No.	RT (min)	Peak m/z	Tentative Identification	Chemical Formula	$[M–H]^-$ /[M–H]+ (m/z) literature	Reference
1	3.94-4.20	366.85	13-Hydroxyoxyberberine	$C_{20}H_{17}NO_6$	367.4	PubChem
2	6.47-6.65	355.15	Hydroprotopine	$C_{20}H_{20}NO_5^+$	354.4	PubChem
3	4.20-4.47	158.65 (-/+)	Ephedrine/hordenine	$C_{10}H_{15}NO$	166.123/165.23	(Hahn et al., 2022); Mass bank
4	4.47-4.60	156.70 (+)	N-methyltyramine		152	NIST
5	6.47-6.65	355.15	Protopine	$C_{20}H_{19}NO_5$	353.13	Lehner et al., 2004
6	3.94-4.20	323.25	Stylopine	$C_{19}H_{17}NO_4$	323.3 (-)	PubChem
7	3.94-4.20	368.85	Cryptopine	$C_{21}H_{23}NO_5$	369.4 (-)	PubChem
8	4.47-4.60	320.80	7-Methoxy-17,19-dioxa-11-azapentacyclo[12.7.0.03,11.04,9.016,20]henicosa-1(21),2,4,6,9,14,16(20)-heptaen-8-on	$C_{19}H_{15}NO_4$	321.3	PubChem
9	6.84-7.18	408.75	alpha-Fagarine hydrochloride	$C_{21}H_{24}ClNO_5$	405.9	PubChem
10	6.84-7.18	408.75	16,17-Dimethoxy-14-(2-oxopropyl)-5,7-dioxa-13-azapentacyclo[11.8.0.02,10.04,8.015,	$C_{23}H_{23}NO_6$	409.4	PubChem

			20]henicosa-2,4(8),9,15(20),16,18-hexaen-21-one			
11	4.47-4.60	364.70	Corycavine	$C_{21}H_{21}NO_5$	367.4 (-)	PubChem
12	6.47-6.65	355.15/344.95	trans-Canadine-N-oxide/ D-Tetrahydropalmatine	$C_{20}H_{21}NO_5$/ $C_{21}H_{25}NO_4$	355.4	PubChem
13	11.42-11.97/7.18-7.38	520.65-529.30 (-)	Dimethyl 14,16,17-trimethoxy-21-oxo-5,7-dioxa-13-azahexacyclo[12.7.2.01,13.02,10.04,8.015,20]tricosa-2,4(8),9,15(20),16,18,22-heptaene-22,23-dicarboxylate	$C_{27}H_{25}NO_{10}$	523.5	PubChem
14	7.18-7.38	363.85	Leptopidinine/Hypecorinine (possible isomers)	$C_{20}H_{17}NO_6$	367.4	PubChem
15	3.94-4.20	366.85	16,17-Dimethoxy-13,21-dimethyl-5,7-dioxa-13-azoniapentacyclo[11.8.0.02,10.04,8.015,20]henicosa-1(21),2,4(8),9,15(20),16,18-heptaene	$C_{22}H_{24}NO_4^+$	366.4	PubChem
16	7.18-7.38/11.42-11.97	355.15/352.90	(S)-N-Methylcanadine	$C_{21}H_{24}NO_4^+$	354.4	PubChem
17	3.94-4.20	366.85	Pachyrrhizone	$C_{20}H_{14}O_7$	366.3	PubChem
18	11.42-11.97	352.90	8-Oxocanadine	$C_{20}H_{19}NO_5$	353.4	PubChem
19	3.94-4.20	368.85	1,2-Dimethoxy-6-methyl-5,7,8,15-tetrahydro-6H-benzo[c][1,3]dioxolo[4',5':4,5]benzo[1,2-g]azecin-14-one	$C_{21}H_{23}NO_5$	369.4	PubChem
20	6.84-7.18	358.50	1-(4-fluorophenyl)-2-[(5R)-4-methoxy-6-methyl-7,8-dihydro-5H-[1,3]dioxolo[4,5-g]isoquinolin-5-yl]ethanone	$C_{20}H_{20}FNO_4$	357.4	PubChem
21	4.47-	366.35(-)	1-Methoxyberberinium	$C_{21}H_{20}NO_5^+$	366.4	PubChem
22		344.95	Mundoserone	$C_{19}H_{18}O_6$	342.3	PubChem
23	3.94-4.20	368.85	2-(4-Methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-5-yl)-1-(3-methoxyphenyl)79xoazeti/ (16,17-Dimethoxy-5,7-dioxa-1-azapentacyclo[11.8.0.03,11.04,8.014,19]henicosa-3(11),4(8),9,14,16,18-hexaen-12-yl)methanol	$C_{21}H_{23}NO_5$	369.4	PubChem

24	3.94-4.20	340.80	16,17-Dimethoxy-5,7-dioxa-13-azoniapentacyclo[11.8.0.02,10.04,8.015,20]henicosa-2,4(8),9,15(20),16,18-hexaene	$C_{20}H_{22}NO_4^+$	340.4	PubChem
25	3.94-4.20	368.85	2-(4-Methoxy-6-methyl-5,6,7,8-tetrahydro[1,3] dioxolo[4,5-g]isoquinolin-5-yl)-1-(2-methoxyphenyl)ethanone	$C_{21}H_{23}NO_5$	369.4	PubChem
26	3.58-3.94	398.85	1-(2,4-Dimethoxyphenyl)-2-(4-methoxy-6-methyl-5,6,7,8-tetrahydro[1,3] dioxolo[4,5-g]isoquinolin-5-yl)ethanone	$C_{22}H_{25}NO_6$	399.4	PubChem
27		384.60(-)	4-Methoxy-5-[2-(2-methoxyphenyl)-2-oxoethyl]-6,6-dimethyl-5,6,7,8-tetrahydro[1,3] dioxolo[4,5-g]isoquinolin-6-ium	$C_{22}H_{26}NO_5^+$	384.4	PubChem
28		446.95	4-Methoxy-6,6-dimethyl-5-[2-oxo-2-(3,4,5-trimethoxyphenyl)ethyl]-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-6-ium	$C_{24}H_{30}NO_7^+$	444.5	PubChem
29	3.94-4.20	340.80(-)	2-(4-Methoxy-6-methyl-5,6,7,8-tetrahydro[1,3]dioxolo[4,5-g]isoquinolin-5-yl)-1-phenylethanone	$C_{20}H_{21}NO_4$	339.4	PubChem
30	4.47-4.60/7.18-7.38	487.05/308.70/308.15	caffeoyl-hexose-deoxy-hexoside	C15H18O9	487.05/308.70/308.15	Chen et al., 2012
31	6.47-6.65	222.95/221.85	N-Methylmescaline	C12H19NO3	225.285	ChEBI
32	11.42-11.97	176/158.65	Salsolidine	$C_{12}H_{17}NO_2$	207.07	
33	6.84-7.18	401.80(-) 401.10(-/+)	Aurantiamide	$C_{25}H_{26}N_2O_3$	402.5	
34	4.47-4.60	441.05	dia-aurantiamide acetate	$C_{27}H_{28}N_2O_4$	444.522	ChemSpider
35	3,58-3.94	245.85	Pyrrolezanthine	$C_{14}H_{15}NO_3$	245.85	ChemSpider
36	3.58-3.94	291.85	Fusarentin 6-Methyl Ether	$C_{15}H_{20}O_6$	296.31	ChemSpider
	LC-MS Analysis: Tentative identification of other compounds in *Alhagi maurorum* extract					
1	3.58-3.98	475	4-((3-chloro-2-(2-hydroxyphenyl)-4- 80xoazetidine-1-yl)(2-hydroxyphenyl)-methyl)-1,2-dihydro-5-methyl2-phenyl-1h-pyrazol-3(2h)-one (antifungal)	C26H22ClN3O4	475.13	10.1002/jhet.2349

2	3.58-3.98	291.85	Diclofenac (NSAID)	C14H11Cl2NO2	292.00	PMID:-20470236
3	3.58-3.98	250.85	Bromocatechol (phenol)	C6H5BrO2	251.05	CN-113480534-A
4	3.58-3.98	627.10	5-(2-Ethoxy-5-((4-methyl-1,4-diazepan-1-yl)sulfonyl)phenyl)-1-methyl-3-propyl-1,6-dihydro7H-pyrazolo[4,3-d]pyrimidin-7-one (Phosphodiesterase inhibitor)	C34H39N6O4S	627.30	WO-2017060874-A1
5	3.58-3.98	459.05	4-((4-chlorophenyl)(4-hydroxy-2-oxo-2H-chromen-3-yl)methyl)-5-methyl-2-phenyl-1Hpyrazol-3(2H)-one (coumarin derivative)	C26H19ClN2O4	460.10	PMID:-9635556
6	3.58-3.98	773.15	(1,3-Dibenzyl-4,5-diphenylimidazol-2-ylidene)gold(I) p-Mercaptobenzoic Acid (Gold compound)	C36H29N2O2SAu	773.25	PMID:-30110951
7	3.58-3.98	795.20	(S)-Azelastine N-Oxide (Antiallergy)	C22H24ClN3O2	794.48	PubChem
8	3.58-3.98	773.15	Posaconazole (Antifungal)	C37H42F2N8O4	687.00	71313564
9	3.58-3.98	308.25	Bisdemethoxycurcumin (curcuminoid)	C19H16O4	308.04	Patent CN-113980063A
10	3.58-3.98	563.95	4-[(3-bromophenyl) methoxy] -N-[(2-hydroxy-5-methoxyphenyl) methylideneamino]benzamide (palladacycle inhibitor)	C22H19BrN2O4 Pd	564.27	Compound CID: 1378937
11	3.58-3.94	727.30	N,N-bis(4-carbazol-9-ylphenyl)-4-(4-phenylphenyl)aniline (Aniline derivative)	$C_{54}H_{37}N_3$	728.01	Compound CID: 158394886
12	3.58-3.94	624.95	Lyngbyabellin M (non-ribosomal peptide synthetase)	C25H34N2O8Cl2S2	624.91	10.3390/molecules25173986
13	3.48-3.96	727.20	Phosphatidylserine (Phospholipid)	C45H84NO9P	726.95	10.7150/thno.45125
14	3.48-3.96	647.05	DL-Thioctamide (lipoamide)	C8H15NOS2	647.50	PubChem CID 863

3.3 Measurement of Cell Viability

Alhagi maurorum 70% ethanolic extract was investigated using cancer breast cell line (MCF-7) and non-cancer breast cell line (MCF-10-A). The first step before assessing the cell viability of extract on cell line, various concentrations of ethanol on MCF-7 were tested. According to the published literature ethanol 0.3% is physiologically considered safe (Figure 27).

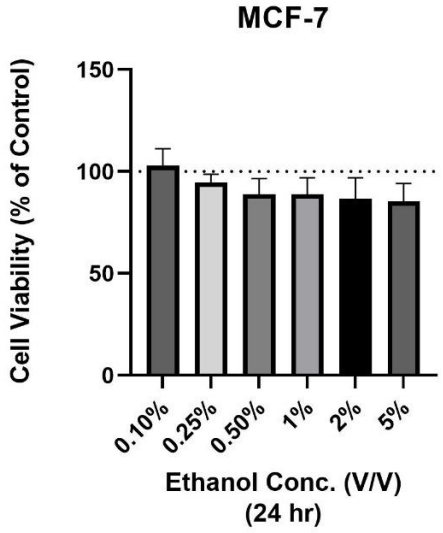


Figure 27: Breast cancer (MCF-7) cells treated with increasing concentrations of ethanol for 24hr. Data is represented as the average of n=4 independent experiments ± SEM. Statistical analysis: One sample t test, * indicates significance $p<0.05$. (ET: Ethanol solvent control)

3.3.1 Effects of *Alhagi maurorum* Extract on Cell Viability

Alhagi maurorum extract prepared in 70% ethanol at different concentrations ranging from 10 µg/ml to 1000 µg/ml were studied using standard MTT assay. The stock of *Alhagi maurorum* extract was prepared in 70% ethanol (stock 1 g /5 ml), then diluted with culture medium and different concentrations for the treatment were prepared by serially diluting stock. The concentration of vehicle control (ethanol 70%) was kept at or below 0.5%. Untreated cells receiving the same volume of culture medium served as control and viability was measured by comparing absorbance of treated and untreated cells. Effects of AM on MCF-7 and MCF-10-A

cells were measured after 24hr of exposure (Figure 28). The formation of purple colour of formazan was the indicator of the assay formed by the activity of mitochondrial dehydrogenase in viable cells.

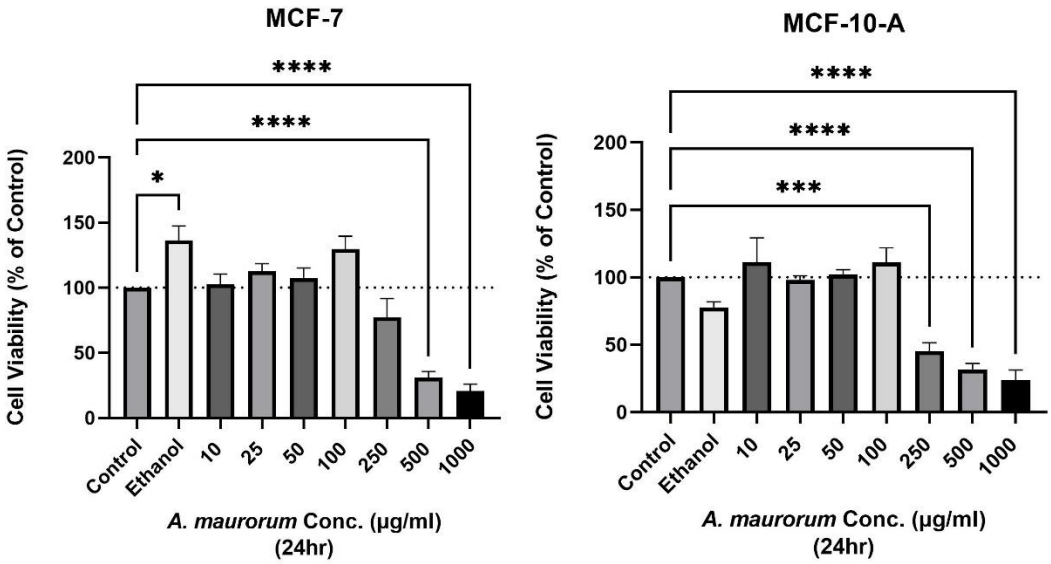


Figure 28: Breast cancer (MCF-7) and normal (MCF-10-A) cells treated with increasing concentrations of *Alhagi maurorum* extract for 24hr. Data is represented as the average of n=5 independent experiments ± SEM. Statistical analysis: One way ANOVA followed by Dunnetts's multiple comparison tests, * indicates significance $p<0.05$; ** indicates $p<0.01$, *** indicates $p<0.001$, ****indicates $p<0.0001$. (ET:Ethanol solvent control)

AM was observed to be toxic at higher concentrations of 500 µg/ml and 1000 µg/ml to MCF-7 cells (up to 60-70%, $p < 0.0001$) and 250 µg/ml, 500 µg/ml and 1000 µg/ml to MCF-10-A cells (up to 60-80%, $p< 0.0001$) relative to control. It was also observed that AM has altered the morphology of cells at higher concentrations when observed under microscope (Figure 29).

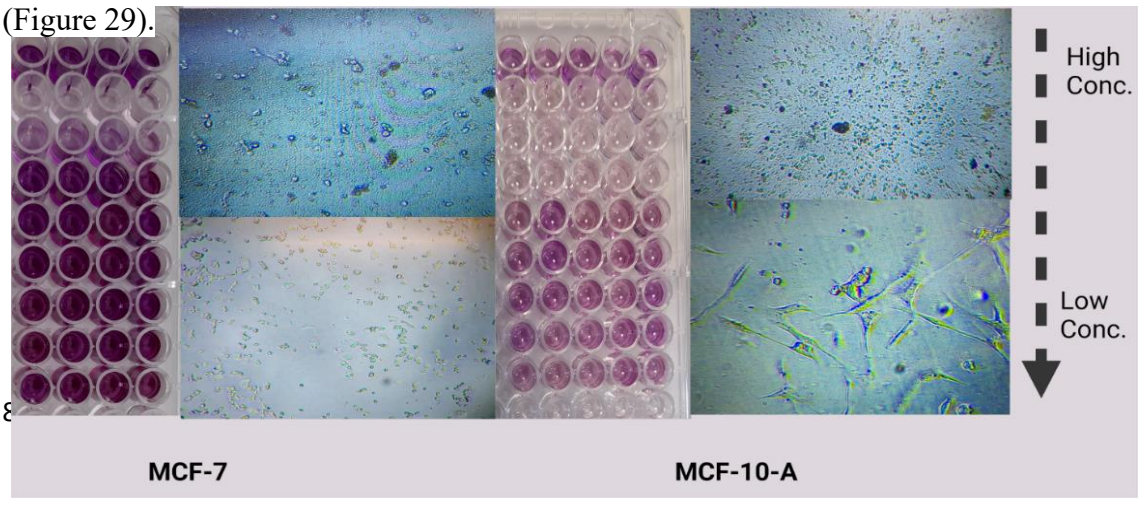


Figure 29: Cell viability Assay of *Alhagi maourorum:* MTT assay used to assess cytotoxic potential. Purple colour observed is proportional to the number of viable cells. Side images are microscopic view where cell death is visible at higher concentrations. (ET:Ethanol solvent control)

3.4 Measurement of Oxidative Stress

ROS signalling molecules are vital to maintain cellular homeostasis and an imbalance in ROS levels results in oxidative stress. In this study the effects of AM prepared in 70% Ethanol were analysed on breast cancer (MCF-7) and normal (MCF-10-A) cell lines. Intracellular ROS levels were measured in this study using a cell-based assay DCFDA used as an indicator of ROS levels (Figure 30).

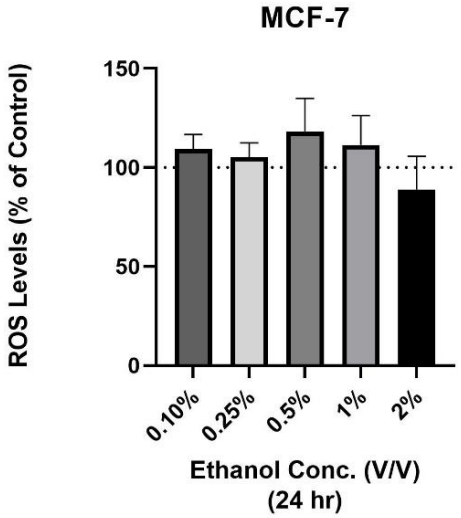


Figure 30: Effect of solvent on ROS levels. Breast cancer (MCF-7) cells treated with increasing concentrations of ethanol for 24hr. Data is represented as the average of n=3 independent experiments ± SEM. Statistical analysis: One sample t test, * indicates significance $p<0.05$. (ET:Ethanol solvent control)

3.4.1 Effects of *Alhagi maurorum* Extract on ROS Levels

AM Extract significantly reduced ROS levels at higher concentrations (100 µg/ml, 250 µg/ml, 500 µg/ml, and 1000 µg/ml) (up to 50%, $p<0.0001$) and at lower concentration (up to

20%, p<0.0053) in case of MCF-7 cell line when compared to untreated control. However, in MCF-10-A, it significantly reduced the ROS levels up to 10-20% at higher concentration of 250 µg/ml, 500 µg/ml, and 1000 µg/ml (up to 20%, p<0.048) (Figure 31).

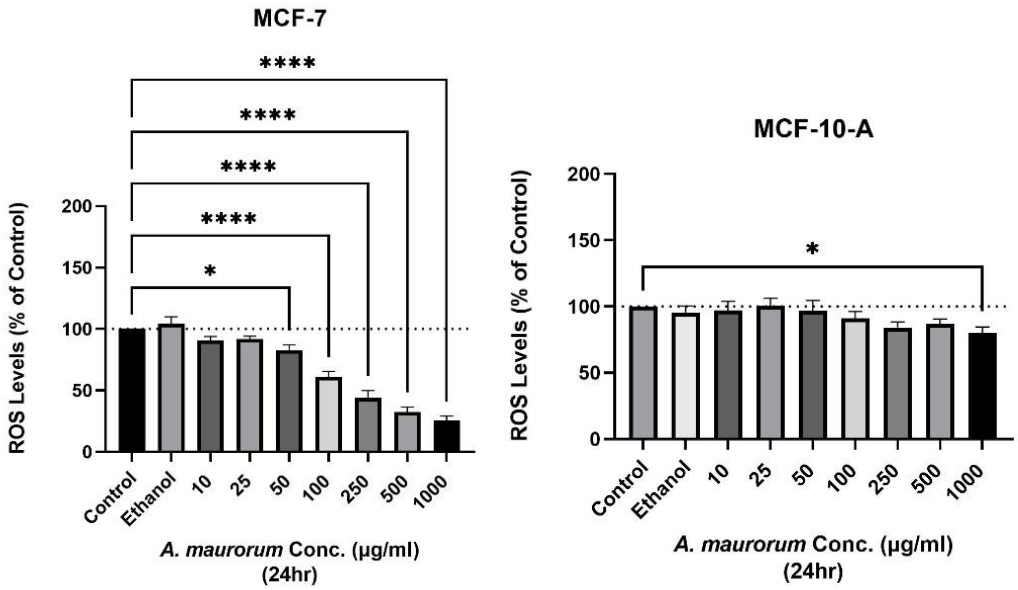


Figure 31: Breast cancer (MCF-7) and normal (MCF-10-A) cells treated with *Alhagi maurorum* ethanolic extract for 24hr. Data is represented as the average of n=5 independent experiments ± SEM. Statistical analysis: One way ANOVA followed by Dunnetts's multiple comparison tests, * indicates significance p<0.05; ** indicates p<0.01, *** indicates p<0.001), ****indicates p<0.0001. (ET:Ethanol solvent control)

3.5 Measurement of Mitochondrial Membrane Potential (MMP)

MMP was measured using a cell-based assay where a fluorescent dye TMRE accumulates in polarized mitochondria due to their relative negative charge of the membrane and high intensity of fluorescence was generated. However, depolarized, or defective mitochondria will not retain dye and generate fluorescence of low intensity.

3.5.1 Effects of *Alhagi maurorum* Extract on MMP

AM extract significantly reduced MMP of MCF-7 and MCF-10-A cells after 24hr of treatment compared to untreated control at high concentrations 250 µg/ml, 500 µg/ml, 1000 µg/ml (up to 50 to 80%, $p < 0.0001$) (Figure 32).

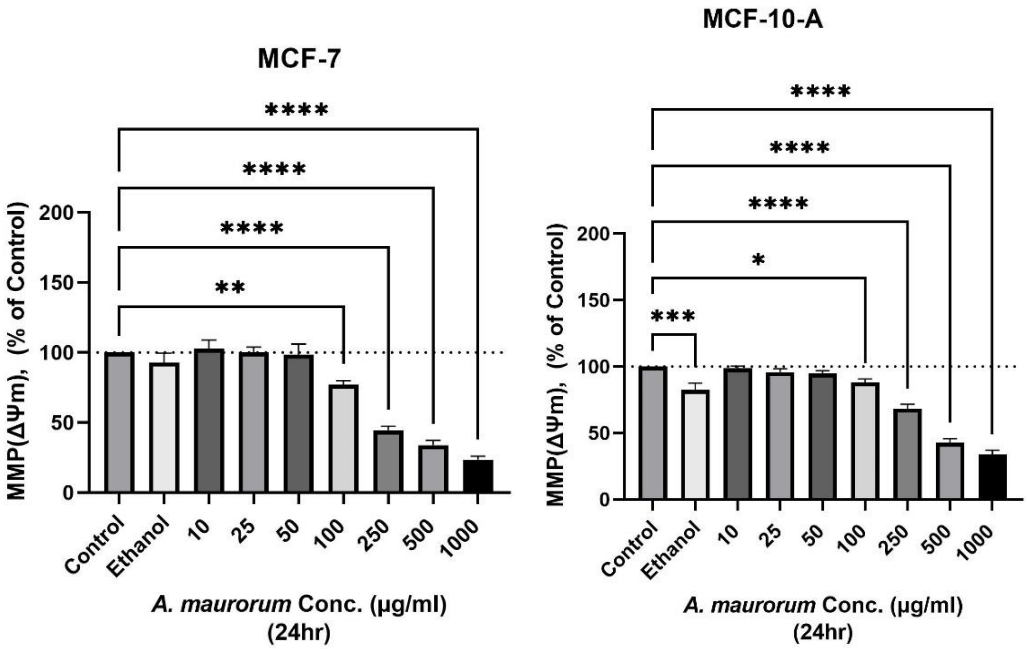


Figure 32: Breast cancer (MCF-7) and normal (MCF-10-A) cells treated with *Alhagi maurorum* ethanolic extract for 24hr. Data is represented as the average of n=5 independent experiments ± SEM. Statistical analysis: One way ANOVA followed by Dunnetts's multiple comparison tests, * indicates significance $p<0.05$; ** indicates $p<0.01$, *** indicates $p<0.001$), ****indicates $p<0.0001$. (ET:Ethanol solvent control)

3.6 Measurement of Intracellular Calcium Concentrations

Ca^{2+} is an abundant intracellular messenger participating in diverse cellular functions. Concentration of Ca^{2+} is maintained by different compartments within a cell. Any change in the level of Ca^{2+} was measured in this study through fluorescence signals generated by labeled molecules used as indicators of Ca^{2+} in the cells. The Rhod-2 AM ester forms are cationic in nature and are selective indicator for mitochondrial Ca^{2+} via membrane potential driven uptake. Exact loading concentration, time and temperature were determined empirically for both cell lines (MCF-7 and MCF-10-A). Incubation time is a critical factor to allow complete de-

esterification of intracellular Rhod-2 AM esters. However, prolonged incubation eliminates cytosolic staining but mitochondrial retained. Further, results could be improved by reducing rhod-2 AM to dihydrorhod-2 AM (non-fluorescent) to distinguish between cytosolic and mitochondrial dye location. Main process involves oxidation of Rhod-2 AM ester of dihydrorhod-2 that led to the release of rhod-2 indicator a process occurs in mitochondrial environment.

3.6.1 Effects of *Alhagi maurorum* Extract on Intracellular Ca^{2+}

Cultured and treated cells with *Alhagi maurorum* extract for 24hr were loaded with the Rhod-2 AM ester forms of the Ca^{2+} indicators. Then fluorescence signals generated from these stained cells were measured. In MCF-7 cells the levels of Ca^{2+} were reduced at higher concentrations 250 µg/ml (up to 30%, $p<0.01$) and 500 µg/ml, 1000µg/ml (up to 50-60%, $p<0.0001$) while no significant change in Ca^{2+} level was observed at lower concentrations compared to untreated control (Figure 33**)**. However, in the case of MCF-10-A the opposite trend was observed that is higher levels of Ca^{2+} at higher concentrations after exposure of 24hr treatment with *Alhagi maurorum* though more repeats are required to draw conclusion.

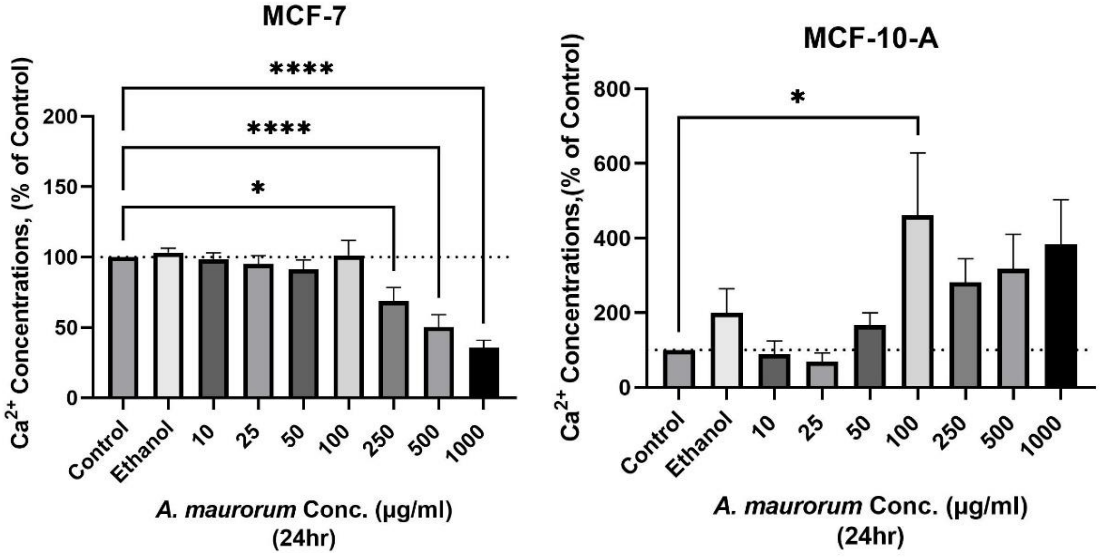


Figure 33: Breast cancer (MCF-7) and normal (MCF-10-A) cells treated with *Alhagi maurorum* extract for 24hr. Data is represented as the average of n=5 independent experiments ± SEM. Statistical analysis: One

way ANOVA followed by Dunnetts's multiple comparison tests, * indicates significance $p<0.05$; ** indicates $p<0.01$, *** indicates $p<0.001$, ****indicates $p<0.0001$. (ET:Ethanol solvent control)

3.7 Measurement of Mitochondrial Respiration

To get a deep insight into the effects of *Alhagi maurorum* on mitochondrial function, Seahorse mitostress assay was performed in which six parameters were measured in one assay: basal respiration, ATP production, proton leak, spare respiratory capacity, maximal respiration, and non-mitochondrial respiration [(For detailed formula see Section 2.2.10: Seahorse cell mito stress assay (Table 11)]. These parameters serve as an indicator of how cells respond to stress induced by mitochondrial ETC modulators/ inhibitors, Oligomycin (Complex V), FCCP (Uncoupler), antimycin A (Complex III) and rotenone (Complex I).

3.7.1 Effects of *Alhagi maurorum* Extract on Mitochondrial Respiration

Alhagi maurorum extract after treatment for 24hr has shown no significant effect on MCF-7 cell line only a significant decrease in proton leaks up to 60% was observed at 250 µg/ml. There is no significant change is observed at lower concentrations of 10µg/ml in MCF-7 cell line (Figure 34**)**. Also, AM extract reduced the basal respiration, ATP production and maximal respiration at 100 µg/ml (up to 70-80%, $p< 0.019$) and at 250 µg/ml (up to 80%, $p<0.008$) in MCF-10-A. In the same way, significant decrease in proton leak at concentration 250 µg/ml (up to 80-90%, $p<0.007$) was also observed whereas, at 100 µg/ml decline was up to 70% though it didn't reach significance. Furthermore, at 10 µg/ml concentration there a significant increased effect of proton leak was observed (up to 10-20%, $p<0.0198$) in MCF-10-A (Figure 36). Additionally, significantly reduced ATP production was observed at 250 µg/ml (up to 80-90%, $p<0.0327$) and at 100 µg/ml (up to 80%, $p< 0.0491$). There is significant reduction in levels of spare respiratory capacity and non-mitochondrial respiration observed at higher concentrations though none reached significance after five repeats. Maximal respiration was also significantly reduced at concentrations 100 µg/ml ($p<0.0126$) and 250 µg/ml up to 70-80% ($p<0.007$) [(For OCR profile of MCF-7 and MCF-10-A see (Figure 35) (Figure 37)].

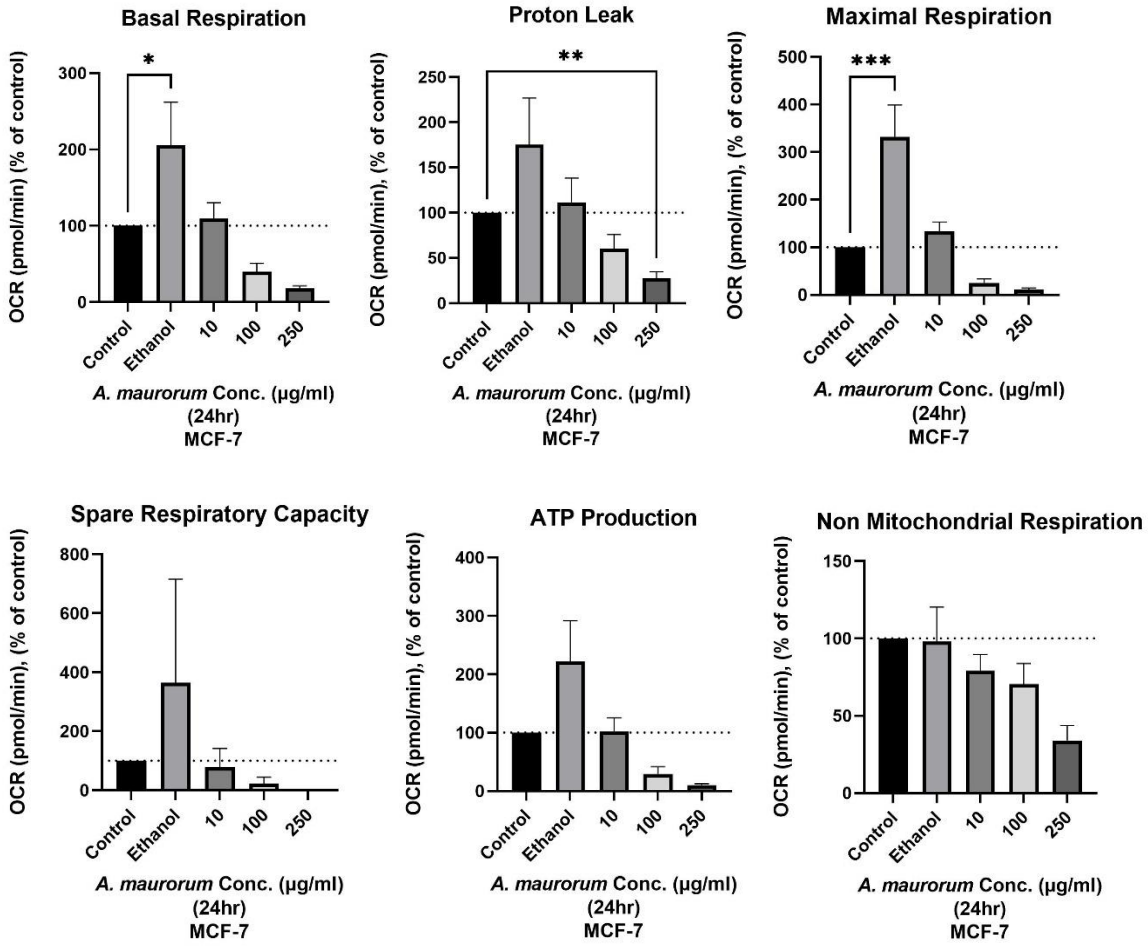


Figure 34: Effect of *Alhagi maurorum* on OXPHOS: Breast cancer (MCF-7) cells treated with *Alhagi maurorum* extract for 24hr. Data is represented as the average of n=5 independent experiments ± SEM. Statistical analysis: One way ANOVA followed by Dunnetts's multiple comparison tests, * indicates significance $p<0.05$; ** indicates $p<0.01$, *** indicates $p<0.001$), ****indicates $p<0.0001$. (ET:Ethanol solvent control)

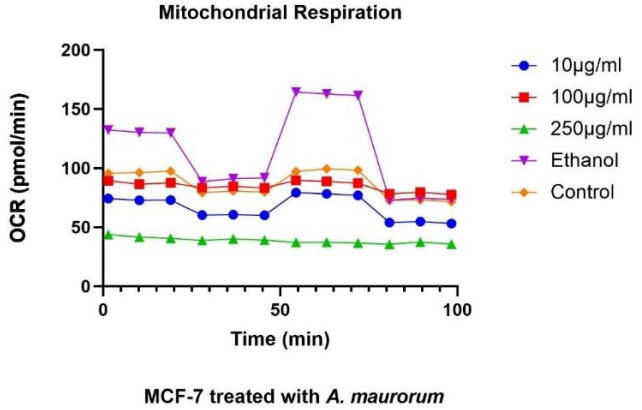


Figure 35: MCF-7 OCR Profile. Seahorse XF Cell Mito Stress test profile of MCF-7 Cell Line treated with *Alhagi maurorum* extract for 24 hr (Agilent, 2021). Schematic, showing interpretation of OCR levels in response to the injection of Oligomycin, FCCP, Rotenone and Antimycin.

