The Ameliorating Effect of Artificial Coffee from Mangrove Fruits (*Rhizophora mucronata*) on T Lymphocyte Cells and Renal Histopathology of BALB/c Mice Induced by Lipopolysaccharide

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Abstract

The potential of *Rhizophora mucronata* mangrove fruits as antioxidants is noteworthy, with promising prospects as nutraceuticals, particularly in their role as immunomodulators for T lymphocyte cells. *R. mucronata* fruits have been utilized by household industries in Indonesia as a coffee-like drink (artificial coffee). This study aims to determine the ability of mangrove fruit artificial coffee *R. mucronata* to stimulate T lymphocyte cells and renal histopathology conditions in LPS-induced mice animal models. This study also characterizes proximate characteristics, antioxidant capacity, and Gas Chromatography-Mass Spectrometry (GC-MS) screening of mangrove fruit artificial coffee compounds. The stages of this research included the process of mangrove fruit artificial coffee; analysis of proximate and caffeine; compounds screening (GC-MS) and toxicity prediction (Pro-Tox II); antioxidant capacity (DPPH); T lymphocyte cell expression (flow cytometry); and renal histopathological evaluation. The results showed moisture content (5.22±0.20%); ash (5.17±0.27%); protein (13.74±0.24%); fat (11.63±0.32%); carbohydrate (64.23±0.15%), caffeine (1.09±0.04%). GC-MS analysis showed that the compounds contained in artificial coffee were derivatives of fatty aldehyde, fatty acid, and cycloalkanes compound classes. Predicted toxicity of artificial coffee LD_{50} 5000 mg/kg. Antioxidant capacity is classified as moderate (IC_{50} 119.41±0.99µg/mL). Mangrove fruit artificial coffee can increase the relative number of $CD4^+CD62L^+$ by 14.68-29.48% and $CD8^+CD62L^+$ T lymphocyte cells by 12.60-30.33%. Artificial coffee can also increase the number of healthy cells and reduce the cells that undergo necrosis in the renal of LPS-induced mice. This study concluded that mangrove fruit artificial coffee *R. mucronata* positively affects T lymphocyte activation and mice renal cells' protection from necrosis due to LPS induction.

Keywords: antioxidant, coffee, lymphocyte, North Sulawesi, Sangihe

Introduction

Mangroves thrive in tropical and subtropical coastal regions and exhibit robust growth in challenging environments characterized by daily tidal fluctuations (Dewanto et al., 2018). These plants demonstrate adaptability to low oxygen levels and brackish salinity conditions (Reef & Lovelock, 2015), often inhabiting waters with salinity conditions 100 times saltier (Santini et al., 2015). Their survival strategies encompass a range of chemo-physiological adaptations (Mitra et al., 2021), including salt secretion mechanisms that aid in coping with high-salinity habitats (Saiyed, 1992; Kathiresan & Bingham, 2001). Moreover, mangroves have evolved specialized root structures to thrive in oxygen-deprived conditions during inundation by high tides (Srikanth et al., 2016).

Mangroves exhibit chemo-physiological adaptability by synthesizing unique compounds (Mitra et al., 2021). These compounds, derived from mangroves, have garnered attention for their potential applications in the pharmaceutical and nutraceutical sectors (Dahibhate et al., 2018; Sadeer et al., 2023). Research indicates that these mangrove-derived compounds possess a

wide range of biological activities, including antimicrobial properties (Manilal et al., 2016; Alhaddad et al., 2019), antioxidant effects (Rumengan et al., 2021; Dewanto et al., 2021), anticancer potential (Dahibhate et al., 2018; Budiyanto et al., 2022), and antidiabetic properties (Hossain et al., 2013; Patra et al., 2015).

Rhizophora mucronata, a prominent mangrove species abundant along the North Sulawesi coast, Indonesia, particularly in the Sangihe Islands, has garnered attention for its potential applications. As of 2014, it was reported that the area of mangrove vegetation in the Sangihe Islands was around 664 ha, with a significant concentration (about 159 hectares) noted in the Central Tabukan sub-district (Fakhrurrozy, 2015). Research has highlighted *R. mucronata* as a predominant species in the Sangihe Islands (Arifin et al., 2019). Notably, all parts of the mangrove *R. mucronata* have been recognized for their pharmaceutical and nutraceutical benefits. Studies have indicated that the leaves possess antibacterial properties (Sahoo et al., 2012; Kannappan et al., 2021), exhibit anticholinesterase activity (Suganthy & Pandima Devi, 2016), and demonstrate potential as antidiabetic and antioxidant agents (Adhikari et al., 2016; Kasitowati et al., 2017; Rumengan et al., 2021), with prospects for anticancer pharmaceutical preparations (Faoziyah & Kurniawan, 2017). The bark and stem also exhibit antibacterial effects (Pradana et al., 2014) and antioxidant properties (Mahmiah et al., 2016; Supriatna et al., 2019). Furthermore, the fruit of R. mucronata is noted for its antioxidant activity (Purwaningsih et al., 2013; Ernawati et al., 2019), potential as an antidiabetic agent (Hardoko et al., 2015), and acetylcholinesterase inhibitory effects (Wahyuni et al., 2015).

Previous studies have reported that *R. mucronata* fruit has the potential as an antioxidant because it contains ascorbic acid, beta-carotene, and phenolic acids (Basyuni et al., 2021). In addition, the phytochemical content contained in *R. mucronata* fruit, such as tannins (Purwaningsih et al., 2013); flavonoids (Ernawati et al., 2019); saponins, steroids (Ramli et al., 2020); and terpenoids (Podungge et al., 2015). Antioxidant substances could prevent and slow cell damage due to free radicals (Dewanto et al., 2021), exposure to carcinogens (Qi et al., 2022), and bacterial infections (Pham-Huy et al., 2008; Lobo et al., 2010). Infection with microorganisms such as bacteria, fungi, and viruses can cause increased production of free radicals due to mitochondrial dysfunction due to oxidative stress and inflammation (Forcados et al., 2021). Antioxidant substances are reported to have immunomodulatory potential and can affect T lymphocytes (Maheshwari et al., 2022). T lymphocyte cells play a role in the immune response system against pathogens by detecting and eliminating antigen threats, regulating immune responses, and providing long-term protection through immunological memory (Kumar et al., 2018). This study examines the effect of artificial coffee of mangrove fruit *R. mucronata* on lipopolysaccharide (LPS)-induced mice. Lipopolysaccharide (LPS) induction is one of the methods used in testing inflammation in animal models in vitro and in vivo studies (Skrzypczak-Wiercioch, 2022). The LPS model is an effective systemic inflammation model used to determine the anti-inflammatory potential of test compounds (Yin et al., 2023).

