Enhancing Immunomodulatory Properties of Javanese Turmeric (Curcuma xanthorrhiza) Kombucha against Diethylnitrosamine in Male Balb/c Mice

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Abstract

Kombucha is a fermented tea beverage, made by the addition of symbiotic culture of bacteria and yeasts (SCOBY). This study utilized Javanese turmeric in a concentration of 0.4% (w/v) as a kombucha fermentation medium and evaluated its immunomodulatory activity, compared to non-fermented Javanese turmeric beverage. 42 healthy male BalB/C mice (20-30 g, 2-3 weeks old) were divided randomly into five groups with seven mice each. The groups were fed: Normal diet; normal diet + Javanese turmeric kombucha; normal diet + diethylnitrosamine (DEN); DEN + non-fermented Javanese turmeric and DEN + Javanese turmeric kombucha. Kombuchas and non-fermented Javanese turmeric were given at dose of 0.3 mL/20 g BW/day. The mice were injected with 100 mg/kg DEN intraperitoneally. The spleen was collected and analyzed for $CD4^+$, tumor necrosis factor α ($TNF\text{-}\alpha^+$), interleukin-6 ($IL\text{-}6^+$), $CD8^+$, $CD11b^+$, and $IL\text{-}10^+$. The statistical analyses included ANOVA and the Fischer's exact test. The percentage of $CD8^+$, $CD11b^+$, and $CD8^+$ $IL\text{-}10^+$ increased significantly ($p<0.05$) among DEN-induced groups, Javanese turmeric kombucha and these values were higher than non-fermented Javanese turmeric. These findings verify that Javanese turmeric kombucha possessed better immunomodulatory activity compared to non-fermented Javanese turmeric.

Keywords: Kombucha, Javanese turmeric, fermentation, immunomodulatory, diethylnitrosamine

1. Introduction

Inflammation is one of the body's defence mechanisms to return its tissues to their initial state. The existence of pathogen or faulty cells is recognized by lymphocytes B and T, causing the cells to signal other immune systems by releasing cytokines and interferons to resolve the damage [1]. However, if the damage persisted, prolonged unresolved inflammation could become chronic and induce fatal consequences to the host organism, therefore it should be prevented. The body provides its self-defence mechanism through the existence of anti-inflammatory mediators that limit exaggerated immune responses [2]. These anti-inflammatory mediators are produced by immune cells. Therefore, immunomodulatory agents, compounds that could modulate immune cells and responses, are needed to strengthen anti-inflammatory mediators to combat inflammation [3].

Javanese turmeric is an Indonesian local rhizome that is mostly used for wound healing, boosting immunity, and as an anti-inflammatory and anti-carcinogenic ingredient [4]. Javanese turmeric extract is known to strongly reduce pro-inflammatory cytokine gene expressions such as tumor necrosis factor α (TNF-α^+), interleukin-6 (IL-6^+), IL-1β, and C-reactive protein (CRP) in the liver, adipose tissue, and muscle [5]. Its functional activity is contributed by its biologically active compounds such as xanthorrhizol, curcuminoid, flavonoid, ar-turmerone, α-turmerone, curcumene, bisacurone, curlone, lactone-germacrone, and germacrone [6]. However, the bioactivity of these compounds is suboptimal due to their binding with plant matrix and other components [7]. To release and enhance these bioactive compounds, processing steps such as fermentation can be carried out.

Kombucha tea is a beverage fermented by symbiotic culture of bacteria and yeasts (SCOBY) which has been gaining popularity owing to its functional properties, one of them being its anti-inflammatory property [8]. Kombucha has been proven to inhibit 5-lipoxygenase activity, an enzyme that is involved with fatty acid conversion to leukotrienes, which induces inflammation [9]. Kombucha has also been found to reduce the levels of TNF-α^+, IL-6^+, and IL-1β, as well as restore T cell levels and macrophages in lipopolysaccharide-challenged mice [10]. Recent research on kombucha has led to the utilization of other bioactive materials [11-18]. This study proposes the usage of Javanese turmeric as a kombucha fermentation medium to further intensify its immunomodulatory activity. The synergistic relation between kombucha microorganisms and Javanese turmeric active compounds is expected to boost Javanese turmeric immunomodulatory function.