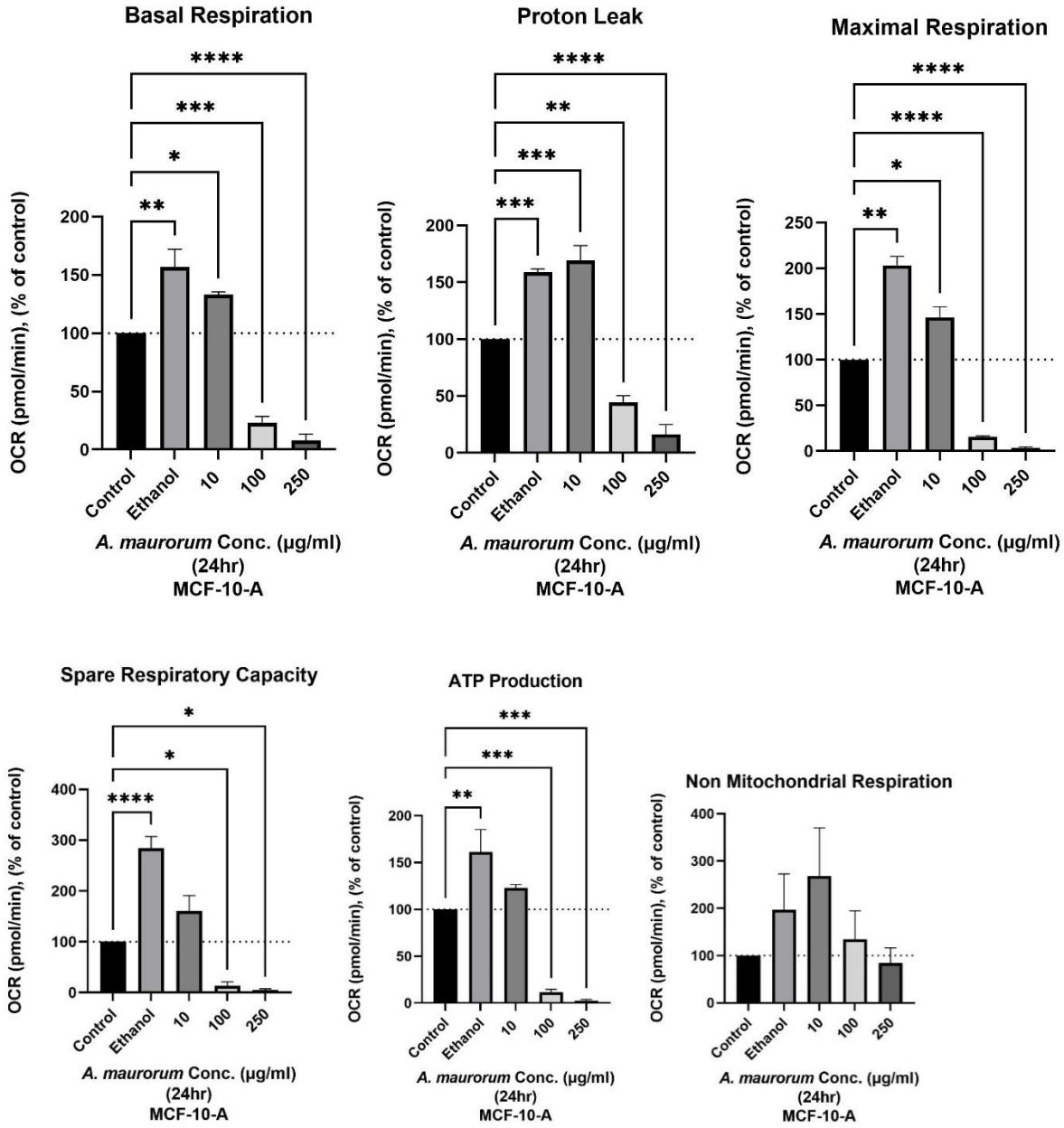


Figure 36 : Effect of *Alhagi maurorum* on OXPHOS. Breast cells (MCF-10-A) treated with *Alhagi maurorum* extract for 24hr. Data is represented as the average of n=5 independent experiments ± SEM. Statistical analysis: One way ANOVA followed by Dunnetts's multiple comparison tests, * indicates significance $p<0.05$; ** indicates $p<0.01$, *** indicates $p<0.001$, ****indicates $p<0.0001$. (ET:Ethanol solvent control).

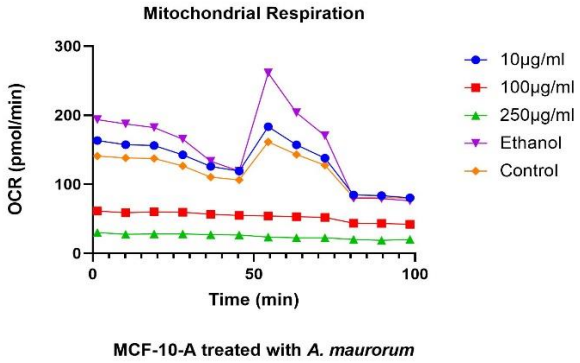


Figure 37: MCF-10-A OCR profile. Seahorse XF Cell Mito Stress test profile of MCF-10-A Cell Line treated with *Alhagi maurorum*e extract for 24 hr (Agilent, 2021). Schematic, showing interpretation of OCR levels in response to the injection of Oligomycin, FCCP, Rotenone and Antimycin.

Chapter 4

Results of Hordenine

4.1 Measurement of Cell Viability

The bioactive compound hordenine was investigated using cancer breast cell line (MCF-7) and non-cancer breast cell line (MCF-10-A).

4.1.1 Effects of Hordenine on Cell Viability

Standard colorimetric MTT assay was used after treating MCF-7 and MCF-10-A cells with a range of concentrations of hordenine (0.1 μM-10,000 μM) for 24hr. Stock solution of hordenine was prepared in DMSO, then diluted serially in culture medium to prepare range of concentrations. However, concentration of vehicle DMSO was kept at and below 1% and untreated cells served as control after receiving the same volume of cell culture medium.

The results suggest that hordenine had no significant effect on cell growth on both cell lines though a trend of slightly lower cell viability compared to normal breast cell line was observed. A significant decrease in cell viability at much higher concentrations (5,000 μM and 10,000 μM) was also observed but that could be the consequence of higher solvent concentration. Furthermore, effects of hordenine by exposing cells (MCF-7 and MCF-10-A) for 6hr and 48hr were also studied. No significant effects observed at most of the concentrations after 6hr and 48hr after five independent experiments in both cell lines (Figure 38).

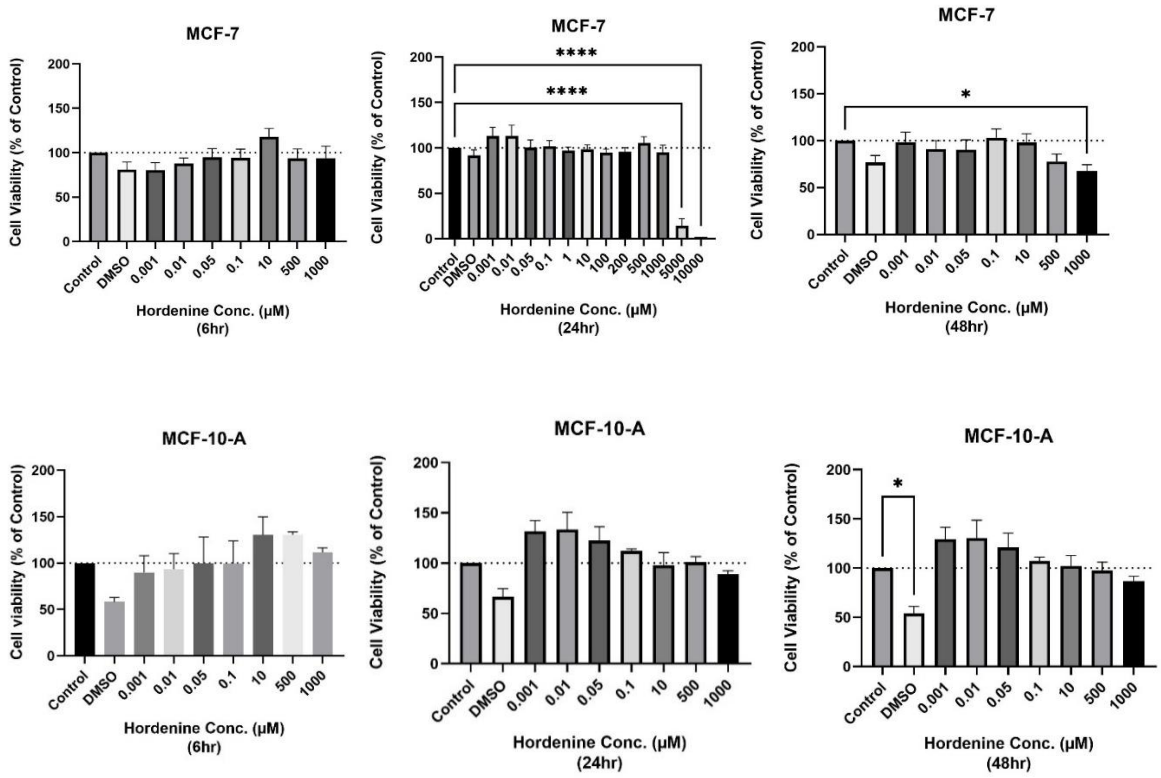


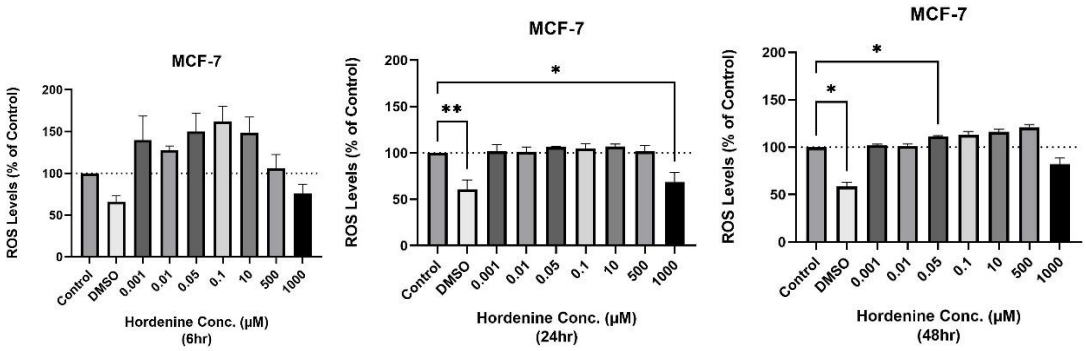
Figure 38: Breast cancer (MCF-7) and normal (MCF-10-A) cells treated with hordenine for 6 hr, 24hr and 48hr. Data is represented as the average of n=5 independent experiments ± SEM. Statistical analysis: One way ANOVA followed by Dunnett's multiple comparison tests, * indicates significance $p<0.05$; ** indicates $p<0.01$, *** indicates $p<0.001$, ****indicates $p<0.0001$.

4.2 Measurement of Oxidative stress

The bioactive compound hordenine was analysed on breast cancer (MCF-7) and normal (MCF-10-A) cell lines.

4.2.1 Effects of Hordenine on ROS Levels

The effects of hordenine on ROS levels were also assessed fluorometrically using DCFDA assay. Hordenine stimulated ROS levels after 6hr of exposure in MCF-7 cell line. There were no significant effects observed after 24hr and 48hr of exposure of cells with hordenine at most of the concentrations but only at very high concentration of 1000 µM after 24hr in MCF-7. However, ROS levels significantly reduced after 6hr in MCF-10-A at concentrations 0.1 µM, 10 µM, 500 µM and 1000 µM (up to 20-30%, p=0.005) while no significant effect after 24hr and 48hr of exposure of cells with hordenine was observed as compared to untreated control (Figure 39) at almost all concentrations.



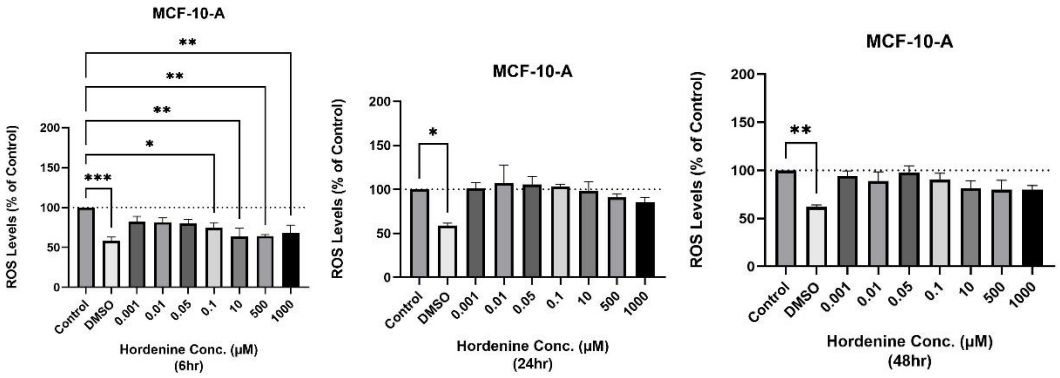


Figure 39: Breast cancer (MCF-7) and normal (MCF-10-A) cells treated with hordenine for 6 hr, 24hr and 48hr. Data is represented as the average of n=5 independent experiments ± SEM. Statistical analysis: One way ANOVA followed by Dunnetts's multiple comparison tests, * indicates significance $p<0.05$; ** indicates $p<0.01$, *** indicates $p<0.001$, ****indicates $p<0.0001$.

4.3 Measurement of Mitochondrial Membrane Potential (MMP)

MMP was measured after treating MCF-7 and MCF-10-A cells with hordenine.

4.3.1 Effects of Hordenine on MMP

Effects of hordenine were assessed on MMP, a key indicator of mitochondrial activity using cell-based assay in which a fluorescent dye was used to measure the intensity of fluorescence generated by polarized and depolarized mitochondria in both cell lines (MCF-7 and MCF-10-A). Cells were exposed to hordenine prepared in DMSO for 6hr, 24hr and 48hr. No significant change in MMP of MCF-7 and MCF-10-A cells was observed compared to untreated control at most of the concentrations used after at least five independent experiments across three timepoints (Figure 40).

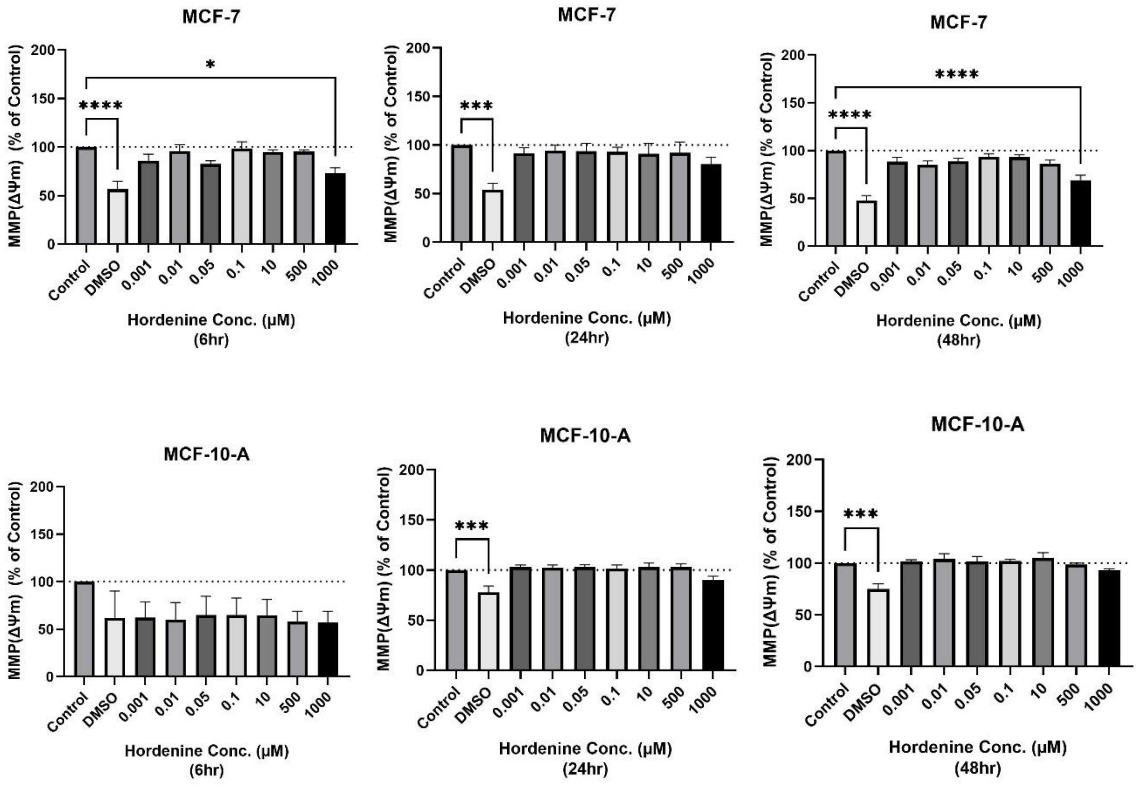


Figure 40: Breast cancer (MCF-7) and normal (MCF-10-A) cells treated with hordenine for 6 hr, 24hr and 48hr. Data is represented as the average of n=5 independent experiments ± SEM. Statistical analysis: One way ANOVA followed by Dunnetts's multiple comparison tests, * indicates significance $p<0.05$; ** indicates $p<0.01$, *** indicates $p<0.001$, ****indicates $p<0.0001$.

4.4 Measurement of Intracellular Ca^{2+}

The effect of hordenine on intracellular Ca^{2+} was analyzed on breast cancer (MCF-7) and normal (MCF-10-A) cell lines.

4.4.1 Effects of Hordenine on Intracellular Ca^{2+}

Cells cultured and treated with hordenine were stained to measure intracellular Ca^{2+} level at three time points, 6hr, 24hr and 48 hr. There is no significant change was observed in the levels of Ca^{2+} at most of the concentrations across three timepoints on MCF-7 cell line (Figure 41).

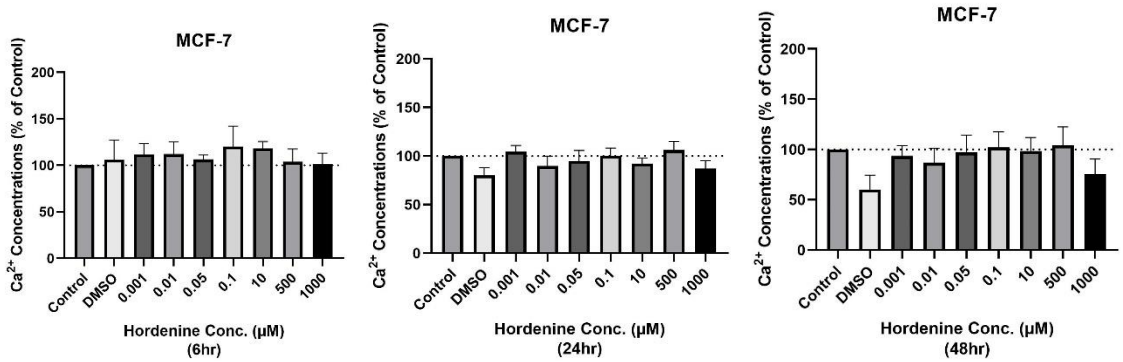


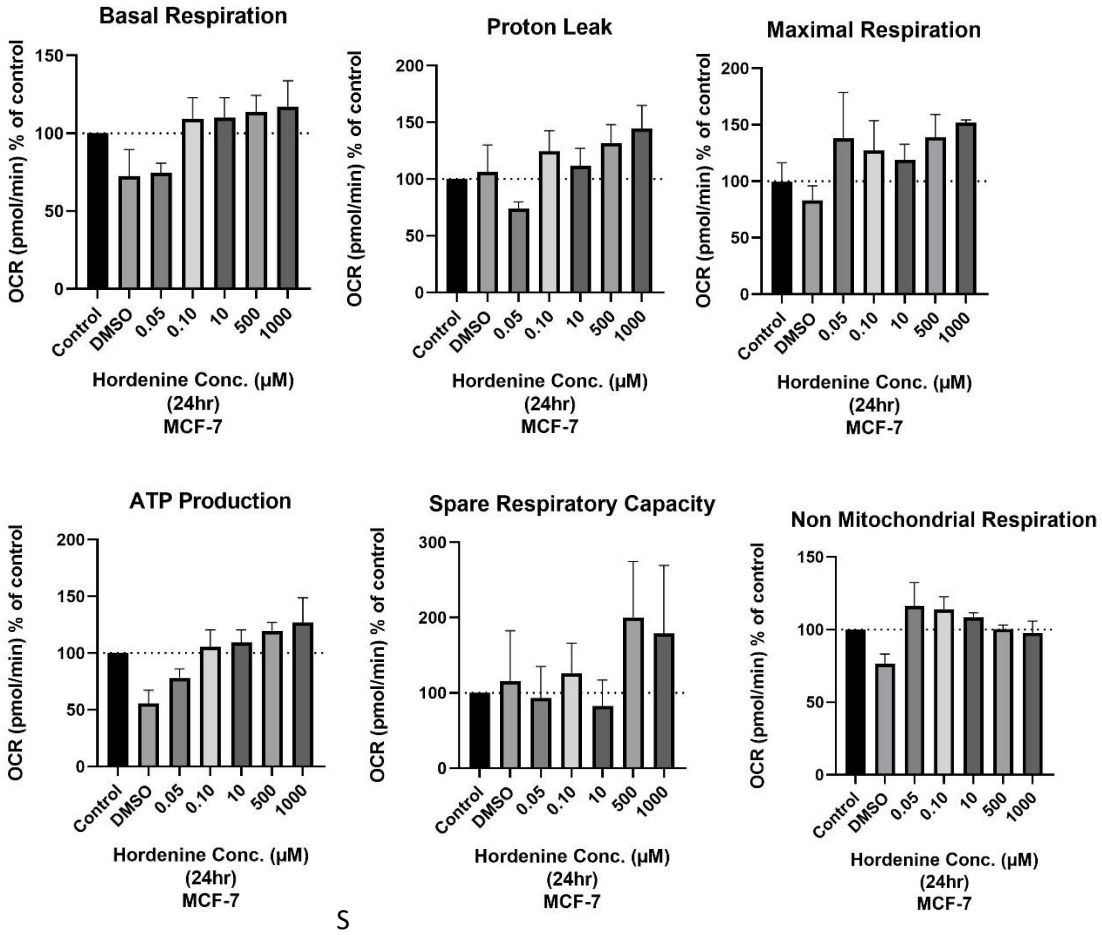
Figure 41: Breast cancer (MCF-7) cells treated with hordenine for 6 hr, 24hr and 48hr. Data is represented as the average of n=5 independent experiments ± SEM. Statistical analysis: One way ANOVA followed by Dunnetts's multiple comparison tests, * indicates significance $p<0.05$; ** indicates $p<0.01$, *** indicates $p<0.001$, ****indicates $p<0.0001$.

4.5 Measurement of Mitochondrial Respiration

Effects of hordenine on mitochondrial respiration Seahorse analyzer was used to perform Seahorse mitostress assay. To get a deep insight into the effect of hordenine on mitochondrial functions. The following six parameters were measured in one assay, basal respiration, ATP production, proton leak, spare respiratory capacity, maximal respiration, and non-mitochondrial respiration. These parameters are an indicator of how cells respond to stress caused by mitochondrial inhibitors, Oligomycin (Complex V), FCCP (Uncoupler), antimycin A (Complex III) and rotenone (Complex I) after exposure of hordenine for 24hr.

4.5.1 Effects of Hordenine on Mitochondrial Respiration

No significant effect is observed after treatment with hordenine at all concentrations in MCF-7 and MCF-10-A on all parameters of OCR (Figure 42).



S

Figure 42: Seahorse mitostress assay. Key parameters of mitochondrial respiration measured after treatment of MCF-7 cell line with hordenine (0.05 µM-1000 µM) for 24hr. Data is presented as percentage of the control ± SEM of n=4 independent experiments. Statistical Analysis: One way ANOVA for pairwise comparison. * Indicates significance ($p<0.05$).

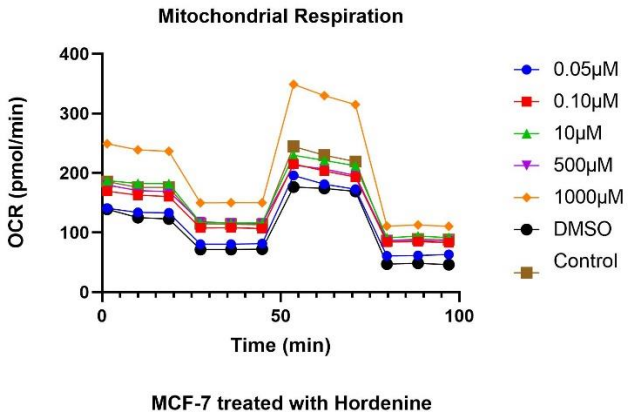


Figure 43: OCR profile of MCF-7 after sequential injection of ETC modulators. Schematic, showing interpretation of OCR levels in response to the injection of Oligomycin, FCCP, Rotenone and Antimycin.

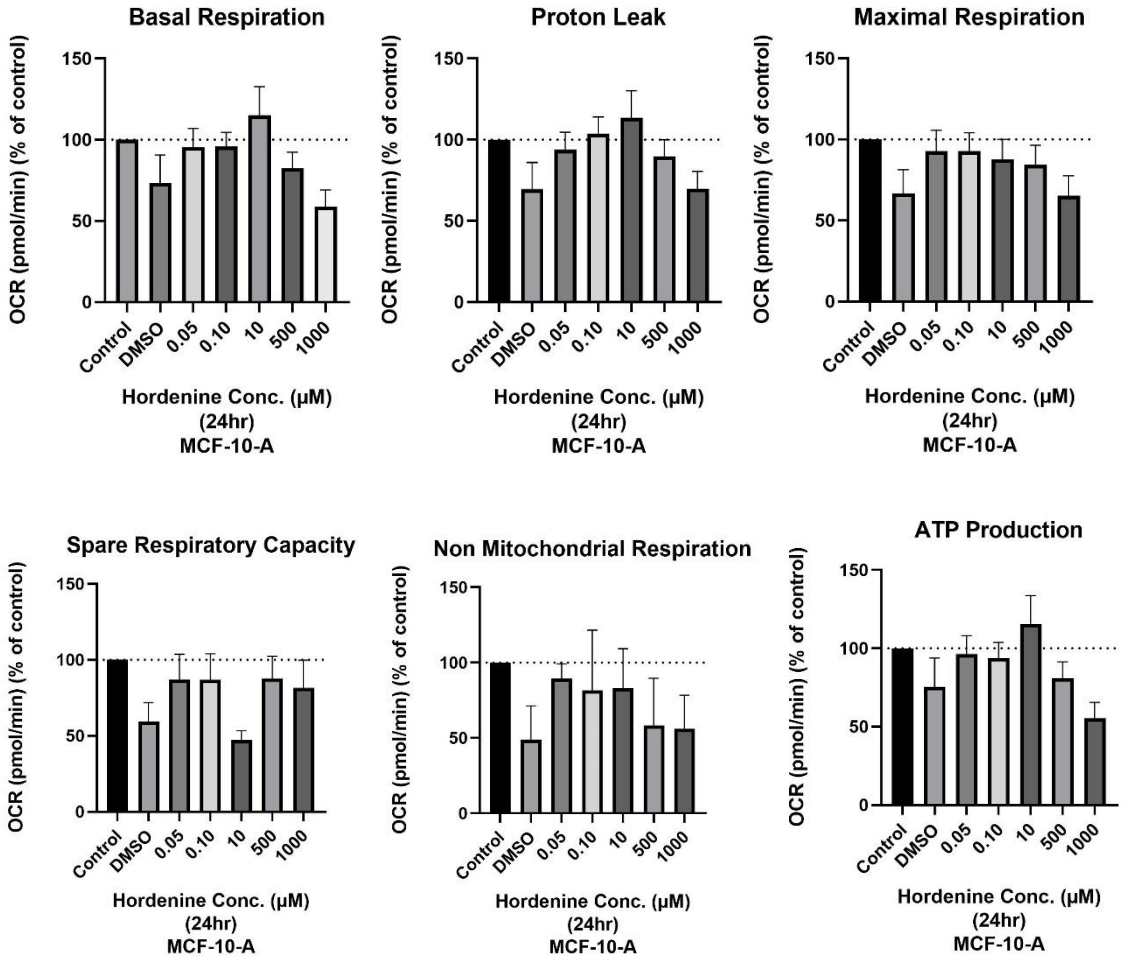


Figure 44: Seahorse Mitostress assay. Key parameters of mitochondrial respiration measured after treatment of MCF-10-A cell line with hordenine (0.05 µM-1000 µM) for 24hr. Data is presented as percentage of the control ± SEM of 3 independent experiments. Statistical analysis: One way ANOVA for pairwise comparison. * Indicates significance ($p < 0.05$).

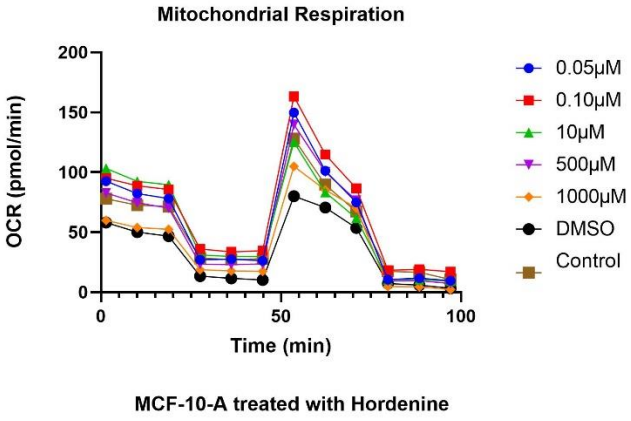
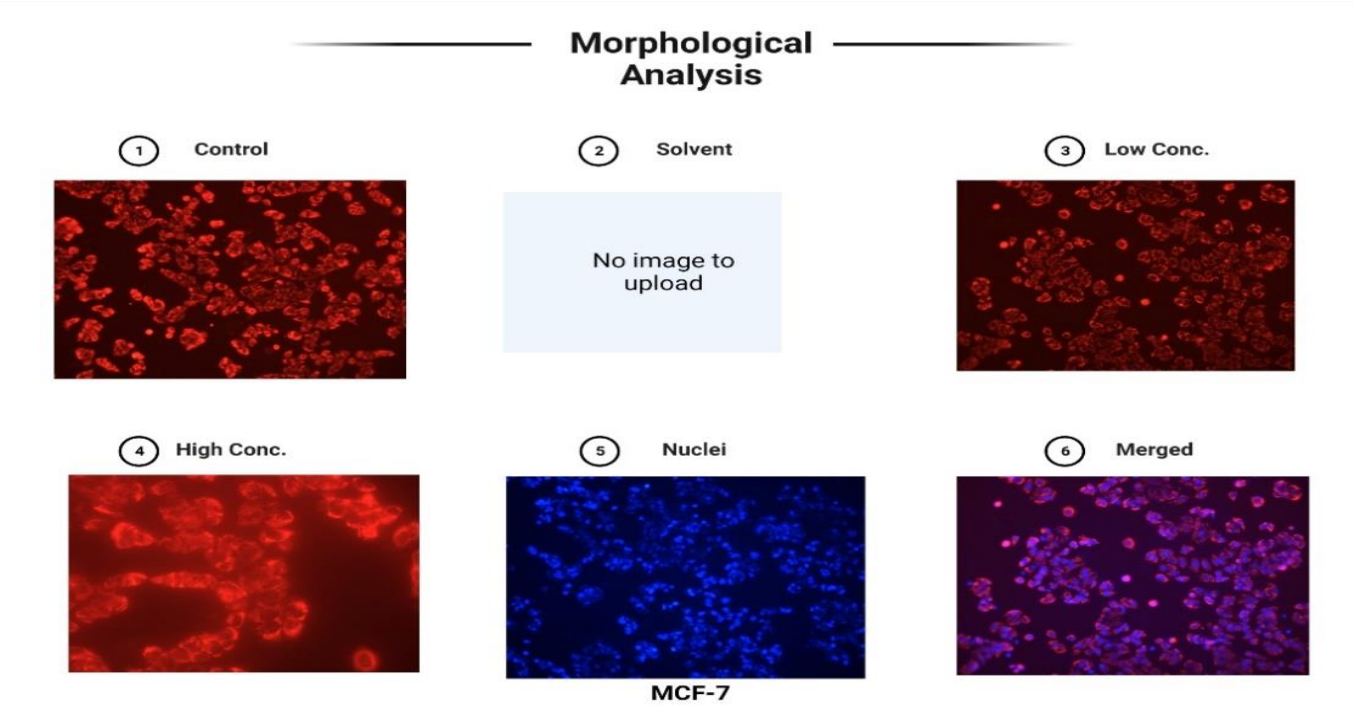


Figure 45: OCR profile of MCF-10-A. Seahorse mitostress assay MCF-10-A OCR profile after sequential injection of ETC modulators. Schematic, showing interpretation of OCR levels in response to the injection of Oligomycin, FCCP, Rotenone and Antimycin.