In Indonesia, the mangrove fruits of Rhizophora have been utilized by household industries as coffee-like drinks (artificial coffee) (Miranti et al., 2018; Sukma & Zahro, 2020). However, no prior research has investigated the effects of this artificial coffee on renal histopathological conditions. The renal organ acts as a blood filter and maintains fluid and electrolyte balance. While artificial coffee products made from mangrove fruit may provide nutraceutical effects (therapeutic benefits) (Alomar, 2020), artificial coffee may also potentially cause adverse effects on the renal. Histopathological analysis serves as a valuable tool for detecting toxicity indicators such as inflammation and necrosis stages, aiding in assessing the safety profile of herbal constituents (Soliman et al., 2020; El-Bassossy et al., 2023). This study examines the ability of mangrove fruit artificial coffee *R. mucronata* to affect the T lymphocyte cell level of LPS-induced mice.

Furthermore, the study evaluates the histopathological condition of the renal of mice given mangrove fruit artificial coffee as an initial screening for evaluating the safety profile of herbal ingredients. The primary objective is to investigate the ameliorating effect of mangrove fruit artificial coffee *R. mucronata* on T lymphocyte cell levels and renal histopathology conditions in LPS-induced mice animal models. The study also characterized the proximate characteristics, antioxidant capacity, and screening of mangrove fruits' artificial coffee compounds by GC-MS.

Material and Methods

Process of Mangrove Fruits Artificial Coffee

The *R. mucronata* fruits were collected from the Miulu coastal village in Sangihe Islands, North Sulawesi, Indonesia, from April to May. The mangrove fruits were soaked in fresh water at room temperature for 20-24 h. Subsequently, the mangrove fruits were halved and separated from the seeds. The mangrove fruit flesh was again soaked for 30-36 h, with fresh

water changes every 4-6 h. Following this, the mangrove fruits were soaked in a 4% calcium hydroxide ($Ca(OH)_2$) solution for 70-72 h at room temperature. The treated fruits were cut into 2-3 cm pieces and dried at 60-70°C for 30-40 h. The dried mangrove fruits were roasted for 30-40 min at 110-120°C. Afterward, the roasted mangrove fruits were ground into powder and sieved through a 100-mesh sieve. The entire process of preparing mangrove fruit artificial coffee is illustrated in Figure 1.

Proximate and Caffeine Analysis

The proximate contents (including moisture, ash, protein, fat, carbohydrate, and energy) were analyzed following the Indonesian National Standard (SNI)

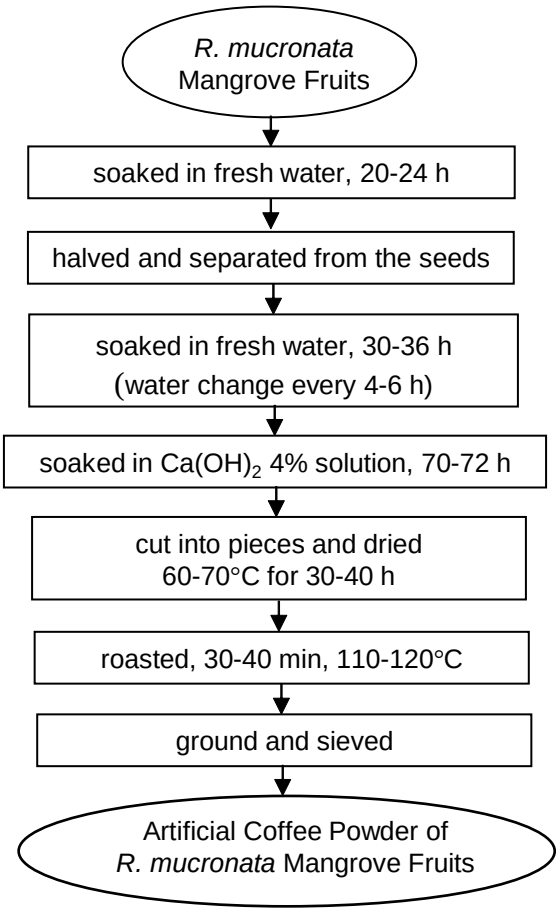


Figure 1. Process of Mangrove Fruits Artificial Coffee

guidelines. The SNI No. 2354.2-2006 determined the moisture content (Indonesia National Standardization Agency [BSN], 2006a). SNI No. 01-2891-1992 determined the ash, protein, and fat content (BSN, 1992). The carbohydrate and energy content was determined by SNI No. 01-3775-2006 (BSN, 2006b). Caffeine content was determined using the isolation method with chloroform, following the instructions outlined by Heèimoviæ et al. (2011).

Screening of Artificial Coffee Compounds and Toxicity Prediction

Mangrove fruit artificial coffee powder compounds were screened using GCMS (HP 6890). The GC-MS column used was Agilent 19091S-433 HP-5MS with p=30 m and #=250 µm. Helium gas was used as the carrier gas, with a 1 mL/minute flow rate at 325°C. The initial temperature was 150°C, with a flow rate of 1°C/min. The screening process started at 10°C/min and then increased to 240°C for 2 min. The total running time was 26 minutes. The scanning range was 50-550 amu. Structural determination was based on analysis of mass spectrum fragmentation patterns and directly comparing mass spectra with NIST (02.L and 17.L) and Wiley (275.L) databases (Tanod et al., 2019). After obtaining the compound name from the GCMS results, toxicity prediction of lethal dosage (LD_{50}) was carried out using Pro-Tox II (https://tox-new.charite.de/protox_II/) (Banerjee et al., 2018; Riyadi et al., 2021).