2. Materials and methods

2.1 Materials

Javanese turmeric of commercial maturity, commercial kombucha starter, and cane sugar were purchased from local distributors and supermarkets in Malang, East Java, Indonesia. Javanese turmeric was peeled, cleaned, and cut into thin slices, then dried in a cabinet drier at 60°C for 5-6 hours. Powdered Javanese turmeric was made by grinding dried Javanese turmeric. Diethylnitrosamine (DEN) was obtained from Tokyo Chemical Industry.

2.2 Kombucha and non-fermented beverage preparation and analysis

Javanese turmeric solution (0.4% w/v) was used as a fermentation medium. The concentration used was chosen as the best optimized treatment, this has been reported in Zubaidah et al., 2022 [19]. Two (2) g of Javanese turmeric powder was placed into teabags and extracted in boiling water (500 mL) for 5 min and added with 10% (w/v) sugar. The solution was poured into sterile glass jars and cooled to room temperature. Kombucha starter (10% v/v) was inoculated to the solution aseptically. The jars were covered with sterile cheese cloths and tightened with rubber bands, then underwent fermentation at room temperature for 12 days.

Non-fermented Javanese turmeric beverage (0.4% w/v) was prepared with the same procedure as Javanese turmeric kombucha but without the addition of a kombucha starter. The preliminary analysis of the physicochemical and microbiological characteristics of the kombucha was outlined in our previous study [19]. The preliminary analysis performed were as follows: pH, total titratable acids [20], total phenolic content (Folin-Ciocalteau method), the IC_{50} value of antioxidant activity (DPPH method) [18], and total microbial cells (total plate count method) [21].

2.3 Analysis of immunomodulatory indicators in experimental animals

Forty-two healthy male BalB/C mice (20-30 g, 2-3 weeks old) were divided randomly into five (5) groups [seven (7) mice each]. The groups were as follows: Normal diet; normal diet + Javanese turmeric kombucha; normal diet + DEN; DEN + non-fermented Javanese turmeric; and DEN + Javanese turmeric kombucha. The mice were acclimated for 7 days and weighing was done once every 3 days. Mice were given Javanese turmeric kombucha and Javanese turmeric non-fermented beverage once a day on a regular basis for 3 weeks at a dose of 0.3 mL/20 g BW/day. The dose was applied in accordance with the average human dose of kombucha, which is 118 mL/day. Based on the data, the dose of kombucha for mice was calculated by multiplying 118 ml/day with 0.0026 (body surface area ratio convertible factor). The mice were given a standard diet and water ad libitum during the experiment. This study was approved by the Brawijaya University Research Ethics Committee (Ethical Clearance No. 109-KEP-UB-2021).

DEN is an extremely potent liver carcinogen in mice and it was introduced intraperitoneally (100 mg/kg) once a week for 2 weeks. During two (2) weeks of induction, Javanese turmeric kombucha and non-fermented Javanese turmeric beverage were also administered. One week of incubation was completed after the induction. Javanese turmeric kombucha and Javanese turmeric non-fermented beverage were still be given during the incubation period. The diet arrangement is detailed in **Table 1**.

After the treatments, the mice fasted for 1 day and scarified 24 hours after fasting. Scarification was performed with 0.2 ml ketamine injection (0.1 mg/g BW). The spleen was taken for immunomodulatory analysis with a flow cytometer. The spleen was chosen because of its important role in mediating the immune response. It also ensures a protective response from the immune system towards harmful stimuli. The proliferation of splenocytes (mixture of various immune cells, such as T cells, B cells, dendritic cells, and natural killer cells) directly indicates cellular immunity [22]. The parameters analyzed were $CD4^+$, $TNF\text{-}\alpha^+$, $IL\text{-}6^+$, $CD8^+$, $CD11b^+$, and $IL\text{-}10^+$.

2.4 Statistical analysis

Statistical analysis was carried out by comparing all the groups data. Analysis of Variance (ANOVA) with Minitab 18.0 was employed in data analysis. Results that showed significant difference ($p<0.05$) were followed by Fisher's exact test.

