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Effects of plant extract powder supplementation on lipopolysaccharide-induced sepsis and hepatic and kidney antioxidant capacity in Wistar rats

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ABSTRACT

Controlling inflammation caused by bacterial invasion or toxic compounds is crucial to preventing sepsis. Supplements containing phenolics, flavonoids, vitamins, chlorophyll, and peptides can serve as sources of antioxidants to help control inflammation. Bforlief supplement diet (BSD) is a powdered drink supplement made from 29.3% peach fruit concentrate, strawberry powder, mulberry extract powder, guava powder, broccoli powder, acai berry fruit extract, soy protein powder, moringa leaf extract, inulin, ascorbic acid, stevia sweetener, algae, and a vitamin and mineral premix. In this study, BSD was administered to male Wistar rats for 14 days, with vitamin E as a comparison. 20 rats were divided into 4 experimental groups consisting of G1: normal rats, G2: negative control, G3: group of rats with vitamin E intake of 80 mg/200 g body weight, and G4: group with BSD intake at a dose of 270 mg/200 g body weight. The G2-G4 rat groups were treated with LPS (10 mg/kg body weight) for 3 days after a 7-day adaptation period. The effects of supplement intake were evaluated using parameters such as malondialdehyde (MDA) levels in blood serum, kidney and liver homogenates, production of interleukin-6 (IL6), interleukin-10 (IL10), and tumor necrosis factor- α (TNF- α), and antioxidant capacity including Superoxide Dismutase Activity (SOD), serum hormone Glutathione (GSH), and Catalase Activity (CAT). The research results showed that BSD exhibits DPPH radical-scavenging activity and an IC50 comparable to that of commercial vitamin E. The administration of BSD supplements for 14 days in male Wistar rats induced by LPS was able to normalize weight gain, reduce MDA levels, decrease IL-6 and TNF- α production, increase serum IL-10 levels, and enhance the antioxidant capacity of SOD and CAT in liver and kidney tissues. BSD supplements are potential sources of antioxidants that can mitigate sepsis induced by LPS.

Keywords: sepsis, LSP, supplements, antioxidants, liver, kidney

INTRODUCTION

The BSD supplement is a blend of fruit extracts claimed to help prevent inflammation. BSD is in the form of green granule powder with pink spots, with a slightly sour sweet taste and a peachy fruit aroma. Various consumer testimonials state that the BSD product aids in treating diseases. According to the product label, the composition of the product consists of 29.3% peach fruit concentrate, strawberry powder, mulberry extract powder, guava powder, broccoli powder, acai berry fruit extract, soy protein powder, moringa leaf extract, inulin, ascorbic acid, stevia sweetener, algae, and a vitamin and mineral premix. Based on the composition, the product is suspected to contain phenolic and flavonoid compounds, ascorbic acid, soy protein, inulin, chlorophyll, and dietary fiber.

Various studies have proven the anti-inflammatory, antioxidant, and immunomodulatory activities of these compounds.

Inflammation, the body's initial immune response, is very important for defense against microbial infections, injuries, and stress. Thus, inflammation plays a crucial role in protecting the host from bacterial and viral infections. However, an excessive or inappropriate inflammatory response often contributes to the onset of diseases, including sepsis, cancer, allergies, and asthma. Sepsis can be caused by wounds, bacteria, or bacterial toxins such as lipopolysaccharides (LPS), which are considered the result of the host's excessive inflammatory response. Prevention and treatment of sepsis require early control of the inflammatory response to prevent further reactions. Macrophages are an important component of the pathophysiology of excessive inflammatory responses. LPS is a glycolipid found in the outer membrane of gram-negative bacteria and activates macrophages by binding to the toll-like receptor 4 (TLR-4). Activated macrophages produce inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and interleukin-1 β (IL-1 β), as well as inflammatory mediators such as nitric oxide (NO). Macrophages play a crucial role in the development of life-threatening sepsis, characterized by multiorgan dysfunction, by producing inflammatory cytokines [1], [2], and [3].

The largest component of BSD consists of fruit and vegetable extracts believed to contain high levels of phenolic and flavonoid compounds. Phenolic and flavonoid compounds have strong anti-inflammatory activity. Various studies show the effects of phenolic compounds on the pro-inflammatory response of macrophages induced by lipopolysaccharides (LPS) and endotoxins. The intake of phenolic compounds can significantly reduce weight loss in mice and improve pathological damage to the liver, lungs, and heart caused by LPS-induced sepsis. Phenolic and flavonoid components are capable of suppressing the inflammatory response by inhibiting the production of the inflammatory cytokine interleukin-6 (IL-6), both in vivo and in vitro [4], [5], and [6].

Another component is algae, which is a source of chlorophyll. Chlorophyll a and pheophytin a from fresh leaves show strong anti-inflammatory activity against foot edema induced by carrageenan in rats and formalin in mice. Chlorophyll a is capable of inhibiting the expression of the TNF- α gene (a pro-inflammatory cytokine) induced by bacterial lipopolysaccharides in HEK293 cells, but does not affect the expression of the inducible nitric oxide synthase and cyclooxygenase-2 genes. Meanwhile, chlorophyll b only slightly inhibits inflammation and TNF- α gene expression. However, both chlorophyll a and chlorophyll b showed the same level of inhibition against NF- κ B activation induced by 12-O-tetradecanoylphorbol-13-acetate. In addition, chlorophyll and pheophytin also have in vitro antioxidant activity. This study indicates that chlorophyll a and its degradation products are highly effective anti-inflammatory agents and are readily available in abundant quantities, suggesting their potential for development as phytomedicines or conventional drugs for the treatment of inflammation and related diseases [7], and [8].