4.6 Observation of Morphology Using Fluorescent Microscopy

Mitochondrial imaging of MCF-7 and MCF-10-A after treating cells for 24hr with hordenine was done to see any effect on morphology of mitochondria (Figure 47). No significant changes in morphology were observed confirming the abovementioned results. Furthermore, low concentrations of *Alhagi maurorum* extract were also tested and morphology was analysed which confirmed the above findings that it has not affected the morphology at lower concentrations.

A.


Morphological Analysis
1 Control
2 Solvent
No image to upload
3 Low Conc.
4 High Conc.
5 Nuclei
6 Merged
MCF-7
Morphological Analysis
1 Control
2 Solvent
3 Low Conc.
4 High Conc.
MCF-10-A

B.

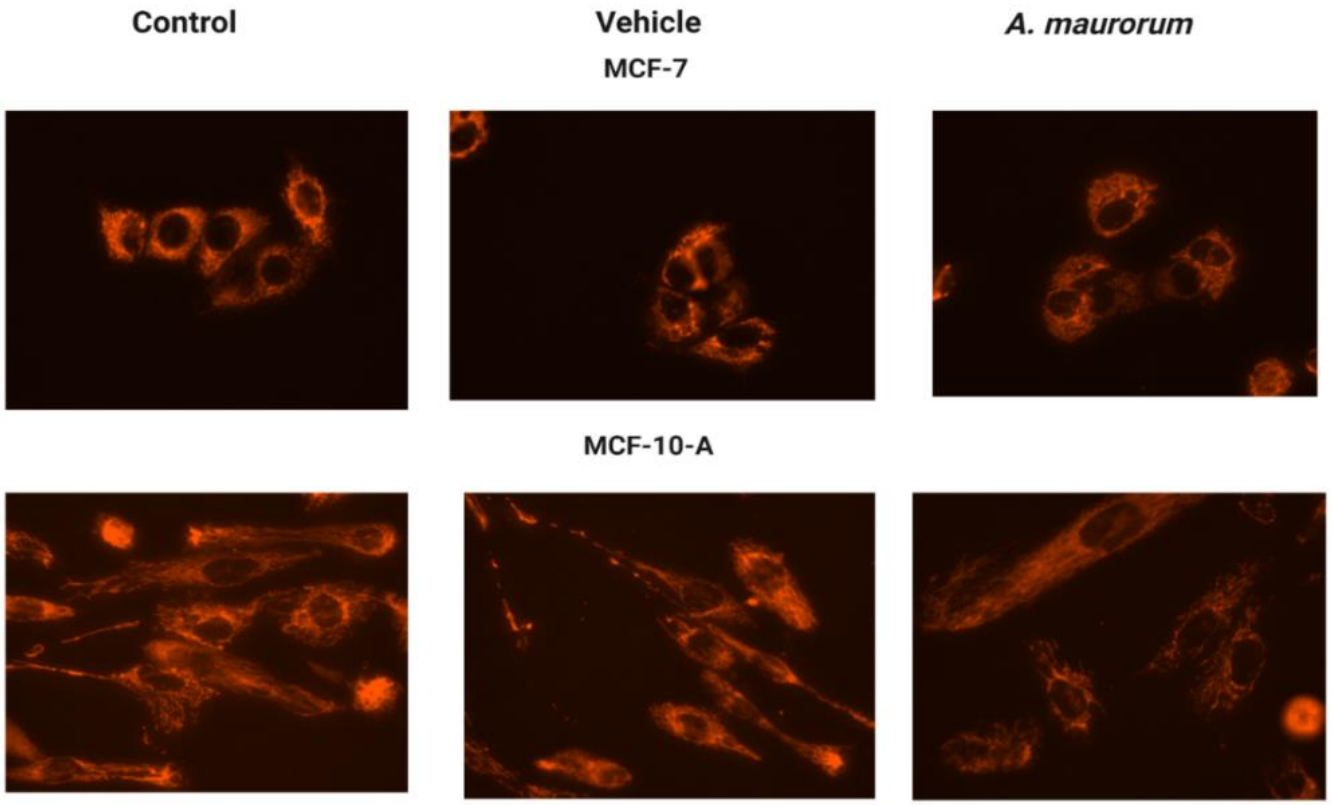


Figure 46: A. Morphological analysis of MCF-7 and MCF-10-A after treatment with hordenine at low and high concentrations for 24hr (top two figures). B. Morphological analysis of MCF-7 and MCF-10-A after treatment with *Alhagi maurorum* for 24hr. Mito Tracker Deep Red Images. Morphology quantified by observing changes in the intensity of Mito Tracker Deep Red (Created with BioRender).

Chapter 5

Discussion

Advance understanding of mitochondrial biology has thrown light on the mitochondrial impact on health and disease beyond ATP and macromolecule production. Mitochondrial involvement is now noticeable in several cellular processes such as redox and Ca^{2+} homeostasis, cell death, critical biosynthetic pathways but mitochondria undergo dramatic changes in morphology as cells grow, divide, and die, and the relationship between mitochondrial structure and functions is still unclear. Although, mitochondrial role in cancer progression has been identified in the last century but it is still not fully understood mechanism how changes in mitochondrial shape and organization can lead to cancer and how altered mitochondrial functions could be manipulated for therapeutic benefit.

Natural compounds and their structural analogues have historically made major contribution to new drug leads and Alhagi species are under study due to their anti-inflammatory and anti-cancer activities (Sarkar et al., 2020; Davinelli et al., 2013; Parvinroo et al., 2014; Ardekani, Rahimi, and Mohammad, 2010). In this study the effects of *Alhagi maurorum* extract (AM extract) on mitochondrial functions of MCF-7 (breast cancer) and MCF-10-A (control) have been evaluated (Irsfeld, Spadafore and Prub, 2013; Rafehi et al., 2021; Tundis et al., 2012; Guan et al., 2020; Thu et al., 2020; Wang et al., 2020; Ghosh and Das, 2010; Zhou et al., 2018; Zhang et al., 2021).

5.1 Effect of AM extract and hordenine on Cell Viability

In the first part of this study the effect of AM extract on the cell viability of cancerous and non-cancerous breast cell lines is assessed. As the main objective is to investigate the impact of different concentrations on cell homeostasis, cell death initiation and induction so range of concentrations from low 10 µg/ml to high up to 1000 µg/ml are tested. Cell viability has not changed at lower concentrations tested and decreased consistently with increase in the concentration of AM extract. AM extract after 24hr of exposure has significantly reduced the cell viability of MCF-7 and MCF-10-A at high concentrations (250 µg/ml, 500

µg/ml, and 1000 µg/ml) up to 70%, though cell viability remained unchanged at low concentrations tested. Increased cell death at high concentrations in MCF-7 suggest cytotoxic effects of the AM extract. The highest cytotoxic activity is observed at 1000 µg/ml concentration (See results 3.3.1 of Chapter 3) and indicate the level of cytotoxic activity is concentration dependent. Mitochondria are the main sites where most of the MTT is reduced, it passes through the mitochondrial inner membrane due to lipophilic nature and presence of positive charge (Ghasemi et al., 2021; Mueller et al., 2004; Riss et al., 2013). Formazan, a water insoluble molecule, is formed in viable cells due to the disruption of the core tetrazole ring by the activity of mitochondrial dehydrogenases, mainly succinate dehydrogenase. Bahamin et al., in his study of *Alhagi maurorum* extract on 4T1 breast cancer cells growth found significant reduction in cell growth up to 70-80% at 130 µg/ml though he used DMSO as a solvent which in our study of hordenine has shown more growth inhibitory effects on MCF-7 and MCF-10-A (Bahamin et al., 2021).

Additionally, the bioactive compound hordenine was also tested on cell viability and it has shown no significant effects on cell viability after exposure for 6hr, 24hr and 48hr on MCF-7 and MCF-10-A cells (see Section 4.1.1). Only at very high physiological concentrations it reduced the cell viability in MCF-7 cell line but, it could be due to high concentrations of DMSO solvent used so these concentrations were excluded in later experiments. Cell viability results of the hordenine are consistent with the work of Anwar et al., 2020 who has also reported no effect of hordenine on the cell viability in lung cancer cell lines. However, unlike this, a study conducted by Zhang et al in 2021, investigated the effects of hordenine on RAW264.7 macrophages cells and showed decrease in cell viability at 100 µg/ml (Wang et al., 2010; Zhao et al., 2018; Lu, Wang, and Zhan, 2019; Zhang et al., 2021). In the most recent study of hordenine by Wang et al., it was shown that hordenine promoted cell proliferation and elevated the activity of primary mouse dermal papilla cells (to treat alopecia) through the Wnt signalling pathway, that promote angiogenesis. (Wang et al., 2023). As, hordenine has not altered the cell viability of different cell models as previously reported at lower concentrations while in this study even higher concentrations were tested in breast cell models and proved to be safe. However, several studies previously have shown hordenine regulate the MAPK signalling which is associated with several physiological processes of development, cellular proliferation, differentiation and apoptosis (Yasi et al., 2019; Zhao et al., 2018; Bertolotto et al., 1998; Busca and Ballotti, 2000; Kim,

et al., 2013 ; Brauers, Steiner and Daldrup, 2013) but due to the nature of the assay, it was not possible to identify any specific effect in this study (Van, Joubert and Cromarty, 2015).

MTT assay is primarily used to assess cellular metabolic activity and increased metabolic activity suggest proliferation and indirectly could be related to increase in mitochondrial mass succinate dehydrogenase complex (SDH-A), primary reducing enzyme of MTT. Succinate dehydrogenase (SDH-A protein) is encoded by the SDH-A gene in humans which has been linked to mitochondrial respiratory chain, citric acid cycle and is also considered as tumor suppressor gene (Rai, et al., 2018; Ghasemi et al., 2021; Alkahtani et al., 2022). Results obtained also suggest the effect of AM extract on glycolytic pathways too which are more active in cancer cells because cancer cells rely more on glycolysis to fulfill their ATP requirements. Interestingly, AM extract has initiated cell death at 250 µg/ml concentration while induced apoptosis at higher concentrations so, different concentrations of AM extract can be used to manipulate cell death processes for example, autophagy, mitophagy and apoptosis and AM is a valuable source of novel anticancer compounds.

There are some limitations that need to be considered to draw full conclusion from the data because of cytotoxicity caused by the MTT assay. Moreover, the full mechanism of action of MTT reduction is still unknown because of the role of other organelles involved, enzymes and several biomolecules (glutathione, cysteine) in this process and need to be explored. So, more supportive experiments are required to confirm the findings, particularly the effect of AM extract on apoptosis or apoptotic proteins. Moreover, the principle behind MTT assay is, every viable cell in given cell line should have same metabolism so, quantification of total metabolism is directly proportional to number of viable cells. However, advanced research in this field also suggests cellular metabolic remodeling under stress meaning chemicals can modify metabolic potential of the cells. So, more relevant experiments must be considered to assess cytotoxic potential, growth inhibition and mitochondrial biogenesis because results simply indicate the metabolic state and not the cell number (Rai,et al., 2018; Mueller et al., 2004).

5.2 Effect of AM extract and hordenine on ROS Levels

AM extract has significantly reduced the ROS levels in MCF-7 at concentrations from 100 µg/ml to 1000 µg/ml in both cell lines. ROS signalling pathway is linked to the phosphorylation of JNK, p38 and ERK1/2. So, reduction in ROS levels in MCF-7 could be

possibly through modulation of JNK, p38 and ERK1/2 proteins. Reduction in ROS levels also indicates AM extract might have affected ETC which is the main source of ROS in mitochondria. Further, reduction in ATP levels while evaluating its effects on mitochondrial respiration confirmed the findings at high concentrations. However, unlike MCF-7 it is noted that AM extract has not affected the ROS levels of MCF-10-A at most of the concentrations tested, only up to 20-30% at higher concentration. Based on previously published studies, ROS species are considered as common and important signalling molecules and lower levels of ROS promote metabolic adaptation, moderate levels are significant to stimulate danger signals and immune responses while high levels activate apoptosis and autophagic cell death pathways so, interestingly AM extract has manipulated ROS levels in MCF-7 differently at different concentrations.

Further, the effect of various concentrations of hordenine on the redox state of both breast cell lines are also studied in this research and changes in ROS levels are quantified in the cells. After treating MCF-7 and MCF-10-A cell lines with hordenine for 24hr and 48hr no significant change in ROS levels is observed as compared to untreated control. Whereas, after 6hr of exposure with hordenine different response is observed in MCF-10-A where, ROS levels significantly reduced at 0.1 µM, 10 µM, 500 µM and 1000 µM and moderately, increased in MCF-7 at 0.05 µM, 0.1 µM and 10 µM suggesting effects are concentration dependent.

ROS induces chronic inflammation through induction of inflammatory cytokines. Activation of NF-kB and chemokine receptors contribute to invasion, and metastasis of various tumor types. Therefore, slight increase in ROS levels activate antioxidant systems such as SOD and GSH. ROS levels can be increased as a therapeutic approach as higher levels of ROS above cellular tolerability threshold initiate cell death response pathways and this feature is very useful to induce apoptosis and even mitophagy. However, normal cell lines have higher capacity to cope with increased ROS levels therefore, not many changes in ROS levels observed in MCF-10-A after treatment with AM extract, only slight reduction in ROS levels at high concentrations (Choi et al., 2020). Anticancer agents could be developed based on dual role of ROS both as pro-oxidant and antioxidant though most of the concentrations have shown some effects though not reached significance, but modulation of ROS levels is not considered ideal as it increases adaptation of cancer cells to ROS and supports cancer stemness. Cancer cells tend to counterbalance ROS levels and steady state of ROS levels in cancer cells are determined by rate of ROS production and rate of ROS

scavenge. High rate of ROS production is counterbalanced by equally high rate of antioxidant activity to maintain redox balance.

Research is ongoing to design ROS therapies where focus is to lower ROS levels to prevent signalling and induce to a certain level to selectively kill cancer cells for example, use of antioxidants, vitamin C in this regard. But the challenge here is if the ROS levels are not sufficiently raised within cancer cells, then therapy would simply further activate NF-kB, P13K, HIFs and MAPK to promote tumorigenesis so, there is need to find compounds who selectively target antioxidant systems. There is also a need to identify which ROS species need to be targeted as ROS is a collective term used to describe various types of molecules produced as byproduct for example superoxide radical ($O\bullet^{-2}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH•) and some other and these are studied but still mechanism is not fully explored. Hydroxyl radical (OH•) have short life span generated from other ROS species and indiscriminately oxidise lipids, proteins and DNA and cause instability whereas, superoxide radical ($O\bullet^{-2}$) do not indiscriminately oxidises lipids, proteins, and DNA. Some information about mechanism of hydrogen peroxide (H_2O_2) mediated oxidation of cysteine residue within protein is known which exist as thiolate anion at physiological pH and is protonated by hydrogen peroxide (H_2O_2) and eventually alter functions of proteins of MAPK pathways (Zorov, Juhaszova and Sollott, 2014).

Furthermore, the site of ROS generation also plays a critical role as ETC process is not perfect and leakage of electrons more likely occur at complex I and Complex III though there are various redox centers on ETC. In a study targeting ROS for the treatment of inflammatory disease and cancer conducted by Li and his coworkers explains complex I leak superoxide radical ($O\bullet^{-2}$) into mitochondrial matrix and Complex III leaks into the intermembrane space and mitochondrial matrix. Superoxide radical ($O\bullet^{-2}$) dismutated to hydrogen peroxide by two dismutases located at different sites, superoxide dismutase 2 (SOD2) in mitochondrial matrix and superoxide dismutase 1 (SOD1) in mitochondrial intermembrane space. So, though both ROS are generated but superoxide radical (O•−2) cannot pass easily through mitochondrial outer membrane due to short half-life and electrophilic nature so very unlikely become the candidate of signalling molecule in the cell however within mitochondria may impact mitochondrial quality control system whereas, hydrogen peroxide (H_2O_2) is more stable and electrophobic in nature makes it an ideal signalling molecule (Li et al., 2013; Han et al., 2003). One more aspect needs consideration in designing ROS therapies, mitochondrial antioxidant enzymes are nuclear encoded and

transported into the mitochondria so this mitochondria-nucleus crosstalk may also have some impact. There are some studies in this field who also reported three SOD isoforms where, Cu, Zn-dependent isoform SOD1 (Cu-Zn-SOD) found in mitochondrial intermembrane space and cytosol, manganese-dependent isoform SOD2 (Mn-SOD, SOD2) only in mitochondrial matrix and Cu, Zn SOD dependent isoform SOD3 in the extracellular space Cu, Zn SOD (EC-SOD). Moreover, impact of other mtROS scavenging enzymes and proteins such as catalase, GPx, peroxiredoxin and thioredoxins proteins need to be studied.

In addition to abovementioned factors, there is also the need to consider the role of metabolic state of mitochondria (that means rest and work state), a concept proposed by Chance and William in 1955. He explained the role of ADP and ATP in regulation of mitochondrial size by metabolism and found ADP as a key regulator of mitochondrial size, later this intramitochondrial regulation of respiratory rate was also confirmed by other scientists (Korzeniewski, 2015) though the impact of the optimal metabolic state of mitochondria is still not completely explored and hypermetabolism is reported in several mitochondrial diseases such as, Luft disease and complex diseases like cancer are some examples. Mitochondrial ROS production is largely dependent on O_2 concentration there is conflicting information available as several studies proposed linear relationship between mtROS production and oxygen concentration though some studies suggest increase in mtROS production even under hypoxic conditions therefore, to address this role of MMP cannot be ignored. It is known based on previous studies, physiological ROS signalling require optimised MMP and any fluctuation will cause oxidative stress the reason is there is an association between redox couples involved in oxidation of NADH and those involved in antioxidant defence systems (NADPH). $NADP^+$ is necessary for mitochondrial antioxidant enzyme systems which is compromised with an increase in mitochondrial uncoupling of the ETC and increased ROS production but still molecular basis of this process needs more understanding as limited data is available to draw any conclusion because under hypoxic conditions increased mtROS production is linked to hypoxia-inducible factor 1α (HIF-1α), high MMP, low electron flow and overexpression of mitochondrial uncoupling proteins (UCPs)(Busiello, Savarese S, Lombardi, 2015).

Mitochondrial quantity is largely dependent on the energy demands of the cell so, if mitochondrial ROS generation increases, there is also an increase in the expression of antioxidant enzymes system GPx and SOD_2, so the role of mitochondrial biogenesis factor peroxisome-proliferator-activated receptor-γ coactivator 1α (PGC1α) needs understanding

in addressing levels of ROS (Li et al., 2013; Zorov, Juhaszova and Sollott, 2014; Chance and William, 1955). Role of intracellular Ca^{2+} in monitoring the levels of ROS cannot be ignored and it regulates the metabolic state of mitochondria by moderately increasing mitochondrial Ca^{2+} concentration, that stimulates flow of electrons in ETC and decrease mtROS production (Peng and Jou, 2010; Li et al., 2013). MtROS are also involved with processes of fusion and fission; under stressed conditions fragmented mitochondrial networks are found but still limited information is available how mtROS affect process of mitochondrial fusions. Both processes of fission and fusion influence several other mitochondrial functions too because fresh mitochondria keep on adding in the system. Several nuclear transcription factors are also found in mitochondria and are known as mitochondrial transcription factors (mitoTFs). For example, p53 and NF-κB influence ROS levels through different mechanisms, p53 induces apoptosis and inhibits SOD2 activity as well. Some of the epigenetic regulatory enzyme proteins like sirtuins, important for suppressing inflammation, are believed to play a role in mtROS regulation, especially mitochondrial SIRT-3, SIRT-4, and SIRT-5.

Also, combination of ROS-generating drugs with pharmaceuticals that can break redox adaptation could also be a better strategy to design new therapies and overcome issues of drug resistance and side effects. Some scientists suggest lack of ROS also contributes to the progression of cancer because some levels of ROS are needed to correctly fold proteins in ER that could be significant for other critical cellular processes (Zorov, Juhaszova and Sollott, 2014). DCFDA is used as a probe for overall oxidative stress measurements in this study and is not ideal to study specific radical species due to indiscriminate nature of the assay. More experiments are required to find which ROS species is mainly reduced and effects on antioxidant systems to interpret accurately the effect of AM extract and hordenine. Study in the field of mtROS is essential before designing ROS therapies because of impact it has on different organs for example hypertension is associated with increased mtROS in organs mainly kidney and central nervous system, more likely caused by angiotensin II hormone, artherosclerosis is caused by excessive mtROS as increased mtDNA damage reported in these conditions which directly encode genes for the protein complexes of ETC and also several inflammatory diseases like rheumatoid arthritis, thyroiditis and type 1 diabetes are also known to be impacted by the levels of mtROS (Togliatto, Lombardo, Brizzi, 1988;Wen, Gwathmey, Xie, 2012).

5.3 Effect of AM extract and hordenine on Mitochondrial Membrane Potential

Optimal MMP is essential for the normal metabolic activity of the mitochondria. It relies on the activity of citric acid cycle and is an intermediate form of energy which is used by ATP synthase (Haddad and Mohiuddin, 2023). The MMP also has significant influence on the process of mitophagy and facilitates the removal of dysfunctional mitochondria. Alternatively, a decline in MMP affects ATP synthesis and eventually arrest growth of the cells, as seen at higher concentrations tested in this study, when cells of both cell lines were treated with AM extract. Adenine nucleotide transporter (ANT) protein located in mitochondria plays an important role in the development of MMP which is the primary site of exchange of ATP and ADP from matrix to the intermembrane space. Another source of MMP is reverse operation of ATP synthase where the ATPase inhibitory factor 1(IF1) is involved and intracellular ATP is utilized to maintain MMP i.e., rotational direction of ATP synthase and reverse operation of ANT importing cytosolic ATP into mitochondria. ATP compound is essential for buffering stable MMP and this is very important feature in clinical conditions associated with decreased OXPHOS for example hypoxia, stroke, lactic acidosis and mitochondrial myopathies (Zorova et al., 2018; Walker, 2013

The results obtained from this study indicate AM extract modulated MMP and reduced it at high concentrations. Mitochondrial depolarization at high concentrations may induce opening of mitochondrial permeability transition pore formation and released cytochrome c, AIF (apoptosis inducing factor) and Smac into the cytosol from the inter membrane space of the mitochondria. Migration of cytochrome c is a significant event in the process of apoptosis. AIF release leads to chromatin condensation as well as DNA fragmentation. However, experiments are necessary to confirm role of AIF and MAPK pathway, though the results indicated intriguing role of AM extract in provoking mitochondrial damage at high doses, modulation of apoptotic proteins in MCF-7 and, in other words affecting chemiosmotic coupling within mitochondria in MCF-7 (Yuan et al., 2012; Xu et al., 2011). Furthermore, MMP after treating cells with hordenine showed no significant change across three time points at all concentrations in both cell lines used in this study. Interaction between mtROS, MMP has already been explained in Section 5.2.

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Although some studies have reported that the MMP typically falls between 150-200 mV, which is higher than the cell membrane potential which values of 50-100 mV, determining the optimal values of MMP remains challenging despite intensive research in this field. Several factors contribute to this challenge, for example, it's understood that a higher MMP corresponds to a greater energy capacity of the IMM due to its role as an electrical insulator. However, the role of various other membrane transport proteins also needs to be investigated as they are part of the membrane mass; processes like ion leakage on the IMM rely on MMP, and a high MMP contributes to oxidative stress by increasing ATP production and ROS levels. Conversely, a low MMP results in reductive stress due to decreased ATP production and ROS levels, thus, there's a need to identify specific uncouplers that can lower MMP to a certain level. This is crucial to produce the required amount of ATP while maintaining lower levels of ROS production.

This feature could be a potential therapeutic target for disease associated with aging, obesity, heart attack, etc (Zorova et al., 2018; Gottlieb et al., 2003; Izyumov et al., 2004). Duration of depolarization and hyperpolarization is also very critical in design of therapies and needs more understanding and less data is available to draw any conclusion. So, data obtained in this study suggest the effect of AM extract on IMM. However, there is a need for more supportive experiments, because even cellular mitochondria have different magnitude of MMP, although the mechanism behind this is still not clear. Additionally, while fluorescent probes might non-specifically bind to phospholipid membranes of mitochondria, the probes themselves can impact the MMP for example, could actively pump other positively charged substances, and undergo intracellular modifications, though TMRE the most suitable probe used in this study based on literature (Zorova et al., 2018; Scaduto and Grotyohann, 1999).

5.4 Effect of AM extract and hordenine on Intracellular Calcium Concentration

Besides, the effect of AM extract and hordenine were also investigated on intracellular Ca^{2+} mobilization process. Ionized Ca^{2+} is a physiologically active form and is a ubiquitous signalling messenger involved in higher mitochondrial activity regions, for

example, skeletal muscle contraction and excitation-contraction in cardiac and smooth muscles.

Intracellular free Ca^{2+} concentration varies within cells, in cytoplasm, nuclear matrix and in mitochondrial matrix and ER serves as the primary Ca^{2+} store. Mitochondrial Ca^{2+} sensors are recently identified and are found in intermembrane space that control mitochondrial Ca^{2+} uptake and mitochondrial surface targeted Ca^{2+} sensors control mitochondrial motility though the distribution mechanism of mitochondrial transport of Ca^{2+} has not been fully explored yet. Mitochondrial Ca^{2+} sensing mechanism is still not fully explored, although the role of mtCU for Ca^{2+} uptake in the inner mitochondrial membrane (pore forming component) and intermembrane space and two proteins anchor mitochondria, Miro 1 target microtubular motor protein kinesin for anterograde movement and Miro 2 dynein for retrograde movement on OMM, has been identified (Bagur and Hajnoczky, 2017).

This signalling modulates vascular smooth muscle tone, stabilise plasma membrane by binding to phospholipids in the lipid bilayer, regulating plasma membranes to sodium ions affecting excitability of tissues in addition to mitochondrial morphology. AM extract significantly affected the intracellular Ca^{2+} levels at high concentrations in MCF-7 though opposite trend is observed in the case of MCF-10-A where there is a trend of an increase in intracellular Ca^{2+} levels at higher concentration. Pottle et al., in his study compared the intracellular basal concentrations of MCF-7 and MCF-10-A and found higher expression of IGF-1 in MCF-7 which increases Ca^{2+} permeability and reduced capacity to release Ca^{2+} suggesting higher concentrations of basal intracellular Ca^{2+} in MCF-7 (Pottle et al., 2013). He further suggested the functional role of T-type Ca^{2+} channels in proliferation of MCF-7 breast cancer cells (Melgari, et al., 2022; Pottle et al., 2013). Our results possibly produced effects through T-type Ca^{2+} channel in MCF-7 which can be a possible therapeutic drug target as it is also found in various other cancers for example, gastric, esophageal, and ovarian cancers are some examples. So, AM extract has potential to design T-type Ca^{2+} channel blockers like mibefradil T-type Ca^{2+} channel blocker. Differences in effects observed in both cell lines used in this study might be due to differences in expression of IGF-I signalling.

The effect of hordenine was also evaluated across three time points and no effect is observed on the intracellular concentration in MCF-7. Mitochondrial Ca^{2+} uniporter located on the IMM is the main site for the transport of Ca^{2+} into the matrix and process takes place

either via sodium- Ca^{2+} exchange protein or via Ca^{2+}-induced Ca^{2+} release pathways. These changes may affect MMP as it initiates Ca^{2+} waves which stimulate second messenger system proteins that performs several functions such as proliferation, mitosis, differentiation and possibly co-ordinate the release of hormones in endocrine cells (Neil et al., 1997; Pratt, Hernandez-Ochoa, and Martin, 2020). Influx of Ca^{2+} into mitochondrial matrix also impact mitochondrial bioenergetics and citric acid cycle (activate isocitrate dehydrogenase). In neurons, an increase in Ca^{2+} levels both in cytosol and mitochondria coordinate neuronal activity although mobilization decelerates with age. As AM extract mobilized Ca^{2+} levels though differently in both cell lines suggest it's impact on downstream signaling pathways including protein kinase (PKA) MAPK and nuclear translocation of NF-KB that are associated to Ca^{2+} mobilization. Under hypoxic conditions intracellular Ca^{2+} concentration increases immediately which is dangerous because it causes the activation of ATPase enzymes and uncontrolled release of neurotransmitters affecting normal synaptic transmission and affecting other cellular functions such as metabolism (Supnet and Bezprozvanny, 2010; Missiaen et al.,2000).

Now, interest is growing to target mitochondrial Ca^{2+} influx and efflux for the treatment of several diseases. Here are few things need to be considered that Ca^{2+} exist in different forms that is, protein bound, ionized Ca^{2+} and complexed form also extracellular and intracellular Ca^{2+} concentrations are closely regulated by different organelles such as, ER, mitochondria and sarcoplasmic reticulum. Mitochondria offer buffering Ca^{2+} activity, most likely to the organelles near OMM. However, targeting this mitochondrial communication is a very interesting area to design new drugs for the treatment of hypocalcaemia caused by hypothyroidism, renal diseases and hypercalcemia caused by bone resorption caused by hyperthyroidism and cancer, but this still needs intensive research (Slater and Cleland, 1953; Boyman, Greiser and Lederer, 2021; Lewis, 2023; Pallagi et al., 2020).

5.5 Effect of AM extract and hordenine on OXPHOS

In addition, the mitochondrial respiration rate of MCF-7 and MCF-10-A cells was also measured after treatment of cells with AM extract and hordenine for 24hr. As the

mitochondrion engages in homeostatic processes because cellular homeostasis depends on the sustained ATP generation, OCR is also recognized as a traditional measure of mitochondrial functions in pathophysiology (Zhang et al., 1993).

Oligomycin inhibitor of ATP-synthase helps to indicate O_2 consumption directly coupled to ATP generation. Carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP), a mitochondrial uncoupler, measures maximal OCR and antimycin A (complex III inhibitor) and rotenone (complex I inhibitor) together affects the ETC. In our study, hordenine has shown no significant effect on OCR in MCF-7 and MCF-10-A cell lines after exposure of 24hr at all concentrations tested (Lawrence et al., 2017; Clark, 1956). The Seahorse analyzer measures and quantifies the rate of ATP production from mitochondria and quantifies mitochondrial dysfunction in complex diseases, and is widely implemented to study the effects of natural compounds, including cytotoxicity study of bio-active components of licorice in HepG2 cells (Qian, et al., 2019), the marine sponge sesquiterpene on lung cancer cell apoptosis (Cheng et al., 2019; Mahmoud, May, and Mohammad, 2021; Rohlena et al., 2011), anti-proliferative effects of T-3-BP-AuNPs (mitochondria-targeted gold nanoparticle T-3-BP-AuNP) (Marrache and Dhar, 2015) and selective cytotoxicity of the natural compound acteoside.

AM extract was also tested on mitochondrial respiration of MCF-7 and MCF-10-A. AM extract has affected mitochondrial respiration which reduced from low to high concentrations on parameters tested such as basal respiration, non-mitochondrial respiration, ATP production, proton leak, though none of the effect reached significance in MCF-7. In the case of MCF-10-A it has significantly affected various parameters of mitochondrial respiration. This assay further shed light on the overall bioenergetics of the MCF-7 and MCF-10-A cell lines after sequential induction of the OXPHOS inhibitors. Drop in ATP linked OCR after addition of oligomycin (ATP synthase inhibitor) suggest inhibition of proton flux and this increases proton gradient across the inner mitochondrial membrane that prevents electron transport across through complexes I-IV eventually reducing oxygen consumption. As high concentrations of AM extract have severely affected ETC so reduction in other parameters indicate mitochondrial integrity is compromised which is previously observed in MMP assays. So, defects in ETC resulted in lower bioenergetics of both cell lines. Similarly, hordenine has not significantly affected most of the parameters after 24hr of exposure some effects observed could be random change as in case of spare reserve capacity.

Based on other reported studies high basal respiration indicates high ATP turnover in cell, increased proton leak, increased non- mtROS and high proton leak indicates increased UCP activity that also damages inner membrane. Furthermore, high maximal capacity suggests high substrate availability, increased mitochondrial mass and good ETC integrity and finally high non-mitochondrial respiration suggests increased cytosolic ROS levels. In this study no significant changes at lower concentrations of AM extract were observed on mitochondrial respiration and differences in parameters observed are due to several factors and need further testing to shed light on specific cellular pathways affected.

According to Warburg theory proposed around 1930, tumour cells were believed to rely on glycolysis even under oxygen rich conditions. So, it was thought that mitochondria might be dysfunctional in cancers, and this was supported by certain studies of renal, melanoma and non-small cell lung cancer, where reduced OXPHOS was observed. However, this hypothesis was later challenged because upregulated OXPHOS, as in case of pancreatic cancer, was also observed in certain cases, whereas some cancer cells use mitochondrial fatty acid oxidation as a source of energy as seen in triple negative breast cancer. So, targeting OXPHOS to deprive cells of energy may be a questionable strategy because mitochondria may reprogram, and cancer cells use glycolysis as a source of energy under stress induced by ETC modulators in this study. There is need to find drug molecules that can specifically target OXPHOS sites as in case of metformin which is used to target complex I. So far, idea of targeting glycolytic pathway along with ETC is proposed and under study (Warburg, 1956; Ward and Thompson, 2012; Evans et al., 2005; Wheaton et al., 2014; Greene, Segaran and Lord, 2022).

5.6 Effect of AM extract and hordenine on mitochondrial morphology

Also mitochondrial morphological analysis is performed to observe any changes, after hordenine treatment mitochondrial morphology is not altered only small, interconnected mitochondrial networks in MCF-7 and MCF-10-A are formed that points to the process of fission rather fusion. Further AM extract at low concentration tested caused no change in the morphology of mitochondria of both cell lines (MCF-7 and MCF-10-A) used in this experiment (Rafelski, 2013; Rohlena et al., 2011).

Findings of this study greatly contributed to the understanding of the effect of AM extract on MCF-7 for any anticancer effect and MCF-10-A for nutritive value though mechanism through which these effects are produced are still not clear, and more work is considered necessary (Perini et al., 2018; Freitas et al., 2017). Alqasoumi reported in 2008 in his study that AM extract administration of 660 mg/kg had significant hepatoprotective effects, and significantly decreased the levels of serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase in mice. In another study, AM extract at a concentration of 4 μg/ml produced skeletal muscle relaxant effect in frogs and a dose of 1.6 g/kg produced sedative effects, while 1 g/kg induced bradycardia and petroleum, while other extract has shown cytotoxic effects in dose and time dependent in human acute myeloid leukemia cell line (HL-60) (16.0 and 22.0 μg/ml) (Abdellatif et l., 2014; Gargoum et al., 2013; Sulaiman, 2013). AM is also a main ingredient of a Uyghur herbal preparation Abnormal Savda Munziq (ASMq) used in Uyghur medicine. Aikemu in 2012 investigated its antitumor activity and suggested affects are possibly through modulation of immune system (Aikemu et al., 2012; Wang et al., 2015).