Antioxidant Capacity Analysis

Antioxidant capacity was evaluated by the DPPH method (Aisiah et al., 2022). Mangrove fruit artificial coffee was dissolved in ethanol (200 µg/mL) and then diluted to a concentration series of 20, 40, 60, 80, and 100 µg/mL. 50 µM DPPH was added to each extract concentration and incubated in a dark room for 30 min at room temperature. The absorbance value of DPPH (A) and the absorbance of extracts from each dilution series (B) were measured by UV-VIS spectrophotometer (Shimadzu 1800) at 517 nm. The 50% inhibition concentration (IC_{50} = µg/mL) was obtained from the linear regression equation between the extract concentration (x-axis) and the percentage value of antioxidant capacity (y-axis). The formula acquired the antioxidant capacity:

$$Antioxidant\ capacity\ (\%) = \frac{A - B}{A} \times 100\%$$

Animal Model Research Procedures

This study used male BALB/c mice aged 6-7 weeks with a body weight of 23-26 g obtained from the Department of Pathology, Faculty of Medicine, Brawijaya University, Indonesia. Five mice were housed per cage box with free access to food and clean water in a 12-hour light/dark cycle with constant temperature and humidity. Mice were adapted for seven days before treatment was given. The Animal Care and

Utilization Committee approved this study; Brawijaya University, Indonesia, approved all animal housing and experiments with an ethical clearance No. 098-KEP-UB-2022 by the Guidelines for the Care and Utilization of Laboratory Animals (National Institutes of Health, USA).

Furthermore, thirty mice were randomly divided into six treatment groups, namely: (I) Normal; (II) LPS 5 mg/kg BW (*Escherichia coli* O127:B8, Sigma-Aldrich); (III). LPS 5 mg/kg BW + Vitamin E 500 mg/kg BW; (IV). LPS 5 mg/kg BW + artificial coffee 500 mg/kg BW; (V) LPS 5 mg/kg BW + artificial coffee 1000 mg/kg BW; and (VI). LPS 5 mg/kg BW + artificial coffee 1500 mg/kg BW. Groups I and II were given saline solution. In contrast, group III was given vitamin E, and groups IV-VI were given artificial coffee of *R. mucronata* fruits for 14 days before the mice were induced with LPS. Thirty minutes after the last treatment, groups II-VI mice were injected with LPS, while group I was injected with saline solution intraperitoneally. The survival rate of mice was observed every hour for 6 h. After 6 h of LPS injection, the mice were sacrificed and harvested spleen (for T lymphocyte cell level observation) and renal (for histopathology evaluation).

Evaluation of T Lymphocyte Cells ($CD4^+CD62L^+$ and $CD8^+CD62L^+$) Expression with Flow Cytometry

Fresh mice spleens were harvested, washed twice with sterile phosphate buffered saline, and isolated into a single-cell suspension. The single-cell suspension, with a density of $2\text{-}3\times10^6$ cells, was centrifuged at 2500 rpm for 5 minutes at 10°C. The supernatant was discarded, and the pellet was stained with FITC anti-mouse CD4 (clone: GK1.5, BioLegend, San Diego, USA); PE anti-mouse CD8 (clone: 53-6.7, BioLegend, San Diego, USA); and PE/Cy5 anti-mouse CD62L (clone: MEL-14, BioLegend, San Diego, USA) as regulatory T lymphocyte cell markers for 30 min at 4°C under low light conditions. Splenocytes were pre-stained and fixed with Cytofix/Cytoperm Kit (BD Biosciences, Pharmingen). The staining combinations used for detecting T-cell activation by flow cytometric were $CD4^+CD62L^+$ and $CD8^+CD62L^+$. Populations were analyzed using flow cytometry (BD FACS CaliburTM) FlowJo v10 for Windows (Riyadi et al., 2022).

Evaluation of Renal Histopathological

Renal mice were fixed in 10% neutral buffered formalin, then dehydrated and embedded in paraffin (Amos AEC 380). Afterward, the renal embedded in paraffin was cut into four sections with a microtome (Thermo Shandon Finesse 325) and stained with hematoxylin-eosin (HE). Stained renal slides were then observed, and histopathological images were taken using an Olympus BX51 equipped with an Olympus XC10 digital camera system. The renal cells counted were healthy, pyknosis, karyorrhexis, and karyolysis (Razik et al., 2015).

Data Analysis

The data were analyzed using a completely randomized design (CRD), with six treatments and three replications. The data were statistically analyzed using Analysis of Variance (ANOVA). If $F_{count} > F_{table}$, the analysis continued with Duncan's further test at the 5% level.

Results and Discussion

The proximate and caffeine levels of mangrove fruit artificial coffee *R. mucronata* were determined to observe its characteristics and compare with the Indonesian National Standard on ground coffee No. 01-3542-2004 (BSN, 2004). Table 1 presents the proximate analysis of mangrove fruit artificial coffee and its caffeine level.

Table 1 shows that *R. mucronata* artificial coffee's moisture content meets the ground coffee's SNI. The proximate composition data in Table 1 closely aligns with findings reported by Mandeno et al. (2023), specifically regarding moisture (4.14%), ash (4.97%),

Table 1. Proximate of artificial coffee from *R. mucronata* fruits

Parameters	Results	Ground Coffee (SNI No. 01-3542-2004)
Moisture (%)	5.22 ± 0.20	Max. 7
Ash (%)	5.17 ± 0.27	Max. 5
Protein (%)	13.74 ± 0.24	–
Fat (%)	11.63 ± 0.32	–
Carbohydrate (%)	64.23 ± 0.15	–
Energy (kcal)	416.60 ± 0.80	–
Caffeine (%)	1.09 ± 0.04	0.45 - 2

protein (14.38%), fat (12.26%), carbohydrate (63.67%), energy content (422.58 kcal), and caffeine content (1.20%). The variation observed between this study and Mandeno et al. (2023) pertains to the concentration of $Ca(OH)_2$ utilized. The moisture content of food is essential to evaluate, to prevent the growth of pathogenic microbes so that the product is more resistant during storage (Vera Zambrano et al., 2019). Previous studies reported that the moisture content of *Rhizophora* sp. ranged between 1.12 and 2.1%. In coffee products, moisture content can affect the flavor and roasting process (Pittia et al., 2007).