Another component of BSD that has anti-inflammatory effects, due to LPS, is ascorbic acid (vitamin C). At high doses, ascorbic acid can cure sepsis by activating the Nrf2/HO-1 signaling pathway [9]. Furthermore, [10] stated that early treatment with vitamin C significantly reduced MDA levels, restored SOD activity, decreased inflammatory biomarkers, and reduced cardiomyocyte damage in rats. Ascorbic acid is capable of reducing the production of cytokines IL-1 β , IL-6, IL-8, and TNF in LPS-induced rats. Ascorbic acid is capable of reducing the production of pro-inflammatory cytokines and decreasing the upregulation of CD16 and CD163, but not CD40 and PDL-1 in LPS-polarized monocytes [9].

Other research has shown that high-fiber supplementation increases survival rates and reduces bacterial growth in sepsis rats using the cecal ligation and puncture (CLP) model. This is because dietary fiber supplementation reduces serum concentrations of pro-inflammatory cytokines such as TNF- α , IL-6, and high mobility group protein 1 (HMG-1), and increases IL-10 levels compared to CLP mice. These findings suggest that high-fiber supplementation could be a potential therapy for sepsis [11]. Author Wang et al. [12] also showed that early intake of medium fiber increases 28-day survival and reduces hospital mortality in sepsis patients. Dietary fiber intake can reduce inflammation associated with obesity and metabolic diseases. Cross-sectional analysis shows that dietary fiber intake and fecal short-chain fatty acid content are inversely associated with lipopolysaccharide-binding protein, a marker of systemic inflammation, whereas adiposity markers are positively associated with plasma IL6 and C-reactive protein [13].

Another component in the BSD product is soybean protein powder. Some authors Yi et al. [14] investigated the potential application of peptides from soy protein as an anti-inflammatory functional food. The research results show that soybean protein can inhibit the increase in toll-like receptor 4 activity by limiting the production of lymphocyte antigen 96 (LY96), while also inhibiting the mitogen-activated protein kinase-c-Jun N-terminal kinase pathway in cells, as well as the NF- κ B activation induced by LPS due to the degradation of nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (I κ B α). As a result, the release of pro-inflammatory cytokines (TNF- α , IL-6, and IL-1 β) is inhibited, thereby preventing LPS-induced inflammation in RAW 264.7 cells.

Based on various studies, this research aims to evaluate the supplementation of the BSD product, which is a mixture of 29.3% peach fruit concentrate, strawberry powder, mulberry extract powder, guava powder, broccoli powder, acai berry fruit extract, soy protein powder, moringa leaf extract, inulin, ascorbic acid, stevia sweetener, algae, and vitamin and mineral premix as antioxidants, anti-inflammatories, and to improve sepsis conditions caused by LPS induction in male Wistar rats.

Scientific Hypothesis

The consumption of BFORLIFE plant extract powder supplementation may improve Lipopolysaccharide-induced sepsis and the hepatic and kidney antioxidant capacity in Wistar rats.

Objectives

This research aims to evaluate the supplementation of the BSD product, which is a mixture of 29.3% peach fruit concentrate, strawberry powder, mulberry extract powder, guava powder, broccoli powder, acai berry fruit extract, soy protein powder, moringa leaf extract, inulin, ascorbic acid, stevia sweetener, algae, and vitamin and mineral premix as antioxidants, anti-inflammatories, and to improve sepsis conditions caused by LPS induction in male Wistar rats.

MATERIAL AND METHODS

The primary material of this research is the BForliefte (BSD) powdered drink supplement product obtained from PT. Bliefe Jaya Abadi Indonesia. Chemicals for the analysis of antioxidant and immunomodulatory activity, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), lipopolysaccharide (LPS), Ethylenediaminetetraacetic acid (EDTA), and vitamin E. Animals for in vivo trials and standard AIN 1993 feed were obtained from the UGM Food and Nutrition Laboratory.

Methods

Chemical analysis and antioxidant activity of BSD: BSD was analyzed for proximate composition, total energy, reducing sugar content, and total sugar content (AOAC, 1995), phenolic content [12] and total flavonoids [15] dietary fiber content [16], total chlorophyll content [17], as well as ascorbic acid content using the titration method [18]. The antioxidant activity of BSD was analyzed using the DPPH radical scavenging method and the IC50 reducing power method.

DPPH Radical scavenging activity: The free radical scavenging activity (RSA) of DPPH was determined using the method of [19]. This method was chosen because the volume of the tested sample is smaller than that of the DPPH solution, thereby minimizing the influence of the chlorophyll sample color on the measurement. A 0.2 ml sample of the methanol extract of *Sambiloto simplicia* powder was added to 3.8 ml of a 0.1 mM DPPH solution in methanol, stirred by vortexing for 1 minute; the resulting filtrate was incubated in the dark at room temperature for 30 minutes. Control was made using methanol as a substitute for the sample and BHT as a comparison at a concentration of 100 ppm. After incubation, the filtrate was measured for absorbance at 515 nm using a UV-Vis spectrophotometer (Shimadzu). The obtained data are A₀: absorbance of DPPH in the absence of the sample, A_S: absorbance of the model with added DPPH, and A_b: absorbance of the extract sample without DPPH. Radical Scavenging Activity is expressed as a percentage (%). The RSA value indicates the sample's ability to decolorize violet DPPH, calculated using Equation 1.

$$\text{RSA (\%)} = [A_0 - (A_S - A_b) / A_0] \times 100 \quad (1)$$

Power Reduction: The reducing power of the powdered drink supplement is determined by its ability to reduce FeCl₃ solution, as explained by [20]. Aliquot 2.5 mL of BSD supplement at various concentrations (0, 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml), mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide, incubated at 50°C for 20 minutes, then add 2.5 mL of 10% trichloroacetic acid. The mixture was centrifuged at 650 g for 10 minutes. Then, 5 mL of supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm. The obtained data were analyzed using regression in Excel. Based on the obtained linear equation, the IC50 values for BSD and commercial vitamin E were calculated for comparison.