Insulin-like growth factor type 1 receptor (IGF-1R) is found in human breast cancers however its expression in triple negative breast cancer is relatively low. IGF-1 is mostly secreted by stromal cells and acts through paracrine signaling to adjacent epithelial tumor cells and once the disease is well established within the primary breast tumor microenvironment, IGF-1 autocrine (within epithelium) and endocrine (via the systemic circulation) activity facilitates disease progression and metastasis respectively. Within the new tumor location, the interplay between metastasized breast cancer cells and host tissue cells drives the last to a more cancerous phenotype via IGF-1 paracrine signaling. PI3K/AKT and RAS-MAPK axes are two well-established downstream pathways of IGF/insulin signalling (Mauro et al., 2000; Peruzzi et al., 1999; Pan et al., 2016; Paradies et al., 2019). Therefore, modulation of AKT phosphorylation is potentially a therapeutic strategy for several diseases, especially inflammation of various organs. Although the exact mechanism has not yet been reported but some molecular docking studies have shown strong binding of hordenine with AKT which suggest hordenine can be a potential inhibitor of AKT signalling pathways which is linked to glucose metabolism, protein synthesis, apoptosis and cell survival and can serve as therapeutic target in breast cancer. IGF signaling is also critical for the maintenance of cellular stress homeostasis particularly ER stress which is caused by misfolded proteins and lead to ROS production so it can also be used to activate endoplasmic

stress response pathways to prevent apoptosis by inhibiting IGF signalling in breast cancer cells furthermore increasing adaptability and cell survival (Gao, et al., 2016).

AM extract has shown different effects compared to hordenine. As extracts are mixtures of several compounds and it is very difficult to find out which constituent is responsible for the purported biological activity. Urabee in 2021 reported in his chemical investigation of 98% ethanolic extract of AM the presence of more than 30 compounds (antioxidant compounds, essential oils, sterols, coumarins, vitamins and phenolic constituents) (Urabee et al., 2021). So, mass spectroscopic analysis was also carried out to find out about the bioactive compounds of the AM extract. HPTLC and LC-MS analysis has shown the presence of more than one compound in the AM extract and suggests further testing of the other compounds to interpret observed effects more appropriately.

Future Work

To address the limitations of this study some future work is required to explore pathways through which *Alhagi maurorum* and hordenine produced effects on mitochondria.

At higher concentrations, *Alhagi maurorum* induced cell death, while reducing ROS levels and altering OXPHOS. Thus, there is a need to examine changes in processes such as apoptosis, mitophagy, glycolysis, citric acid cycle of these cells. A complete footprint of mitochondrial morphology is required to complete morphological analysis in addition to quantification of mitochondrial markers of apoptosis for example mitofilin, which is the most abundant protein and maintains morphology of cristae, AIF, P53 (up-regulator of apoptosis) , cytochrome c, Kras that require ROS for proliferation, VDAC that allows entry and exit between cytosol and mitochondria and PDK4 enzyme (metabolic switch between glycolysis and citric acid cycle) through Real Time Polymerase Chain Reaction (RT-PCR). Investigating the effects of *Alhagi maurorum* on mitophagy processes could be beneficial to remove dysfunctional mitochondria. Similarly, based on given claims of anti-inflammatory properties of this plant, a complete *in vitro* and *in vivo* analysis of key inflammatory markers may be of interest. ER being the most significant site of Ca^{2+} storage and *Alhagi maurorum* altered mitochondrial Ca^{2+} concentrations in both cell lines so, there is need to study interplay between the mitochondrion and ER with respect to Ca^{2+}.

Hordenine, an important constituent of *Alhagi maurorum* was also tested in this study and showed no prominent effects on mitochondrial functions, but with some specific differences such as effect on ROS levels after 6hr of exposure in both cell lines used in this study. These differences may arise from what is known as the additive, synergistic or antagonistic effects as seen in case of entourage effect observed in cannabis where mixtures of compounds are associated with modulation of the principal bioactive compound and work by Pan, showed combining baicalein and taxol induce mitochondria mediated apoptosis (Pan et al., 2016).

Mass spectroscopic analysis of *Alhagi maurorum* clearly indicates the presence of more than one compound in the mixture. Plant extracts with ability to regulate mitochondrial functions can offer new opportunities to treat various complex diseases that require more than one bioactive compound and to address issues of drug resistance and side effects by choosing combinations of different bioactive compounds (Caesar and Cech, 2019; Ferber et

al., 2020). Furthermore, there is also needed to carry out LC-MS analysis of hordenine to compare with the fragmentation pattern of hordenine observed in the LC-MS analysis of *Alhagi maurorum* extract.

Data gathered from this study must be supported by the above-mentioned experiments to get further in-depth insight into the observed effects and potential cellular pathways involved. This will open several therapeutic targets for the treatment of pathologies mainly due to altered metabolism, for example, diabetes, cancer, obesity, and neurodegenerative disorders**.**

Lastly, this study has utilized only breast cell models which are very valuable for the preliminary investigation due to the ease of handling, replication and consistency which makes them good model for comparison of results between experiments. However, it is well known there are several disadvantages such as possible genetic and phenotypic drift over extended passage numbers of cell lines, possible contamination, and lack of key characteristics of cancers such as complex microenvironment and cancer stem cells so, it is worth looking at more complex models, such as 3D cellular models.

Conclusion

In conclusion, the results from my PhD work indicate *Alhagi maurorum* has potential to regulate mitochondrial functions and is a potential source of compounds targeting mitochondrial functions. At this stage my work focused on the metabolic aspect of mitochondrial functions only and it would be interesting to undertake in depth study of proteomics and transcriptomics to get better understanding of the molecular mechanism underpinning the effects of *Alhagi maurorum* and its other constituents on associated mitochondrial and cellular functions. This work provided new insight into the effects of *Alhagi maurorum* on mitochondrial functions and will attract more in-depth study and may contribute to the design of more effective and targeted drug candidates considering metabolic flexibility of the cellular system.

References

Abdellatif, A.G., Gargoum, H.M., Debani, A.A., Bengleil, M., Alshalmani, S., El., Zuki, N., et al., (2014). Camel thorn has hepatoprotective activity against carbon tetrachloride or acetaminophen induced hepatotoxicity but enhances the cardiac toxicity of Adriamycin in rodents. World Acad Sci Eng Technol., 8(2), 58-62. 43. https://doi: 10.13140/2.1.1895.9041.

Abdel, R. M.A., and Nidal, O., (2010). Use of sorghum and maize allelopathic properties to inhibit germination and growth of wild barley. Not Bot Hort Agrobot Cluj, 38(3), 124-127.

Achan, J., Talisuna, A. O., Erhart, A., Yeka, A., Tibenderana, J. K., Baliraine, F. N., Rosenthal, P. J. and D'Alessandro, U., (2011). Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria. Malaria journal, 10, 144. https://doi: org/10.1186/1475-2875-10-144.

Aghvami, M., Ebrahimi, F., Zarei, M.H., Salimi, A., Jaktaji, R.P., Pourahmad, J., (2018). Matrine induction of ROS mediated apoptosis in human B-lymphocytes Via mitochondrial targeting. APJCP, 19, 555–560. http://doi: 10.22034/APJCP.2018.19.2.555

Agilent, (2021). Seahorse XF cell Mito stress test kit. https://www.agilent.com/en/product/cell-analysis/real-time-cell-metabolic-analysis/xf-assay-kits-reagents-cell-assay-media/seahorse-xf-cell-mito-stress-test-kit-740885.

Ahmed, Measer. (2019). Protective effect of aqueous extract of Alhagi maurorum in spermatogenesis and antioxidant status of adult rats exposed to carbon tetrachloride. Iraqi J Vet Sci, 33, 1-7. https://doi: 10.33899.125509.1031.

Ahmad S., Riaz N., Saleem M., Jabbar A., Rehman N. and Ashraf M., (2010). Antioxidant flavonoids from *Alhagi maurorum* M., J Asian Nat Prod Res, 12(2), 138–143. https://doi:10.1080/10286020903451724.

Ahmed, M., (2019). Protective effect of aqueous extract of *Alhagi maurorum* M., in spermatogenesis and antioxidant status of adult rats exposed to carbon tetra chloride. Iraqi J. Vet Sci, 33, 1-7. https://doi: 10.33899/IJVS.2019.125509.1031.

Aikemu, A., Umar, A., Yusup, A., Upur, H., Berke, B., Begaud, B., Moore, N., (2012). Immunomodulatory and antitumour effects of abnormal Savda Munziq on S180 tumour-bearing mice. BMC Compl Altern Med, 12(157). https://doi: 10.1186/1472-6882-12-157.

Ait-Aissa, K., Blaszak, S.C., Beutner, G. et al., (2019). Mitochondrial oxidative phosphorylation defect in the heart of subjects with coronary artery disease. Sci Rep, 9, 7623. https://doi.org/10.1038/s41598-019-43761-y

Alkhazraji, A., Elgamal, M., Hui Ang, S. and Shivarov, V., (2019). Review: Article all cancer hallmarks lead to diversity. Int J Clin Exp Med, 12(1):132-157. ISSN:1940-5901/IJCEM0072581.

Al-Askar, A., (2012). In vitro antifungal activity of three saudi plant extracts against some phytopathogenic fungi. Quimica Agricola y Biotecnologia, 3 (8), 277–284. _https://doi.org/10.2478/v10045-012-0073-9.

Al-Snafi, A., (2015). *Alhagi maurorum* M. as a potential medicinal herb: An Overview. Int J Pharm Rev Res. 5. 130-136.

Alchetron, (2018). *Alhagi maurorum* M., [image]. Available from https://alchetron.com/Alhagi.

Ali E.S., Hua J., Wilson C.H., Tallis G.A., Zhou F.H., Rychkov G.Y., Barritt G.J., (2016). "The glucagon-like peptide-1 analogue exendin-4 reverses impaired intracellular Ca2+ signalling in steatotic hepatocytes". Biochim Biophys Acta, Mol Cell Res, 1863 (9), 2135–46. Doi: 10.1016/j.bbamcr.2016.05.006.

Alqasoumi, S.I., Al-Rehaily, A.J., AlSheikh, A.M., Abdel-Kader, M.S., (2008). Evaluation of the hepatoprotective effect of *Ephedra foliate* F. & C., *Alhagi maurorum* M., *Capsella bursa-pastoris* M., and *Hibiscus sabdariffa* L., against experimentally induced liver injury in rats. Nat Prod Sci., 14(2), 95-9. https://doi: 10.210.101.116.28/W_files/kiss2/05004355_pv.

Alqasoumi, S. I., (2007). Isolation and chemical structure elucidation of hepatoprotective constituents from plants used in traditional medicine in Saudi Arabia. Riyadh: College of Pharmacy, King Saud University.http:// https://faculty.ksu.edu.sa/sites/default/files/my_c.v._english_dr._saleh_alqasoumi_0.pdf

Antico Arciuch, V.G., Elguero, M.E., Poderoso, J.J. and Carreras, M.C. (2012). Mitochondrial regulation of cell cycle and proliferation. Antioxi Redox Signal, 16(10), 1150–1180. https://doi: 10.1089/ars.2011.4085.

Anders S. and Schroeter C., (2017). The impact of nutritional supplement intake on diet behavior and obesity outcomes. PLoS ONE 12(10), 185-258. https://doi.org/10.1371/journal.pone.0185258.

Andersen T., and Fogh J., (2001). Weight loss and delayed gastric emptying following a South American herbal preparation in overweight patients. J Hum Nutr Diet, 14(3), 243-50. https://doi: 10.1046/j.1365-277x.2001.00290. x.

Angajala, A., Lim, S., Phillips, J.B., Kim, J.H., Yates, C., You, Z., Tan, M., (2018). Diverse roles of mitochondria in immune responses: Novel insights into immuno-metabolism. Front Immunol. 9, Al1605. https://doi: 10.3389/fimmu.2018.01605.

Anwar, S., Mohammad, T., Shamsi, A., Queen, A., Parveen, S., Luqman, S., Hasan, G. M., Alamry, K. A., Azum, N., Asiri, A. M., and Hassan, M. I. (2020). Discovery of hordenine as a potential inhibitor of pyruvate dehydrogenase kinase 3: Implication in lung cancer therapy. Biomedicines, 8(5), 119. https://doi.org/10.3390/biomedicines8050119

Arnold, I., Wagner-Ecker, M., Ansorge, W. and Langer, T., (2006). Evidence for a novel mitochondria-to-nucleus signalling pathway in respiring cells lacking i-AAA protease and the ABC-transporter Mdl1. Gene, 367, 74-88. https://doi: 10.1016/j.gene.2005.09.044

Ara, I.Y., S., Bukhari, B., Raja, W., and Maqbool, M., (2020). Managing anxiety in Unani system of medicine, IJMPR, 4,143-148. http:// doi: 10.1111/tbj.12355.

Arauna, D., Furrianca, M.; Espinosa-Parrilla, Y., Fuentes, E., Alarcon, M. and Palomo, I., (2019). Natural bioactive compounds as protectors of mitochondrial dysfunction in cardiovascular diseases and aging. Mol, 24 (23). https://doi: 10.3390/molecules24234259.

Arnold, C. R., Mangesius, J., Skvortsova, I. I., and Ganswindt, U., (2020). The role of cancer stem cells in radiation resistance. Front Oncol, 10, 164. https://doi.org/10.3389/fonc.2020.00164.

Arlen W.F., and Leo M., (1956). The biogenesis of alkaloids: XVI. Hordenine metabolism in barley. Can J Chem, 34(11), 1641-1646. https://doi.org/10.1139/v56-212.

Arno H., Justin T. F., Monica L.D., Andrea L., Renee L. R., (2010). Chemistry of Cannabis, Editor(s): Hung-Wen (Ben) Liu, Lew Mander, Comp Nat Pro II, Elsevier, 1033-1084.

Ardekani, R.R., Rahimi, R., and Mohammad, M.A., (2010). A review of the efficacy of traditional Iranian medicine for inflammatory bowel disease. World J Gastroenterol, 16, 4504-14. https://doi: 10.3748/wjg.v16.i36.4504.

Aslam, M. and Ahmad, M.S., (2016). Alhagi maurorum M., and Tamarix aphylla H., two medicinal weeds mentioned in Holy Quran and Hadith and their ethanomedicine uses in district Rajhanpur of Pakistan. Univ J Pharm, 1, 81-85. https://doi: 10.22270/ujpr.v1i1.RW5.

Asghari, M. H., Fallah, M., Moloudizargari, M., Mehdikhani, F., Sepehrnia, P., and Moradi, B. (2016). A systematic and mechanistic review on the phytopharmacological properties of Alhagi Species. Anc Sci Life, 36(2), 65–7. https://doi: 10.4103/asl.ASL_37_16.

Asghari J., and Tewari J.P., (2007). Allelopathic potentials of eight barley cultivars on *Brassica juncea* C., and *Setaria viridis* L.. J Agric Sci Technol., 9, 165-176. https://doi: 20.1001.1.16807073.2007.9.2.3.0.

Ashkenazi, A., Fairbrother, W. J., Leverson, J. D., and Souers, A. J., (2017). From basic apoptosis discoveries to advanced selective BCL-2 family inhibitors. Nat Rev Drug Discov, 16(4), 273–284. https://doi: 10.1038/nrd.2016.253.

Atta, A.H., and Abo E. K., (2004). The antinociceptive effect of some Egyptian medicinal plant extracts. J Ethnopharmacol, 95 (2), 235-238. https://doi: 10.1016/j.jep.2004.07.006.

Audano, M., Pedretti, S., Ligorio, S., Crestani, M., Caruso, D., De Fabiani, E., Mitro, N., (2020) "The Loss of Golden Touch": Mitochondria-organelle interactions, metabolism, and cancer. Cells, 21, 9(11), 2519. https://doi: 10.3390/cells9112519.

Avois, L., Robinson, N., Saudan, C., Baume, N., Mangin, P., and Saugy, M., (2006). Central nervous system stimulants and sport practice. Br J Sport Med, 40, (Suppl 1), 16–20. https://doi.org/10.1136/bjsm.2006.027557.

Awaad, A. S., El-Meligy, R. M., Qenawy, S. A., Atta, A. H., and Soliman, G. A., (2011). Anti-inflammatory, antinociceptive and antipyretic effects of some desert plants. J Saudi Chem Soc, 15(4), 367-373. https://doi.org/10.1016/j.jscs.2011.02.004

Ayman, M. M., May B., Mohammad, H. A., (2021). 9-Anti-inflammatory natural products from marine algae. Inflammation and natural products, 175-203. https://doi:10.1016/B978-0-12-819218-4.00012-2.

Ayob, A.Z., Ramasamy, T.S., (2018). Cancer stem cells as key drivers of tumors progression. J Biomed Sci, 25, 20. https://doi.org/10.1186/s12929-018-0426-4.

Baggetto, L. G., and Testa-Parussini, R., (1990). Role of acetoin on the regulation of intermediate metabolism of Ehrlich ascites tumor mitochondria: its contribution to membrane cholesterol enrichment modifying passive proton permeability. Arch Biochem Biophys, 283(2), 241-248. https://doi: 10.1016/0003-9861(90)90638-f.

Baik, J.H., (2013). Dopamine signalling in food addiction: role of dopamine D2 receptors. BMB reports, 46(11), 519. https:// doi: 10.5483/bmbrep.2013.46.11.207

Bao J., Huang B., Zou L., Chen S., Zhang C., Zhang Y., He C., (2015). Hormetic effect of berberine attenuates the anticancer activity of chemotherapeutic agents. PLoS One,10(9),1–13. https://doi.org/10.1371/journal.pone.0139298.

Bartel, K., Pein, H., Popper, B. et al., (2019). Connecting lysosomes and mitochondria – a novel role for lipid metabolism in cancer cell death. Cell Commun Signal 17**(**87). https://doi:10.1186/s12964-019-03

Barwell, C.J., Basma, A.N., Lafi, M.A., Leake L.D., (1989). Deamination of hordenine by monoamine oxidase and its action on vasa deferentia of the rat. J Pharm Pharmacol. 41(6):421-3. https:// doi: 10.1111/j.2042-7158.1989.tb06492.x.

Barcena, C., Mayoral, P. and Quiros, P.M. (2018). Chapter Two – Mitohormesis, an antiaging paradigm. Int Rev Cell Mol Biol., 340, 35-77. https://doi: 10.1016/bs.ircmb.2018.05.002.

Beattie, D.T. and Smith, J.A., (2008). "Serotonin pharmacology in the gastrointestinal tract: a review". Naunyn-Schmied Arch Pharmacol, 377 (3), 181–203. https:// doi:10.1007/s00210-008-0276-9.

Bedoui, S., Herold, M.J., and Strasser, A., (2020). Emmerging connectivity of programmed cell death pathways and it's physiological implications. Nat Rev Mol Cell Biol, 21, 678-695. https://doi: 10.1038/s41580-020-0270-8.

Bell, A., Dorsch, K.D., Mccreary, D.R. and Hovey, R., (2004). A look at nutritional supplement use in adolescents. J Adolesc Health, 34(6),508-516. https://doi: 10.1016/j.jadohealth.2003.07.024.

Benard et al., 2007, G., Bellance, N., James, D., Parrone, P., Fernandez, H., Letellier, T. and Rossignol, R., (2007). Mitochondrial bioenergetics and structural network organization. J Cell Sci, 120, 838-848. https:// doi: 10.1242/jcs.03381.

Ben-Jonathan N., and Hnasko R., (2001). "Dopamine as a prolactin (PRL) inhibitor,". Endocr Rev, 22, 6, 724–763. https:// doi: 10.1210/edrv.22.6.0451

Bertheloot, D., Latz, E. and Franklin, B.S., (2021). Necroptosis, pyroptosis and apoptosis: an intricate game of cell death. Cell Mol Immunol, 18, 1106-1121. https://doi.org/10.1038/s41423-020-00630-3.

Bertolotto, C., Abbe, P., Hemesath, T. J., Bille, K., Fisher, D. E., Ortonne, J. P., and Ballotti, R., (1998). Microphthalmia gene product as a signal transducer in cAMP-induced differentiation of melanocytes. J Cell Bio, 142(3), 827-835. https:// doi: 10.1083/jcb.142.3.827

Bernal, S. D., Lampidis, T. J., McIsaac, R. M., and Chen, L. B., (1983). Anticarcinoma activity in vivo of rhodamine 123, a mitochondrial-specific dye. Science, 222(4620), 169-172. https://doi: 10.1126/science.6623064.

Bernardino de Sihagun, (1880). "Historia General de las Cosas de Nueva Espaiia," 1560, Bustamente, Ed., Paris, France.

Bentley, K.W., (2006). β-Phenylethylamines and the isoquinoline alkaloids. Nat Prod Rep, 23(3), 444-463. https://doi.org/10.1039/B509523A

Birsoy, K., Wang, T., Chen, W.W., Freinkman, E., Abu-Remaileh, M., and Sabatini D.M., (2015). An essential role of the mitochondrial electron transport chain in cell proliferation is to enable aspartate synthesis. Cell 162, 540–551. https://doi: 10.1016/j.cell.2015.07.016.

Blacker, T. S., Mann, Z. F., Gale, J. E., Ziegler, Mathias, B. A J., Szabadkai, G., Duchen, M. R., (2014). "Separating NADH and NADPH fluorescence in live cells and tissues using FLIM". Nature Communications. Springer Science and Business Media LLC. **5** (1), https://doi:10.1038/ncomms4936. ISSN 2041-1723. PMC 4046109.

Bobrovnikova, E. and Hurov, J.B., (2014). Targeting metabolic changes in cancer: Novel therapeutic approaches. Annu Rev Med, 65(1), 157-170. https://doi: 10.1146/annurev-med-092012-112344.

Bonjar, G. S., (2004). Inhibition of three isolates of Staphylococcus aureus mediated by plants used by Iranian native people. J Med Sci, 4(2), 136-141. http://doi: 10.3923/jms.2004.136.14.

Bos, J. L., (2006). "Epac proteins: multi-purpose cAMP targets". Trends in Biochemical Sciences. 31 (12), 680–686. https://doi:10.1016/j.tibs.2006.10.002.

Boyman, L., Greiser, M., Lederer, W.J., (2021). Calcium influx through the mitochondrial calcium uniporter holocomplex, MCU_{cx}. J Mol Cell Cardiol, 151,145-154.http://doi: 10.1016/j.yjmcc.2020.10.015.

Bryant, K.G., Chae, Y.C., Martinez, R.L., Gordon, J.C., Elokely, K.M., Kossenkov, A.V., Grant, S., Childers, W.E., Abou-Gharbia, M., Altieri, D.C., (2017). A mitochondrial-targeted purine-based HSP90 antagonist for leukemia therapy. Oncotarget, 8, 112184–112198. http://doi: 10.18632/oncotarget.23097.

Brady, R.L., and Tyler, V.E., (1958). Biosynthesis of hordenine in tissue homogenates of Panicum miliaceum l. Plant Physiol, 334-338. http:// doi: 10.1104/pp.33.5.334

Braymer, J.J., and Lill, R., (2017). Iron–sulfur cluster biogenesis and trafficking in mitochondria. J Bio Chem, 292(31), pp.12754-12763.

Bridson, D., and Forman L., (1998). The herbarium handbook, 3rd ed. Royal botanic gardens, kew, Great Britain.

British Columbia Ministry of Forests., (1996). Techniques and procedures for collecting, preserving, and storing botanical specimens. Research branch, British Columbia ministry of forests, victoria, BC, Canada. Working paper 18.

Brady L.R. and Tyler V.E., (1958). Biosynthesis of hordenine in tissue homogenates of *Panicum miliaceum* L., Plant Physiol, 33, 334-340. https:// doi: 10.1104/pp.33.5.334.

Brauers, G., Steiner, I. and Daldrup, T. (2013). Quantification of the biogenic phenethylamine alkaloid hordenine by LC-MS / MS in beer. Toxichem Krimtech, 80(Special Issue),323. http://doi: 10.1016/s0021-9673(99)00644-5.

Brand, M.D., (2016). Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. Free Radic Biol Med, 100,14-31. http://doi: 10.1016/j.freeradbiomed.2016.04.001.

Busca, R., and Ballotti, R., (2000). Cyclic AMP a key messenger in the regulation of skin pigmentation. Pigment Cell Res, 13(2), 60-69. https:// doi: 10.1034/j.1600-0749.2000

Buajitti E, Rosella L.C., Zabzuni E., Young L.T., Andreazza A.C., (2022). Prevalence and health care costs of mitochondrial disease in Ontario, Canada: A population-based cohort study. PLoS ONE, 17(4), e0265744. https://doi.org/10.1371/journal.pone.0265744.

Busiello RA, Savarese S, Lombardi A., (2015). Mitochondrial uncoupling proteins and energy metabolism. Front Physiol, 6(36).http://doi: 10.3389/fphys.2015.00036.

Caesar, L.K., and Cech, N.B., (2019). Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2. Nat Prod Rep., 36(6):869-888. http:// doi: 10.1039/c9np00011a.

Calvo, R.Y., Araneta, M.R., Kritz-Silverstein, D., Laughlin, G.A., Barrett-Connor, E., (2014). Relation of serum uric acid to severity and progression of coronary artery calcium in postmenopausal White and Filipino women (from the Rancho Bernardo study). Am J Cardiol, 113(7),1153-8. http:// doi: 10.1016/j.amjcard.2013.12.022.

Calvo, S. E. and Mootha, V. K. (2010). The mitochondrial proteome and human disease. Annu Rev Genomics Hum Genet, 11, 25–44. http://doi: 10.1146/annurev-genom-082509-141720

Camarillo, I.G., Xiao, F., Madhivanan, S., Salameh, T., Nichols, M., Reece, L.M., et al., (2014). 4—Low and high voltage electrochemotherapy for breast cancer: an in vitro model study. In: Sundararajan R, editor. Electroporation-based therapies for cancer: Woodhead Publishing, 55–102. https:// doi: 10.1533/9781908818294.55

Cao, X., Lin, X., Wu, C., Zhang, M., Wang, M., (2022). Green extraction-assisted pseudo-targeted profile of alkaloids in lotus seed epicarp based on UPLC-QTOF MS with IDA. Foods,11(7),1056. http:// doi: 10.3390/foods11071056

Carafoli, E., (2003). Historical review: mitochondria and calcium: ups and downs of an unusual relationship. Trends Biochem Sci, 28(4),175-181. http://doi: 10.1016/S0968-0004(03)00053-7.

Cardenas, C., Miller, R.A., Smith, I., Bui, T., Molgo, J., Muller, M., Vais, H., Cheung, K.H., Yang, J. and Parker, I., et al., (2010). Essential regulation of cell bioenergetics by

constitutive InsP3 receptor Ca^{2+} transfer to mitochondria. Cell, 142, 270-283. http://doi: 10.1016/j.cell.2010.06.007.

Cardinale, D.A, Larsen, F.J., Schiffer, T.A., Morales-Alamo, D., Ekblom, B., Calbet, J.A.L., Holmberg, H.C. and Boushel, R. (2018). Superior intrinsic mitochondrial respiration in women than in men. Front Physiol, 17 (9),1133. http:// doi: 10.3389/fphys.2018.01133

Chao, C.C., Hou, S.M., Huang, C.C., Hou, C.H., Chen, P.C., Liu, J.F., (2017). Plumbagin induces apoptosis in human osteosarcoma through ROS generation, endoplasmic reticulum stress and mitochondrial apoptosis pathway. Mol Med Rep, 16(4), 5480-5488. http://doi: 10.3892/mmr.2017.7222.

Chance, B. and Williams, G.R., 1955. Respiratory enzymes in oxidative phosphorylation: III. The steady state. J Biol Chem, *217*(1), 409-427.

Chae, Y.C., Caino, M.C., Lisanti, S., Ghosh, J.C., Dohi, T., Danial, N.N., Villanueva, J., Ferrero, S., Vaira, V., Santambrogio, L., et al., (2012). Control of tumor bioenergetics and survival stress signaling by mitochondrial HSP90s. Cancer Cell, 22, 331–344. http://doi: 10.1016/j.ccr.2012.07.015

Chemat, F., Vian, M.A., Cravotto, G., (2012). Green extraction of natural products: concept and principles. Int J Mol Sci,13(7), 8615-8627. http://doi: 10.3390/ijms13078615.

Cheng, G., Zielonka, J., Dranka, B.P., McAllister, D., Mackinnon, A.C., Joseph, J., Kalyanaraman, B., (2012). Mitochondria-targeted drugs synergize with 2-deoxyglucose to trigger breast cancer cell death. Cancer Res, 72, 2634–2644. http://doi: 10.1158/0008-5472.CAN-11-3928

Cui, Q., Wen, S., and Huang, P., (2017). Targeting cancer cell mitochondria as a therapeutic approach: Recent updates. Future Med Chem, 9, 929–949. http://doi: 10.4155/fmc-2017-0011.

Chinnery, P.F. (2021). Primary mitochondrial disorders Overview: In: Adam MP, Ardinger HH, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1224.

Chang, H.W., Shtessel, Lb., and Lee, S.S., (2015). Collaboration between mitochondria and the nucleus is key to long life in Caenorhabditis elegans. Free Radic Biol Med, 78, 168-178. http://doi: 10.1016/j.freeradbiomed.2014.10.576

Collins, E.J., Cervantes-Silva, M.P., Timmons, G.A., O'Siorain, J.R., Curtis, A.M., Hurley, J.M., (2021). Post-transcriptional circadian regulation in macrophages organizes temporally distinct immunometabolic states. Genome Res 31,171–185. http:// doi: 10.1101/gr.263814.120

Collins, L.V., Hajizadeh, S., Holme, E., Jonsson, I.M. and Tarkowski, A., (2004). Endogenously oxidized mitochondrial DNA induces in vivo and in vitro inflammatory responses. J Leukoc Biol 75, 995-1000. http://doi: 10.1189/jlb.0703328

Collins, T.J., Berridge, M.J., Lipp, P., Bootman, M.D., (2002). Mitochondria are morphologically and functionally heterogeneous within cells. EMBO J, 2,21(7),1616-27. http://doi: 10.1093/emboj/21.7.1616

Collins, H.E., Kane, M.S., Litovsky, S.H., Darley-Usmar, V.M., Young, M.E., Chatham, J.C. and Zhang, J., (2021). Mitochondrial morphology and mitophagy in heart diseases: Qualitative and quantitative analyses using transmission electron microscopy. Front Aging 2,670267. http://doi: 10.3389/fragi.2021.670267

Currie, E., Schulze, A., Zechner, R., Walther, T.C. and Farese Jr, R.V., (2013). Cellular fatty acid metabolism and cancer. Cell Metab, 18(2),53-161. http://doi: 10.1016/j.cmet.2013.05.017

Chen, Z., Zhang, H., Lu, W., and Huang, P., (2009). Role of mitochondria-associated hexokinase II in cancer cell death induced by 3-bromopyruvate. Biochim Biophys Acta (BBA)-Bioenergetics, 1787(5), 553-560. http://doi: 10.1016/j.bbabio.2009.03.003

Cheng, G., Zielonka, J., Dranka, B.P., McAllister, D., Mackinnon, A.C., Joseph, J., et al., (2012). Mitochondria-targeted drugs synergize with 2-deoxyglucose to trigger breast cancer cell death. Cancer Res,72(10), 2634–44. http://doi: 10.1158/0008-5472.CAN-11-3928

Chipuk, J.E., Moldoveanu, T., Llambi, F., Parsons, M.J., and Green, D.R. (2010). The BCL-2 family reunion. Mol Cell, 37, 299-310. http://doi: 10.1016/j.molcel.2010.01.025

Chase C.R., and Pratt R.J., (1949). Fluorescence of powdered vegetables drugs with reference to development of a system of identification, J Am Pharm Assoc, 33(6), 428-432. https://doi: 10.1002/jps.3030380612

Cheng, L., Shi, L., Wu, J., Zhou, X., Li, X., Sun, X., Zhu, L., Xia, T.S. and Ding, Q., (2018). A hederagenin saponin isolated from Clematis ganpiniana induces apoptosis in breast cancer cells via the mitochondrial pathway. Oncol Lett, 15(2), 1737-1743. https:// doi: 10.3892/ol.2017.7494.

Cheng, G., Zielonka, J., Dranka, B. P., McAllister, D., Mackinnon, A. C., Jr, Joseph, J. and Kalyanaraman, B. (2012). Mitochondria-targeted drugs synergize with 2-deoxyglucose to trigger breast cancer cell death. Cancer Res, 72(10), 2634–2644. https://doi.org/10.1158/0008-5472.CAN-11-3928

Cheng, M., Huang, H., Lin, Y., Tsui, K., Chen, P., Cheng, S., Chong, I., Sung, P., Tai, M., Wen, Z., Chen, N. and Kuo, H. (2019). BA6 Induces apoptosis via stimulation of reactive oxygen species and inhibition of oxidative phosphorylation in human lung cancer cells. Article ID 6342104,21. Oxid Med Cell Longev. https:// doi: 10.1155/2019/6342104.

Chen, W., Calvo, P.A., Malide, D., Gibbs, J., Schubert, U., Bacik, I., Basta, S., O'Neill, R., Schickli, J., Palese, P., Henklein, P., Bennink, J.R., Yewdell, J.W., (2001). A novel influenza A virus mitochondrial protein that induces cell death. Nat Med. (12), 1306-12. https://doi: 10.1038/nm1201-1306.

Cho, B., Kim, T., Huh, Y. J., Lee, J., and Lee, Y. I., (2019). Amelioration of mitochondrial quality control and proteostasis by natural compounds in parkinson's disease models. Int J Mol Sci, 20 (20), 5208. https://doi: 10.3390/ijms20205208.

Choi, H.D., Kim, K.Y., Park, K.I., Kim, S.H., Park, S.G., Yu, S.N., Kim, Y.W., Kim, D.S., Chung, K.T., Ahn, S.C., (2020). Dual role of reactive oxygen species in autophagy and apoptosis induced by compound PN in prostate cancer cells. Mol Cell Toxicol, 17, 41–50. https://doi.org/10.1007/s13273-020-00107-4

Ciccarelli A., Daly A.F. and Beckers A., (2005). "The epidemiology of prolactinomas," Pituitary, 8(1), 3–6. https:// doi: 10.1007/s11102-005-5079-0.

Clark, L.C., (1956). Monitoring and control blood and tissue oxygen. Trans Am Soc Artif Intern Organs 2:41–48. https://doi:10.4236/jbise.2014.712094

Coleman, V., Sa-Nguanmoo, P., Koenig, J. et al., (2018). Partial involvement of Nrf2 in skeletal muscle mitohormesis as an adaptive response to mitochondrial uncoupling. Sci Rep, 8, 2446. https:// doi: 10.1038/s41598-018-20901-4

Collet C., Schiltz C., Geoffroy V., et al., (2008). "The serotonin 5-HT2B receptor controls bone mass via osteoblast recruitment and proliferation". FASEB Journal. 22 (2): 418–27. https:// doi:10.1096/fj.07-9209com.