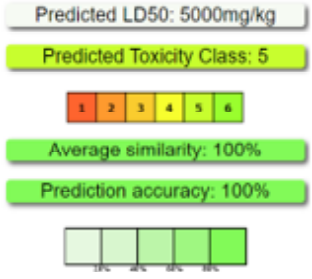
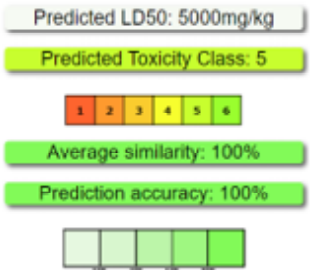
Table 1 depicts that the ash content of artificial coffee does not meet the SNI for ground coffee. Ash content indicates the mineral content of a coffee product (Pigozzi et al., 2018). Research on *Rhizophora* sp. has indicated an ash content ranging from 1.13% to 4.2% (Sukma & Zahro, 2020). Previous studies have also noted the presence of calcium (Ca), copper (Cu), iron (Fe), potassium (K), manganese (Mn), sodium (Na), and zinc (Zn) in *R. mucronata* fruits (Rout et al., 2015). In addition, *R. mucronata* artificial coffee contains protein, as mangrove fruit is also reported to have protein ranging from 1.2-45.48% (Ray et al., 2015). These fruits are also known to have a low-fat content, typically around 4.4% to 4.9% (Siddik & Hossain, 2023).

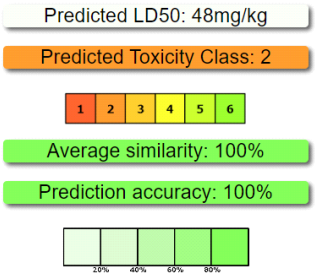
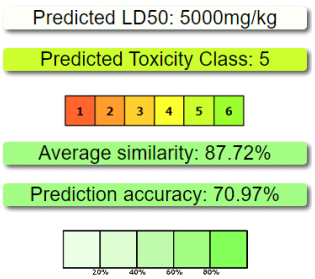
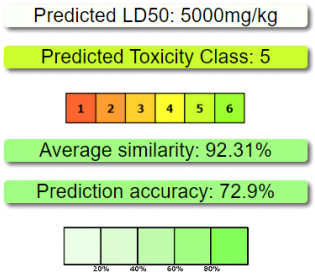
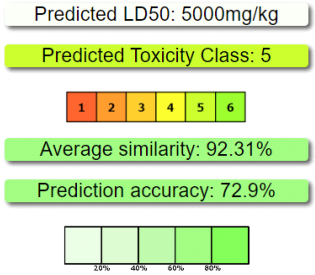
Table 1 further reveals that carbohydrates constitute the predominant component in *R. mucronata* artificial coffee, contributing to its high energy content (Talib et al., 2018). These carbohydrates, categorized as non-reducing sugars, impart a sweet taste to the artificial coffee products, aligning with findings on mangrove fruits (Rout et al., 2015; Basyuni et al., 2021). Caffeine is one of the parameters evaluated in ground coffee products. Caffeine levels in artificial coffee products are still within the limits of the SNI for ground coffee. Caffeine is a substance found in some leaves and fruits of plants (Ashihara & Crozier, 2001). Caffeine is a substance that stimulates increased activity in the human brain and nervous system (Meeusen et al., 2013; Cappelletti et al., 2014) and increases the circulation of chemicals such as cortisol and adrenaline in the human body (Lovallo et al., 2005).

The GC-MS method also evaluated the classes of compounds in artificial coffee of *R. mucronata* fruit. The compounds read were then predicted for toxicity using the Pro-Tox II database. Table 2 presents the results of compound screening and toxicity prediction of mangrove fruit artificial coffee *R. mucronata* with GC-MS with quality above 85%.

Table 2 shows that 95.78% of the compounds in artificial coffee of *R. mucronata* fruit fall into the slightly toxic category. According to the Canadian Center for Occupational Health and Safety, compounds with an LD_{50} value of 300mg/kg are considered highly toxic; moderately toxic 300d"LD_{50}<100mg/kg, and slightly toxic 1000d"LD_{50}<5000mg/kg (Siddik &

Table 2. Toxicity prediction of screening compounds from artificial coffee of fruit *R. mucronata* with GC-MS

Area (%)	Compounds	RT (Min)	Class	Toxicity Prediction	Bioactivity
50.95	9(Z)-Hexadecenal	15.064 15.243 15.728 19.457 20.160	Fatty alde-hyde		- Antifungal (Hoda et al., 2020) - Antioxidant, anticholinesterase, antidiabetic (Nazir et al., 2021) - Antiproliferative (Dasuki Sulain, 2017)
33.97	cis-9-Octadecenal	20.313	Alde-hyde		- Antioxidant (Nazir et al., 2021) - Antibacterial (Hatami et al., 2016)

17.17	**cis-9-Octadecenoic acid**	**13.203 18.101 19.236**	**Fatty acid**		- **Antimicrobial, anti-inflammatory (Dhayanithi et al., 2012; Machado et al., 2017)** - **Analgesic, antidiarrhoeal, and cytotoxic (Hossain et al., 2017)** - **Antioxidant (Cho et al., 2010)**
7.08	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	15.338 15.492	Fatty acid ester		- Anti-arthritic, anti-acne, antihistaminic, anti-androgenic, 5-reductase inhibitor, nematicide, anti-coronary, anti-eczemic, anti-cholesterolemic, and insectifuge agent (Sudha et al., 2013)
2.70	2-octyl-cyclopropane-octanal	19.571	Cyclo-propane fatty acid		- Anti-obesity (Momoh et al., 2022) - Antimicrobial and antioxidant (Joshi et al., 2020; Yahaya et al., 2020)
1.08	2-Nonylcyclopro-paneundecanal	24.353	Cyclo-alkanes		- Antidiabetic (Aloke et al., 2021)

Hossain, 2023). Therefore, it is necessary to evaluate the safety of this artificial coffee by histopathologic examination.