Preparation of Test Animals: The test animals were 20 male Wistar rats, aged 2 months and weighing 179.70±3.30 g. Housed individually in a battery system at room temperature and natural lighting with a 12-hour light/dark cycle. All the rats were given water and food in the form of pellets ad libitum. The Research Ethics Committee has recommended this research under reference no. KE/AA/III/10111548/EC/2024. Male Wistar rats were divided into 4 groups: group 1 (G1) was the normal control group with standard feed (AIN 1993), group 2 (G2) was the negative control group with standard feed without supplementation, group 3 (G3) was the positive control group with standard feed and vitamin E supplementation, and group 4 was the group with standard feed and BSD supplementation. All groups of rats underwent a 7-day adaptation period on standard feed, after which the G2, G3, and G4 groups received LPS 10 mg/kg bw for 3 days. The rats' blood was collected the day after LPS

treatment (day 0) for analysis. Then, for 14 days, the rats were treated according to the experiment. BSD intake was given to group G4 at a dose of 15 g/day for adults or 270 mg/200 g body weight of the rats, while vitamin E was administered to group G3 at 80 mg/200 g body weight. On the 14th day, blood samples were taken for analysis. At the end of the study, the rats were euthanized according to clinical animal trial procedures, and liver and kidney tissues were collected for histopathological examination. In addition, on days 0, 7, and 14, the rats' weight gain was observed.

Histological Preparation and Immunohistochemical (IHC) Analysis: The kidney and liver tissues obtained and isolated were cleaned, fixed in 10% formalin, dehydrated, infiltrated, and embedded in paraffin. After that, the kidney and liver tissues were sliced to 7 μ m using a microtome; the slices were placed on glass slides and stained with Hematoxylin–Eosin. Histopathological examination of liver and kidney tissue preparations from 4 groups of rats was observed using a microscope with 400x magnification. Changes observed included the degree of inflammation, the number of inflammatory cells, the condition of hemorrhage, fatty degeneration, and necrosis.

Analysis of blood serum includes IL-6, IL-10, and TNF α (ELISA kits), as well as malondialdehyde (MDA) levels using the thiobarbituric acid reactive substance (TBARS) assay kit (Cayman, USA). Evaluation of antioxidant capacity in kidney and liver homogenates includes the antioxidant capacity activities of Superoxide Dismutase Activity (SOD), serum hormone Glutathione (GSH), and Catalase Activity (CAT) analyzed using the method described by [21].

Experimental Design: The experimental design in this study is a simple completely randomized design with sample type treatments tested on 4 groups of rats, namely group 1 (G1) which is the normal control group with standard feed (AIN 1993), group 2 (G2) which is the negative control group with standard feed without supplementation, group 3 (G3) which is the positive control group with standard feed and vitamin E supplementation, and group 4 which is the group with standard feed with BSD supplementation. Data were analyzed using ANOVA and followed by DMRT if there was a significant effect, using SPSS Statistics 24 software version 31 by IBM SPSS.

RESULTS AND DISCUSSION

Chemical composition of BSD supplements

The results of the chemical composition analysis of BSD are presented in Table 1.

Table 1 Chemical composition of BSD.

No	Component	Total
1.	Total energy	3.95 KKal
2.	Water	2.44%
3.	Fat	nd
4.	Protein	3.41%
5.	Ash	0.22%
6.	Total carbohydrate (by difference)	93.92%
7.	Total sugar	20.80%
8.	Reduction sugar	2.21%
9.	Dietary fiber	0.057%
10.	Crude fiber	0.44%
11.	Total phenolic	414 mg/100 g
12.	Total flavonoid=	138 mg/100 g
13.	Total chlorophyll	729 mg/100 g
14.	Ascorbic acid	617.63 mg/100 g

Note: nd – not detected.

It is known that the BSD supplement does not contain fat and has a total energy of 3.95 KCal, a protein content of 3.41%, ash of 0.22%, and carbohydrates of 93.93%, including total sugars of 20.80%, reducing sugars of 2.21%, and dietary fiber of 0.057% (w/w). In addition to these components, the supplement also contains 414 mg/100 g of total phenolic compounds, 138 mg/100 g of total flavonoids, 729 mg/100 g of total chlorophyll, and ascorbic acid at 617.63 mg/100 g. According to the product label, the other type of carbohydrate besides sugar and dietary fiber is inulin. It is suspected that these components will contribute to the BSD supplement's antioxidant and anti-inflammatory activities.

In vitro antioxidant activity

The results of the antioxidant activity test of BSD are presented in Table 2. The DPPH radical scavenging activity of the BSD supplement is 91.22%, while that of vitamin E is 89.38%. Statistical results show that the DPPH radical scavenging activity of the BSD supplement is higher than that of commercial vitamin E (P<0.05). This is suspected because BSD contains various components that can act as antioxidants, such as phenolics and flavonoids, chlorophyll, peptides, and ascorbic acid. Previous research results show that phenolic and flavonoid compounds have a high ability to capture DPPH radicals [22], [23], [24], [25] and chlorophyll also has the ability to capture DPPH radicals [17]. Other researchers have also shown that peptides can capture DPPH radicals [26], [27], and [28].

Unlike the DPPH radical scavenging activity, the reducing power of the BSD supplement is lower than that of commercial vitamin E. This is evidenced by the BSD supplement's IC50 being higher than that of vitamin E (P<0.05), as also shown in Figure 1, where the BSD curve's slope is lower than that of the vitamin E curve. IC50 indicates the amount of a pharmacological agent or specific compound required to inhibit biological activity by half. In this case, the IC50 is the amount of substance required to reduce the reducing ability or absorbance to half [29]. The IC50 value of the BSD supplement (17.32 mg/ml) is higher than that of vitamin E (10.94 mg/ml), indicating that vitamin E is more effective in reducing FeCl3. This is suspected to be because vitamin E has a higher purity than the BSD supplement, which is a combination of various natural extracts [30].

Table 2 Antioxidant activity of BSD supplements.

Sampel	RSA (%)	IC50 (mg/ml)
Vitamin E komersial	89.38±0.04	10.94±0.18
Produk BSD	91.22±0.79	17.32±0.21

Note: n = 3.