Cui, Q., Wen, S. and Huang, P., (2017). Targeting cancer cell mitochondria as a therapeutic approach: recent updates. Future Med Chem., 9(9),929–49. https:// doi: 10.4155/fmc-2017-0011.

Cushnie T.P., Cushnie B., and Lamb A.J., (2014). Alkaloids: an overview of their antibacterial, antibiotic-enhancing and antivirulence activities. Int J Antimicrob Agents, 44(5), 377-86. https://doi: 10.1016/j.ijantimicag.2014.06.001.15.

Cuyas, E., Sara V., Nuria F., Cristian, B., Angel G. Martind, T. Alarconc, E., and Javier A., (2018). Mitostemness. Cell Cycle, 17(8), 918-926. https://doi.org/10.1080/15384101.2018.1467679.

Dai, D.F. Rabinovitch, P.S., and Ungvari, Z., (2012). Mitochondria and cardiovascular aging. Circ. Res., 110. https://doi: 10.1161/CIRCRESAHA.111.246140.

Daly A.F., Tichomirowa M.A. and Beckers A., (2009). "The epidemiology and genetics of pituitary adenomas," Best Pract Res Clin Endocrinol Metab, 23(5), 543–554. https:// doi: 10.1016/j.beem.2009.05.008.

Das A., Jawed J.J., Das M.C., Sandhu P., De U.C., Dinda B., Akhter Y., Bhattacharjee S., (2017). Antileishmanial and immunomodulatory activities of lupeol, a triterpene compound isolated from Sterculia villosa. Int J Antimicrob Agents, 50(4), 512-522. doi: 10.1016/j.ijantimicag.2017.04.022.

Daurio, N.A.,Tuttle, S.W., Worth, A.J., Song, E.Y., Davis, J.M., Snyder, N.W., Blair, I.A., Koumenis, C., (2016). AMPK activation and metabolic reprogramming by tamoxifen through estrogen receptor-independent mechanisms suggests new uses for this therapeutic modality in cancer treatment. Cancer Res, 76, 3295–3306. http://doi: 10.1158/0008-5472.CAN-15-2197

De Francesco, E. M., Sotgia, F., and Lisanti, M. P. (2018). Cancer stem cells (CSCs): metabolic strategies for their identification and eradication. Biochem J, 475(9), 1611-1634. https://doi: 10.1042/BCJ20170164.

DeFronzo, R.A. and Tripathy, D., (2009). Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes care, 32(suppl-2), S157-S163. https://doi: 10.2337/dc09-S302.

Demers-Lamarche, J., Guillebaud, G., Tlili, M., Todkar, K., Bélanger, N., Grondin, M., Nguyen, A.P., Michel, J. and Germain, M., (2016). Loss of mitochondrial function impairs lysosomes. J Biol Chem, 291(19), 10263-10276. https://doi: 10.1074/jbc.M115.695825.

Desagher S., and Martinou J.C., (2000). Mitochondria as the central control point of apoptosis. Trends Cell Biol, 10(9), 369-77. https:// doi: 10.1016/s0962-8924(00)01803-1.

De Stefani, D., Bononi, A., Romagnoli, A., Messina, A., De Pinto, V., Pinton, P., Rizzuto, R., (2012). VDAC1 selectively transfers apoptotic Ca^{2+} signals to mitochondria. Cell Death Differ, 19(2), 267-73. https://doi: 10.1038/cdd.2011.92.

De Vivo, D. C., Hirano, M., and DiMauro, S., (1996). Mitochondrial disorders. In H. W. Moser (ed.), Handbook of Clinical Neurology, vol. 22. Amsterdam: Elsevier, 389–446.

DiMauro, S., (2013). Luft's Disease, Editor(s): William J. Lennarz, M. Daniel Lane, Encyclopedia of Biological Chemistry (Second Edition), Academic Press, 760-761, https://doi.org/10.1016/B978-0-12-378630-2.00122-5.

DiMauro, S., Bonilla, E., Lee, C.P., Schotland, D.L., Scarpa, A., Conn, H. and Chance, B., (1976). Luft's disease: Further biochemical and ultrastructural studies of skeletal muscle in the second case, J Neurol Sci, 27(2), 217-232, https://doi.org/10.1016/0022-510X(76)90063-0.

DiDonato, S., (1997). Diseases associated with defects of beta-oxidation. In R. N. Rosenberg, S. B. Prusiner, S. DiMauro, and R. L. Barchi (eds.), The molecular and genetic basis of neurological disease. Boston: Butterworth-Heinemann, 939–956.

Dong, L., Gopalan, V., Holland, O., and Neuzil, J., (2020). Mitocans Revisited: Mitochondrial targeting as efficient anti-cancer therapy. Int J Mol Sci, 21(21), 7941. https://doi.org/10.3390/ijms21217941.

Dong, L.F., Low, P., Dyason, J., Wang, X.F., Prochazka, L., Witting, P.K., Freeman, R., Swettenham, E., Valis, K., Liu, J., et al., (2008). α-Tocopheryl succinate induces apoptosis by targeting ubiquinone-binding sites in mitochondrial respiratory complex II. Oncogene, 27, 4324–4335. http://doi: 10.1038/onc.2008.69.

Dong, L.F., Jameson, V.J.A., Tilly, D., Cerny, J., Mahdavian, E., Marin-Hernandez, A., Hernandez-Esquivel, L., Rodriguez-Enriquez, S., Stursa, J., Witting, P.K., et al., (2011). Mitochondrial targeting of vitamin E succinate enhances its pro-apoptotic and anti-cancer activity via mitochondrial complex II. J Biol Chem, 286, 3717–3728. http://doi: 10.1074/jbc.M110.186643.

Dowling, J.K., Afzal, R., Gearing, L.J., Cervantes-Silva, M.P., Annett, S., Davis, G.M., De Santi, C., Assmann N., Dettmer, K., Gough, D.J., et al., (2021). Mitochondrial arginase-2

is essential for IL-10 metabolic reprogramming of inflammatory macrophages. Nat Commun 12, 1–14. https:// doi: 10.1038/s41467-021-21617-2.

Dojindo, (2022). Reagent for Monitoring Calcium Ion. https://www.dojindo.com/EUROPE/products/R001/.

Du, J., Cleghorn, W.M., Contreras, L., Lindsay, K., Rountree, A.M., Chertov, A.O., Turner, S.J., Sahaboglu, A., Linton, J., Sadilek, M. and Satrustegui, J., (2013). Inhibition of mitochondrial pyruvate transport by zaprinast causes massive accumulation of aspartate at the expense of glutamate in the retina. J Biol Chem, 288(50), 36129-36140. https:// doi: 10.1074/jbc.M113.507285

Du, X., Zhang, P., Fu, H., Ahsan, H.M., Gao, J. and Chen, Q. (2019). Smart mitochondrial-targeted cancer therapy: subcellular distribution, selective TrxR2 inhibition accompany with declined antioxidant capacity. Int J Pharm, 555, 346–55. https://doi: 10.1016/j.ijpharm.2018.11.057.

Dunbar, E.M., Coats, B.S., Shroads, A.L., Langaee, T., Lew, A., Forder, J.R., Shuster, J.J., Wagner, D.A., Stacpoole, P.W., (2014). Phase 1 trial of dichloroacetate (DCA) in adults with recurrent malignant brain tumors. Invest New Drugs, 32, 452–464. http://doi: 10.1007/s10637-013-0047-4.

Didier R., Remesy C., Demigne C., and Fafournoux P., (1985). Hepatic proliferation of mitochondria in response to a high protein diet. Nutr Res, 5(10), 1093-1102. https://doi.org/10.1016/S0271-5317(85)80141-X

Diebold, L.P., Gil, H.J. and Gao, P. et al. (2019). Mitochondrial complex III is necessary for endothelial cell proliferation during angiogenesis. Nat Metab 1, 158–171. https:// doi: 10.1038/s42255-018-0011-x.

Domino E.F. and Khanna S.S., (1976). "Decreased blood platelet MAO activity in unmedicated chronic schizophrenic patients". The American Journal of Psychiatry. 133 (3), 323–6. https://doi:10.1176/ajp.133.3.323.

Dong L. F., Jameson V. J., Tilly D., Prochazka L., Rohlena J., Valis K., et al., (2011). Mitochondrial targeting of alpha-tocopheryl succinate enhances its pro-apoptotic efficacy: a new paradigm for effective cancer therapy. Free Radic Biol Med., 50(11),1546–55. https :// doi: 10.1016/j.freeradbiomed.2011.02.032.

Dong, L., and Neuzil, J., (2019). Targeting mitochondria as an anticancer strategy. Cancer Commun 39, 63. https ://doi: 10.1186/s40880-019-0412-6.

Dong L.F., Swettenham E., Eliasson J., Wang X.F., Gold M., Medunic Y., Stantic M., Low P., Prochazka L., Witting P.K., Turanek J., Akporiaye E.T., Ralph S.J., Neuzil J, (2007). Vitamin E analogues inhibit angiogenesis by selective induction of apoptosis in proliferating endothelial cells: the role of oxidative stress. Cancer Res, 67(24), 11906-13. https://doi: 10.1158/0008-5472.CAN-07-3034.

Du, X., Zhang, P., Fu, H., Ahsan, H.M., Gao, J. and Chen, Q., (2019). Smart mitochondrial-targeted cancer therapy: subcellular distribution, selective TrxR2 inhibition accompany with declined antioxidant capacity. Int J Pharm, 555,346–55. https ://doi: 10.1016/j.ijpharm.2018.11.057

Dulloo, A.G., Duret, C., Rohrer, D., Girardier, L., Mensi, N., Fathi, M., Chantre, P. and Vandermander, J., (1999). Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. Am J Clin Nutr, 70(6), 1040- 1045. https:// doi: 10.1093/ajcn/70.6.1040.

Dwyer, J. T., Coates, P. M. and Smith, M. J. (2018). Dietary supplements: Regulatory challenges and research resources. Nutrients, 10(1), 41.
https ://doi.org/10.3390/nu10010041

Dyer N.L., Surdam J., Dusek J.A., (2021). A systematic review of practiced-based research of complementary and integrative health therapies as provided for pain management in clinical settings: Recommendations for the future and a call to action. Pain med, 151.
https ://doi: 10.1093/pm/pnab151.

Efimova, S.S., Zakharova, A.A. and Ostroumova, O.S., (2020). Alkaloids modulate the functioning of ion channels produced by antimicrobial agents via an influence on the lipid host. Front. Cell Dev. Biol. 8,537. https:// doi: 10.3389/fcell.2020.00537.

Egusquiza-Alvarez, C.A., Castaneda-Patlan, M.C., Albarran-Gutierrez, S., Gonzalez-Aguilar, H., Moreno-Londono, A.P., Maldonado, V., Melendez-Zajgla, J., Robles-Flores, M., (2021). Overexpression of multifunctional protein p32 promotes a malignant phenotype in colourectal cancer cells. Front Oncol, 11:642940. http://doi: 10.3389/fonc.2021.642940.

Ekor M., (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol, 4, 177.
https://doi.org/10.3389/fphar.2013.00177

Encyclopedia of the life (EOL), (2014). *Alhagi maurorum* Medik. Synonyms, The Plant List. Available from http://eol.org/pages/703028/ names/synonyms. [Accessed 10th May 2020]

Ernst E., (2000). The role of complementary and alternative medicine. BMJ (Clinical research ed.), 321(7269), 1133–1135. https://doi.org/10.1136/bmj.321.7269.1133.

Ernster, L., and Schatz, G., (1981). Mitochondria: A historical review. J Cell Bio, 91 (3), 2276-255s. https://rupress.org/jcb/article-pdf/91/3/227s/1075582/227s.pdf.

Etebari, M., Ghannadi, A., Jafarian-Dehkordi, A. and Ahmadi, F., (2012). Genotoxicity evaluation of aqueous extracts of Cotoneaster discolor and Alhagi pseudalhagi by comet assay. J Res Med Sci, 17(2), S237-41. Corpus ID: 97672728.

European Food Safety Authority, “Food supplements,” 2017, https://www.efsa.europa.eu/en/topics/topic/food-supplements.

Evans, J.M., Donnelly, L.A., Emslie-Smith, A.M., Alessi, D.R. and Morris, A.D., (2005). Metformin and reduced risk of cancer in diabetic patients. Bmj, 330(7503),1304-1305. https://doi.org/10.1136/bmj.38415.708634.F7.

Fagervall, I. and Ross, S.B., (1986). A and B forms of monoamine oxidase within the monoaminergic neurons of the rat brain. J Neurochem. 47(2), 569-76. https://doi: 10.1111/j.1471-4159. 1986. tb04537.x.

Faitg, J., Davey, T., Turnbull, D.M., White, K. and Vincent, A.E., (2020). Mitochondrial morphology and function: two for the price of one! J Microsc, 278,89-106. https:// doi: 10.1111/jmi.12891.

Falkowska, A. et al., (2015). Energy metabolism of the brain, including the cooperation between astrocytes and neurons, especially in the context of glycogen metabolism. Int J Mol Sci, 16, 25959–25981. https:// doi: 10.3390/ijms161125939

FAOSTAT , (2018). Barley crops.UN Food and Agriculture Organization Corporate Statistical Database (FAOSTAT).http://www.fao.org/in-action/inpho/crop-compendium/cereals-grains/en/.

Feinman, R.D., and Fine, E.J., (2013). Fructose in perspective. Nutr Metab (Lond),10(1),45. https://doi: 10.1186/1743-7075-10-45.

Fernandez-Vizarra E. and Zeviani M., (2015). Nuclear gene mutations as the cause of mitochondrial complex III deficiency. Front. Genet. 6,134. https://doi: 10.3389/fgene.2015.00134.

Ferber, S.G., Namdar, D., Hen-Shoval, D., Eger, G., Koltai, H., Shoval, G., Shbiro, L., Weller, A., (2020). The “Entourage Effect”: terpenes coupled with cannabinoids for the treatment of mood disorders and anxiety disorders. Curr Neuropharmacol,18(2),87-96. https://doi: 10.2174/1570159X17666190903103923.

Fernandes, G., Barone, A.W., Dziak, R. and Winder, S., (2017). The effect of ascorbic acid on bone cancer cells in vitro. Cogent Biology 3,1. https:// https://doi.org/10.1080/23312025.2017.1288335.

Ferrarini, I., Louie, A., Zhou, L., and El-Deiry, W. S., (2021). ONC212 is a novel mitocan acting synergistically with glycolysis inhibition in pancreatic cancer. Mol Cancer Ther, 20, 1572-83. https:// doi: 10.1158/1535-7163.MCT-20-0962.

Ferrarini, M.G., Nisimura, L.M., Girard, R.M.B.M., Alencar, M.B., Fragoso, M.S.I., Araujo-Silva, C.A, Veiga, A.A., Abud, A.P.R., Nardelli, S.C., Vommaro, R.C., Silber, A.M., France-Sagot, M., Ávila, A.R., (2021). Dichloroacetate and pyruvate metabolism: Pyruvate dehydrogenase kinases as targets worth investigating for effective therapy of toxoplasmosis. msphere, 6(1),e01002-20. https://doi: 10.1128/mSphere.01002-20.

Filic, V., Vladic, A., Stefulj, J., Cicin-Sain, L., Balija, M., Sucic, Z., and Jernej B., (2005). "Monoamine oxidases A and B gene polymorphisms in migraine patients". J Neurol Sci, 228 (2), 149–53. https:// doi:10.1016/j.jns.2004.11.045.

Fisher R., Pusztai L., Swanton C., (2013). Cancer heterogeneity: implications for targeted therapeutics. Br J Cancer, 108(3):479-85.http;// doi: 10.1038/bjc.2012.581.

Fransen, M., Lismont, C. and Walton, P., (2017). The Peroxisome-Mitochondria Connection: How and Why? International journal of molecular sciences, 18(6), 1126. https://doi.org/10.3390/ijms18061126.

Franze, E., Stolfi, C., Troncone, E., Scarozza, P. and Monteleone, G. (2020). Role of Interleukin-34 in Cancer. Cancers,12, 252. https:// doi: 10.3390/cancers12010252

Frank M., Weckman T.J., Wood T., Woods W.E., Tai C.L., Chang S.L., Ewing A., Blake J.W., Tobin T., (1990). Hordenine: pharmacology, pharmacokinetics and behavioural effects in the horse. Equine Vet J., 22(6),437-41. https:// doi: 10.1111/j.2042-3306. 1990.tb04312. x.

Freitas, D.D.S.; Morgado-Diaz, J.A.; Gehren, A.S.; Vidal, F.C.B.; Fernandes, R.M.T.; Romao, W.; Tose, L.V.; Frazao, F.N.S.; Costa, M.C.P.; Silva, D.F.; et al., (2017). Cytotoxic analysis and chemical characterization of fractions of the hydroalcoholic extract of the *Euterpe oleracea* M. seed in the MCF-7 cell line. J Pharm Pharmacol, 69, 714–721. https:// doi: 10.1111/jphp.12679

Fullerton, M., McFarland, R., Taylor, R. W., and Alston, C. L., (2020). The genetic basis of isolated mitochondrial complex II deficiency. Molecular genetics and metabolism, 131(1-2), 53–65. https://doi.org/10.1016/j.ymgme.2020.09.009.

Fu, L.L, Zhou, C.C., Yao, S., Yu, J.Y., Liu, B. and Bao, J.K., (2011). Plant lectins: targeting programmed cell death pathways as anti-tumor agents. Int J Biochem Cell Biol, 43, 1442–1449. https:// doi: 10.1016/j.biocel.2011.07.004.

Galloway, C. A., Lee, H., and Yoon, Y. (2012). Mitochondrial morphology-emerging role in bioenergetics. Free Radical Bio Med, 53(12), 2218–2228. https://doi.org/10.1016/j.freeradbiomed.2012.09.035.

Gallo, M., (2022). Extraction and isolation of natural products. Separation, 9, 287. https:// doi.org/10.3390/separations9100287.

Galitzky J., Taouis M., Berlan M., Rivière D., Garrigues M., Lafontan M., (1988). Alpha 2-antagonist compounds and lipid mobilization: evidence for a lipid mobilizing effect of oral yohimbine in healthy male volunteers. Eur J Clin Invest,18(6),587-94.http:// doi: 10.1111/j.1365-2362.1988.tb01272.x.

Gao, Z., Cai, L., Lu, J., Wang, C., Li, Q., Chen, J., Song, X., Chen, X., Zhang, L., Zheng, W. and Su, Z., (2017). Expression of stem cell markers and dopamine D2 receptors in human and rat prolactinomas. Med Sci Monit, 23, 1827. https://doi: 10.12659/msm.901154.

Gao, S., Sun, D., Wang, G., Zhang, J., Jiang, Y., Li, G., Zhang, K., Wang, L., Huang, J. and Chen, L., (2016). Growth inhibitory effect of paratocarpin E, a prenylated chalcone isolated from *Euphorbia humifusa* W., by induction of autophagy and apoptosis in human breast cancer cells. Bioorg Chem, 69, 121-128. https://doi: 10.1016/j.bioorg.2016.10.005

Gargoum, H.M., Muftah, S.S., Al Shalmani, S., Mohammed, H.A., Alzoki, A.N., Debani, A.H., et al., (2013). Phytochemical screening and investigation of the effect of *Alhagi maurorum* M., on carbon tetrachloride, acetaminophen and adriamycin induced toxicity in experimental animals. J Sci Innov Res, 2(6), 1023-33. ISSN 2320-4818

Garcia, G.C., Bartol, T.M., Phan, S. et al., (2019). Mitochondrial morphology provides a mechanism for energy buffering at synapses. Sci Rep 9, 18306. https://doi.org/10.1038/s41598-019-54159-1

Garcia-Berumen, C. I., Ortiz-Avila, O., Vargas-Vargas, M. A., Rosario-Tamayo, B. A., Clotilde, G., Lopez, C., Saavedra-Molina, A., Rodriguez-Orozco, A. R. and Cortes-Rojo, C., (2019). The severity of rat liver injury by fructose and high fat depends on the degree

of respiratory dysfunction and oxidative stress induced in mitochondria. Lipids Health Dis 18, 78. https:// doi: 10.1186/s12944-019-1024-5.

Gasecka, M., Siwulski, M., Magdziak, Z., Budzynska, S., Stuper-Szablewska, K., Niedzielski, P., Mleczek, M., (2020). The effect of drying temperature on bioactive compounds and antioxidant activity of *Leccinum scabrum* G. and Hericium erinaceus P. J Food Sci Technol, 57(2), 513-525. https:// doi: 10.1007/s13197-019-04081-1

Gaude E. and Frezza C., (2014). Defects in mitochondrial metabolism and cancer. Cancer Metab, 17(2),10. https:// doi: 10.1186/2049-3002-2-10.

Geoffrey M. T., (1963). PhD thesis: The biosynthesis of some phenolic alkaloids. The University of London.

Gerald W. Dorn, (2013). Mitochondrial dynamics in heart disease, Biochimica et Biophysica Acta (BBA) – Mol Cell Res, 1833, (1), 233-241.
https ://doi.org/10.1016/j.bbamcr.2012.03.008.

Gernot B., Irina S. and Thomas D., (2013). Quantification of the biogenic phenethylamine alkaloid hordenine by LC-MS/MS in beer. Toxichem Krim Tech, 80(Special Issue), 323.

Ghasemi, M., Turnbull, T., Sebastian, S., Kempson, I., (2021). The MTT assay: Utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. Int J Mol Sci, 22(23),12827.http:// doi: 10.3390/ijms222312827.

Ghosal, S. and Srivastava, R.S., (1973). Chemical investigation of *Alhagi pseudalhagi* B: β-phenethylamine and tetrahydroisoquinoline alkaloids. J Pharm Sci, 62: 1555-1556. https://doi: 10.3109/07357900903287006

Ghosh, A.K. and Das, S.S., (2010). Alkaloid hordenine in *Tamarindus indica* L., Indian Drugs. 47. 5-10.

Ghosal S., Srivastan RS, et al., (1972). Desmodium Alkaloids. IV Chemical and Pharmacological Evaluation of D. Triflouin. Planta Med 23,321 328. https:// doi: 10.1055/s-0028-1099451

Ghosh, A.K. and Das, S.S., (2010). Alkaloid hordenine in *Tamarindus indica* L. Indian Drugs. 47, 5-10.

Ghoneum, A. and Said, N., (2019). PI3K-AKT-mTOR and NFκB Pathways in ovarian cancer: Implications for targeted therapeutics. Cancers, 11(7), 949. https://doi: 10.3390/cancers11070949

Gibellini, L., Bianchini, E., De Biasi, S., Nasi, M., Cossarizza, A., Pinti, M., (2015). Natural compounds modulating mitochondrial functions. Evid Based Complement Alternat Med, 527209.http:// doi: 10.1155/2015/527209.

Giorgi, C., Marchi, S., and Pinton, P., (2018). The machineries, regulation, and cellular functions of mitochondrial calcium. Nat Rev Mol Cell Biol 19**,** 713–730.
https://doi.org/10.1038/s41580-018-0052-8.

Giraldo P., Elena B., Francisco M., and Estela G., (2019). "Worldwide Research Trends on wheat and barley: A Bibliometric Comparative Analysis". Agronomy, 9(7), 352.
https://doi.org/10.3390/agronomy9070352.

Glienke, W., Maute, L., Wicht, J., and Bergmann, L., (2009). Curcumin inhibits constitutive STAT3 phosphorylation in human pancreatic cancer cell lines and down regulation of 137urviving/BIRC5 gene expression. Cancer Invest. 28, 166–171.

Gnaiger, E., (2014). Mitochondrial pathways and respiratory control: An introduction to OXPHOS analysis. 4th ed. Mitochondr Physiol Network 19.12. Oroboros MiPNet Publications, Innsbruck, 80. Innsbruck, Austria: OROBOROS Instruments.

Goncharov M.Y., Yakovlev G.P., and Vitovskaya G.A., (2001). Composition of polysaccharides from above-ground part of *Alhagi maurorum* M. Rastitel'nye Resursy, 37,60-63. https://eurekamag.com/research/003/689/003689967.php.

Gong, J., Zhang, W.G., Feng, X.F., Shao, M.J. and Xing, C., (2017). Aesculetin (6, 7-dihydroxycoumarin) exhibits potent and selective antitumor activity in human acute myeloid leukemia cells (THP-1) via induction of mitochondrial mediated apoptosis and cancer cell migration inhibition. J BUON, 22(6), 1563-1569.

Gong G., Tao J.,Wang Y., Wu, J., An J., Meng J.,Wang X., Chen Y., Zou J., (2021). Total barley maiya alkaloids inhibit prolactin secretion by acting on dopamine D2 receptor and protein kinase A targets. J Ethnopharmacol, 273, 113- 994, https://doi.org/10.1016/j.jep.2021.113994.

Golub, D., Iyengar, N., Dogra, S., Wong, T., Bready, D., Tang, K., Modrek, AS., Placantonakis, D.G., (2019). Mutant isocitrate dehydrogenase inhibitors as targeted cancer therapeutics. Front Oncol, 17(9), 417. http://doi: 10.3389/fonc.2019.00417.

Gorman, G.S., Schaefer, A.M., Ng, Y., Gomez, N., Blakely, E.L., Alston, C.L., Feeney, C., Horvath, R., Yu-Wai-Man, P., Chinnery, P.F., Taylor, R.W., Turnbull, D.M., and McFarland, R., (2015). Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. Ann Neurol, 77, 753-759. https://doi.org/10.1002/ana.24362.

Gottlieb, E., Armour, S., Harris, M. et al., (2003). Mitochondrial membrane potential regulates matrix configuration and cytochrome c release during apoptosis. Cell Death Differ, 10, 709–717. https://doi.org/10.1038/sj.cdd.4401231.

Graziani, V., Scognamiglio, M., Belli, V., Esposito, A., D'Abrosca B., Angela C., Rosita R., Marta P., Aniello R., Fortunato C., Teresa T., Nicoletta P. and Antonio F., (2018). Metabolomic approach for a rapid identification of natural products with cytotoxic activity against human colourectal cancer cells. Sci Rep 8, 5309. https:// doi: 10.1038/s41598-018-23704-9

Graf, T., and Stadtfeld, M., (2008). Heterogeneity of embryonic and adult stem cells. Cell Stem Cell, 3(5), 480-483. https://doi: 10.1016/j.stem.2008.10.007.

Greene, J., Segaran, A., and Lord, S., (2022). Targeting OXPHOS and the electron transport chain in cancer; Molecular and therapeutic implications. Sem Can Bio, 86(2), 851-859. https://doi.org/10.1016/j.semcancer

Gucek, M., and Murphy, E., (2010). Review: What can we learn about cardio protection from the cardiac mitochondrial proteome? Cardiovasc Res, 88(2) ,211-8. https:// doi: 10.1093/cvr/cvq277.

Gutierrez, S. P., Sanchez, M. A. Z., Gonzalez, C. P., and Garcia, L. A. (2007). Antidiarrhoeal activity of different plants used in traditional medicine. Afr J Biotech, 6(25). http://doi: 10.5897/AJB2007.000-2465.

Guzzo, G., Sciacovelli, M., Bernardi, P., and Rasola, A., (2014). Inhibition of succinate dehydrogenase by the mitochondrial chaperone TRAP1 has antioxidant and anti-apoptotic effects on tumor cells. Oncotarget, 5(23), 11897. https:// doi: 10.18632/oncotarget.2472

Guo, M., Peng, Y., Gao, A., Du C. and Herman, J.G. (2019). Epigenetic heterogeneity in cancer. Biomark Res 7, 23. https://doi: 10.1186/s40364-019-0174-y

Guo R.Z., Wang X. Ma L., Chen Y.G. and Wu J.H., (2018). "Inhibition effects of hordenine on prolactin secretion in hyperprolactinemia rats," Chinese Traditional Patent Medicine, 40, 2386–2389.

Gul, R., Jan, S.U., Faridullah, S., Sherani, S., Jahan, N., (2017). Preliminary phytochemical screening, quantitative analysis of alkaloids and antioxidant activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan. Sci World J, 2017:5873648. https://doi: 10.1155/2017/5873648.

Haddad, A., and Mohiuddin, S.S., (2023). Biochemistry, citric acid cycle. In: StatPearls [Internet]. Treasure Island (FL): Stat Pearls Available from: https://www.ncbi.nlm.nih.gov/books/NBK541072/

Hahn, M., Lindemann, V., Behrens, M., Mulac, D., Langer, K., Esselen, M., et al., (2022). Permeability of dopamine D2 receptor agonist hordenine across the intestinal and blood-brain barrier *in vitro*. PLoS ONE 17(6), e0269486. https://doi.org/10.1371/journal.pone.0269486

Hanahan, D. and Weinberg, R. A. (2011). "Hallmarks of cancer: the next generation." cell 144(5), 646-674. http://doi: 10.1016/j.cell.2011.02.013.

Han, D., Canali, R., Rettori, D., Kaplowitz, N., (2003). Effect of glutathione depletion on sites and topology of superoxide and hydrogen peroxide production in mitochondria. Mol Pharmacol, 64(5), 1136-44. http://doi: 10.1124/mol.64.5.1136

Hanif F., Muzaffar K., Perveen K., Malhi S.M., Simjee Sh.U., (2017). Glioblastoma multiforme: A review of its epidemiology and pathogenesis through clinical presentation and treatment. Asian Pac J Cancer Prev, 18(1),3-9. http:// doi: 10.22034/APJCP.2017.18.1.3.

Hapke, H.J., Strathmann, W. (1995). Pharmakologische Wirkungen des Hordenine [Pharmacological effects of hordenine]. Dtsch Tierarztl Wochenschr. 102(6),228-32.

Harding, J.L., Pavkov, M.E., Magliano, D.J. *et al.* Global trends in diabetes complications: a review of current evidence. Diabetologia, **62**, 3–16. https://doi.org/10.1007/s00125-018-4711-2

Haskett, Dorothy R., (2014). "Mitochondrial Diseases in Humans". Embryo Project Encyclopedia. ISSN: 1940-5030 http://embryo.asu.edu/handle/10776/8031.

Hashim, S., Beh, H.K., Hamil, M.S., Ismail, Z., Majid, A.M., (2016). High-performance Thin-layer Chromatography method development, validation, and simultaneous quantification of four compounds identified in standardized extracts of orthosiphon stamineus. Pharmacog Res 8(4), 238-243.http:// doi: 10.4103/0974-8490.188872.

Hart B.L., (1983). "Flehmen behavior and vomeronasal organ function". In Muller-Schwarz, Dietland; Silverstein, Robert M. (eds.). Chemical Signals in Vertebrates 3. Springer, 87. https:// doi:10.1007/978-1-4757-9652-0_5.

Hashim, N.M., Ibrahim, M.Y., Yahayu, M., Omer, F.A.E., Abd Rahman, M., Nordin, N. and Lian, G.E.C., (2017). In vitro assessment of anti-proliferative effect induced by α-mangostin from *Cratoxylum arborescens* B., on HeLa cells. PeerJ, 5, p.e3460. http:// doi: 10.7717/peerj.3460.

He, W., Newman, J.C., Wang, M.Z., Ho, L. and Verdin, E. (2012). Mitochondrial sirtuins: regulators of protein acylation and metabolism. Trends Endocrinol Metab, 23(9), 467-476. http://doi: 10.1016/j.tem.2012.07.004.

He, L., Han, J., Li, B., Huang, L., Ma, K., Chen, Q., Liu, X., Bao, L. and Liu, H., (2016). Identification of a new cyathane diterpene that induces mitochondrial and autophagy-dependent apoptosis and shows a potent in vivo anti-colorectal cancer activity. Eur J Med Chem, 111, 183-192.http:// doi: 10.1016/j.ejmech.2016.01.056

Heck, C.I., and De Mejia, E.G., (2007). Yerba Mate Tea (Ilex paraguariensis): a comprehensive review on chemistry, health implications, and technological considerations. J Food Sci, 72(9), R138-R151. https:// doi: 10.1111/j.1750-3841.2007.00535.x.

Heffter A., (1894). Arch Exp Pathol Pharmukol 34, 65.

Henley, A.B., Yang, L., Chuang, K.L., Sahuri-Arisoylu, M., Wu, L.H., Bligh, S.W.A., et al. (2017). *Withania somnifera* L., root extract enhances chemotherapy through 'Priming'. PLoS ONE 12(1),e0170917. https://doi.org/10.1371/journal.pone.017091

Heyman, H.M. and Meyer, J.J.M., (2012). NMR-based metabolomics as a quality control tool for herbal products. S Afr J Bot. 82. 21-32. https://doi.org/10.1016/j.sajb.2012.04.001.

Hintz, H.F., (1988). FDA Poisonous plant database (FDA: F22087). Equine Pract, 10(2), 17.https://www.cfsanappsexternal.fda.gov/scripts/plantox/detail.cfm?id=12465

Hi-Tech Pharmaceuticals Thermo-Rx® brand *Senegalia Berlandieri* Extract is Most Effective Weight Loss Ingredient Available!, 2019. https://www.biospace.com/article/releases/hi-tech-pharmaceuticals-thermo-rx-brand-senegalia-berlandieri-extract-is-most-effective-weight-loss-ingredient-available-/

Hoang, l. S., Tran, M.H., Lee, J.S., To, D.C., Nguyen, V.T., Kim, J.A., Lee, J.H., Woo, M.H., Min, B.S., (2015). Anti-inflammatory activity of pyrrolizidine alkaloids from the leaves of *Madhuca pasquieri* D. Chem Pharm Bull, 63(6),481-4. https:// doi: 10.1248/cpb.c14-00855.

Hoch, T., Kreitz, S., Gaffling, S., Pischetsrieder, M. and Hess, A., (2015). Fat/carbohydrate ratio but not energy density determines snack food intake and activates brain reward areas. Sci Rep, 5(1), 1-9. https://doi.org/10.1038/srep10041

Hoffman, J.R., Kang, J., Ratamess, N.A. et al., (2009). Thermogenic effect of an acute ingestion of a weight loss supplement. J Int Soc Sports Nutr 6, 1. https://doi.org/10.1186/1550-2783-6-

Hoffmann, T., Krug, D., Huttel, S., Muller, R., (2014). Improving natural products identification through targeted LC-MS/MS in an untargeted secondary metabolomics workflow. Anal Chem, 86(21), 10780-8. http:// doi: 10.1021/ac502805w.