The artificial coffee of mangrove fruits *R. mucronata* was evaluated for its antioxidant capacity using the DPPH method. According to Blois, the antioxidant capacity of an extract is classified into four categories: very strong (IC_{50}d”50 µg/mL), strong (50<IC_{50}d”100 µg/mL), moderate (100<IC_{50}d”150 µg/mL), weak (150<IC_{50}d” 200 µg/mL), and very weak (IC_{50}d”200 µg/mL) (Dewanto et al., 2021). The evaluation showed that the antioxidant capacity of artificial coffee of mangrove fruit *R. mucronata* was moderate, while vitamin E was very strong. The percentage of antioxidant capacity and IC_{50} of mangrove fruit artificial coffee *R. mucronata* and vitamin E can be seen in Figure 2.

Furthermore, to observe the effect of mangrove fruit artificial coffee *R. mucronata* on the immune system response due to LPS induction, artificial coffee was given to mice for 14 days, which were then induced with LPS *E. coli*. The parameters evaluated were helper T lymphocyte cells expressed by $CD4^+CD62L^+$ (Figure 3) and suppressor T lymphocyte cells expressed by $CD8^+CD62L^+$ (Figure 4).

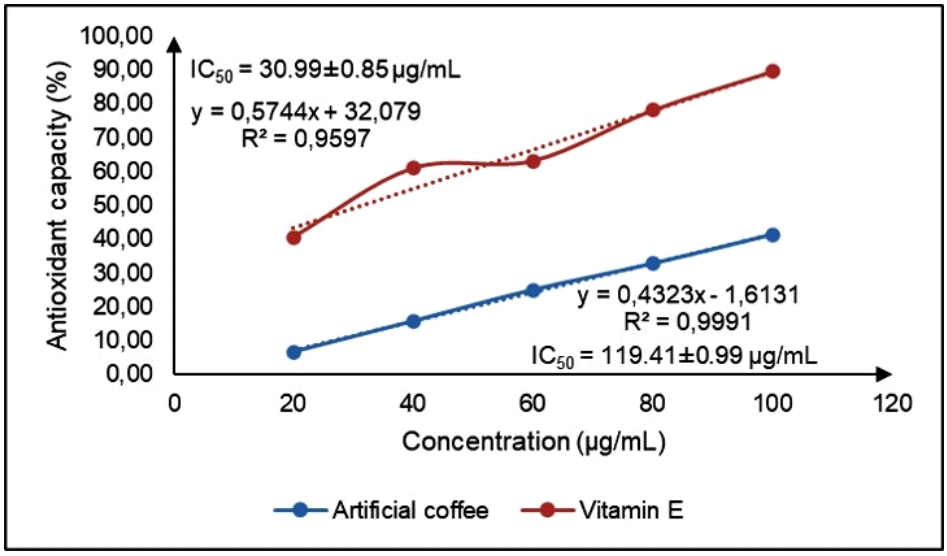


Figure 2. Antioxidant capacity and IC_{50} artificial coffee of *R. mucronata* fruit and vitamin E

The expression of unactivated T lymphocyte cells can be recognized through the $CD4^+CD8^+CD62L^+$ profile. When the profile of naïve T lymphocyte cells decreases due to infection from microbes or foreign bodies as antigens, T lymphocyte cells have been activated or become effector cells. Figure 3 shows that there is a decrease in the relative number of $CD4^+CD62L^+$ T lymphocyte cells in mice induced by LPS *E. coli* by 27.42% ($P<0.05$), while in mice induced by LPS and given mangrove fruit artificial coffee increased the relative number of $CD4^+CD62L^+$ T lymphocyte cells by 14.68 to 29.48% (depending on the concentration of mangrove fruit artificial coffee). In comparison, the group of mice given vitamin E experienced an increase of 25.82%. Duncan's test showed the relative number of $CD4^+CD62L^+$ T lymphocyte cells in the group of mice given vitamin E and artificial coffee 1000 and 1500 mg/kg, almost the same as the normal mice group. Duncan's test also showed the relative number of $CD4^+CD62L^+$ T lymphocyte cells in the group of mice given 500 and 1000 mg/kg artificial coffee, almost the same as those given vitamin E.

Figure 3. Level of helper T lymphocyte cells ($CD4^+CD62L^+$) in LPS-induced mice due to *R. mucronata* fruit artificial coffee. All data are shown as mean±SD (n=5). Based on Duncan's test, the means with different notations (a-c) are significantly different, and vice versa at $p<0.05$.

Figure 4. illustrates a decrease in the relative number of $CD8^+CD62L^+$ T lymphocyte cells in mice induced by LPS *E. coli* by 30.46% ($P<0.05$). In comparison, mice induced by LPS and given mangrove fruit artificial coffee increased the relative number of $CD8^+CD62L^+$ T lymphocyte cells by 12.60 to 30.33% (depending on the concentration of mangrove fruit artificial coffee). In comparison, the group of mice given vitamin E as a positive control experienced an increase of 21.63%. Duncan's test showed the relative number of $CD8^+CD62L^+$ T lymphocyte cells in the group of mice given vitamin E and artificial coffee 1000 and 1500 mg/kg, almost the same as the normal mice group. Duncan's test also showed the relative number of $CD8^+CD62L^+$ T lymphocyte cells in the group of mice given 500 and 1000 mg/kg artificial coffee, almost the same as those given vitamin E.

The analysis shown in Figures 3 and 4 indicates that the antioxidant compound components in mangrove fruit artificial coffee can increase the relative number of $CD4^+CD8^+CD62L^+$ T lymphocyte cells in mice induced by LPS *E. coli* (Kesarwani et al., 2013). These results support the screening of compounds in mangrove fruit artificial coffee, which showed antioxidant, anti-inflammatory, and antimicrobial properties. CD62L, or L-selectin, is an adhesion molecule that can attach and roll on blood vessel endothelial cells. T lymphocyte cells circulate in the blood and lymph nodes. T lymphocyte cells will be activated if they encounter an antigen and lose the CD62L molecule. As a result, L-selectin moves towards the antigen to prevent inflammation (Ley, 2003). This result is shown by the decreased amount of CD62L in LPS-induced individuals. The CD62L molecule is up-regulated on T lymphocyte cells that will push into the lymph node in endothelial cells. This process encourages the activation of CD8 T lymphocyte cells as suppressor T cells to fight the incoming antigen (Tedder et al., 1995).