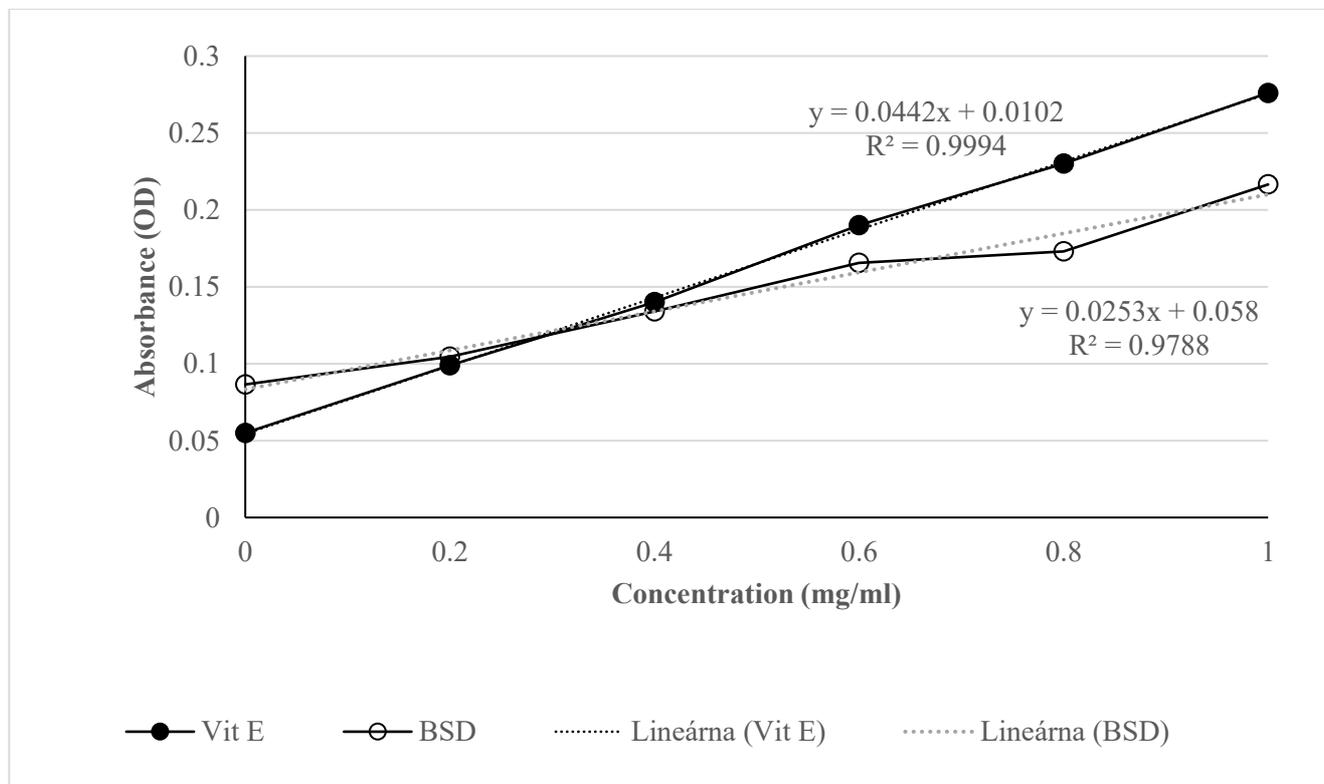


Figure 1 Reducing power of commercial vitamin E and BSD supplements.

The weight of the rats during the trial period

To determine the effect of BSD supplement intake, an experimental study was conducted using 20 male Wistar rats aged 2 months, divided into 4 groups: group 1 (G1) is the normal control group with standard feed (AIN 1993), group 2 (G2) is the negative control group with standard feed without supplementation, group 3 (G3) is the positive control group with standard feed and vitamin E supplementation, and group 4 is the group with standard feed and BSD supplementation. BSD supplements and vitamin E were administered orally every day for 14 days. The treatment was administered after the rats underwent a 7-day adaptation period and LPS treatment at 10 mg/kg body weight for 3 days in the G2-G4 groups. Body weight was observed on days 0, 7, and 14 (Figure 2). Based on the data in Figure 3, it is evident that LPS induction will affect the mice's weight development during the experimental period. On day 0, the rats' weights across the treatment groups G1, G2, G3, and G4 did not differ significantly, but on days 7 and 14, the weights did differ significantly. In the G2 treatment group, body weight was lowest, whereas in the G3 and G4 groups it was higher. The weight gain of the G4 group rats approached that of the normal group rats (G1). This indicates that BSD supplementation can normalize rat growth, as evidenced by weight development resembling that of normal rats. It is suspected that components in BSD, such as phenolic compounds, can alleviate inflammatory conditions that disrupt body development, as shown in the study by [31].

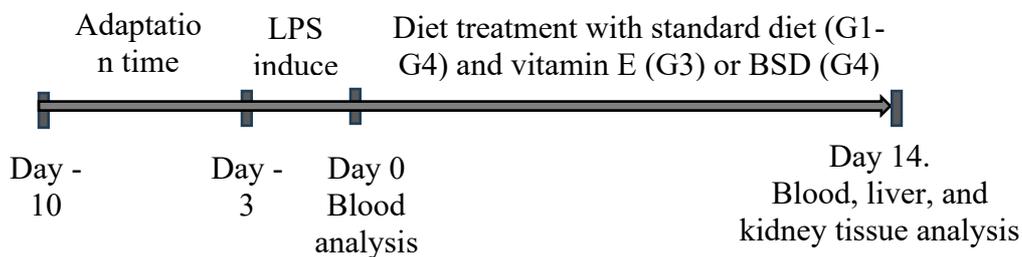


Figure 2 Treatment of experimental animals.