3. Results and discussion

3.1 Characteristics of kombucha versus non-fermented beverage

The therapeutic properties of Javanese turmeric kombucha was investigated and compared to non-fermented Javanese turmeric beverage in the treatment for mice. According to the analysis conducted and reported by Zubaidah and collaborators [19], the non-fermented Javanese turmeric beverage (8.17 ± 0.01) had a much lower pH value after kombucha fermentation (3.45 ± 0.02). This correlates to the formation of titratable acid (0.21 ± 0.02%) previously not detected in the Javanese turmeric beverage before kombucha fermentation. Total microbial cells in Javanese turmeric kombucha were 1.45 x 10^8 CFU/mL. Kombucha fermentation was found to enhance the total phenolic content by 58.67% and improve Javanese turmeric beverage's antioxidant activity by 38.93%.

The bioactive compounds of Javanese turmeric beverage and Javanese turmeric kombucha were also identified using LC/MS which tracked down compound changes due to kombucha fermentation. The results are shown in **Table 2.**

Javanese turmeric kombucha possessed more identified bioactive compounds compared to non-fermented Javanese turmeric. Organic acids (acetic acid, carbonic acid, pyruvic acid, glucuronic acid), D-saccharic acid-1,4-lactone (DSL), acetate, conjugated curcuminoids, and niacinamide were present due to microbial activity in kombucha fermentation. According to Martínez-Leal and collaborators [39], glucuronic acid was produced by yeasts and *Gluconoacetobacter sp.* This acid was conjugated with curcuminoid during fermentation and formed curcumin glucuronide. Tetrahydrocurcumin (THC) glucuronide was also found in Javanese turmeric kombucha due to lactic acid bacteria (LAB) activity [40, 41]. THC-glucuronide was formed due to the reduction of curcumin and conjugation with glucuronic acid. Curcumin monoacetate was formed due to *Candida* spp. lipase enzyme. Esparan and collaborators [42] mentioned that *Candida* spp. lipase catalyzed the conjugation of acetic ions with -OH moieties of curcumin. Yeasts and bacteria also produced niacinamide during fermentation [9].

More variety of terpenoids were detected in kombucha compared to the non-fermented beverage. Xanthorrhizol was the only terpenoid found in the non-fermented beverage, while xanthorrhizol and bisacurol were detected in kombucha. Bisacurol naturally exists in Javanese turmeric [7]. However, this compound was only identified in kombucha. It is predicted that microbes released bisacurol from plant matrix or other compounds. This ability enabled bisacurol to exist in its free form and became easier to be detected [43].

3.2 Immunomodulatory Activity of Javanese Turmeric Kombucha

The immune response analysis was conducted on the T cells' adaptive immune response from the spleen. The T cells which were used as parameters are $CD4^+$ and $CD8^+$. TNF-α^+ and IL-6^+ were used as the inflammatory indicators, while $CD11b^+$ and IL-10^+ were used as the anti-inflammatory parameters. The results of the analysis are presented in **Table 3.**

The result of TNF-α^+ analysis showed a significant difference between the normal diet group and the DEN + normal diet group ($p<0.05$). There were no significant differences on the result of IL-6^+ analysis, although an increase of IL-6^+ was found in the normal diet + DEN group. The introduction of DEN increased TNF-α^+. This proved that inflammation did occur. TNF-α^+ and IL-6^+ are pro-inflammatory cytokines that are responsible for the inflammatory signalling pathway. These cytokines were released in order to increase the amount of T helper cells (Th cells), cytotoxic cells, lymphocyte B, or other immune cells in the problematic site [1]. Between the groups, the normal diet + DEN group possessed the highest percentage of $CD4^+$ TNF-α^+. DEN is a toxicant that undergoes biotransformation in the liver. This process resulted in reactive intermediates that induced DNA methylation, which subsequently induced depurination and guanine transversion into thymine [44]. If the cell is unable to repair itself due to DNA structural changes, cells are considered damaged and the immune system will be recruited to resolve the problem. It was reported that DEN induced the activation of macrophages and neutrophils. This activation was followed by the production of pro-inflammatory cytokines such as IL-6^+, IL-8, IL-1β, TNF-α^+, CCL2, COX-2, and CCL20, causing inflammation [45]. However, as the analysis was performed 14 days after the last induction, it was predicted that the mice's immune system was beginning to resolve the inflammation in the given time. Thus, the data of IL-6^+ did not show any significant difference ($p>0.05$).