Previous studies reported that LPS is a natural adjuvant synthesized by gram-negative bacteria, affects

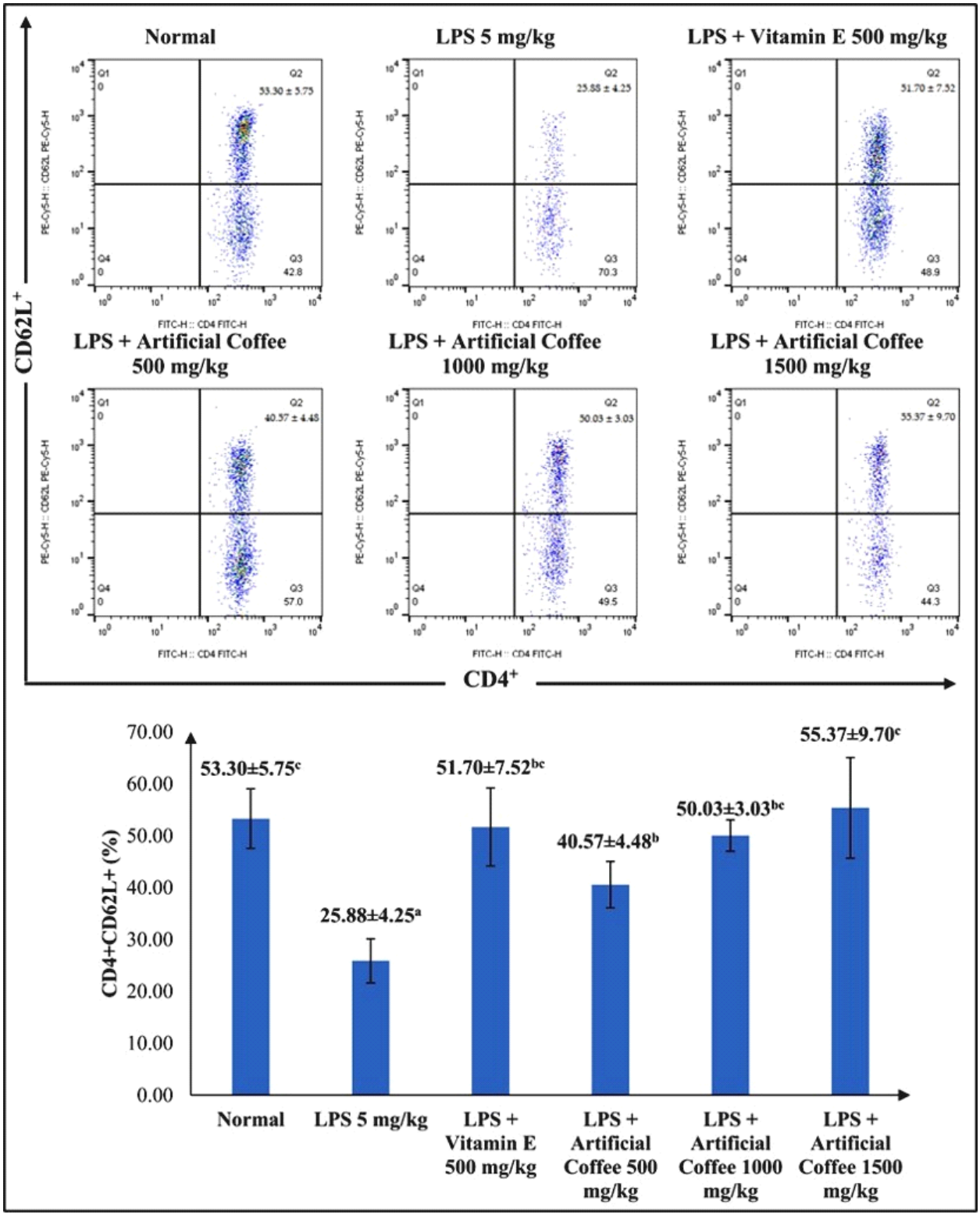


Figure 3. Level of helper T lymphocyte cells ($CD4^+CD62L^+$) in LPS-induced mice due to *R. mucronata* fruit artificial coffee. All data are shown as mean±SD (n=5). Based on Duncan's test, the means with different notations (a-c) are significantly different, and vice versa at $p<0.05$.