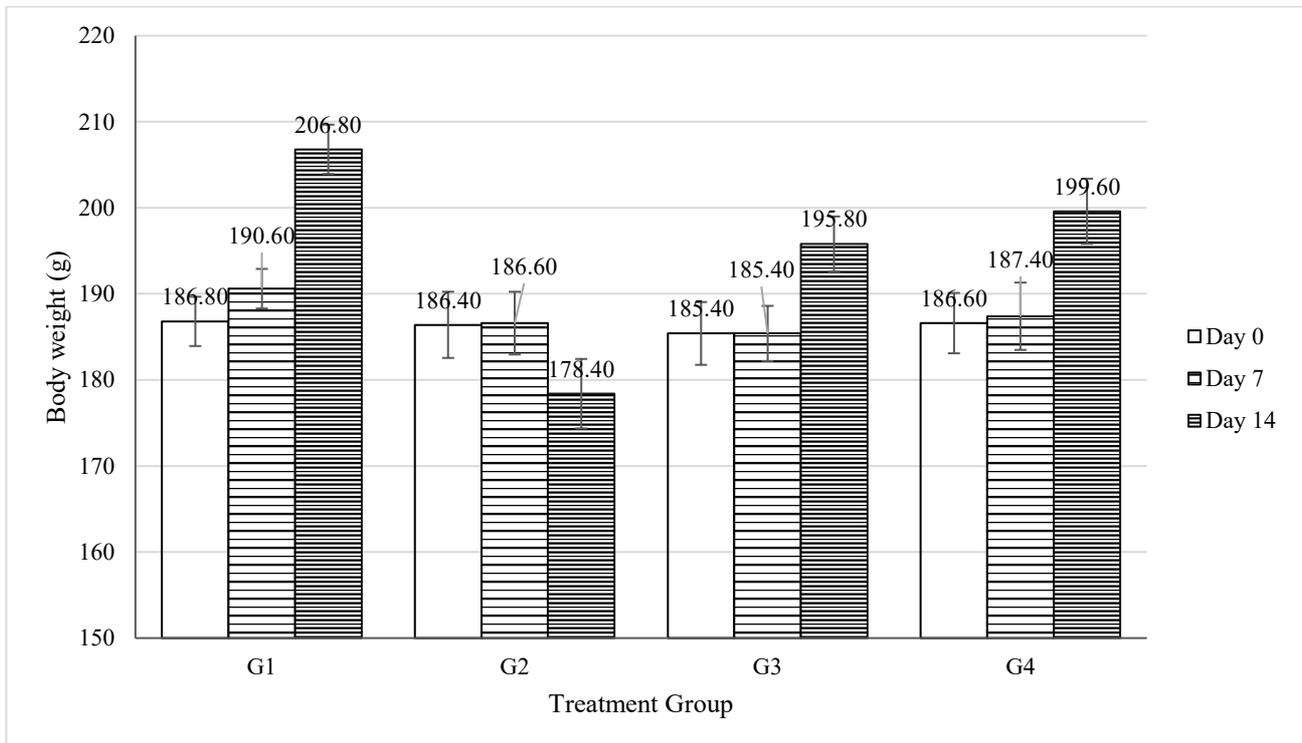


Figure 3 Body weight of rats. Note: days 0, 7, and 14 in group 1 (G1) is the normal control group with standard feed (AIN 1993), group 2 (G2) is the negative control group with standard feed without supplementation, group 3 (G3) is the positive control group with standard feed and vitamin E supplementation, and group 4 is the group with standard feed and BSD supplementation.

In Vivo Antioxidant Activity

MDA serum blood level: Figure 4.A shows that treatment with LPS at 10 mg/kg body weight for 3 days increases serum MDA levels in rats on day 0 in groups G2, G3, and G4. This indicates that LPS induction causes oxidative stress in the rat's body. The serum MDA levels in the rats indicate the extent of damage caused by free-radical-induced lipid peroxidation [32]. The higher the serum MDA levels, the greater the blood lipid peroxidation. In the G1 group of mice that were not induced with LPS, there was no increase in serum MDA levels. After 14 days of receiving commercial vitamin E (G3) and BSD supplements (G4), serum MDA levels decreased. This indicates that the intake of commercial vitamin E and BSD supplements can prevent blood fat oxidation. Statistically, the ability to inhibit lipid peroxidation in vivo in LPS-induced rats between commercial vitamin E and BSD supplements is not significantly different ($P>0.05$).