Javanese turmeric kombucha and Javanese turmeric beverage showed lower percentages of $CD4^+$ TNF-α^+ and $CD4^+$ IL-6^+ compared to normal diet + DEN. This meant that Javanese turmeric beverage and Javanese turmeric kombucha were able to reduce the inflammation of DEN-induced mice. Javanese turmeric contained xanthorrhizol and calebin-A. These compounds are known for their anti-inflammatory activities. Xanthorrhizol was able to suppress the phosphorylation of c-Jun N-terminal kinase (JNK) in the MAPK signalling pathway [28]. Thus, it prevented the transcription of COX-iNOS, c-fos, and p50, which then inhibited the binding of NF-κB and AP-1 with DNA and lowered inflammation. Calebin-A lowered the regulation of TNF-α, which consequently prevented the NF-κB activation [26]. Calebin-A also non selectively inhibited COX, an enzyme that contributes to inflammation [27].

The results also showed that anti-inflammatory parameters noted the immunomodulatory activity of the treatments. The percentage of $CD8^{+}$ $CD11b^{+}$ and $CD8^{+}$ $IL\text{-}10^{+}$ significantly differed among groups ($p<0.05$). The induction of Javanese turmeric kombucha increased CD8+ CD11b+ significantly, with the normal diet + Javanese turmeric kombucha and DEN + Javanese turmeric kombucha groups reaching the highest percentages 1.46 ± 0.04% and 1.05 ± 0.20% respectively. Similar results are reported with $CD8^{+}$ $IL\text{-}10^{+}$, where adding Javanese turmeric beverage and kombucha increased the $CD8^{+}$ $IL\text{-}10^{+}$ percentage significantly ($p>0.05$). The $CD8^{+}$ $IL\text{-}10^{+}$ analysis is illustrated in **Fig. 1.**

Both $CD8^{+}$ $CD11b^{+}$ and $CD8^{+}$ and $IL\text{-}10^{+}$, showed the same trends, where normal diet and DEN + normal diet showed the least percentage of $CD11b^{+}$ and $IL\text{-}10^{+}$, while Javanese turmeric beverage and kombucha showed higher percentage. $CD11b^{+}$ is an integrin family member which is highly expressed on monocytes, macrophages, neutrophils, dendritic cells (DCs), natural killer cells (NK cells), and subsets of lymphocyte B and T cells. Higher $CD11b^{+}$ is associated with higher $IL\text{-}10^{+}$ production which can prevent T-cell activation and subsequently reduce pro-inflammatory cytokines [46]. The high percentage of $CD8^{+}$ that could bind into $CD11b^{+}$ indicated that there was a high amount of specific immune cells on the site. The normal diet + Javanese turmeric kombucha group showed significantly higher ($p<0.05$) percentages of $CD11b^{+}$ and $IL\text{-}10^{+}$ compared to the normal diet group. This proved that Javanese turmeric kombucha stimulated the production of specific immune cells. The existence of $CD11b^{+}$ also increased the production of $IL\text{-}10^{+}$, which might inhibit inflammation.

The normal diet + DEN group showed the lowest percentages of CD11b+ and IL-10+ (0.33 ± 0.20% and 0.48 ± 0.19% respectively]. This indicated that there were fewer specific immune cells on the inflammatory site to combat inflammation. Consequently, the number of pro-inflammatory cytokines was higher in the DEN + normal diet group. While DEN decreased the percentage of $CD11b^{+}$, Javanese turmeric beverage and Javanese turmeric kombucha increased the percentages of $CD8^{+}$ $CD11b^{+}$. Javanese turmeric possessed curcuminoid fractions, crude polysaccharides (glucose, galactose, arabinose, xylose, mannose, rhamnose), germacrone, curzerenone, curcumenol, and xanthorrhizol that may reduce proinflammatory cytokines such as IL-1β, NF-κB, and $TNF\text{-}\alpha^{+}$ [4]. Javanese turmeric also possessed calebin-A, a curcumin derivative that is able to lower the regulation of $TNF\text{-}\alpha^{+}$ [26].