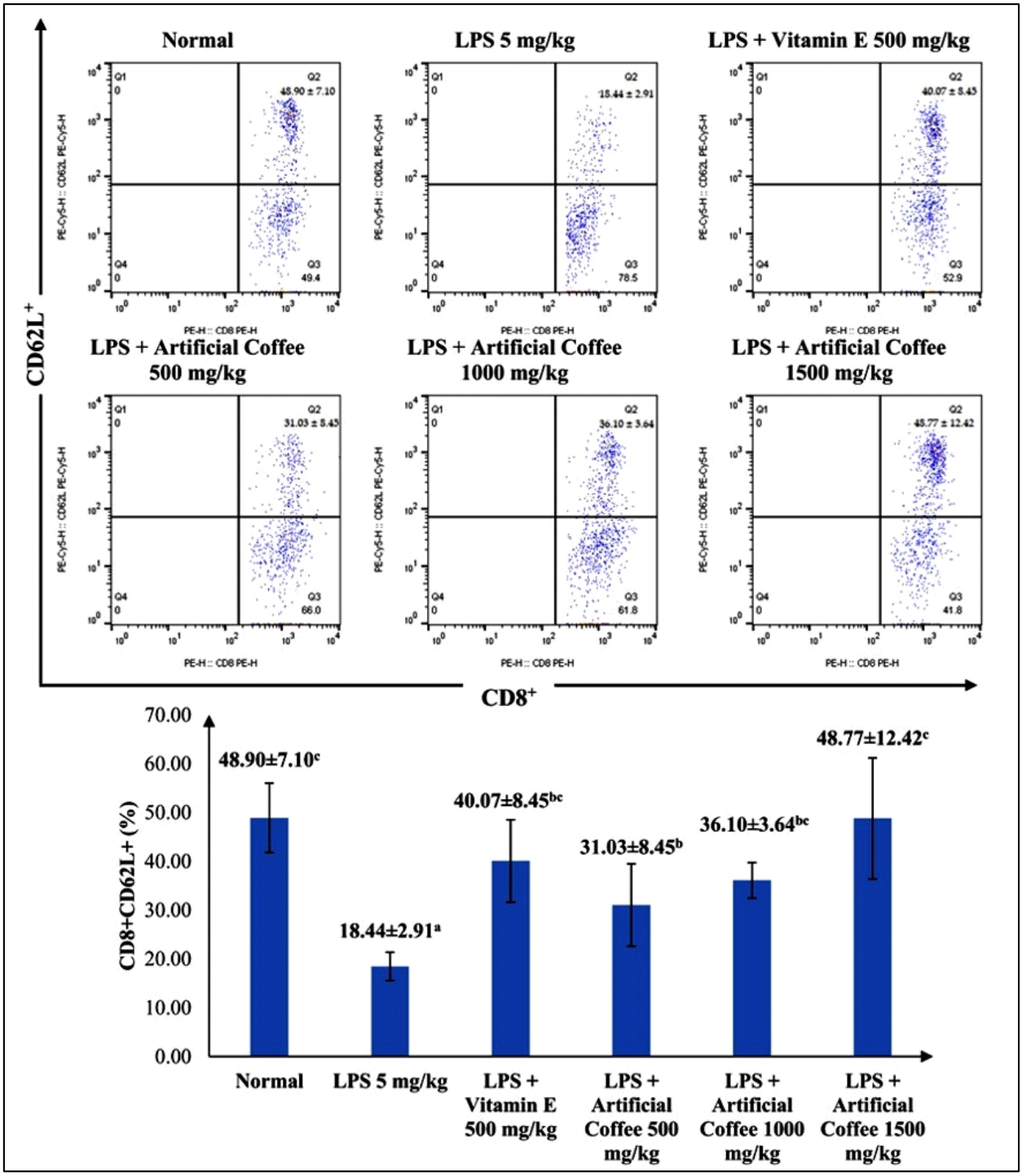


Figure 4. Level of suppressor T lymphocyte cells ($CD8^+CD62L^+$) in LPS-induced mice due to *R.mucronata* fruit artificial coffee. All data are shown as mean±SD (n=5). Means with different notations (a-c) are significantly different, and vice versa at $p<0.05$ based on Duncan's test.

CD4 T lymphocyte cell responses (McAleer & Vella, 2008), and promotes CD8 T lymphocyte cell activation (Tripathy et al., 2017). CD4 T lymphocyte cells act as coordinators of the immune response by stimulating other immune cells, such as macrophages, B cells, and CD8 T lymphocyte cells, to fight infection (Swain et al., 2012). Antioxidant plant substances can stimulate T lymphocyte cell proliferation with an immunomodulatory mechanism (Lin et al., 2013). Antioxidant substances derived from flavonoids and phenolics are also reported to stimulate T lymphocyte cell proliferation (Safriani et al., 2021). Antioxidant

Table 3. Histopathologic evaluation of mice renal due to LPS induction

Treatments	Cells (%)			
	Healthy	Pyknosis	Karyorrhexis	Karyolysis
Normal	76.86±2.91^{c}	1.92±0.93^{a}	15.26±3.48^{a}	5.96±0.73^{a}
LPS 5 mg/kg	47.08±1.14^{a}	9.65±0.95^{b}	29.46±0.47^{c}	13.81±0.26^{c}
LPS + Vitamin E 500 mg/kg	64.52±2.35^{b}	1.75±1.09^{a}	22.07±3.03^{b}	11.66±1.71^{b}
LPS + Artificial coffee 500 mg/kg	67.51±3.71^{b}	2.64±1.52^{a}	19.87±1.54^{b}	9.97±0.78^{b}
LPS + Artificial coffee 1000 mg/kg	65.82±2.66^{b}	2.72±0.46^{a}	21.10±2.10^{b}	10.36±0.59^{b}
LPS + Artificial coffee 1500 mg/kg	64.83±0.67^{b}	2.66±0.62^{a}	21.81±0.81^{b}	10.70±0.83^{b}

All data are shown as mean±SD (n=5). Means with different notations (a-c) on the table are significantly different, and vice versa at $p<0.05$ based on Duncan's test.

substances in artificial mangrove fruit coffee are thought to affect T lymphocyte cell proliferation and immune response positively.

In this study, cellular changes in renal tissue were also observed to evaluate the safety of artificial coffee from mangrove fruit. The histopathologic examination provided preliminary information on structural changes in the renal caused by herbal administration. The histopathological examination was performed to understand the effects of herbs on renal health and identify potential toxic or beneficial effects (Alomar, 2020; El-Bassossy et al., 2023). The parameters were observed in the histopathological examination, namely healthy cells and cells undergoing stages of necrosis, namely pyknosis, karyorrhexis, and karyolysis (Berber et al., 2021).

The stage of necrosis in cells, pyknosis, is a morphological change that occurs in the cell nucleus and is characterized by condensation of the nucleus, with smaller and darker cells (Hou et al., 2016). Pyknosis can occur in the renal is characterized by parenchymal cell loss. Specifically, 30 to 50% of renal tubules may show degeneration due to pyknosis (Priante et al., 2019). Previous studies reported bacterial infections could cause pyknosis in the renal, characterized by swelling and rupture of cells, the release of cell contents, and a strong inflammatory response (Xia et al., 2019). After pyknosis, the next stage of necrosis is karyorrhexis. Karyorrhexis is a destructive fragmentation of the nucleus of a dying cell; the chromatin in the nucleus breaks into fragments that are irregularly distributed throughout the cytoplasm (Takada et al., 2020). The next stage of necrosis, karyolysis, is characterized by the nucleus losing its structure and eventually being uniformly stained with eosin. Karyolysis is the complete dissolution of the dying cell's chromatin due to enzymatic degradation by endonuclease (Berber et al., 2021). These sequential changes in the nucleus cause damage to the nucleus during the necrosis process. Histopathologic observations of the renal can be seen in Table 3 and Figure 5.