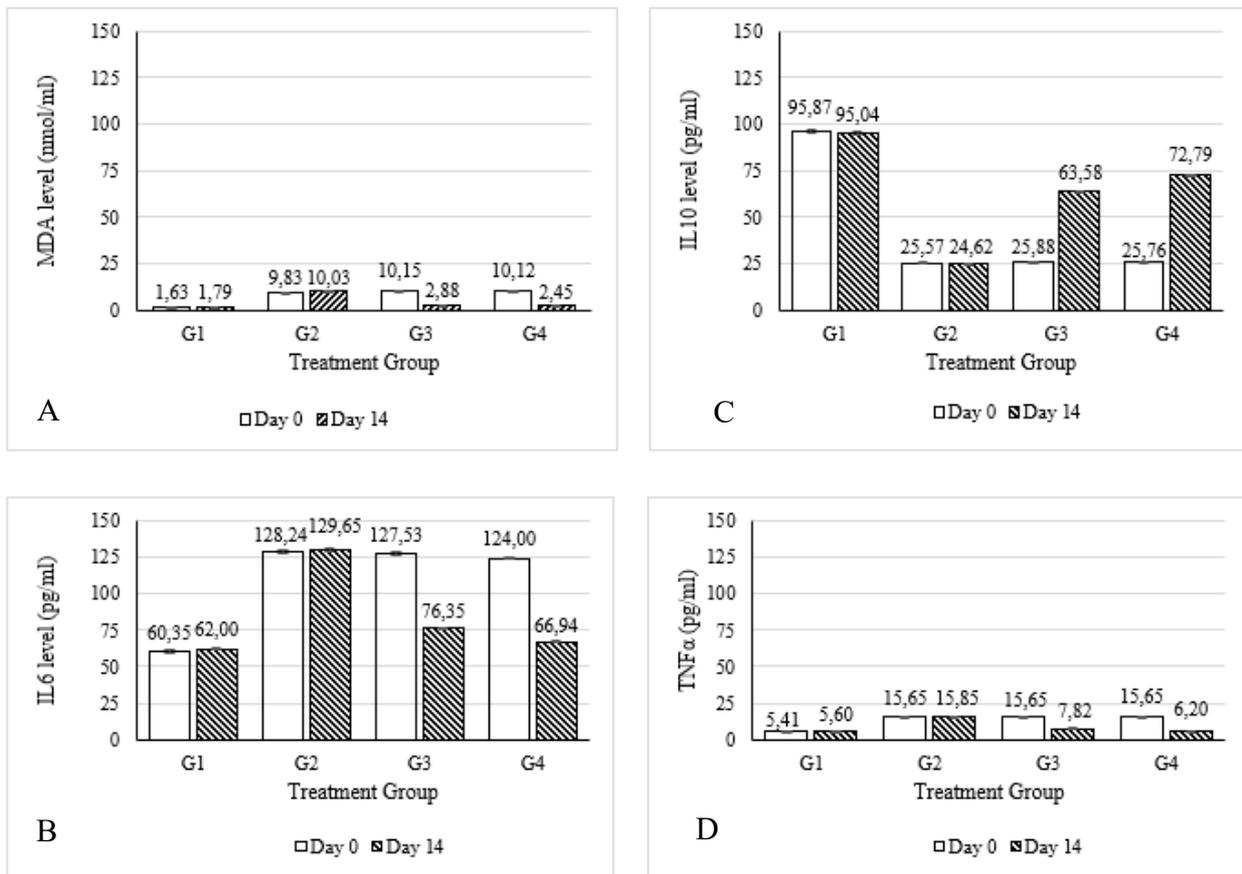


Figure 4 MDA (A), IL6 (B), IL10 (C), and TNFα (D) serum blood levels of mice. Note: days 0 and 14 from group 1 (G1) which is the normal control group with standard feed (AIN 1993), group 2 (G2) which is the negative control group with standard feed without supplementation, group 3 (G3) which is the positive control group with standard feed and vitamin E supplementation, and group 4 which is the group with standard feed and BSD supplementation.

The data in Figure 4.A also shows that serum MDA levels in the negative control group of rats were high and remained elevated after 14 days on a standard diet. Whereas in the group of rats given commercial vitamin E and BSD supplements, after 14 days of treatment, the serum blood MDA levels returned to nearly normal. The intake of commercial vitamin E and BSD supplements for 14 days can inhibit lipid peroxidation, because vitamin E and the phenolic components, flavonoids, and chlorophyll in BSD can act as antioxidants in vivo [33].

IL6, IL10, and TNFα serum blood levels: Figure 4.B, C, and D show the results of the analysis of IL6, IL10, and TNFα serum levels on days 0 and 14. It was observed that treatment with LPS at 10 mg/kg body weight for 3 days in groups G2, G3, and G4 resulted in increased IL-6 and TNF-α levels and decreased IL-10 levels. This indicates that LPS induction will enhance the local inflammatory response and rapidly spread throughout the body after invasion [34]. LPS-induced inflammation activates monocytes, macrophages (M4), and neutrophils in the blood, spleen, and liver, which subsequently produce inflammatory cytokines (including IL-1, -6, and -8, as well as tumor necrosis factor (TNF)). Monocytes are known as the leading promoters of acute and chronic inflammatory responses [35]. Monocytes are activated and attracted to the site of inflammation to enhance and

prolong the response to microbial stimulation initiated by tissue macrophages [36]. The initial monocyte response begins with rapid TNF production. These pro-inflammatory cytokines are the main mediators of the inflammatory response. TNF- induces the release of other cytokines (IL-1, IL-6), eicosanoids, reactive oxygen species, and the activation of the complement and coagulation cascades. The second phase of the monocyte response involves the production of IL-10, a potent immunosuppressive cytokine detectable within 8 hours of LPS exposure. IL-10 reduces the production of pro-inflammatory cytokines and plays a role in the development of LPS tolerance following initial LPS stimulation, making monocytes tolerant to LPS-induced injury [37].

After being given commercial vitamin E (G3) and BSD supplements (G4) for 14 days, the levels of IL6 (Figure 4.B) and TNF α (Figure 4.C) in the rat serum decreased, while IL10 (Figure 4.D) increased again. It appears that the BSD supplement is more effective at reducing IL6 and TNF α levels and increasing IL10 levels than commercial vitamin E. Meanwhile, in the G2 group, IL6 and TNF α levels remained high, and IL10 remained low, similar to the G2 negative control group, which received only standard feed. This shows that if the body experiences excessive oxidative stress from external attacks, its internal antioxidant capacity cannot counteract it without exogenous antioxidants [38]. The intake of BSD, which contains phenolic and flavonoid components, soy protein, inulin, chlorophyll, dietary fiber, and ascorbic acid, will reduce the inflammatory response, as indicated by decreases in IL6 and TNF α levels and increases in IL10. The intake of BSD, a complex of various compounds, is more effective than vitamin E alone.

Based on Figure 5.B, it is known that under normal conditions, serum IL-6 levels in mice range around 60.35 \pm 1.83 pg/ml. This is similar to the results of [39] study, which showed that the IL6 levels in normal male Wistar rats were 69.41 \pm 11.39 pg/ml, and in [4] study, they were 83.02 \pm 21.46. It is also known that LPS injection for 3 days increases serum IL6 levels in rats across all groups on day 10, with values that are not significantly different, except in the normal group. After receiving commercial vitamin E and BSD supplements for 14 days, IL-6 levels decreased again on day 14. Based on the statistical analysis of IL6 levels on day 14, BSD supplements were more effective at reducing IL6 levels than vitamin E [40]. This indicates that BSD supplementation is more effective at inhibiting inflammation induced by LPS injection.