This study proved that fermentation into kombucha increases Javanese turmeric immunomodulatory activity. Javanese turmeric kombucha was able to increase the percentage of $CD11b^{+}$ and $IL\text{-}10^{+}$ in comparison to non-fermented Javanese turmeric beverage. It is also important to note that the $CD11b^{+}$ and $IL\text{-}10^{+}$ percentages of DEN +

Javanese turmeric kombucha group were not significantly different from normal diet + Javanese turmeric kombucha. This showed that Javanese turmeric kombucha strengthened specific immune cells and increased anti-inflammatory mediators. Javanese turmeric kombucha might have helped reduce inflammation in DEN-induced groups and restore the system into a normal condition. In accordance with the findings, fermentation of Javanese turmeric into ciders by the addition of *Acetobacter xylinum* was able to downregulate the gene expression of IL-1β, TNF-α^+, and chemokines and inhibit inflammation better than curcuminoid fractions [34].

Javanese turmeric kombucha increased immunomodulatory function due to bioactive compounds and enzymes [such as reductase and cellulase] that were released during fermentation [47, 48]. THC, a metabolite of curcumin, was formed after curcumin reduction with reductases from SCOBY as its catalyst. THC underwent glucuronidation catalyzed by conjugative enzymes to produce THC-glucuronide [39, 49, 50]. THC-glucuronide exhibits competent activity in the inhibition of TNF-α^+, IL-6^+, and NF-κB translocation to the nucleus [51]. Glucuronidation also produced curcumin glucuronide, another curcumin metabolite with anti-inflammatory properties [39]. These metabolites enhanced the immunomodulatory activity of Javanese turmeric kombucha.

4. Conclusions

Kombucha fermentation enhanced the immunomodulatory activity of Javanese turmeric due to the release of bioactive compounds (THC-glucuronide). Javanese turmeric kombucha produced superior outcomes in reducing $CD4^+$ TNF-α^+ and strengthening $CD8^+$ $CD11b^+$ and $CD8^+$ IL-10^+ compared to non-fermented Javanese turmeric.

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Figure legends

Fig. 1 Innate immunomodulatory activity of kombucha: CD8+ IL-10+ macrophage at different treatments: normal diet, normal diet + Javanese turmeric kombucha, normal diet + DEN, DEN + Javanese turmeric beverage, and DEN + Javanese turmeric kombucha

Table 1 Diet arrangement of mice based on groups of treatment

Day	Groups	Diet
0-7	All groups	Standard feed + water
8-28	Normal diet, DEN + normal diet	Standard feed + water
	Normal diet + Javanese turmeric kombucha, DEN + non-fermented Javanese turmeric, DEN + Javanese turmeric kombucha	Standard feed + water + treatments (0.3 mL/20 g BW/day of Javanese turmeric/non-fermented Javanese turmeric)
29-49	Normal diet	Standard feed + water
	Normal diet + Javanese turmeric kombucha	Standard feed + water + 0.3 mL/20 g BW/day of Javanese turmeric kombucha
	DEN + normal diet	Standard feed + water + injection of DEN (100 mg/kg, 1x/week)
	DEN + non-fermented Javanese turmeric, DEN + Javanese turmeric kombucha	Standard feed + water + treatments (0.3 mL/20g BW/day of Javanese turmeric/non-fermented Javanese turmeric) + injection of DEN (100 mg/kg, 1x/week)
50-56	Normal diet, DEN + normal diet	Standard feed + water
	Normal diet + Javanese turmeric kombucha, DEN + non-fermented Javanese turmeric, DEN + Javanese turmeric kombucha	Standard feed + water + treatments (0.3 mL/20g BW/day of Javanese turmeric/non-fermented Javanese turmeric)

Table 2 Identified compounds of Non-fermented Javanese Turmeric Beverage and Javanese Turmeric Kombucha with LC/MS