Table 3 and Figure 5 show that LPS can reduce the number of healthy cells (29.78%) and can increase the number of cells that experience pyknosis (7.73%), karyorrhexis (14.20%), and karyolysis (7.85%) in the renal's of normal mice. Mangrove fruit artificial coffee can increase the number of healthy cells (17.75-20.43%), and reduce the number of cells that experience pyknosis (6.93-7.01%), karyorrhexis (7.65-9.59%) and karyolysis (3.10-3.83%) in the renal's of LPS-induced mice ($P<0.05$). Duncan's analysis showed that vitamin E and mangrove fruit artificial coffee had the same effect. Although mangrove fruit artificial coffee has not been able to reduce the number of cells that experience necrosis equal to the normal group of mice, this decrease indicates that the antioxidant substance in mangrove fruit artificial coffee works to protect mice renal cells due to LPS induction.

When related to the compound class of mangrove fruit artificial coffee detected by GC-MS analysis, previous studies have reported antioxidant compounds of the fatty aldehyde class can provide protective effects against oxidative stress-related damage in the renal (Zhang et al., 2021). The phenolic aldehyde-like class of compounds from bee pollen extract was reported to exert protective effects against histological changes in the renal, including crystal deposition, collecting tubule dilatation, tubular epithelial necrosis, inflammation, edema, and interstitium congestion (Elghouizi et al., 2022). The fatty acid class of compounds was also reported to attenuate toxicity in the renal by modulating oxidative stress and cell apoptosis(Elsafty et al., 2023). The cycloalkanes class positively affected renal histopathology by reducing oxidative stress and lipid peroxidation in rats (Jalili et al., 2019). The artificial coffee of *R. mucronata* mangrove fruit has the potential to be developed as a nutraceutical beverage because it provides therapeutic effects. Antioxidant substances in mangrove fruit

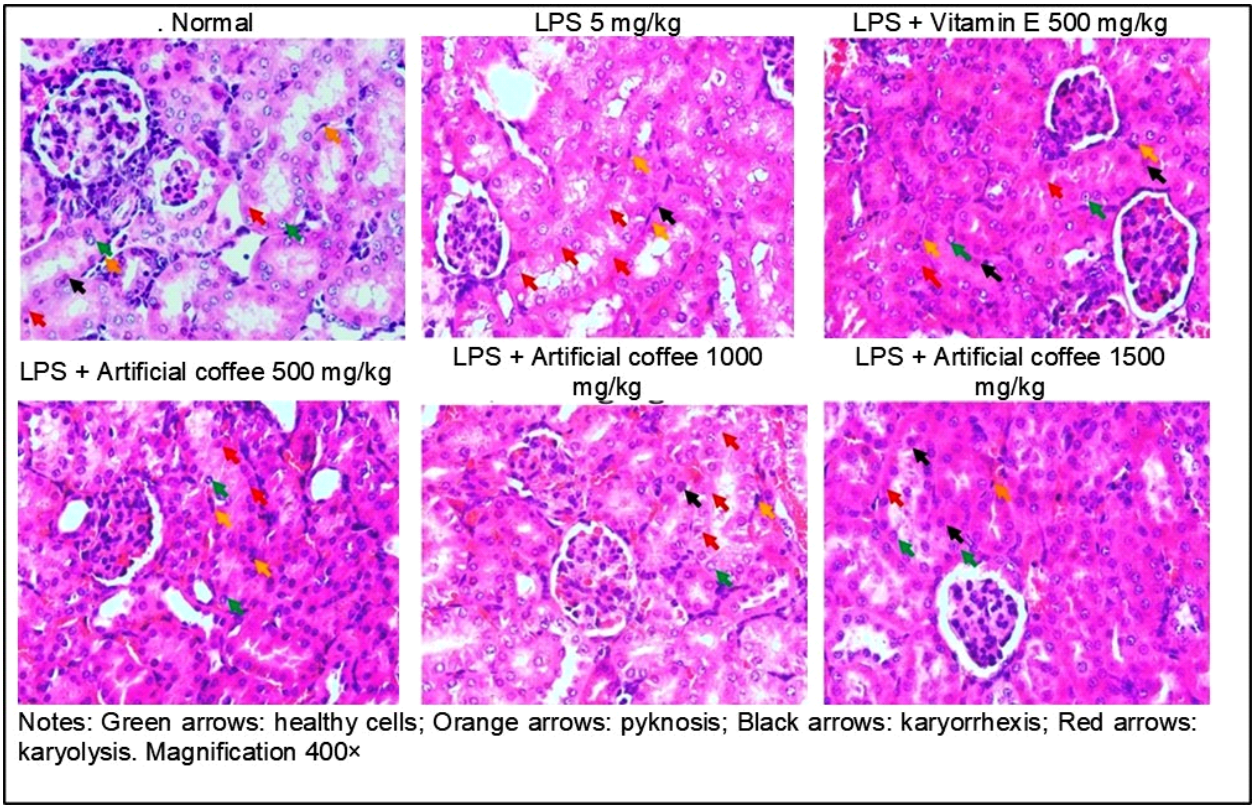


Figure 5. Renal histopathological after 6 h LPS injection

artificial coffee are thought to play a role in stimulating the immune response by encouraging the proliferation of T lymphocyte cells. In addition, the compounds in mangrove fruit artificial coffee can also protect mice renal cells from necrosis due to LPS induction.

Conclusion

These research findings demonstrate that artificial coffee derived from *R. mucronata* mangrove fruits from the Sangihe Islands has the potential as a nutraceutical drink. Mangrove fruit artificial coffee concentrations of 500 and 1000 mg/kg exhibit comparable efficacy to vitamin E in stimulating T lymphocyte cells and positively affecting renal cells. Antioxidant substances in mangrove fruit artificial coffee contribute to stimulating T lymphocyte cells and protecting renal cells in LPS-induced mice from necrosis. However, further assessment of the subacute toxicity response of mangrove fruit artificial coffee still needs to be evaluated.

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Supplementary Materials

Supplementary materials is not available for this article

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