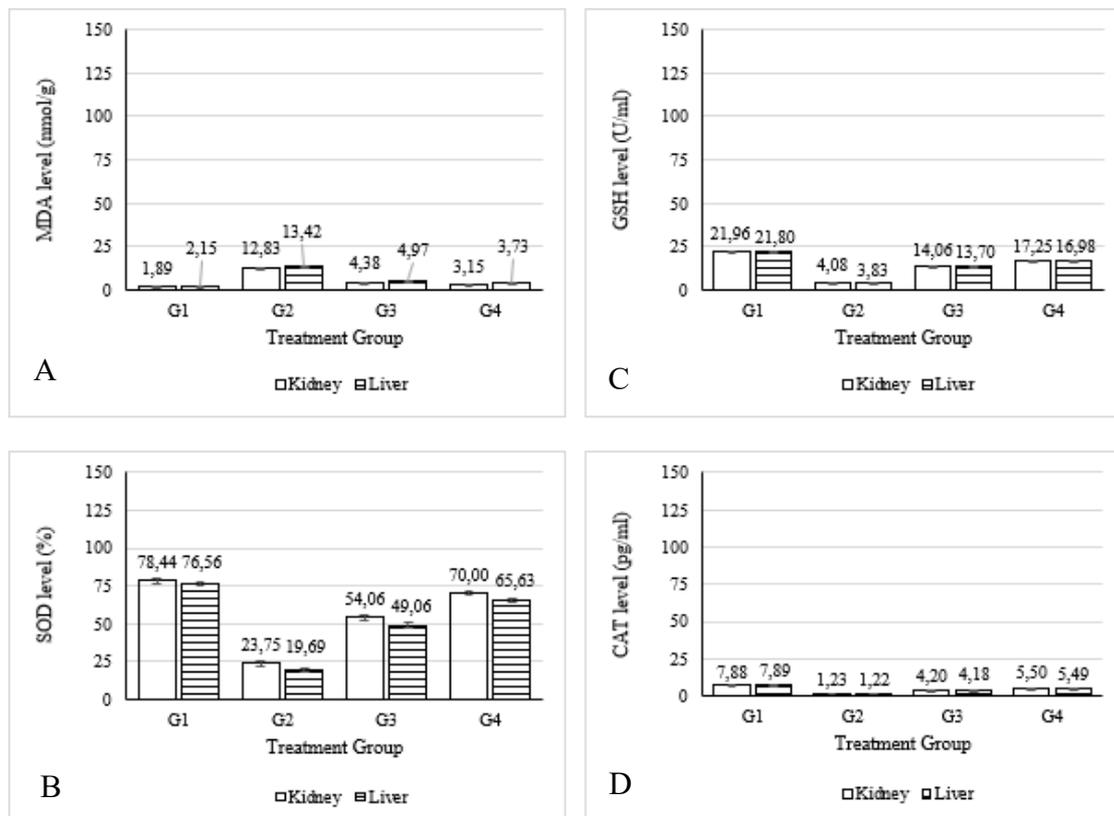


Figure 5 Levels of MDA (A), SOD (B), GSH (C), and CAT (c) in the kidneys and liver of rats. Note: days 0 and 14 from group 1 (G1) which is the normal control group with standard feed (AIN 1993), group 2 (G2) which is the negative control group with standard feed without supplementation, group 3 (G3) which is the positive control group with standard feed and vitamin E supplementation, and group 4 which is the group with standard feed and BSD supplementation.

Based on the analysis of serum TNF α levels, treatment with LPS 10 mg/kg body weight for 3 days increases serum TNF α levels in rats in groups G2, G3, and G4. After 14 days of receiving commercial vitamin E and BSD supplements, TNF α levels in the rats will decrease to near-normal levels. It appears that the intake of BSD supplements is more effective in lowering TNF α levels in LPS rats compared to commercial vitamin E. The reduction in TNF- α production in LPS-stimulated cells is mainly independent of NF- κ B. Still, it is preceded by an increase in the expression and secretion of the anti-inflammatory cytokine IL-10. The increases in IL-10 and the decreases in TNF- α both occur after reductions in LPA5 and LPA6 receptors in J774 cells and may be associated with LPA-mediated p38 activation. Binding of LPA to LPA5 and LPA6 enhances the LPS-induced inflammatory response by activating p38, increasing IL-10 regulation, and decreasing TNF- α production [41]. Based on the data in Figure 4, intake of vitamin E and BSD supplements for 14 days resulted in a decrease in serum TNF α levels. It appears that BSD supplementation is more effective than vitamin E. The decrease in TNF- α levels indicates improvement or recovery from the necrotic condition induced by LPS injection.

The balance between pro- and anti-inflammatory cytokine production by monocytes determines the effectiveness of the inflammatory response [42]. The cytokine profile of monocytes is recognized as an essential indicator of survival in sepsis patients, with a high IL-10/TNF α ratio in febrile patients associated with increased mortality. Monocytes also play a crucial role in the pathogenesis of chronic inflammation. This results in increased monocyte recruitment from the bloodstream to the site of inflammation, leading to a sustained inflammatory cascade that causes tissue damage [43]. Monocyte-derived cytokine inhibitors, such as TNF, have been effective in treating this condition when targeted with inhibitory antibodies. These cytokines enter the bloodstream and can then be distributed throughout the body, triggering systemic inflammatory response syndrome (SIRS). The continuous and excessive inflammatory response (e.g., SIRS) is also complicated by immune dysfunction and infection, leading to multiple organ failure (MOF) and severe sepsis [44], and [45].

Levels of MDA, SOD, GSH, and CAT in the kidneys and liver

Based on the MDA analysis results for liver and kidney tissues shown in Figure 5A, treatment with LPS 10 mg/kg body weight and standard feed intake alone (G2) increases MDA levels in the liver and kidney tissues. This indicates that LPS induction causes oxidative stress in the liver and kidney tissues. It also appears that the intake of BSD supplements (G4) and vitamin E (G3) for 14 days can reduce MDA levels in the liver and kidney tissues. Statistically, the ability to inhibit lipid peroxidation in the liver and kidney tissues of male Wistar rats induced with LPS from the BSD supplement at 270 mg/200 g body weight is more effective than vitamin E at 80 mg/200 g body weight. LPS induction results in acute liver and kidney damage and involves inflammatory responses, oxidative stress, and protein synthesis, which ultimately leads to multiorgan damage. Repairing disturbances in metabolites and metabolic pathways can help prevent and/or treat acute liver and kidney damage caused by LPS [46].