No.	Compound Name	Classification	Non-fermented Javanese Turmeric Beverage		Javanese Turmeric Kombucha		Potential Function
			m/z	Retention time	m/z	Retention time	
1.	Keto-curcumin	Curcuminoid	381.32	1.256	N/D	N/D	Antioxidant, anti-inflammatory, inhibit liver cells apoptosis [23], anti-mutagenic, anticancer, antimicrobial, anti-arthritic, neuroprotective [24]
2.	Hydroxy curcumin	Curcuminoid	383.35	1.737	N/D	N/D	Antibacterial [25]
3.	Calebin-A	Curcuminoid	381.35	2.081	381.32	1.978	Anti-inflammatory [26], anticancer [27]
4.	[13C]-Curcumin	Curcuminoid	381.28	2.597	N/D	N/D	Antioxidant, anti-inflammatory, inhibit liver cells apoptosis [23], anti-mutagenic, anticancer, antimicrobial, anti-arthritic, neuroprotective [24]
5.	Curcumin	Curcuminoid	365.41	14.293	365.34	3.354	Antioxidant, anti-inflammatory, inhibit liver cells apoptosis [23], anti-mutagenic, anticancer, antimicrobial, anti-arthritic, neuroprotective [24]
6.	Demethoxycurcumin	Curcuminoid	343.54	17.905	343.57	17.974	Antioxidant, anti-mutagenic, anti-inflammatory, anticancer, antimicrobial, anti-arthritic, neuroprotective [24]

7.	Xanthorrhizol	Terpenoid	218.33	19.213	217.26	16.22	Antibacterial, hepatoprotective, anti-inflammatory, antioxidant, antiplatelet, anti-hyperglycemic [28]
8.	Piperidine	Alkaloid	85.43	24.269	N/D	N/D	Antimicrobial [29]
9.	Tetrahydrocurcumin (THC)-glucuronide	Curcuminoid	N/D	N/D	544.68	1.393	Antioxidant, anti-inflammatory, anti-allergic, antidiabetic, hepatoprotective, antihypertensive [30]
10.	Curcumin monoacetate	Conjugated curcuminoid	N/D	N/D	381.35	2.632	Inhibit colon cancer [31]
11.	Curcumin monoglucoside	Conjugated curcuminoid	N/D	N/D	533.57	2.735	Neuroprotective, inhibit Parkinson disease, antioxidant [32], antibacterial, anticancer [33]
12.	Curcumin glucuronide	Conjugated curcuminoid	N/D	N/D	541.7	3.044	Antioxidant, anti-inflammatory, inhibit liver cells apoptosis [23], anti-mutagenic, anticancer, antimicrobial, anti-arthritic, neuroprotective [24]
13.	Bisacurol	Terpenoid	N/D	N/D	221.33	13.055	N/A
14.	Acetic Acid	Organic acid	N/D	N/D	60.7	14.947	Antioxidant [34], antimicrobial [35]
15.	Niacinamide	Vitamin B	N/D	N/D	122.52	17.561	Antioxidant, neuroprotective, geroprotector [36]
16.	Carbonic Acid	Organic acid	N/D	N/D	60.66	18.353	Antioxidant [34]
17.	D-saccharic acid-1,4-lactone (DSL)	Carboxylic acid	N/D	N/D	192.22	18.972	Facilitate detoxification [37], antioxidant [38]
18.	Acetate	Carboxylic acid	N/D	N/D	59.73	22.274	Antioxidant [34]

19.	Pyruvic Acid	Organic acid	N/D	N/D	85.47	25.545	Antioxidant [34]
20.	Glucuronic acid	Organic acid	N/D	N/D	198.46	36.069	Help detoxification, facilitate liver absorption, metabolism, and excretion [39]