Hoffman J.R., Kang, J., Ratamess, N.A., Jennings, P.F., Mangine, G., and Faigenbaum, A.D., (2006). Thermogenic effect from nutritionally enriched coffee consumption. J Int Soc Sports Nutr, 3(1), 1-7. https://doi.org/10.1186/1550-2783-3-1-35

Hoffman, J.R., Kang, J., Ratamess, N.A. et al., (2009). Thermogenic effect of an acute ingestion of a weight loss supplement. J Int Soc Sports Nutr 6, 1. https://doi.org/10.1186/1550-2783-6-1.

Holmstrom K.M. and Finkel, T., (2014). Cellular mechanisms and physiological consequences of redox-dependent signalling. Nat Rev Mol Cell Biol, 15(6), 411-21. http://doi: 10.1038/nrm3801.

Holper, L., Ben-Shachar, D. and Mann, J. J. (2019). Multivariate meta-analyses of mitochondrial complex I and IV in major depressive disorder, bipolar disorder, schizophrenia, Alzheimer disease, and Parkinson disease. Neuropsychopharmacology: official publication of the American college of neuropsychopharmacology, Neuropsychopharmacology, 44(5), 837–849. http:// doi: 10.1038/s41386-018-0090-0

Hopp, A., Gruter, P., Hottiger, M.O., (2019). "Regulation of glucose metabolism by NAD + and ADP-Ribosylation". Cells, 8 (8): 890.http:// doi:10.3390/cells8080890.

Horwitz, K.B., Costlow, M.E., McGuire, W.L., (1975). MCF-7; a human breast cancer cell line with estrogen, androgen, progesterone, and glucocorticoid receptors. Steroids, 26(6), 785-95. https:// doi: 10.1016/0039-128x(75)90110-5.

Horan, M. P., Nicolas, P., William, J. and Ballard, O., (2012). Review: Quantifying mitochondrial dysfunction in complex diseases of aging, J Gerontol A Biol Sci Med Sci, 67(10), 1022–1035.http:// doi: 10.1093/gerona/glr263

Huang, H.J., Lee, C.C., Chen, C.Y., (2014). Pharmacological chaperone design for reducing risk factor of Parkinson's disease from Traditional Chinese Medicine. Evid Based Complement Alternat Med, 830-490.http:// doi: 10.1155/2014/830490.

Hu D.Q., Chen Y.G., Wu J.H., Wang, X. and Cao, J.H., (2012). Effect of malt alkaloid extracts on the excitatory autacoid levels in HPRL rats. Journal of Guangdong Pharmaceutical University, 5. https://doi.org/10.1590/s2175-979020200004181104

Hudaib, M., Mohammad, M., Bustanji, Y., Tayyem, R., Yousef, M., Abuirjeie, M,, Aburjai, T., (2008). Ethnopharmacological survey of medicinal plants in Jordan, Mujib Nature Reserve and surrounding area. J Ethnopharmacol, 120(1),63-71. http://doi: 10.1016/j.jep.2008.07.031.

Hyde, B.B., Twig, G. and Shirihai, O.S., (2010). Review: Organellar vs cellular control of mitochondrial dynamics. Semin Cell Dev Biol, 21(6), 575-81.http:// doi: 10.1016/j.semcdb.2010.01.003.

Imbe, A., Tanimoto, K., Inaba, Y., Sakai, S., Shishikura, K., Imbe, H., Tanimoto, Y., Terasaki, J., Imagawa, A., Hanafusa, T., (2018). Effects of L-carnitine supplementation on the

quality of life in diabetic patients with muscle cramps. Endocr J, 65(5),521-526.http:// doi: 10.1507/endocrj.EJ17-0431.

Irawati, Lovett, John V., Roberts J., (1997). Thesis masters research: Barley allelochemicals as self defence properties against vertebrate animals.

Irsfeld, M., Spadafore, M.and Prub, B. M., (2013). β-phenylethylamine, a small molecule with a large impact. Webmed Central, 4(9), 4409. PMID: 24482732; PMCID: PMC3904499.

Islam, M.K., Sostaric, T., Lim, L.Y., Hammer, K., Locher, C., (2021). Development of an HPTLC-based dynamic reference standard for the analysis of complex natural products using Jarrah honey as test sample. PLoS ONE 16(7): e0254857. https://doi.org/10.1371/journal.pone.0254857.

Izyumov D.S., Avetisyan A.V., Pletjushkina O.Y., Sakharov D.V., Wirtz K.W., Chernyak B.V., Skulachev V.P., (2004). "Wages of fear": transient threefold decrease in intracellular ATP level imposes apoptosis. Biochim Biophys Acta,1658(1-2),141-7. doi: 10.1016/j.bbabio.2004.05.007.

Jason K. A., Guillermo A., John B.S., (2018). US Patent Application: Nutraceutical capsule and tablet formulations providing enhanced mental clarity, concentration and stamina while minimizing adrenaline and dopamine concentration perturbations associated with withdrawal. Publication no. 20180055849. https://patents.justia.com/patent/20180055849.

Jaune E, Cavazza E, Ronco C, Grytsai O, Abbe P, Tekaya N, Zerhouni M, Beranger G, Kaminski L, Bost F, Gesson M, Tulic M, Hofman P, Ballotti R, Passeron T, Botton T, Benhida R, Rocchi S., Discovery of a new molecule inducing melanoma cell death: dual AMPK/MELK targeting for novel melanoma therapies. Cell Death Dis, 12(1),64.http:// doi: 10.1038/s41419-020-03344-6.

Jensen, O., Rafehi, M., Gebauer, L. and Brockmoller, J., (2021). Cellular uptake of psychostimulants are high-and low affinity organic cation transporters drug traffickers? Front Pharmacol, 2294. https://doi.org/10.3389/fphar.2020.609811.

Jianshe M., Shuanghu W., Xueli H., Peiwu G., Congcong W., Yunfang Z., Linsheng Y., Xianqin W., (2015). Validated UPLC–MS/MS method for determination of hordenine in rat plasma and its application to pharmacokinetic study. J Pharm Biomed Anal , 111, 131-137. http://doi: 10.1016/j.jpba.2015.03.032.

Johnson E. R. and Matthay M. A., (2010). Acute lung injury: epidemiology, pathogenesis, and treatment. J Aerosol Med Pulm Drug Deliv, 23(4), 243–252. https://doi.org/10.1089/jamp.2009.0775

Kalghatgi, S., Spina, C.S., Costello, J.C., et al., (2013). Bactericidal antibiotics induce mitochondrial dysfunction and oxidative damage in Mammalian cells. Sci Transl Med, 5(192).http:// doi: 10.1126/scitranslmed.3006055.

Kaltner, F.; Rychlik, M.; Gareis, M.; Gottschalk, C., (2020). Occurrence and risk assessment of pyrrolizidine alkaloids in spices and culinary herbs from various geographical origins. Toxins, 12, 155. https://doi.org/10.3390/toxins12030155.

Kalyanaraman, B., Cheng, G., Hardy, M., Ouari, O., Lopez, M., Joseph, J. et al., (2018). A review of the basics of mitochondrial bioenergetics, metabolism, and related signaling

pathways in cancer cells: therapeutic targeting of tumor mitochondria with lipophilic cationic compounds. Redox Biol,14,316.http:// doi: 10.1016/j.redox.2017.09.020

Kamboj, A. and Saluja, A.K., (2011). Isolation of stigmasterol and B-sitosterol from petroleum ether extract of aerial parts of *Ageratum conyzoides* (Asteraceae). International Journal of Pharmacy and Pharmaceutical Sciences. 3. 94-96. ISSN- 0975-1491

Kanwal, H. and Sherazi, B.A., (2017). Herbal medicine: Trend of practice, perspective, and limitations in Pakistan. Asian Pacific Journal of Health Sciences. 4. 6-8. http://doi: 10.21276/apjhs.2017.4.4.2

Kapadia G. J., and Fayez M.B.E., (1970). Review article: Peyote constituents: Chemistry, biogenesis, and biological effects. J Pharm Sci, 59(12), 1699-1727. https://doi: 10.1002/jps.2600591202.

Karthika, K. and Paulsamy S., (2015). TLC and HPTLC fingerprints of various secondary metabolites in the stem of the traditional medicinal climber, *Solena amplexicaulis* L. Indian J Pharm Sci, 77(1),111-6. http:// doi: 10.4103/0250-474x.151591.

Karolinska Institutet. (2021). Mitochondrial function can play significant part in serious disease. ScienceDaily. Retrieved from www.sciencedaily.com/releases/2021/02/210219155928.htm

Kazemi Noureini, S., Fatemi, L., and Wink, M. (2018). Telomere shortening in breast cancer cells (MCF7) under treatment with low doses of the benzylisoquinoline alkaloid chelidonine. PloS one, 13(10), e0204901. https://doi.org/10.1371/journal.pone.0204901

Kerr, D.S., (2013). Review of clinical trials for mitochondrial disorders: 1997–2012. Neurotherapeutics, 10(2), 307-319.http:// doi: 10.1007/s13311-013-0176-7.

Keston, A.S. and Brandt, R., (1965). The fluorometric analysis of ultra micro quantities of hydrogen peroxide. Anal Biochem, 11, 1–5.http:// doi: 10.1016/0003-2697(65)90034-5

Kim M. Sook, Gernapudi R., Cedeno Y. Cedeno, Polster B. M., Martinez R., Shapiro P., Kesari S., Nurmemmedov E., Passaniti A., (2020). Targeting breast cancer metabolism with a novel inhibitor of mitochondrial ATP synthesis. Oncotarget., 11, 3863-3885. Retrieved from https://www.oncotarget.com/article/27743/text/.

Kim S., Lee J., Kim M., Lee J., Kim Y., Jung E., Kim Y., Lee J., Park D., (2013). Hordenine, a single compound produced during barley germination, inhibits melanogenesis in human melanocytes, Food Chemistry, 141(1), 174-181, https://doi.org/10.1016/j.foodchem.

Khushbaktova, Z.A., Syrov, V.N., Kuliev, Z., Bashirova, N.S., Shadieva, Z., Gorodeyskaia, E.A., Medvedev, O.S., (1992). The effect of proanthocyanidins from Alhagi pseudoalhagi (M. B.) Desv on the course of experimental myocardial infarct]. Eksp Klin Farmakol, 55(6),19-21.

Korzeniewski, B., (2015). 'Idealized' state 4 and state 3 in mitochondria vs. rest and work in skeletal muscle. PLoS One, 10(2), e0117145.http:// doi: 10.1371/journal.pone.0117145.

Kosgodage, U. S., Mould, R., Henley, A. B., Nunn, A. V., Guy, G. W., Thomas, E. L., Inal, J. M., Bell, J. D. and Lange, S., (2018). Cannabidiol (CBD) is a novel inhibitor for exosome and microvesicle (EMV) release in cancer. Front Pharmacol, 9, 889.http:// doi: 10.3389/fphar.2018.00889.

Koo, Y. E., Song, J., and Bae, S., (2018). Use of plant and herb derived medicine for therapeutic usage in cardiology. Medicines (Basel, Switzerland), 5(2), 38.http:// doi: 10.3390/medicines5020038

Kotnik, K. Z., Jurak, G., Starc, G., and Golja, P., (2017). Faster, stronger, healthier: adolescent-stated reasons for dietary supplementation. Journal of nutrition education and behavior, 49(10), 817-826.http://doi: 10.1016/j.jneb.2017.07.005.

Kozakiewicz, P., Grzybowska-Szatkowska, L., Ciesielka, M., and Rzymowska, J., (2021). The role of mitochondria in carcinogenesis. Int J Mol Sci, 22(10), 5100. https://doi.org/10.3390/ijms22105100

Kuriyama A, Maeda H., (2019). Topical application of licorice for prevention of postoperative sore throat in adults: a systematic review and meta-analysis. *J Can Anesth*, 54,25-32. https://doi.org/10.1007/s12630-019-01396-7

Kulkarni, K., Patil, L., Khanvilkar, V. and Kadam, V. J. (2014). Fingerprinting techniques in herbal standardization. Indo American J Pharm Res, 4, 1049-1062.http:// doi: 10.3389/fphar.2022.853023

Kotzamani, A., Vasilakoglou, I., Dhima, K. et al., (2021). Impact of soil salinity on barley allelopathic potential and main secondary metabolites gramine and hordenine. J Plant Growth Regul, 40, 137–146. https://doi.org/10.1007/s00344-020-10084-6.

Korneev K.V., Atretkhany, K.S.N., Drutskaya, M.S., Grivennikov, S.I., Kuprash, D.V. and Nedospasov, S.A., (2017). TLR-signaling and proinflammatory cytokines as drivers of tumorigenesis. Cytokine, 89,127-135. http:// doi: 10.1016/j.cyto.2016.01.021

Kowalczyk, E., Kwiatek, K., (2018). Use of a new LC-MS Method for the determination of pyrrolizidine alkaloids in feeds. J Vet Res, 62(2),183-191. http:// doi: 10.2478/jvetres-2018-0028.

Kowaltowski, A.J., Cosso, R.G., Campos, C.B., Fiskum, G., (2002). Effect of Bcl-2 overexpression on mitochondrial structure and function. J Biol Chem,8,277(45),42802-7.http:// doi: 10.1074/jbc.M207765200.

Krieg, T., and Murphy, M.P., (2014). Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature, 515(7527), 431-435.http:// doi: 10.1038/nature13909.

Kreiter, J., Rupprecht, A., Zimmermann, L., Moschinger, M., Rokitskaya, T.I., Antonenko, Y.N., Gille, L., Fedorova, M. and Pohl, E.E., (2019). Molecular mechanisms responsible for pharmacological effects of genipin on mitochondrial proteins. Biophys J, 117(10), 1845-1857.http:// doi: 10.1016/j.bpj.2019.10.021.

Lagouge M, Argmann C, Gerhart-Hines Z, et al., (2006). Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. Cell,127(6),1109-1122. http:// doi: 10.1016/j.cell.2006.11.013

Laghari, A. H., Memon, S., Nelofar, A. and Khan, K. M., (2011). *Alhagi maurorum* M., A convenient source of lupeol. Industrial Crops and Products, 34, (1), 1141-1145.http:// doi: 10.1016/j.indcrop.2011.03.031.

Laghari, A.H., Ali Memon, A., Memon, S., Nelofar, A., Khan, K.M., Yasmin, A., (2012). Determination of free phenolic acids and antioxidant capacity of methanolic extracts obtained from leaves and flowers of camel thorn (*Alhagi maurorum*). Nat Prod Res, 26(2),173-6. http://doi: 10.1080/14786419.2010.538846.

Lan, B., He, Y., Sun, H., Zheng, X., Gao, Y., Li, N., (2019). The roles of mitochondria-associated membranes in mitochondrial quality control under endoplasmic reticulum stress. Life Sci, 231, 116587.http://doi: 10.1016/j.lfs.2019.116587.

Latimer H.R., and Veal E.A., (2016). Peroxiredoxins in regulation of MAPK signalling Pathways; sensors and barriers to signal transduction. Mol Cells, 39(1), 40-5. http://doi: 10.14348/molcells.2016.2327.

Lawrence, R. Gray et al., (2017). Measuring mitochondrial pyruvate oxidation. Springer protocols Sci, 15, 231, 116587.http:// doi: 10.1016/j.lfs.2019.116587.

LeBel, C.P., Ischiropoulos, H. and Bondy, S.C., (1992). Evaluation of the probe 2',7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. Chem Res Toxicol, 5, 227– 31. http:// doi:10.1021/tx00026a012.

Lebecque S., Crowet J.M., Lins L. et al., (2018). Interaction between the barley allelochemical compounds gramine and hordenine and artificial lipid bilayers mimicking the plant plasma membrane. Sci Rep 8, 9784. https://doi.org/10.1038/s41598-018-28040-6.

Leete and Marion, ibid., 1954, 32, 646.

Leete E., Kirkwood S., and Marion L., (1952). The biogenesis of alkaloids. VI. The formation of hordenine and N-methyltyramine from tyramine in barley. Can Jour Chem, 30, 749-760. https://doi.org/10.1139/v52-090

Leete E., and Marion L., (1953). The biogenesis of alkaloids. VII. The formation of hordenine and Nmethyltyramine from tyrosine in barley. Can Jour Chem, 31, 126-128. http:// doi: 10.1139/v53-017

Lenaz, G., D'Aurelio, M., Merlo, P. M., et al., (2000). Mitochondrial bioenergetics in aging. Biochim Biophys Acta, 1459,397-404. http:// doi: 10.1016/s0005-2728(00)00177-8

Letts, J.A., Fiedorczuk, K., Degliesposti, G., Skehel, M. and Sazanov, L.A., (2019). Structures of respiratory supercomplex I+ III2 reveal functional and conformational crosstalk. Molecular Cell, 75(6), 1131-1146. http:// doi: 10.1016/j.molcel.2019.07.022.

Lewis, M.R., and Lewis, W.H., (1914). Mitochondria in tissue culture. Science, 27,39(1000),330-3. http:// doi: 10.1126/science.39.1000.330.

Lewis, J.L., (2023). Overview of disorders of calcium concentration. MSD manuals professional. https://www.msdmanuals.com/professional/endocrine-and-metabolic-disorders/electrolyte-disorders/overview-of-disorders-of-calcium-concentration

Li, C., He, C., Xu, Y., Xu, H., Tang, Y., Chavan, H., Duan, S., Artigues, A., Forrest, M.L., Krishnamurthy, P. and Han, S., (2019). Alternol eliminates excessive ATP production by

disturbing Krebs cycle in prostate cancer. Prostate, 79(6), 628-639. http:// doi: 10.1002/pros.23767.
Li X., Rousseau J. F., Ding Y., Song M. and Lu W., (2020). Understanding drug repurposing from the perspective of biomedical entities and their evolution:bibliographic research using aspirin. JMIR Medical Informatics, 8(6), e16739. https://doi.org/10.2196/16739.

Liang, C., Ahmad, K., and Sue, C. M. (2014). The broadening spectrum of mitochondrial disease shifts in the diagnostic paradigm. Biochim Biophys Acta (BBA)-General Subjects, 1840(4), 1360-1367. http:// doi: 10.1016/j.bbagen.2013.10.040.

Liu B., Min M.W., and Bao J.K., (2009). Induction of apoptosis by concanavalin A and its molecular mechanisms in cancer cells. Autophagy, 5, 432–433.http:// doi: 10.4161/auto.5.3.7924

Liu H., Ye M., and Guo H., (2020). An updated review of randomized clinical trials testing the improvement of cognitive function of *Ginkgo biloba* L., extract in healthy people and alzheimer's patients. Front Pharmacol, 10,1688. http:// doi: 10.3389/fphar.2019.01688.

Liu L, Zhu H, Wu W, Shen Y, Lin X, Wu Y, Liu L, Tang J, Zhou Y, Sun F and Lin H-W (2019). Neoantimycin F, a Streptomyces-Derived natural product induces mitochondria-related apoptotic death in human non-small cell lung cancer cells. Front Pharmacol, 10,1042.http:// doi: 10.3389/fphar.2019.01042.

Liu, X., Wang, J., Sun, B., Zhang, Y., Zhu, J. and Li, C., (2007). Cell growth inhibition, G2M cell cycle arrest, and apoptosis induced by the novel compound Alternol in human gastric carcinoma cell line MGC803. Investigational new drugs, 25(6), 505-517.

http:// doi: 10.1007/s10637-007-9057-4

Li, G.B., Fu, R.Q., Shen, H.M., Zhou, J., Hu, X.Y., Liu, Y.X., Li, Y.N., Zhang, H.W., Liu, X., Zhang, Y.H., and Huang, C., (2017). Polyphyllin, I induce mitophagic and apoptotic cell death in human breast cancer cells by increasing mitochondrial PINK1 levels. Oncotarget, 8(6),10359. http:// doi: 10.18632/oncotarget

Lima, E.B., Sousa, C.N., Meneses, L.N., Ximenes, N.C., Santos, Junior, M.A., Vasconcelos, G.S., Lima, N.B., Patrocinio, M.C., Macedo, D., Vasconcelos, S.M., (2015). *Cocos nucifera* L.,: A phytochemical and pharmacological review. Braz J Med Biol Res, 48(11),953-64. http:// doi: 10.1590/1414-431X20154773.

Lingappan K., (2018). NF-κB in Oxidative Stress. Curr Opin Toxicol, 7,81-86. http:// doi: 10.1016/j.cotox.2017.11.002.

Liu, Y., Patra, B., Singh, S.K. et al., (2021). Terpenoid indole alkaloid biosynthesis in *Catharanthus roseus* L.: effects and prospects of environmental factors in metabolic engineering. Biotechnol Lett 43, 2085–2103. http:// doi: 10.1007/s10529-021-03179-x.

Liu D. L. and Lovett J.V., (1993a). Biologically active secondary metabolites of barley. 1. Developing techniques and assessing allelopathy in barley. J Chem Ecol, 19(10), 2217-2230. http:// doi: 10.1007/BF00979659.

Liu D. L. and Lovett J.V., (1993b). Biologically active secondary metabolites of barley. II. Phytotoxicity of barley allelochemicals. J Chem Ecol, 19(10):2231-2244.http:// doi: 10.1007/BF00979660.

Li, X., Fang, P., Mai, J., Choi, E.T., Wang, H., and Yang, X., (2013). Targeting mitochondrial reactive oxygen species as novel therapy for inflammatory diseases and cancers. J Hematol Oncol **6**, 19. https://doi.org/10.1186/1756-8722-6-19.

Locher, C., Neumann, J., Sostaric, T., (2017). Authentication of honeys of different floral origin via high-performance thin-layer chromatographic fingerprinting. J Planar Chromatogr 30, 57–62. http:// doi: 10.1556/1006.2017.30.1.8

Lopez, de M. A., (1998). Clasificacion de las enfermedades mitocondriales [Classification of mitochondrial diseases]. Rev Neurol, 26 (1:S9-14).

Longo, N., Amat di San Filippo, C. and Pasquali, M., (2006). Disorders of carnitine transport and the carnitine cycle. Am J Med Genet, 142C: 77-85. https://doi.org/10.1002/ajmg.c.30087.

Lorenzo G., Oliver K., and Guido K., (2016). Mitochondrial regulation of cell death: a phylogenetically conserved control. Microbial Cell 3(3), 101-108. http:// doi: 10.15698/mic2016.03.483.

Lotz, C., Lin, A. J., Black, C. M., Zhang, J., Lau, E., Deng, N., Wang, Y., Zong, N. C., Choi, J. H., Xu, T., Liem, D. A., Korge, P., Weiss, J. N., Hermjakob, H., Yates, J. R., 3rd, Apweiler, R. and Ping, P. (2014). Characterization, design, and function of the mitochondrial proteome: from organs to organisms. J Proteome Res, 13(2), 433–446. http:// doi: 10.1586/14789450.2015.1024227.

Loizzo M.R., Rashed K., Said A., Bonesi M., Menichini F., Tundis R., (2014). Antiproliferative and antioxidant properties of *Alhagi maurorum* M., aerial parts. Ind Crops Prod, 53, 289-95. http:// doi: 10.1016/j.indcrop.2013.12.049.

Lovett J. V., Hoult A.H., and Christen O., (1994). Biologically active secondary metabolites of barley. IV. Hordenine production by different barley lines. J Chem Ecol, 20(8),1 945-1954. http:// doi: 10.1007/BF02066235

Lu M., Wang Y., and Zhan X., (2019). The MAPK pathway-based drug therapeutic targets in pituitary adenomas. Front, Endocrinol, 10, 330. http:// doi: 10.3389/fendo.2019.00330.

Luft, R., Ikkos, D., Pnlmieri, G., Ernster, L., Afzelius, B., (1962). A case of severe hypermetabolism of nonthyroid origin with a defect in the maintenance of mitochondrial respiratory control: A correlated clinical, biochemical, and morphological study. J Clin Invest 41,1776-1804.http:// doi: 10.1172/JCI104637

Macakova K., Afonso R., Saso L., Mladenka P., (2019). The influence of alkaloids on oxidative stress and related signaling pathways. Free Radic Biol Med., 134,429-444. http:// doi: 10.1016/j.freeradbiomed.2019.01.026.

Mackin, C.J., Widlansky, M.E., Hamburg, N.M., et al., (2007). Effect of combined treatment with alpha-Lipoic acid and acetyl-L-carnitine on vascular function and blood pressure in patients with coronary artery disease. J Clin Hypertens (Greenwich), 9(4), 249-255.http:// doi: 10.1111/j.1524-6175.2007.06052.x.

Man, P.Y.W., Griffiths, P.G., Brown, D.T., Howell, N., Turnbull, D.M. and Chinnery, P.F., (2003). The epidemiology of leber hereditary optic neuropathy in the Northeast of England. Am J Hum Genet, 72(2), 333-339. http://doi: 10.1086/346066.

Manzo-Avalos S, Saavedra-Molina A., (2010). Cellular and mitochondrial effects of alcohol consumption. Int J Environ Res Public Health, 7(12), 4281-4304.http:// doi: 10.3390/ijerph7124281

Map of Pakistan, (2022). (https://www.mapsofworld.com/pakistan/pakistan-political-map.html).

Marra, F., Lunetti, P., Curcio, R., Lasorsa, F.M., Capobianco, L., Porcelli, V., Dolce, V., Fiermonte, G., Scarcia, P., (2021). An overview of mitochondrial protein defects in neuromuscular diseases. Biomolecules, 4,11(11),1633.http:// doi: 10.3390/biom11111.

Martinez, R.M.; Guimaraes, D.A.B.; Berniz, C.R.; Abreu, J.P.; Rocha, A.P.M.; De Moura, R.S.; Resende, A.C.; Teodoro, A.J., (2018). Açai (*Euterpe oleracea* M.) Seed extract induces cell cycle arrest and apoptosis in human lung carcinoma cells. Foods, 7, 178.http:// doi: 10.3390/foods7110178

Marchi, S., and Pinton, P., (2014). The mitochondrial calcium uniporter complex: molecular components, structure and physiopathological implications. J Physiol, 592(5), 829-839.

http:// doi: 10.1113/jphysiol.2013.268235.

Maria, D., Elsa, D., Carole, M., Marilena, D., Laurie, G., Fanny, P., Lucile, B., Martine, G., Diane, H., Noelle, D., Philippe, H., Josep, M., Canals, J., Thierry, D., Nicole, D., Flint B., Emmanuel, B., (2013). A role of mitochondrial complex II defects in genetic models of Huntington's disease expressing N-terminal fragments of mutant huntingtin. Hum Mol Genet, 19(1), 3869–3882, https://doi.org/10.1093/hmg/ddt242.

Marrache, S., and Dhar, S., (2015). The energy blocker inside the powerhouse: mitochondria targeted delivery of 3-bromopyruvate. (Edge Article) Chem. Sci.,6, 1832-1845.

https://doi.org/10.1039/C4SC01963F

Martínez-D.M., Santamaría G., Ortega A.D., and Cuezva J.M., (2006). Biogenesis and dynamics of mitochondria during the cell cycle: significance of 3'UTRs. PLoS One. 31(4),189-91. http:// doi: 10.1371/journal.pone.0000107.

Marambaud, P., Zhao, H., Davies, P., (2005). Resveratrol promotes clearance of Alzheimer's disease amyloid-beta peptides. J Biol Chem, 280(45), 37377- 3738.http:// doi: 10.1074/jbc.M508246200.

Martinez-Reyes, I., Chandel, N.S., (2020). Mitochondrial TCA cycle metabolites control physiology and disease. Nat Commun 11**,** 102. https://doi.org/10.1038/s41467-019-13668-3.

Marashdah, M. S., and Al-Hazimi, H. M., (2010). Pharmacological activity of ethanolic extract of Alhagi maurorum roots. Arab J Chem, 3(1), 39-42. https://doi.org/10.1016/j.arabjc.2009.12.007.

Massicot and Marion, L., (1957). The biogenesis of alkaloids. XVIII. The formation of hordenine from phenylalanine in barley. Can Jour Chem, 35, 1-4. http:// https://doi.org/10.1139/v57-001.

Masahiro M., Simon-Pierre G., Laura H., Ola L., Michael P., Julie S., and Ivan T., (2014). mTOR coordinates protein synthesis, mitochondrial activity and proliferation, Cell cycle, 14(4), 473-480. http:// doi: 10.4161/15384101.2014.991572

Matsumura, T., Uryu, O., Matsuhisa, F., Tajiri, K., Matsumoto, H. and Hayakawa, Y., (2020). N-acetyl-l -tyrosine is an intrinsic triggering factor of mitohormesis in stressed animals. EMBO Reports, 21: e49211.http:// doi: 10.15252/embr.201949211.

Mauro, L., Salerno, M., Morelli, C., Boterberg, T., Bracke, M.E. and Surmacz, E. (2003), Role of the IGF-I receptor in the regulation of cell–cell adhesion: Implications in cancer development and progression. J Cell Physiol, 194,108-116. http:// doi: 10.1002/jcp.10207.

Maver, M., Miras-Moreno, B., Lucini, L., Trevisan, M., Pii, Y., Cesco, S., and Mimmo, T. (2020). New insights in the allelopathic traits of different barley genotypes: Middle Eastern and Tibetan wild-relative accessions vs. cultivated modern barley. PloS one, 15(4), e0231976. https://doi.org/10.1371/journal.pone.0231976

Mayr, J. A., Haack, T. B., Freisinger, P., Karall, D., Makowski, C., Koch, J., Feichtinger, R. G., Zimmermann, F. A., Rolinski, B., Ahting, U., Meitinger, T., Prokisch, H. and Sperl, W., (2015). Spectrum of combined respiratory chain defects. J Inherited Metab Disease, 38(4), 629–640. https://doi.org/10.1007/s10545-015-9831-y.

McCormick, A.L., Skaletskaya, A., Barry, P.A., Mocarski, E.S. and Goldmacher, V.S., (2003). Differential function and expression of the viral inhibitor of caspase 8-induced apoptosis (vICA) and the viral mitochondria-localized inhibitor of apoptosis (vMIA) cell death suppressors conserved in primate and rodent cytomegaloviruses. Virology, 316(2), 221-233.http:// doi: 10.1016/j.virol.2003.07.003.

McCarron J. G., Wilson C., Sandison M. E., Olson M. L., Girkin J., M, Saunter C., and Chalmers S., (2013). From structure to function: Mitochondrial morphology, motion and shaping in vascular smooth muscle. J Vasc Res, 50,357-371.http:// doi: 10.1242/jcs.104.3.917

McFarland, R., Taylor, R.W., and Turnbull, D.M., (2010). A neurological perspective on mitochondrial disease. Lancet Neurol, 9(8), 829-840.http:// doi: 10.1016/S1474-4422(10)70116-2.

Measer, A., (2019). Protective effect of aqueous extract of *Alhagi maurorum* M., in spermatogenesis and antioxidant status of adult rats exposed to carbon tetrachloride. Iraqi J Vet Sci, 33. 1-7. http:// doi: 10.33899/ijvs.2019.125509.1031.

Medzhitov, R. (2007). Recognition of microorganisms and activation of the immune response. Nature 449, 819-826. http:// doi: 10.1038/nature06246.

Melgari, D., Frosio, A., Calamaio, S., Marzi, G.A., Pappone, C., Rivolta, I., (2022). T-Type calcium channels: A mixed blessing. Int J Mol Sci, 23(17), 9894. http:// doi: 10.3390/ijms23179894.

Meylan, E., Curran, J., Hofmann, K., Moradpour, D., Binder, M., Bartenschlager, R. and Tschopp, J., (2005). Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature 437, 1167-1172. http:// doi: 10.1038/nature04193

Meyer J.H., Ginovart N., Boovariwala A., Sagrati S., Hussey D., Garcia A., et al., (2006). Elevated monoamine oxidase a level in the brain: an explanation for the monoamine imbalance of major depression". Arch Gen Psych, 63 (11): 1209–16. http:// doi:10.1001/archpsyc.63.11.1209.

Milojkovic, O.D., Ristivojevic, P., Trifkovic, J., Vovk, I., Lusic, D., Tesic, Z., (2016). TLC fingerprinting and pattern recognition methods in the assessment of authenticity of poplar-type propolis. J Chromatogr Sci 54,1077–1083. http:// doi: 10.1093/chromsci/bmw024

Mimaki, M., Wang, X., McKenzie, M., Thorburn, D.R. and Ryan, M.T., (2012). Understanding mitochondrial complex, I assembly in health and disease. Biochim Biophys Act (BBA)-Bioenergetics, 1817(6), 851-862. http:// doi: 10.1016/j.bbabio.2011.08.010.

Missiaen, L., Robberecht, W., van den Bosch, L., Callewaert, G., Parys, J.B., Wuytack, F., Raeymaekers, L., Nilius, B., Eggermont, J., De Smedt, H., (2000). Abnormal intracellular Ca $^{(2+)}$ homeostasis and disease. Cell Calcium, 28(1), 1-21. http:// doi: 10.1054/ceca.2000.0131

Mizushima, N., Levine, B., Cuervo, A. M. and Klionsky, D. J., (2008). Autophagy fights disease through cellular self-digestion. Nature, 451, 1069-1075.http:// doi: 10.1038/nature06639

Mils V., Bosch S., Roy J., Bel-Vialar S., Belenguer P., Pituello F., et al. (2015). Mitochondrial reshaping accompanies neural differentiation in the developing spinal cord. PLoS ONE 10(5). https://doi.org/10.1371/journal.pone.0128130

Miller V.J., Villamena F.A., and Volek J.S., (2018). Nutritional ketosis and mitohormesis: Potential implications for mitochondrial function and human health. J Nutr Metab, 27.http://doi: 10.1155/2018/5157645.

Min, H.Y., Jung, Y., Park, K.H., and Lee, H.Y., (2020). Papuamine inhibits viability of non-small cell lung cancer cells by inducing mitochondrial dysfunction. Anticancer Res, 40, 323–333. http://doi: 10.21873/anticanres.13956.

Mirdeilami, S. Z., Barani, H., Mazandarani, M., and Heshmati, G. A., (2011). Ethnopharmacological survey of medicinal plants in Maraveh Tappeh region, north of Iran.

Missiroli, S., Perrone, M., Genovese, I., Pinton, P., Giorgi, C., (2020). Cancer metabolism and mitochondria: Finding novel mechanisms to fight tumors. Ebiomedicine, 59, 102943. http:// doi: 10.1016/j.ebiom.2020.102943.

Mosmann, T., (1983). Rapid colourimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 65, 55–63. http:// doi: 10.1016/0022-1759(83)90303-4.

Mo, W., Xu, X., Xu, L., Wang, F., Ke, A., Wang, X., and Guo, C., (2011). Resveratrol inhibits proliferation and induces apoptosis through the hedgehog signaling pathway in pancreatic cancer cell. Pancreatology. 11, 601–609. http://doi: 10.1159/000333542.

Mongre, R.K., Mishra, C.B., Prakash, A., Jung, S., Lee, B.S., Kumari, S., Jin Hong, T., Lee, M.S., (2019). Novel carbazole-piperazine hybrid small molecule induces apoptosis by

targeting BCL-2 and inhibits tumor progression in lung adenocarcinoma *In Vitro* and xenograft mice model. Cancers, 11, 1245. http://doi: 10.3390/cancers11091245.

Muhammad, G., Hussain, M. A., Anwar, F., Ashraf, M. and Gilani, A.-H., (2015). *Alhagi maurorum* M: A plant genus rich in bioactives for pharmaceuticals, Phytother Res, 29, 1–13. http:// doi: 10.1002/ptr.5222.

Mueller, H., Kassack, M.U., Wiese, M., (2004). Comparison of the usefulness of the MTT, ATP, and calcein assays to predict the potency of cytotoxic agents in various human cancer cell lines. J Biomol Screen, 9(6),506-15. doi: 10.1177/1087057104265386.

Mundekkad, D. and Padmaja, C. (2014). Preliminary phytochemical analysis and TLC of the extracts of *Excoecaria agallocha* L. Int J Pharma Med, 5. E-ISSN: 0975-8232; P-ISSN: 2320-5148

Mullins, C., and Bonifacino, J.S., (2001). The molecular machinery for lysosome biogenesis. Bioessays, 23(4), 333-343.http:// doi: 10.1002/bies.1048

Moens, U., Kostenko, S., and Sveinbjørnsson, B., (2013). The role of Mitogen-Activated Protein Kinase-Activated Protein Kinases (MAPKAPKs) in Inflammation. Genes, 4(2), 101–133. https://doi.org/10.3390/genes4020101.

Morita M, Prudent J, Basu K, Goyon V, Katsumura S, Hulea L, Pearl D, Siddiqui N, Strack S, McGuirk S, St-Pierre J, Larsson O, Topisirovic I, Vali H, McBride HM, Bergeron JJ, Sonenberg N, (2017). mTOR Controls mitochondrial dynamics and cell survival via MTFP1. Mol Cell, 67(6), 922-935.e5. http:// doi: 10.1016/j.molcel.2017.08.013.

Mostafa, R. E., Heba, B., M., (2019). Potency of *Alhagi maurorum* M., plant extracts as phytoacaricidal against *Panonychus citri* (Acari: Tetranychidae). Egyp Acad J Biological Sci, H. Botany. 10. 41-53. http://doi: 10.21608/eajbsh.2019.93239.

Nabatchian, F., Moradi, A., Aghaei, M., Ghanadian, M., Jafari, S.M. and Tabesh, S., (2017). New 6 (17)-epoxylathyrane diterpene: aellinane from *Euphorbia aellenii* R. induces apoptosis via mitochondrial pathway in ovarian cancer cell line. Toxicol Mech Methods, 27(8), 622-630. http:// doi: 10.1080/15376516.2017.

Nabavizadeh, S.H., and Nabavi, M., 2010. The effect of herbal drugs on neonatal jaundice. Iran J Pharm Res, 2, 39-40. http://doi: 10.22038/IJP.2020.54279.4293

Nahin, R. L., and Straus, S. E., (2001). Research into complementary and alternative medicine: problems and potential. BMJ (Clinical research ed.), 322(7279), 161–164. https://doi.org/10.1136/bmj.322.7279.161.

Narendra, D., Tanaka, A., Suen, D. F. and Youle, R. J., (2008). Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J. Cell Biol. 183, 795-803.http:// doi: 10.1083/jcb.200809125.

National Center for Complementary and Integrative Health NCCIHT, (2021). Natural doesn't necessarily mean safer or better. https://www.nccih.nih.gov/health/know-science/natural-doesnt-mean-better.

Navarro, A., and Boveris A., (2010). Brain mitochondrial dysfunction in aging, neurodegeneration, and Parkinson's disease. Front Aging Neurosci, 2, 34.http:// doi: 10.3389/fnagi.2010.00034.

Navaratnarajah T., Anand R., Reichert A.S., Distelmaier F., (2021). The relevance of mitochondrial morphology for human disease. Int J Biochem Cell Biol., 134:105951. http://doi: 10.1016/j.biocel.2021.105951.

Neamah, N. F., (2012). A pharmacological evaluation of aqueous extract of Alhagi maurorum. Global Journal of Pharmacology, 6(1), 41-46. http://doi: 10.1016/j.chmed.2019.09.007.

Neal, J.M., Sato, P.T., Johnson, C.L., McLaughlin, J.L., (1971). Cactus Alkaloids X: Isolation of Hordenine and N-Methyltyramine from *Ariocarpus kotschoubeyanus*. J Pharma Sci, 60(3), P477-478. http:// doi: 10.1002/jps.2600600334

Neubauer S. (2007). Review: The failing heart – an engine out of fuel. N Engl J Med., 356(11), 40-51. http:// doi: 10.1056/NEJMra063052

Neuzil J., Dong L.F., Ramanathapuram L., Hahn T., Chladova M., Wang X.F., Zobalova R., Prochazka L., Gold M., Freeman R., Turanek J., Akporiaye E.T., Dyason J.C., Ralph S.J., (2007). Vitamin E analogues as a novel group of mitocans: anti-cancer agents that act by targeting mitochondria. Mol Aspects Med,28(5-6):607-45. http://doi: 10.1016/j.mam.2007.02.003.

Neuzil J., Dong L.F., Rohlena J., Truksa J., Ralph S.J., (2013). Classification of mitocans, anti-cancer drugs acting on mitochondria. Mitochondrion,13(3):199-208. http://doi: 10.1016/j.mito.2012.07.112.

Neuzil, J., Dong, L.F., Rohlena, J., Truksa, J., Ralph, S.J., (2013). Classification of mitocans, anti-cancer drugs acting on mitochondria. Mitochondrion,13(3),199–208.http:// doi: 10.1016/j.mito.2012.07.112.

Ng, J. Y., (2021). Insight into the characteristics of research published in traditional, complementary, alternative, and integrative medicine journals: a bibliometric analysis. BMC Compl Med Therap, 21(1), 185. https://doi.org/10.1186/s12906-021-03354-7.

Ng YS, Turnbull D.M., (2016). Mitochondrial disease: genetics and management. J Neurol, 263(1), 179-91.http:// doi: 10.1007/s00415-015-7884

NHS, (2022). Rare mitochondrial disorders service https://mitochondrialdisease.nhs.uk/patient-area/disease-information/.

Nie, L., Oishi, Y., Doi, I., Shibata, H., Kojima, I., (1997). Inhibition of proliferation of MCF-7 breast cancer cells by a blocker of $Ca^{(2+)}$-permeable channel. Cell Calcium, 22(2),75-82.http:// doi: 10.1016/s0143-4160(97)90107-x.

Ohara, M., Ogawa, K., Suda, G., Kimura, M., Maehara, O., Shimazaki, T., Suzuki, K., Nakamura, A., Umemura, M., Izumi, T., Kawagishi, N., Nakai, M, Sho T., Natsuizaka, M., Morikawa, K., Ohnishi, S., Sakamoto, N., (2018). L-Carnitine suppresses loss of skeletal muscle mass in patients with liver cirrhosis. Hepatol Commun, 6, 2(8), 906-918.http:// doi: 10.1002/hep4.1207.

Omar, H.R., Komarova, I., El-Ghonemi, M., Fathy, A., Rashad, R., Abdelmalak, H.D., Yerramadha, M.R., Ali, Y., Helal, E., Camporesi, E.M., (2012). Licorice abuse: time to send a warning message. Ther Adv Endocrinol Metab, 3(4), 125-38.http:// doi: 10.1177/2042018812454322.

Osellame, L.D., Singh, A.P., Stroud, D.A., Palmer, C.S., Stojanovski, D., Ramachandran, R., Ryan, M.T., (2016). Cooperative and independent roles of the Drp1 adaptors Mff, MiD49 and MiD51 in mitochondrial fission. J Cell Sci 129, 2170–2181. http://doi: 10.1242/jcs.185165

Owusu-Ansah E., Yavari A., Mandal S. and Banerjee U. (2008). Distinct mitochondrial retrograde signals control the G1-S cell cycle checkpoint. Nat Genet, 40(3), 356-61.http:// doi: 10.1038/ng.2007.50

Pagliarini, D.J., Rutter, J., (2013). Hallmarks of a new era in mitochondrial biochemistry. Genes Dev, 27(24), 2615-27.http:// doi: 10.1101/gad.229724.113

Pallagi, P.; Madacsy, T.; Varga, A.; Maleth, J., (2020). Intracellular Ca^{2+} signalling in the pathogenesis of acute pancreatitis: Recent advances and translational perspectives. Int J Mol Sci, 21, 4005. https://doi.org/10.3390/ijms21114005.

Palmeira, C.M., Teodoroab, J.S., Amorim, J.A., Steegborne, C., Sinclair, D.A and Rolo A.P., (2019). Mitohormesis and metabolic health: The interplay between ROS, cAMP and sirtuins. Free Radic Biol Med, 141, 483-49.http:// doi: 10.1016/j.freeradbiomed.2019.07.017

Pan, Q., Xue, M., Xiao, S.S., Wan, Y.J., and Xu, D.B., (2016). A combination therapy with baicalein and taxol promotes mitochondria-mediated cell apoptosis: involving in Akt/β-catenin signaling pathway. DNA Cell Biol, 35(11), 646-656.http:// doi: 10.1089/dna.2016.3312.

Paradies, G., Paradies, V., Ruggiero, F.M., Petrosillo, G., (2019). Role of cardiolipin in mitochondrial function and dynamics in health and disease: Molecular and pharmacological aspects. Cells, 8(7), 728.http:// doi: 10.3390/cells8070728

Parvinroo, S., Zahediasl, S., Sabetkasaei, M., Kamalinejad, M., and Naghibi, F., (2014). The effects of selected hot and cold temperament herbs based on Iranian traditional medicine on some metabolic parameters in normal rats. Iran J Pharm Res: IJPR, 13(Suppl), 177–184. PMID: 24711844; PMCID: PMC3977068.

Peitzsch, C., Tyutyunnykova, A., Pantel, K. and Dubrovska, A. (2017). Cancer stem cells: the root of tumor recurrence and metastases. Sem Cancer Biol, 44, 10-24. Academic Press.http:// doi: 10.1016/j.semcancer.02.011.

Peng, T.I., Jou, M.J., (2010). Oxidative stress caused by mitochondrial calcium overload. Ann N Y Acad Sci. 12(1), 183-8.http://doi: 10.1111/j.1749-6632.2010.05634.x.

Perini, J.A.; Rodrigues-Baptista, K.C.; Machado, D.E.; Nasciutti, L.E.; Perini, J.A., (2018). Anticancer potential, molecular mechanisms, and toxicity of *Euterpe oleracea* M. extract (açai): A systematic review. PLoS ONE, 13, e0200101. http:// doi: 10.1371/journal.pone.0200101

Peruzzi, F., Prisco, M., Dews, M., Salomoni, P., Grassilli, E., Romano, G., Calabretta, B., and Baserga, R. (1999). Multiple signaling pathways of the insulin-like growth factor 1 receptor in protection from apoptosis. Mol Cel Biol, 19(10), 7203–7215. https://doi.org/10.1128/MCB.19.10.7203.

Perciavalle R.M., Stewart D.P., Koss B., Lynch J., Milasta S., Bathina M., Temirov J., Cleland M.M., Pelletier S., Schuetz J.D., Youle R.J., Green D.R., Opferman J.T., (2012). Anti-

apoptotic MCL-1 localizes to the mitochondrial matrix and couples mitochondrial fusion to respiration. Nat Cell Biol, 14(6), 575-83.http:// doi: 10.1038/ncb2488.

Perillo, B., Di Donato, M., Pezone, A., Di Zazzo, E., Giovannelli, P., Galasso, G., Castoria, G, Migliaccio, A., (2020). ROS in cancer therapy: the bright side of the moon. Exp Mol Med, 52(2):192-203.http:// doi: 10.1038/s12276-020-0384-2.

Pfanner, N., Warscheid, B. and Wiedemann, N., (2019). Mitochondrial proteins: from biogenesis to functional networks. Nat Rev Mol Cell Biol, 20, 267–284. https://doi.org/10.1038/s41580-018-0092-0.

Picard, M., Wallace, D.C., and Burelle, Y., (2016). The rise of mitochondria in medicine. Mitochondrion, 30, 105-116.http:// doi: 10.1016/j.mito.2016.07.003

Piomboni, P., Focarelli, R., Stendardi, A., Ferramosca, A. and Zara, V., (2012). The role of mitochondria in energy production for human sperm motility. Int J Andrology, 35,109-124. https://doi.org/10.1111/j.1365-2605.2011.01218.x

Pittler, M.H., and Ernst, E., (2004). Dietary supplements for body-weight reduction: a systematic review. Am J Clin Nutr, 79(4), 529-536.http:// doi: 10.1093/ajcn/79.4.529.

Pittelli, M., Felici, R., Pitozzi, V., Giovannelli, L., Bigagli, E., Cialdai, F., Romano, G., Moroni, F. and Chiarugi, A., (2011). Pharmacological effects of exogenous NAD on mitochondrial bioenergetics, DNA repair, and apoptosis. Mol Pharmacol, 80(6), 1136-1146.http:// doi: 10.1124/mol.111.073916

Pizzorno, J., (2014). The Path Ahead: Mitochondria—Fundamental to Life and Health Joseph Pizzorno, ND, Editor in Chief, integrative Medicine, 13(2). PMID: 26770084; PMCID: PMC4684129.

Plitzko, B., and Loesgen, S., (2018). Measurement of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in culture cells for assessment of the energy metabolism. Bio-protocol 8(10). http:// doi: 10.21769/BioProtoc.2850.

Plitzko, B., Kaweesa, E.N., and Loesgen, S., (2017). The natural product mensacarcin induces mitochondrial toxicity and apoptosis in melanoma cells. J Biol Chem, 292(51), 21102-21116.http:// doi: 10.1074/jbc.M116.774836

Pottle, J., Sun, C., Gray, l., and Li, M., (2013). Exploiting MCF-7 cells' calcium dependence with interlaced therapy. J Can Ther, 4(7A), 32-40. http://doi: 10.4236/jct.2013.47A006.

Poillet-Perez, L., Despouy, G., Delage-Mourroux, R., Boyer-Guittaut, Mn., (2015). Couples'play between ROS and autophagy in cancer cells, from tumor initiation to cancer therapy. Redox Biol, 4, 184–192.http:// doi: 10.1016/j.redox.2014.12.003.

Pramanik K.C., and Srivastava S.K., (2012). Apoptosis signal-regulating kinase 1-thioredoxin complex dissociation by capsaicin causes pancreatic tumor growth suppression by inducing apoptosis. Antioxid Redox Signal 17, 1417–1432. http:// doi: 10.1089/ars.2011.4369.

Pratt, S.J., Hernandez-Ochoa, E., and Martin, S.S., (2020). Calcium signaling: breast cancer's approach to manipulation of cellular circuitry. Biophys Rev, 12, 1343–1359. https://doi.org/10.1007/s12551-020-00771-9

Pumiputavon, K., Chaowasku, T., Saenjum, C., Osathanunkul, M., Wungsintaweekul, B., Chawansuntati, K., Wipasa, J. and Lithanatudom, P., (2017). Cell cycle arrest and apoptosis induction by methanolic leaves extracts of four Annonaceae plants. BMC Compl Alter Med,17(1), 294.http:// doi: 10.1186/s12906-017-1811-3.

Qanungo, S., Das M., Haldar, S. and Basu, A. (2005). Epigallocatechin-3- gallate induces mitochondrial membrane depolarization and caspase-dependent apoptosis in pancreatic cancer cells. Carcinogenesis 26, 958–967. https://doi.org/10.1093/carcin/bgi040

Qiao L., Ting Z., Chang L., Xiao-Yu W., Ji-Quan Z., Fei W., Ge L., Yue-Ming M. and Bing-Liang M. (2019). Mitochondrial membrane potential played crucial roles in the accumulation of berberine in HepG2 cells. Biosci Rep, 39 (4).http:// doi: 10.1042/BSR20190477

Qian, Y., Yang, Y., Wang, K., Zhou, W., Dang,Y., Zhu, M., Li, F. and Ji, G., (2019). 2′-Hydroxychalcone induced cytotoxicity via oxidative stress in the lipid-loaded Hepg2 cells Front Pharmacol, 10:1390. http://doi: 10.3389/fphar.2019.01390.

Qu, Y., Han, B., Yu, Y., Yao, W., Bose, S., Karlan, B. Y., Giuliano, A. E. and Cui, X., (2015). Evaluation of MCF10A as a reliable model for normal human mammary epithelial cells. PloS one, 10(7), e0131285. https://doi.org/10.1371/journal.pone.0131285.

Quiros, P. M., Prado M.A, Zamboni N., D Amico D., Williams R., Finley D., Gygi S.P., Auwerx J., (2017). Multi-omics analysis identifies ATF4 as a key regulator of the mitochondrial stress response in mammals. J Cell Biol, 216 (7), 2027–2045.http:// doi: 10.1083/jcb.201702058.

Rahman, S.M.A., Abd-Ellatif, S.A., Deraz, S.F., Khalil, A.A., (2011) Antibacterial activity of some wild medicinal plants collected from western Mediterranean coast. Egypt. Natural alternatives for infectious disease treatment. African J Biotechnol, 10(52), 10733-10743.http:// doi: 10.5897/AJB11.007

Ralph, S., Low, P., Dong, L., Lawen, A., Neuzil, J., (2006). Mitocans: Mitochondrial targeted anti-cancer drugs as improved therapies and related patent documents. Recent patents on anti-cancer drug discovery. 1(3), 327-46. http://doi. 10.2174/1574892067787769.

Rao G.S., (1970). Identity of Peyocactin, an antibiotic from Peyote (*Lophophora williamsii* L.), and hordenine. J Pharm Pharmacol, 22,544 545. http:// doi: 10.1111/j.2042-7158

Rastogi, A., Joshi, P., Contreras, E., and Gama, V., (2019). Remodeling of mitochondrial morphology and function: an emerging hallmark of cellular reprogramming. Cell Stress, 3(6), 181–194. https://doi.org/10.15698/cst2019.06.189.

Rafelski, S.M., (2013). Mitochondrial network morphology: building an integrative, geometrical view. BMC Biol 11**,** 71. https://doi.org/10.1186/1741-7007-11-71.

Rafehi, M., Faltraco, F., Matthaei, J., Prukop, T., Jensen, O., Grytzmann, A., Blome, F.G., Berger, R.G., Krings, U., Vormfelde, S.V., Tzvetkov, M.V., Brockmoller, J., (2019). Highly variable pharmacokinetics of Tyramine in humans and polymorphisms in OCT1, CYP2D6, and MAO-A. Front Pharmacol, 10:1297. http://doi: 10.3389/fphar.2019.01297.

Ray, K.K., Seshasai, S.R., Erqou, S., et al., (2010). Statins and all-cause mortality in high risk primary prevention: a meta-analysis of 11 randomized controlled trials involving 65,229

participants. Arch Intern Med, 170(12), 1024-1031.http://doi: 10.1001/archinternmed.2010.182.

Recouvreux M.V., Camilletti M.A., Rifkin D.B. and Díaz-Torga G., (2016). The pituitary TGFβ1 system as a novel target for the treatment of resistant prolactinomas. J Endcrinol, 228(3), 73-83.http:// doi: 10.1530/JOE-15-0451

Reda, A., Refaat, A., Abd-Rabou, A. A., Mahmoud, A. M., Adel, M., Sabet, S., and Ali, S. S. (2019). Role of mitochondria in rescuing glycolytically inhibited subpopulation of triple negative but not hormone-responsive breast cancer cells. Sci Rep, 9(1), 13748. https://doi.org/10.1038/s41598-019-50141-z.

Reichert A.S. and Neupert W., (2004). Mitochondriomics or what makes us breathe. Trends Genet, 20(11), 555-62. http://doi: 10.1016/j.tig.2004.08.012.

Reilly P.J., (1981). The detection and identification of hordenine in the horse. Fourth intern conf control of the use of drugs in racehorses. Melbourne, Conference Papers, 165 166.

Rietschel H.G., (1937). Arch. Exp. Pathol. Pharmakol. 186, 387

Rietschel H.G., (1937). Klin. Wochenschr.1937. 16, 714.

Riss, T.L., Moravee, R.A., Niles, A.L., et al., (2013). Cell viability assays, assay guidance manual. http:// PMID:23805433

Ristow, M. and Schmeisser, K. (2014). Mitohormesis: Promoting health and lifespan by increased levels of reactive oxygen species (ROS). Dose-Response: a publication of International Hormesis Society, 12(2), 288–341.http:// doi: 10.2203/dose-response.

Ro, S.H., Semple I., Ho., Park, H.W. and Lee, J.H. (2015). Sestrin2, a regulator of mitohormesis in brown adipose tissue. Front Endocrinol, 6,114. http:// doi: 10.3389/fendo.2015.00114.

Roberts A.T., de Jonge-Levitan L., Parker C.C. and Greenway F.L., (2005). The effect of an herbal supplement containing black tea and caffeine on metabolic parameters in humans. Altern Med Rev, 10(4), 321.Available at http://www.anaturalhealingcenter.com/documents/Thorne/articles/caffeine10-4.pdf.

Rogalinska M., (2016). The role of mitochondria in cancer induction, progression and changes in metabolism. Mini Rev Med Chem, 16(7), 524-30.http:// doi: 10.2174/1389557515666151016124331.

Rohlena J., Dong L.F., Ralph S.J., Neuzil J., (2011). Anticancer drugs targeting the mitochondrial electron transport chain. Antioxid Redox Signal, 15(12), 2951-74.http:// doi: 10.1089/ars.2011.3990.

Rohlenova, K., Sachaphibulkij, K., Stursa, J., Bezawork-Geleta, A., Blecha, J., Endaya, B., et al. (2017). Selective disruption of respiratory supercomplexes as a new strategy to suppress Her2(high) breast cancer. Antioxid Redox Signal, 26(2), 84–103. http://doi: 10.1089/ars.2016.6677

Roman M. C., Betz J. M. and Hildreth J., (2007). Determination of synephrine in bitter orange raw materials, extracts, and dietary supplements by liquid chromatography with ultraviolet detection: single-laboratory validation. Journal of AOAC International, 90(1), 68–81. PMID: 17373438 PMCID: PMC3207213

Rosen C.J., (2009). "Breaking into bone biology: serotonin's secrets". Nature Medicine, 15 (2,145–6.http:// doi:10.1038/nm0209-145.

Roshanak, S., Rahimmalek, M., Goli, S.A., (2016). Evaluation of seven different drying treatments in respect to total flavonoid, phenolic, vitamin C content, chlorophyll, antioxidant activity and colour of green tea (*Camellia sinensis* or *C. assamica* L.,) leaves. J Food Sci Technol, 53(1), 721-9. http:// doi: 10.1007/s13197-015-2030-x.

Ruhoy, I. S. and Saneto, R. P. (2014). The genetics of Leigh syndrome and its implications for clinical practice and risk management. App Clin Genet,7, 221–234.

http:// doi: 10.2147/TACG.S46176

Sacconi, S., Trevisson, E., Salviati, L., Ayme, S., Rigal, O., Redondo, A.G., Mancuso, M., Siciliano, G., Tonin, P., Angelini, C. and Aure, K., (2010). Coenzyme Q10 is frequently reduced in muscle of patients with mitochondrial myopathy. Neuromuscul Disord, 20(1), 44-48. http:// doi: 10.1016/j.nmd.2009.10.014.

Samejo M, Memon S, Bhanger M, Khan K., (2012). Chemical composition of essential oils from *Alhagi maurorum* M. Chem Nat Compd, 48 (5), 898-900. https://doi.org/10.1007/s10600-012-0417-8

Sanchez-Alvarez R., De Francesco E.M., Fiorillo M., Sotgia F. and Lisanti M.P., (2020). Mitochondrial Fission Factor (MFF) inhibits mitochondrial metabolism and reduces breast cancer stem cell (CSC) activity. Front Oncol, 10,1776.http:// doi: 10.3389/fonc.2020.01776.

Sanz A., (2016). Mitochondrial reactive oxygen species: Do they extend or shorten animal lifespan? Biochim Biophys Acta, 1857(8), 1116-1126. http://doi: 10.1016/j.bbabio.2016.03.018.

Sarkar, N., Das, B., Bishayee, A. and Sinha, D., (2020). Arsenal of phytochemicals to combat against arsenic-induced mitochondrial stress and cancer. Antioxid Redox Signa, 33(17), 1230-1256. http://doi: 10.1089/ars.2019.7950

Saxena, M., Prabhu, S.V, Mohseen, M., Pal, A.K., Alarifi, S., Gautam, N., Palanivel, H., (2022). Antidiabetic effect of *Tamarindus indica* and *Momordica charantia* and downregulation of TET-1 gene expression by saroglitazar in glucose feed adipocytes and their involvement in the Type 2 Diabetes-associated inflammation *in vitro*. Biomed Res Int, 9565136.http://doi: 10.1155/2022/9565136.

Scaduto, R.C., and Grotyohann, L.W., (1999). Measurement of mitochondrial membrane potential using fluorescent rhodamine derivatives. Biophys J, 76(1), 469-77.http://doi: 10.1016/S0006-3495(99)77214-0.

Schultes R.E., (1937). “Peyote and plants used in the peyote ceremony,” Harvard Unio. Mus. Leap., 4, l29. Available at https://www.samorini.it/doc1/alt_aut/sz/schultes-peyote-and-plants-confused-with-it.pdf.

Schweitzer A., and Wright S., (1938). Action of hordenine compounds on the central nervous system. The Journal of physiology, 92(4), 422–438. https://doi.org/10.1113/jphysiol.1938.sp003613

Schutte, B., Nuydens, R., Geerts, H. and Ramaekers, F. (1998). Annexin V binding assay as a tool to measure apoptosis in differentiated neuronal cells. J Neurosci Methods, 86, 63–69. http:// doi: 10.1016/s0165-0270(98)00147-2.

Schockel, L., Glasauer, A., Basit, F. et al., (2015). Targeting mitochondrial Complex I using BAY 87-2243 reduces melanoma tumor growth. Cancer Metab 3,11.http:// doi: 10.1186/s40170-015-0138-0.

Schmidt, O., Pfanner, N. and Meisinger, C., (2010). Mitochondrial protein import: from proteomics to functional mechanisms. Nat Rev Mol Cell Biol, 11, 655–667. https://doi.org/10.1038/nrm2959.

Scialò, F., Fernandez-Ayala, D. J., and Sanz, A., (2017). Role of mitochondrial reverse electron transport in ROS signalling: Potential roles in health and disease. Front Physiol, 8, 428. https://doi.org/10.3389/fphys.2017.00428

Scheibye-Knudsen, M., Scheibye-Alsing, K., Canugovi, C., et al., (2013). A novel diagnostic tool reveals mitochondrial pathology in human diseases and aging. Aging, 5(3),192-208.http:// doi: 10.18632/aging.100546.

Scorrano, L., De Matteis, M.A., Emr, S., Giordano, F., Hajnoczky, G., Kornmann, B., Lackner, L.L., Levine, T.P., Pellegrini, L., Reinisch, K., Rizzuto, R., Simmen, T., Stenmark, H., Ungermann, C., Schuldiner, M., (2019). Coming together to define membrane contact sites. Nat Commun, 20, 10(1),1287.http:// doi: 10.1038/s41467-019-09253-3.

Seifert, E. L., Ligeti, E., Mayr, J. A., Sondheimer, N., and Hajnoczky, G. (2015). The mitochondrial phosphate carrier: role in oxidative metabolism, calcium handling and mitochondrial disease. Biochem Biophys Res Comm, 464(2), 369-375.http:// doi: 10.1016/j.bbrc.2015.06.031.

Sena, L. A., and Chandel, N. S., (2012). Physiological roles of mitochondrial reactive oxygen species. Mol cell, 48(2), 158–167. https://doi.org/10.1016/j.molcel.2012.09.025.

Seneca, (2007). Chapter 2 - Alkaloid chemistry. Alkaloids - Secrets of life, Elsevier, Ed. (s): Tadeusz A., 61-139, https://doi.org/10.1016/B978-044452736-3/50004-0.

Shati, A.A., Alkahtani, M.A., Alfaifi, M.A., Elbehairi, S.A., Elsaid, F.G., Prasanna, R. and Mir, M.A. (2020). Secondary metabolites of Saussurea costus leaf extract induce apoptosis in breast, liver, and colon cancer cells by caspase-3-dependent intrinsic pathway. Biomed Res Intern, 11,1608942. http:// doi: 10.1155/2020/1608942.

Shaker, E., Mahmoud, H., and Mnaa, S., (2010). Anti-inflammatory and anti-ulcer activity of the extract from Alhagi maurorum (camelthorn). Food Chem Toxicol, 48(10), 2785-90. http://doi: 10.1016/j.fct.2010.07.007.

Sheweita, S.A., Mashaly, S., Newairy, A., Abdou, H. and Eweda, S. (2016). Changes in oxidative stress and antioxidant enzyme activities in streptozotocin-induced diabetes mellitus in rats: Role of *Alhagi maurorum* M., extracts. Oxid Med Cell Longe, 1-8. http:// doi: 10.1155/2016/5264064.

Shimizu, H., Schredelseker, J., Huang, J., Lu, K., Naghdi, S., Lu, F., Franklin, S., Fiji, H.D., Wang, K., Zhu, H. and Tian, C., (2015). Mitochondrial Ca2+ uptake by the voltage-dependent anion channel 2 regulates cardiac rhythmicity. Elife, 4, p.e04801. http:// doi: 10.7554/eLife.04801.

Shiau, J.P., Chuang, Y.T., Tang, J.Y., Yang, K.H., Chang, F.-R., Hou, M.F., Yen, C.Y., Chang, H.-W., (2022). The impact of oxidative stress and AKT pathway on cancer cell functions and its application to natural products. Antioxidants, 11, 1845. https://doi.org/10.3390/antiox11091845.

Shonberg, J., Lopez, L., Scammells, P.J., Christopoulos, A., Capuano, B. and Lane, J.R., (2014). Biased agonism at G protein-coupled receptors: the promise and the challenges—a medicinal chemistry perspective. Med Res Rev, 34(6), 1286-1330.http:// doi: 10.1002/med.21318.

Signes, A. and Fernandez-Vizarra, E., (2018). Assembly of mammalian oxidative phosphorylation complexes I–V and supercomplexes. Essays Biochem, 62(3), 255-270.http:// doi: 10.1042/EBC20170098.

Singh, G., Pachouri, U. C., Khaidem, D. C., Kundu, A., Chopra, C., and Singh, P. (2015). Mitochondrial DNA damage and diseases. Research, 4, 176. https://doi.org/10.12688/f1000research.6665.1.

Singh V.P, Bineeta Y., and Pandey, V.B. (1999). Flavanone glycosides from *Alhagi pseudalhagi* B. Phytochemistry, 51, (4), 587-590.http:// doi: 10.1016/s0031-9422(99)00010-2.

Sinha, K., Chowdhury, S., Banerjee, S., Mandal, B., Mandal, M., Majhi, S., Brahmachari, G., Ghosh, J., Sil, P.C., (2019). Lupeol alters viability of SK-RC-45 (Renal cell carcinoma cell line) by modulating its mitochondrial dynamics. Heliyon, 5(8), e02107. http:// doi: 10.1016/j.heliyon.2019.e02107.

Sivitz, W.I. and Yorek, M.A., (2010). Mitochondrial dysfunction in diabetes: from molecular mechanisms to functional significance and therapeutic opportunities. Antioxid Redox Signal, 12(4), 537-77. http:// doi: 10.1089/ars.2009.2531.

Skrtic, M., Sriskanthadevan, S., Jhas, B., Gebbia, M., Wang, X., Wang, Z., Hurren, R., Jitkova, Y., Gronda, M., Maclean, N., et al., (2011). Inhibition of mitochondrial translation as a therapeutic strategy for human acute myeloid leukemia. Cancer Cell, 20, 674–688. http://doi: 10.1016/j.ccr.2011.10.015.

Slater, E.C. and Cleland, K.W., (1953). The effect of calcium on the respiratory and phosphorylative activities of heart-muscle sarcosomes. Biochem J, 55(4), 566. http:// doi: 10.1042/bj0550566.

Smith T.A., (1977). Phenethylamine and related compounds in plants. Phytochemistry 16:9 18.

Sobiech M., Giebułtowicz, J. and Luliński, P., (2019). Theoretical and experimental proof for selective response of imprinted sorbent - analysis of hordenine in human urine. J Chrom. 460677

Sobiech M., Giebułtowicz J. and Lulinski P., (2020). Application of magnetic core–shell imprinted nanoconjugates for the analysis of Hordenine in human plasma-preliminary data on pharmacokinetic study after oral administration. J Agri Food Chem, 68 (49), 14502-14512.http:// doi: 10.1021/acs.jafc.0c05985.

Sommer, T., Hubner, H., El Kerdawy, A. et al., (2017)**.** Identification of the beer component Hordenine as food-derived dopamine D2 receptor agonist by virtual screening a 3D compound database. Sci Rep 7, 44201. https://doi.org/10.1038/srep44201

Sommer T., Goen, T., Budnik N. and Pischetsrieder M., (2020). Absorption, biokinetics, and metabolism of the Dopamine D2 receptor agonist Hordenine (N, N-Dimethyltyramine) after beer consumption in humans. J Agri Food Chem, 68 (7), 1998-2006.http:// doi: 10.1021/acs.jafc.9b06029.

Soto-Urquieta, M.G., Lopez-Briones, S., Perez-Vazquez, V., Saavedra-Molina, A., Gonzalez-Hernandez, G.A., Ramirez-Emiliano, J., (2014). Curcumin restores mitochondrial functions and decreases lipid peroxidation in liver and kidneys of diabetic db/db mice. Biol Res, 47(1), 74. http:// doi: 10.1186/0717-6287-47-74.

Sotgia, F., Martinez-Outschoorn, U.E., Pavlides, S. et al., (2011). Understanding the Warburg effect and the prognostic value of stromal caveolin-1 as a marker of a lethal tumor microenvironment. Breast Cancer Res 13, 213. https://doi.org/10.1186/bcr2892

Soule, H.D., Maloney, T.M., Wolman, S.R., Peterson, W.D., Brenz, R., McGrath, C.M., Russo, J., Pauley, R.J., Jones, R.F. and Brooks, S.C. (1990). Isolation and characterization of a spontaneously immortalized human breast epithelial cell line, MCF-10. Cancer Res, 50, 6075–86. PMID: 1975513.

Spath E., (1919). Monatsh. Chem., 40, 129(1919).

Spath E. and Roder H., (1922). Monatsh. Chem., 43, 93(1922).

Spinelli, J.B. and Haigis, M.C., (2018). The multifaceted contributions of mitochondria to cellular metabolism. Nat Cell Biol, 20(7), 745-754.http:// doi: 10.1038/s41556-018-0124-1.