Table 3 Immunomodulatory activity of Javanese Turmeric Kombucha

Treatments	$CD4^+$ TNF-α^+ (%)	$CD4^+$ IL-6^+ (%)	$CD8^+$ $CD11b^+$ (%)	$CD8^+$ $IL10^+$ (%)
Normal diet	0.06 ± 0.04^{b}	0.27 ± 0.35^{a}	0.48 ± 0.14^{c}	0.59 ± 0.09^{b}
Normal diet + Javanese turmeric kombucha	0.14 ± 0.04^{ab}	0.25 ± 0.08^{a}	1.46 ± 0.04^{a}	2.50 ± 0.42^{a}
DEN + normal diet	0.20 ± 0.09^{a}	0.65 ± 0.34^{a}	0.33 ± 0.20^{c}	0.48 ± 0.19^{b}
DEN + Javanese turmeric beverage	0.16 ± 0.04^{ab}	0.25 ± 0.07^{a}	1.05 ± 0.20^{b}	1.65 ± 0.75^{a}
DEN + Javanese turmeric kombucha	0.14 ± 0.09^{ab}	0.28 ± 0.12^{a}	1.47 ± 0.17^{a}	2.44 ± 0.71^{a}

*TNF-α^+: tumor necrosis factor α^+; IL-6^+: interleukin-6; IL-10^+: interleukin-10

**data followed by different letters shows significant difference on Fischer's exact test (confidence interval 95%)

Figure 1

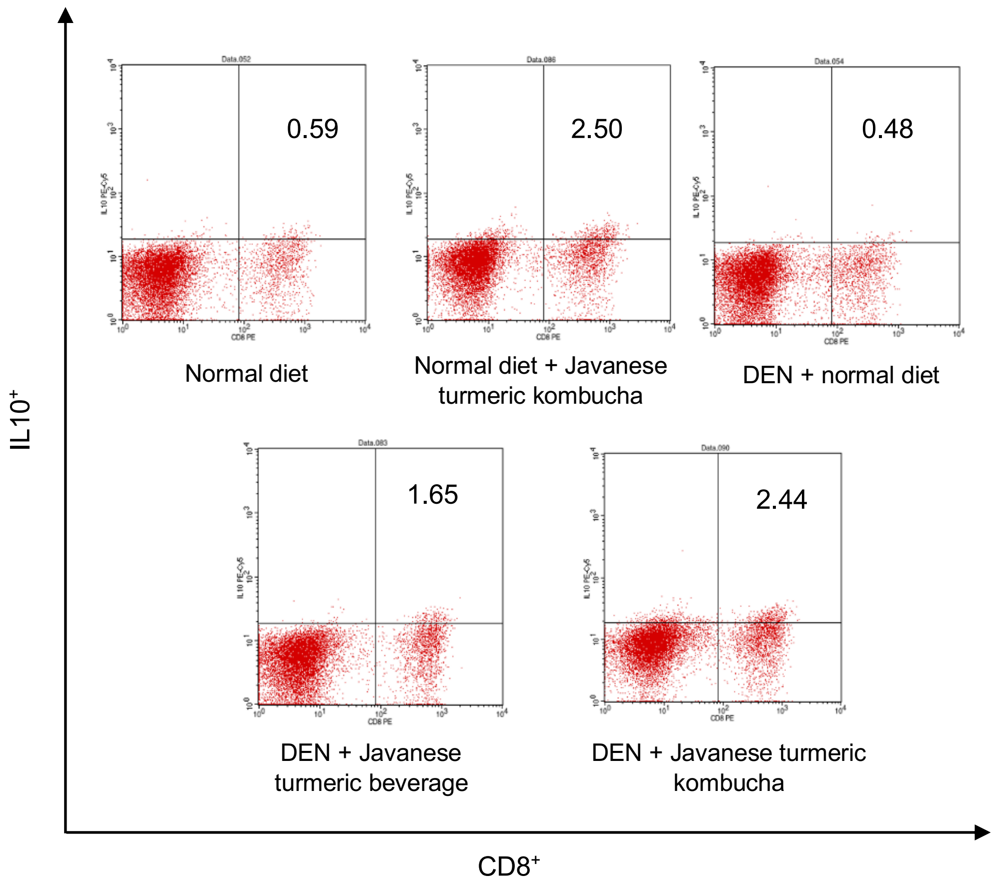

Fig. 1 Innate immunomodulatory activity of kombucha: CD8+ IL-10+ macrophage at different treatments: normal diet, normal diet + Javanese turmeric kombucha, normal diet + DEN, DEN + Javanese turmeric beverage, and DEN + Javanese turmeric kombucha