Spivak A.Y., Nedopekina D.A., Gubaidullin R.R., Dubinin M.V., Belosludtsev K.N., (2021). Conjugation of natural triterpenic acids with delocalized lipophilic cations: Selective targeting cancer cell mitochondria. J Pers Med., 11(6), 470.http:// doi: 10.3390/jpm11060470.

Srivastava, S., (2016). "Emerging therapeutic roles for NAD(+) metabolism in mitochondrial and age-related disorders". Clin Tran Med, 5 (1), 25.http:// doi:10.1186/s40169-016-0104-7.

Stanley, C.A. (2004). Carnitine deficiency disorders in children. Annals of the New York Acad Sci, 1033: 42-51. https://doi.org/10.1196/annals.1320.004

Stein, L. R., and Imai, S. (2012). The dynamic regulation of NAD metabolism in mitochondria. Trend Endocrinol Metab: TEM, 23(9), 420–428. https://doi.org/10.1016/j.tem.2012.06.005

Stribney and Kirkwood, ibid., 1954, 32, 918

Stroud D.A., Surgenor E.E., Formosa L.E., Reljic B., Frazier A.E., Dibley M.G., Osellame L.D., Stait T, Beilharz T.H., Thorburn D.R., Salim A. and Ryan M.T., (2016). Accessory subunits are integral for assembly and function of human mitochondrial complex I. Nature. 538 (7623), 123-126.http:// doi: 10.1038/nature19754.

Su S., Cao M., Wu, G., Long Z., Cheng Y., Fan J., Xu Z., Su H., Hao Y., Li G., Peng J., Li S., Wang X., (2018). Hordenine protects against hyperglycemia-associated renal complications in streptozotocin-induced diabetic mice. Biomed and Pharmaco,104, 315-324. https://doi.org/10.1016/j.biopha.05.036.

Suhm, T., Kaimal, J.M., Dawitz, H., Peselj, C., Masser, A.E., Hanzen, S., Ambrozic, M., Smialowska, A., Bjorck, M.L., Brzezinski, P. and Nystrom, T., (2018). Mitochondrial translation efficiency controls cytoplasmic protein homeostasis. Cell Metab, 27(6), 1309-1322.http:// doi: 10.1016/j.cmet.2018.04.011.

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clinic, 71, 209- 249. https://doi.org/10.3322/caac.21660

Supnet, C., and Bezprozvanny, I., (2010). The dysregulation of intracellular calcium in Alzheimer disease. Cell Calcium, 47(2):183-9. http:// doi: 10.1016/j.ceca.2009.12.014.

Sussman C.R., Wang X., Chebib F.T. and Torres V.E., (2020). Modulation of polycystic kidney disease by G-protein coupled receptors and cyclic AMP signaling. Cellular Signalling, 72, 109649.http:// doi: 10.1016/j.cellsig.2020.109649

Sulaiman, G.M., (2013). Antimicrobial and cytotoxic activities of methanol extract of Alhagi maurorum. Afr J Microbiol Res., 7(16):1548-57. http://doi: 10.5897/AJMR12.1795.

Sultana, B., Anwar, F., Ashraf, M., (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules, 15; 14(6):2167-80.http:// doi: 10.3390/molecules14062167.

Sulaiman G.M., (2013). Antimicrobial and cytotoxic activities of methanol extract of *Alhagi maurorum* M., Afr J Microbiol Res, 7(16), 1548-57.http://doi: 10.5897/AJMR12.1795

Sulaiman, M., Tijani, H.. Abubakar, B. and Saidu, H., Hindatu, Y. N., Mohammed, A. I., (2013). An overview of natural plant antioxidants: analysis and evaluation. Advances in Biochemistry, 1(4), 64-72. http:// doi: 10.11648/j.ab.20130104.12.

Sun, L. et al., (2015). cMyc-mediated activation of serine biosynthesis pathway is critical for cancer progression under nutrient deprivation conditions. Cell Res 25, 429–444. http:// https://doi.org/10.1038/cr.2015.33

Tait, S. W., and Green, D. R., (2010). Mitochondria and cell death: outer membrane permeabilization and beyond. Nat Rev Mol Cell Biol, 11, 621-632.http:// doi: 10.1038/nrm2952

Tait, S. W., and Green, D. R., (2013). Mitochondrial regulation of cell death. Cold spring harbor perspectives in biology, 5(9). http:// doi: 10.1101/cshperspect.a008706

Tait S. W. G., and Green D. R., (2012). Mitochondria and cell signalling. Journal of Cell Science , 125: 807-815. http:// doi: 10.1242/jcs.099234

Takuma Y., Hiromi I., Tomohiro T., Takahiro K., Atsushi K., Kaori, I., Kazuto N. and Takumi, K., (2017). RLR-mediated antiviral innate immunity requires oxidative phosphorylation activity. Sci Rep 7 (1), 5379. https://doi.org/10.1038/s41598-017-05808-w

Tan, F. J., Fire, A. Z., and Hill, R. B., (2007). Regulation of apoptosis by *C. elegan s/ Alpinia elegans* C. CED-9 in the absence of the C-terminal transmembrane domain. Cell Death Differ. 14, 925-1935.http:// doi: 10.1038/sj.cdd.4402215

Tavassoli, A.P., Anushiravani, M., Hoseini, S. M., Nikakhtar, Z., Naghedi, H.B., Ramezani, M., Ayati, Z., Amiri, M.S., Sahebkar, A., and Emami, S. A., (2020). Phytochemistry and therapeutic effects of Alhagi spp. and tarangabin in the traditional and modern medicine: a review. J Herbmed Pharmacol, 9. 86-104. http://doi 10.34172/jhp.2020.13.

Taylor, R.W., and Turnbull, D.M., (2005). Mitochondrial DNA mutations in human disease. Nat Rev Genet, 6(5), 389-402.http:// doi: 10.1038/nrg1606.

Tennant, D.A., Duran, R.V. and Gottlieb, E., (2010). Targeting metabolic transformation for cancer therapy. Nature Rev Cancer, 10(4), 267-277. http:// doi: 10.1038/nrc2817.

Thu, Z.M., Myo, K.K., Aung, H.T., Clericuzio, M., Armijos, C. and Vidari, G., (2020). Bioactive phytochemical constituents of wild edible mushrooms from Southeast Asia. Molecules, 25(8), 1972. https://doi.org/10.3390/molecules25081972

Tirichen, H., Yaigoub, H., Xu, W., Wu, C., Li, R. and Li, Y., (2021). Mitochondrial reactive oxygen species and their contribution in chronic kidney disease progression through oxidative stress. Front Physiol 12:627837. http:// doi: 10.3389/fphys.2021.627837

Thomas, L.W., Esposito, C., Stephen, J.M. et al. (2019). CHCHD4 regulates tumour proliferation

Togliatto, G., Lombardo, G., Brizzi, M.F., (2017). The future challenge of reactive oxygen species (ROS) in hypertension: From bench to bed side. Int J Mol Sci, 18(9),1988.http:// doi: 10.3390/ijms18091988.

Tilokani, L., Nagashima, S., Paupe, V. and Prudent, J., (2018). Mitochondrial dynamics: overview of molecular mechanisms. Essays Biochem 20, 341–360. http:// doi: 10.1042/EBC20170104

Timmers S, Konings E, Bilet L, et al., (2011). Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. Cell Metab,14(5),612-622. http:// doi: 10.1016/j.cmet.2011.10.002.

Tilokani, L., Nagashima, S., Paupe, V., Prudent, J., (2018). Mitochondrial dynamics: overview of molecular mechanisms. Essays Biochem, 62(3), 341-360.http:// doi: 10.1042/EBC20170104.

Tundis, R., Menichini, F., Conforti, F., Loizzo, M.R., Bonesi, M., Statti, G and Menichini, F., (2009). A potential role of alkaloid extracts from Salsola species (Chenopodiaceae) in the treatment of Alzheimer's disease, J Enz I Med Chem, 24(3), 818-824. http://doi: 10.1080/14756360802399662.

Tundis, R., Loizzo, M.R., Bonesi, M., Menichini, F., Mastellone, V., Colica, C. and Menichini, F., (2012). Comparative study on the antioxidant capacity and cholinesterase inhibitory activity of Citrus aurantifolia Swingle, *Citrus aurantium* L., and *Citrus bergamia* R. and Poit. peel essential oils. J Food Sci, 77(1), 40-46.http:// doi: 10.1111/j.1750-3841.2011.02511.

The Ludwig Center, (2021). The stem cell theory of cancer. Ludwig center. Stanford medicine. https://med.stanford.edu/ludwigcenter/overview/theory.html.

Thammarat P., Sirilun S., Phongpradist R., Raiwa A., Pandith H., Jiaranaikulwanitch J., (2021). Validated HPTLC and antioxidant activities for quality control of catechin in a fermented tea (*Camellia sinensis* var. assamica R.,). Food Sci Nutr, 9(6), 3228-3239. http:// doi: 10.1002/fsn3.2285.

Unknown author, (1906). The pharmacology of hordenine, an alkaloid obtained from barley. The Lancet, 167(4304), 536. https://doi.org/10.1016/S0140-6736(01)80440-2.

University of Texas M. D. Anderson Cancer Center. (2021). Mitochondrial enzyme found to block cell death pathway points to new cancer treatment strategy: Study finds targeting DHODH enzyme can induce ferroptosis, suppress tumor growth. ScienceDaily.www.sciencedaily.com/releases/2021/05/210512143542.htm

Urra, F. A., Cordova-Delgado M., Pessoa-Mahana H., Ramirez-Rodriguez O., Weiss-Lopez B., Ferreira J. and Araya-Maturana R. (2013). Mitochondria: A promising target for anticancer alkaloids. Curr Top Med Chem 13, 2171–2183.http:// doi: 10.2174/15680266113139990150

Urabee, M.C., Abdulsattar, J.O., Nasif, Z., and Al-Garawi, Z.S., (2021). Extraction methods of *Alhagi maurorum* M. (camel thorn) and its therapeutic applications. J Phy: Conference Series, 1853.http://doi: 10.1016/j.phytochem.2007.07.011

Valera-Alberni M. and Canto C. (2018). Mitochondrial stress management: a dynamic journey. Cell stress, 2(10), 253–74. http:// doi: 10.15698/cst2018.10.158.

Valavanidis, A., Vlachogianni, T., Fiotakis, C., (2009). 8-hydroxy-2'-deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 27(2), 120-139. http:// doi: 10.1080/10590500902885684

Vandenabeele, P., Galluzzi, L., Vanden Berghe, T. and Kroemer, G. (2010). Molecular mechanisms of necroptosis: an ordered cellular explosion. Nat Rev Mol Cell Biol, 11, 700-714.https://www.ncbi.nlm.nih.gov/pubmed/?term=Veltri KL[Author]&cauthor=true&cauthor_uid=2318903

Van T. A., Joubert, A. M. and Cromarty, A. D., (2015). Limitations of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. BMC research notes, 8, 47. https://doi.org/10.1186/s13104-015-1000-8.

Van der Windt, G., Chang, C. H., and Pearce, E. L. (2016). Measuring bioenergetics in T cells using a seahorse extracellular flux analyzer. Curr Protoc Immunol, 113, 3.16B.1–3.16B.14. http:// doi: 10.1002/0471142735.im0316bs113

Varghese, Alka E., (2013). "A comparative phytochemical analysis and screening of anti-microbial activities of three different extracts of leaves of *Uvaria narum* (Dunal) W. and their HPTLC analysis." Int J Adv Res Pharm Bio Sci, 2 (4),31. https://link.gale.com/apps/doc/A357968828/AONE?u=anon~fc313518&sid=google-Scholar&xid=83071e3d

Vassiliadis, S., Elkins, A.C., Reddy, P., Guthridge, K.M., Spangenberg G.C., Rochfort, S.J., (2019). A simple LC-MS method for the quantitation of alkaloids in endophyte-infected perennial ryegrass. Toxins (Basel), 11(11),649.http:// doi: 10.3390/toxins11110649.

Veeresh, P., Kaur, H., Sarmah, D., Mounica, L., Verma, G., Kotian, V., Kesharwani, R., Kalia, K., Borah, A., Wang, X., Dave, K.R., Rodriguez, A.M., Yavagal, D.R., Bhattacharya, P., (2019). Endoplasmic reticulum-mitochondria crosstalk: from junction to function across neurological disorders. Ann Acad Sci., 1457(1), 41-60.http:// doi: 10.1111/nyas.14212.

Veltri K.L., Espiritu M., and Singh G., (1990). Distinct genomic copy number in mitochondria of different mammalian organs. J Cell Physiol, 143(1), 160-4.http:// doi: 10.1002/jcp.1041430122.

Vringer E., and Tait S.W.G., (2019). Mitochondria and inflammation: Cell death heats up. Front Cell Dev Biol, 7, 100. http:// doi: 10.3389/fcell.2019.00100

Vyas S., Zaganjor E., Haigis M.C., (2016). Mitochondria and Cancer. Cell, 166(3), 555-566. http://doi: 10.1016/j.cell.2016.07.002.

Wachtel-Galor S and Benzie IFF. (2011). Chapter 1: Herbal Medicine: An introduction to its history, usage, regulation, current trends and research needs. In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Bio molecular and clinical aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor and Francis.

Walker, J.E., (2013). The ATP synthase: the understood, the uncertain and the unknown. Biochem Soc Trans, 41(1), 1-16. http:// doi: 10.1042/BST20110773

Wallace, D.C., (2010). Review mitochondrial DNA mutations in disease and aging. Environ Mol Mutagen. 51(5):440-50.http:// doi: 10.1002/em.20586.

Wallace, D.C. (2012). Review Mitochondria and cancer. Nat Rev Cancer, 12(10),685-98.http:// doi: 10.1038/nrc3365

Wang, C. and Youle, R. J. (2009). The role of mitochondria in apoptosis. Annual Rev Genet, 43, 95–118. http:// doi: 10.1146/annurev-genet-102108-134850.

Wang, L., Liu, Q., Li, F., Qiu, J., Fan, H., Ma, H., Xia, H., (2013). Apoptosis induced by PGC-1β in breast cancer cells is mediated by the mTOR pathway. Oncology Reports, 30, 1631-1638. https://doi.org/10.3892/or.2013.2628.

Wang, C., Zang, K., Tang, Z., Yang, T., Ye, X., Dang, Y., (2023). Hordenine activated dermal papilla cells and promoted hair regrowth by activating Wnt signalling pathway. Nutrients, 15(3), 694. http://doi: 10.3390/nu15030694

Wang, C., and Youle, R. J., (2009). The role of mitochondria in apoptosis. Ann Rev Genet, 43, 95–118. https://doi:org/10.1146/annurev-genet-102108-134850

Wang, X.D., Li, C.Y., Jiang, M.M., Li, D., Wen, P., Song, X., Chen, J.D., Guo, L.X., Hu, X.P., Li, G.Q. and Zhang, J., (2016). Induction of apoptosis in human leukemia cells through an intrinsic pathway by cathachunine, a unique alkaloid isolated from *Catharanthus roseus* L., Phytomed, *23*(6), 641-653. http:// doi: 10.1016/j.phymed.2016.03.003

Wang, J., Zhang, Q.B., Zhang, Z.S., Song, H.F., Li, P.C., (2010). Potential antioxidant and anticoagulant capacity of low molecular weight fucoidan fractions extracted from *Laminaria japonica*. Int J Biol Macromol 46: 6–12. http:// doi: 10.1016/j.ijbiomac.2009.10.015.

Wang, M.; Carver, J.J.; Phelan, V.V.; Sanchez, L.M.; Garg, N.; Peng, Y., (2016). Sharing, and community curation of mass spectrometry data with global natural products social molecular networking. Nat Biotechnol 34, 828–837.http:// doi: 10.1038/nbt.3597.

Wang L., Gao P., Zhang M., Huang Z., Zhang D., Deng Q., Li Y., Zhao Z., Qin X., Jin D., Zhou M., Tang X., Hu Y., W ang L., (2017). Prevalence and ethnic pattern of diabetes and prediabetes in China in 2013 Jama, 317 (24), 2515-2523.http:// doi: 10.1001/jama.2017.7596.

Wang, X., Guo, R. Z., Ma, L., Ding, Q. Y., Meng, J. H., Chen, Y. G., and Wu, J. H. (2020). Antiprolactinoma effect of hordenine by inhibiting MAPK signaling pathway activation in rats. Evidence-based complementary and alternative medicine: eCAM, 2020, 3107290. https://doi.org/10.1155/2020/3107290.

Wang X., Ma L., Zhang E.J., et al., (2014).“Water extract of Fructus Hordei Germinatus shows antihyperprolactinemia activity via dopamine D2 receptor,” Evid Based Complement Alternat Med, 7.http:// doi: 10.1155/2020/3107290

Wang, X.D., Li, C.Y., Jiang, M.M., Li, D., Wen, P., Song, X., Chen, J.D., Guo, L.X., Hu, X.P., Li, G.Q. and Zhang, J., (2016). Induction of apoptosis in human leukemia cells through an intrinsic pathway by cathachunine, a unique alkaloid isolated from *Catharanthus roseus* L. Phytomedicine, 23(6), 641-653. http:// doi: 10.1016/j.phymed.2016.03.003.

Wang, C.W., Hsu, W.H. and Tai, C.J., (2017). Antimetastatic effects of cordycepin mediated by the inhibition of mitochondrial activity and estrogen-related receptor α in human ovarian carcinoma cells. Oncotarget, 8(2), 3049. http:// doi: 10.18632/oncotarget.13829

Wang, J., Li, H., Wang, X., Shen, T., Wang, S. and Ren, D., (2018). Alisol B-23-acetate, a tetracyclic triterpenoid isolated from Alisma orientale, induces apoptosis in human lung cancer cells via the mitochondrial pathway. Biochem Biophys Res Commun, 505(4), 1015-1021.http:// doi: 10.1016/j.bbrc.2018.10.022

Wang, X., Lu, X., Zhu, R., Zhang, K., Li, S., Chen, Z. and Li, L., (2017). Betulinic acid induces apoptosis in differentiated PC12 cells via ROS-mediated mitochondrial pathway. Neurochemical research, 42(4), 1130-1140.http:// doi: 10.1007/s11064-016-2147-y

Wang, Y.J., Li, Q., Xiao, H.B., Li, Y.J., Yang, Q., Kan, X.X., Chen, Y., Liu, X.N., Weng, X.G., Chen, X. and Cai, W.Y., (2015). Chamaejasmin B exerts anti-MDR effect in vitro and in vivo via initiating mitochondria-dependant intrinsic apoptosis pathway. Drug Design, Development and Therapy, Anti-cancer Drugs, 9, 5301. http:// doi: 10.1097/cad.0000000000000184

Warburg, O., 1956. On the origin of cancer cells. Science, 123(3191), 309-314. http:// doi: 10.1126/science.123.3191.309

Warburg, O., Wind, F. and Negelein, E., (1927). The metabolism of tumors in the body. J Gen Physio, 8(6), 519–530. https://doi.org/10.1085/jgp.8.6.519.

Ward, P.S. and Thompson, C.B., 2012. Metabolic reprogramming: a cancer hallmark even warburg did not anticipate. Cancer cell, 21(3), 297-308. https://doi.org/10.1016/j.ccr.2012.02.014.

Watson, E., Davis, R., Sue, C.M., (2020). New diagnostic pathways for mitochondrial disease. J Transl Genet Genom, 4,188-202. http://dx.doi.org/10.20517/jtgg.2020.31.

Weaton T.A. and Stewart I., (1970). The distribution of tyramine, N methyltyramine, hordenine, octopamine and synephrine in higher plants. Lloydia 33,244 250. PMID: 5495514

Webmed, 2022. Hordenine, uses, side effects and more. Vitamins and supplements. https://www.webmd.com/vitamins/ai/ingredientmono-1528/hordenine

Weichert, D., Banerjee, A., Hiller, C., Kling, R.C., Hübner, H. and Gmeiner, P., (2015). Molecular determinants of biased agonism at the dopamine D2 receptor. J Med Chem, 58(6), 2703-2717.http:// doi: 10.1021/jm501889t.

Wei, X., Ruan, W., and Vrieling, K., (2021). Current knowledge and perspectives of pyrrolizidine alkaloids in pharmacological applications: A mini-review. Molecules (Basel, Switzerland), 26(7), 1970. https://doi.org/10.3390/molecules26071970.

Welz, A.N., Emberger-Klein, A. and Menrad, K. (2018). Why people use herbal medicine: insights from a focus-group study in Germany. BMC Complement Altern Med 18, 92. http:// doi: 10.1186/s12906-018-2160-6.

Wen, H., Gwathmey, J.K., Xie, L.H., (2012). Oxidative stress-mediated effects of angiotensin II in the cardiovascular system. World J Hypertens, 2(4), 34-44.http:// doi: 10.5494/wjh.v2.i4.34.

Wheaton, W.W., Weinberg, S.E., Hamanaka, R.B., Soberanes, S., Sullivan, L.B., Anso, E., Glasauer, A., Dufour, E., Mutlu, G.M., Budigner, G.S. and Chandel, N.S., (2014). Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis. elife, 3, p.e02242. https://doi.org/10.7554/eLife.02242.

Wiedemann, N., and Pfanner, N., (2017). Mitochondrial machineries for protein import and assembly. Annu Rev Biochem 20 (86),685-714. http:// doi: 10.1146/annurev-biochem-060815-014352.

Williamson, D.H., Lund, P., Krebs, H.A., (1967). "The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver". Biochem. J. 103 (2), 514–27.http:// doi:10.1042/bj1030514.

Wong A., Eloy J.A., Couldwell W.T. and Liu J.K., (2015). "Update on prolactinomas. Part 1: clinical manifestations and diagnostic challenges," J Clin Neur, 22(10), 1562–1567, 2015.http:// doi: 10.1016/j.jocn.2015.03.058

Xia, M., Zhang, Y., Jin, K. et al., (2019). Communication between mitochondria and other organelles: a brand-new perspective on mitochondria in cancer. Cell Biosci 9, 27. https://doi.org/10.1186/s13578-019-0289-8.

Xiao, W. and Loscalzo, J., (2020). Metabolic Responses to Reductive Stress. Antioxid Redox Signal, 32(18), 1330-1347.http:// doi: 10.1089/ars.2019.7803.

Xie, N., Zhang, L., Gao, W. et al. (2020). NAD^+ metabolism: pathophysiologic mechanisms and therapeutic potential. Sig Transduct Target Ther 5, 227 (2020). https://doi.org/10.1038/s41392-020-00311-7

Xiong W., Li M., Jin-Hu W., (2014). Therapeutic effects of total alkaloids of Fructus Hordei Germinatus in hyperprolactinemis rats. Pak J Pharm Sci. 27(6 Suppl), 2087-93. PMID: 25410078.

Xu, T., Pang, Q., Wang, Y. and Yan, X., (2017). Betulinic acid induces apoptosis by regulating PI3K/Akt signaling and mitochondrial pathways in human cervical cancer cells. Int J Mol Med, 40(6), 1669-1678. http:// doi: 10.3892/ijmm.2017.3163

Xu J., Wang L., Zhang L., Zheng F., Wang F., Leng J., Wang K., Héroux P., Shen H.M., Wu Y., Xia D., (2021). Mono-2-ethylhexyl phthalate drives progression of PINK1-parkin-mediated mitophagy via increasing mitochondrial ROS to exacerbate cytotoxicity. Redox Biol,101776. http:// doi: 10.1016/j.redox.2020.101776.

Xu, J., Shi, Q., Xu, W., Zhou, Q., Shi, R., Ma, Y., Chen, D., Zhu, L., Feng, L., Cheng, A. S., Morrison, H., Wang, X., and Jin, H. (2019). Metabolic enzyme PDK3 forms a positive feedback loop with transcription factor HSF1 to drive chemo-resistance. Theranostics, 9(10), 2999–3013. https://doi.org/10.7150/thno.31301.

Xu, X., Zhang, J., Chen, L., Sun, Y., Qing, D., Xin, X. and Yan, C., (2021) *Alhagi pseudalhagi* B., extract exerts protective effects against intestinal inflammation in ulcerative colitis by affecting TLR_4-Dependent NF-κB signalling pathways. Front Pharmacol, 12:764602. http:// doi: 10.3389/fphar.2021.764602

Xu, Y., Xin, Y., Diao, Y., Lu, C., Fu, J., Luo, L., Yin, Z., (2011). Synergistic effects of apigenin and paclitaxel on apoptosis of cancer cells. PLoS ONE, 6, e29169. https://doi.org/10.1371/journal.pone.0029169.

Xu, Y., Gao, W., Zhang, Y., Wu, S., Liu, Y., Deng, X., Xie, L., Yang, J., Yu, H., Su, J., et al., (2018). ABT737 reverses cisplatin resistance by targeting glucose metabolism of human ovarian cancer cells. Int J Oncol, 53, 1055–1068. http://doi: 10.3892/ijo.2018.4476.

Xu, Z., Zhang, Q., Ding, C., Wen, F., Sun, F., Liu, Y., Tao, C., Yao, J., (2023). Beneficial effects of hordenine on a model of ulcerative colitis. Molec, 28(6), 2834.http://doi: 10.3390/molecules28062834.

Yang, J., Hu, S., Bian, Y., Yao, J., Wang, D., Liu, X., Guo, Z., Zhang, S. and Peng, L., (2022). Targetting cell death: pyroptosis, ferroptosis, apoptosis and necroptosis in osteoarthritis. Front Cell Dev Biol, 9, 789948.http:// doi:10.3389/fcell.2021.s

Yang H., Yang T., Baur J.A., Perez E., Matsui T., Carmona, J.J., Lamming, D.W., Souza-Pinto, N.C., Bohr, V.A., Rosenzweig, A., de Cabo R., Sauve A.A., Sinclair, D.A., (2007). "Nutrient-sensitive mitochondrial NAD^+ levels dictate cell survival". Cell. 130 (6): 1095 107.http:// doi:10.1016/j.cell.2007.07.035.

Yang, Y., He, P. Y., Zhang, Y., and Li, N., (2020). Natural Products Targeting the Mitochondria in Cancers. Molecules (Basel, Switzerland), 26(1), 92. https://doi.org/10.3390/molecules26010092.

Yang, G., Liu, G.L., Wang, S.J., Chi, Z.M. and Chi, Z., (2020). Pullulan biosynthesis in yeast-like fungal cells is regulated by the transcriptional activator Msn2 and cAMP-PKA signaling pathway. Int J Bio Macromolec, 157, 591-603. http://doi: 10.1016/j.ijbiomac.2020.04.174

Yang, J., Anzo, M. and Cohen, P., (2005). Control of aging and longevity by IGF-I signaling. Exper Geronto, 40(11), 867-872. http:// 10.1007/978-94-017-7587-8_5

Yasi, E. A., Allen, A. A., Sugianto, W. and Peralta-Yahya, P., (2019). Identification of three antimicrobials activating serotonin receptor 4 in colon cells. ACS synthetic biology, 8(12), 2710–2717. https://doi.org/10.1021/acssynbio.9b00310.

Yenugonda, V., Nomura, N., Kouznetsova, V. et al., (2017). A novel small molecule inhibitor of p32 mitochondrial protein overexpressed in glioma. J Transl Med, 15, 210. https://doi.org/10.1186/s12967-017-1312-7

Yuan, L., Wang, J., Xiao, H., Xiao, C., Wang, Y., Liu, X., (2012). Isoorientin induces apoptosis through mitochondrial dysfunction and inhibition of PI3K/Akt signaling pathway in HepG2 cancer cells. Toxicol Appl Pharm, 265, 83–92.http:// doi: 10.1016/j.taap.2012.09.022.

Yun, J. and Finkel, T., (2014). Mitohormesis. Cell metabolism, 19(5), 757–766. http:// doi: 10.1016/j.cmet.2014.01.011.

Yu, Bian., Yuan, Z., Yu, Z., Guo-Hui L., Xue-Song F., (2022). Progress in the Pretreatment and Analysis of Flavonoids: An Update since 2013. Separation and purification reviews 51(1), 11-37.http:// doi: 10.1080/15422119.2020.1801469

Zhang, N. and Sauve, A. A. (2018). "Regulatory effects of NAD + metabolic pathways on Sirtuin activity". Sirtuins in health and disease. Progress in molecular biology and translational science, 154, 71–104.http:// doi:10.1016/bs.pmbts.2017.11.012. ISBN 9780128122617.

Zhang J., Merkle H., Hendrich K., Garwood M., From A. H., Ugurbil K. and Bache R. J., (1993). Bioenergetic abnormalities associated with severe left ventricular hypertrophy. J Clin Invest. 92(2), 993-1003.http:// doi: 10.1172/JCI116676

Zhang, Z., Zhang, H., Chen, S., Xu, Y., Yao, A., Liao, Q., Han, L., Zou, Z. and Zhang, X., (2017). Dihydromyricetin induces mitochondria-mediated apoptosis in HepG2 cells through down-regulation of the Akt/Bad pathway. Nutr Res, 38, 27-33. http:// doi: 10.1016/j.nutres.2017.01.003

Zhang, Z., Teruya, K., Eto, H., Shirahata, S., (2011). Fucoidan extract induces apoptosis in MCF-7 cells via a mechanism involving the ROS-dependent JNK activation and mitochondria-mediated pathways. PLoS One, 6(11):e27441. http:// doi: 10.1371/journal.pone.0027441

Zhao, J., Zhang, J., Yu, M., Xie, Y., Huang, Y., Wolff, D.W., Abel P.W., and Tu, Y., (2012). Mitochondrial dynamics regulates migration and invasion of breast cancer cells. Oncogene, E-pub ahead of print, 2(40):4814-24. http://doi: 10.1038/onc.2012.494.

Zorova, L. D., Popkov, V. A., Plotnikov, E. Y., Silachev, D. N., Pevzner, I. B., Jankauskas, S. S., Babenko, V. A., Zorov, S. D., Balakireva, A. V., Juhaszova, M., Sollott, S. J., and Zorov, D. B., (2018). Mitochondrial membrane potential. Analy Biochem, 552, 50–59. https://doi.org/10.1016/j.ab.2017.07.009.

Zorov, D.B., Juhaszova, M., Sollott, S.J., (2014). Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiol Rev, 94(3), 909-50. http://doi: 10.1152/physrev.00026.2013

Zhou J.W., Luo G.H., Jiang H., Jian T., Chen Z., and Jia A., (2018). Hordenine: A novel quorum sensing inhibitor and antibiofilm agent against *Pseudomonas aeruginosa*. J Agri Food Chem, 66 (7), 1620-1628. http://doi: 10.1021/acs.jafc.7b05035

Zhu, X., Wei, Y., Yang, B. et al., (2020). The mitohormetic response as part of the cyto-protection mechanism of berberine. Mol med, 26, 10. https://doi.org/10.1186/s10020-020-0136-8

Zhao, S., Ghosh, A., Lo, C.S., Chenier, I., Scholey, J.W., Filep, J.G., Ingelfinger, J.R., Zhang, S.L. and Chan, J.S., (2018). Nrf2 deficiency upregulates intrarenal angiotensin-converting enzyme-2 and angiotensin 1-7 receptor expression and attenuates hypertension and nephropathy in diabetic mice. Endocrinology, 159(2), 836-852. http:// doi: 10.1210/en.2017-00752.

Zhang X , Du L., Zhang J., Li C Zhang J. and Xuejiao, L., (2021). Hordenine protects against lipopolysaccharide-induced acute lung injury by inhibiting inflammation. Front Pharmacol, 12, 712232. http:// doi: 10.3389/fphar.2021.712232.

Zhou J.W., Luo G.H., Jiang H., Jian T., Chen Z., and Jia A., (2018). Hordenine: A novel quorum sensing inhibitor and antibiofilm agent against *Pseudomonas aeruginosa*. J Agri Food Chem, 66 (7), 1620-1628. http:// doi: 10.1021/acs.jafc.7b05035

Zhang, J., Liu, C., Huang, R.Z., Chen, H.F., Liao, Z.X., Sun, J.Y., Xia, X.K. and Wang, F.X., (2017). Three new C-27-carboxylated-lupane-triterpenoid derivatives from Potentilla discolour Bunge and their in vitro antitumor activities. PLoS One, 12(4), p.e0175502.http:// doi: 10.1371/journal.pone.0175502

Zhang, X.L., Wang, B.B. and Mo, J.S., (2018). Puerarin 6"-O-xyloside possesses significant antitumor activities on colon cancer through inducing apoptosis. Oncol Lett, 16(5), 5557-5564. http:// doi: 10.3892/ol.2018.9364.

Zhang, L., Tong, X., Zhang, J., Huang, J. and Wang, J., (2015). DAW22, a natural sesquiterpene coumarin isolated from *Ferula ferulaeoides* (Steud.) K. that induces C6 glioma cell apoptosis and endoplasmic reticulum (ER) stress. Fitoterapia, 103, 46-54.

Zhang, M., Du, H., Huang, Z., Zhang, P., Yue, Y., Wang, W., Liu, W., Zeng, J., Ma, J., Chen, G. and Wang, X., (2018). Thymoquinone induces apoptosis in bladder cancer cell via endoplasmic reticulum stress-dependent mitochondrial pathway. Chemico-biological interactions, 292, 65-75. http:// doi: 10.1016/j.cbi.2018.06.013

Zhang, Q.W., Lin, L.G. and Ye, W.C., (2018). Techniques for extraction and isolation of natural products: a comprehensive review. Chin Med 13. https://doi.org/10.1186/s13020-018-0177-x.

Zhang, L., Dong, X., Feng, X., Ibrahim, S.A., Huang, W., Liu, Y., (2021). Effects of drying process on the volatile and non-volatile flavor compounds of *Lentinula edodes* S.,. Foods. 10(11), 2836.http:// doi: 10.3390/foods10112836.

Zhang, X., Fryknas, M., Hernlund, E., Fayad, W., Milito, A.D., Olofsson, M.H., Gogvadze, V., Dang, L., Pahlman, S., Schughart, L.A.K., et al., (2014). Induction of mitochondrial dysfunction as a strategy for targeting tumour cells in metabolically compromised microenvironments. Nat Commun, 5, 3295. http://doi: 10.1038/ncomms4295.

Zohary D. and Hopf M., (2000). Domestication of plants in the old world: The origin and spread of cultivated plants in West Asia, Europe, and the Nile Valley (3rd ed.). Oxford University Press., 59–69. ISBN 978-0-19-850357-6.

Zorova, L. D., Popkov, V. A., Plotnikov, E. Y., Silachev, D. N., Pevzner, I. B., Jankauskas, S. S., Babenko, V. A., Zorov, S. D., Balakireva, A. V., Juhaszova, M., Sollott, S. J., and Zorov, D. B., (2018). Mitochondrial membrane potential. Analy Biochem, 552, 50–59. https://doi.org/10.1016/j.ab.2017.07.009