The MDA levels are consistent with the results of SOD, GSH, and CAT analyses in the kidneys and liver (Figures 5.B, C, and D). It was observed that treatment with LPS at 10 mg/kg body weight for 3 days, and standard feed intake alone, resulted in decreased SOD, GSH, and CAT levels in the liver and kidney tissues. It was proven that the intake of commercial vitamin E (G3) and BSD supplements (G4) for 14 days could restore SOD and CAT levels in the liver and kidney tissues to near normal levels in rats. The intake of BSD supplements is more effective in increasing the levels of SOD and CAT in the kidney and liver tissues compared to the intake of commercial vitamin E [47].

Superoxide dismutase (SOD) enzymes play an essential role in converting superoxide into hydrogen peroxide, which is then neutralized by catalase or glutathione peroxidase [48]. SOD is a secondary antioxidant enzyme that has a complex structure with metal ions such as copper or zinc at its active center. SOD catalyzes the dismutation of superoxide radicals (O₂⁻) into hydrogen peroxide (H₂O₂) and oxygen (O₂), thereby reducing free radical levels in the cell [49], and [50]. Catalase plays a vital role in degrading hydrogen peroxide into water and oxygen, thereby reducing the potential for oxidative damage to cells. Catalase enzymes play a role in protecting cells from excessive oxidative stress caused by hydrogen peroxide accumulation [51].

The decrease in MDA levels in liver and kidney tissues is associated with increases in SOD, GSH, and CAT levels in those tissues. It is observed that vitamin E intake in group G3 and BSD supplement intake in group G4 will increase SOD, GSH, and CAT levels, followed by a decrease in MDA levels, indicating reduced lipid peroxidation. SOD plays a vital role in preventing cellular oxidative stress by serving as the first line of defense [52]. Then, using CAT, SOD can convert ROS into hydrogen peroxide, thereby producing an oxidizing effect. GSH is an essential biological free-radical scavenger and antioxidant that helps maintain endogenous redox homeostasis. At the same time, MDA is the end product of polyunsaturated fatty acid peroxidation, driven by oxidative stress [53].

In this study, LPS injection for 3 days and standard feed intake alone (G2) increased MDA levels and decreased SOD, GSH, and CAT levels compared with the normal group (G1). This proves that LPS induction has caused oxidative stress in the liver and kidneys. The activity of various enzymes that inhibit oxidative stress, including SOD, GSH, and CAT, significantly decreased after LPS exposure. The administration of commercial vitamin E (G3) and BSD supplements (G4) can improve sepsis caused by LPS-induced oxidative stress. This is evidenced by the significant inhibition of MDA increase following LPS induction in rats (Figure 5A) and the restoration of SOD, GSH, and CAT levels in groups G3 and G4 (Figures 5C and D). LPS induction also resulted in kidney damage [54].

The data in Figures 5.B, 5.C, and 5.D show that the levels of SOD, GSH, and CAT in the kidneys and liver on day 14 in the group of rats with commercial vitamin E intake (G3) and BSD supplement (G4) were higher compared to the negative control (G2). This indicates that the intake of commercial vitamin E and BSD supplements can improve the oxidative stress in kidney and liver tissues following LPS injection. Based on the statistical analysis, BSD supplementation was more effective than vitamin E supplementation at increasing SOD, GSH, and CAT levels in the kidneys and liver. Although BSD supplement is lower in antioxidant activity using the RSA and IC50 methods, *in vivo* BSD intake is more effective in reducing MDA levels and increasing SOD, GSH, and CAT levels than vitamin E intake. This is suspected to be related to the ease of absorption of functional components such as phenolics, flavonoids, and chlorophyll compared with vitamin E, as well as the greater variety of elements that can act as antioxidants in BSD supplements compared with single vitamins [55].

Table 3 Summary of the effectiveness of commercial vitamin E intake and BSD supplements in improving serum blood profiles and kidney and liver tissue of rats induced by LPS (% compared to normal rats).

Parameter	Type of intake	
	Vitamin E	Supplement BSD
MDA	86.79	91.94
IL6	78.78	92.69
IL10	66.70	76.58
TNF α	78.34	94.19
MDA Liver	74.99	86.00
MDA Kidney	77.24	88.45
GSH Liver	62.79	77.63
GSH Kidney	63.50	77.59
SOD Liver	64.08	85.71
SOD Kidney	68.93	89.25
CAT Liver	52.91	69.54
CAT Kidney	53.25	69.81

This is supported by data in Table 3, which shows a comparison of the effectiveness of commercial vitamin E intake and BSD supplements over 14 days in improving the serum blood profile and kidney and liver tissues of male Wistar rats treated with LPS compared to normal rats (%). It appears that the intake of BSD supplements can more effectively improve the serum blood profile and the kidney and liver tissues of rats induced by LPS (69.54-92.69% compared to normal rats), compared to vitamin E intake (52.91-86.79% compared to normal rats). Overall, the intake of Bforliefe powder is more effective at reducing fat oxidation in blood and liver and kidney tissues and at increasing endogenous antioxidant capacity than the intake of commercial vitamin E. However, in this study, BSD supplements were administered as a single dose of 15 g/day for adults, or equivalent to 270 mg/200 g body weight in rats; therefore, it is necessary to determine the effects of higher doses.

Histopathology Liver and Kidney

The condition of sepsis due to oxidative stress can also be observed in the histopathological results of liver and kidney tissues (Figure 6) and in the degree of inflammation and the number of inflammatory cells, as presented in Table 6. Based on that data, it can be concluded that:

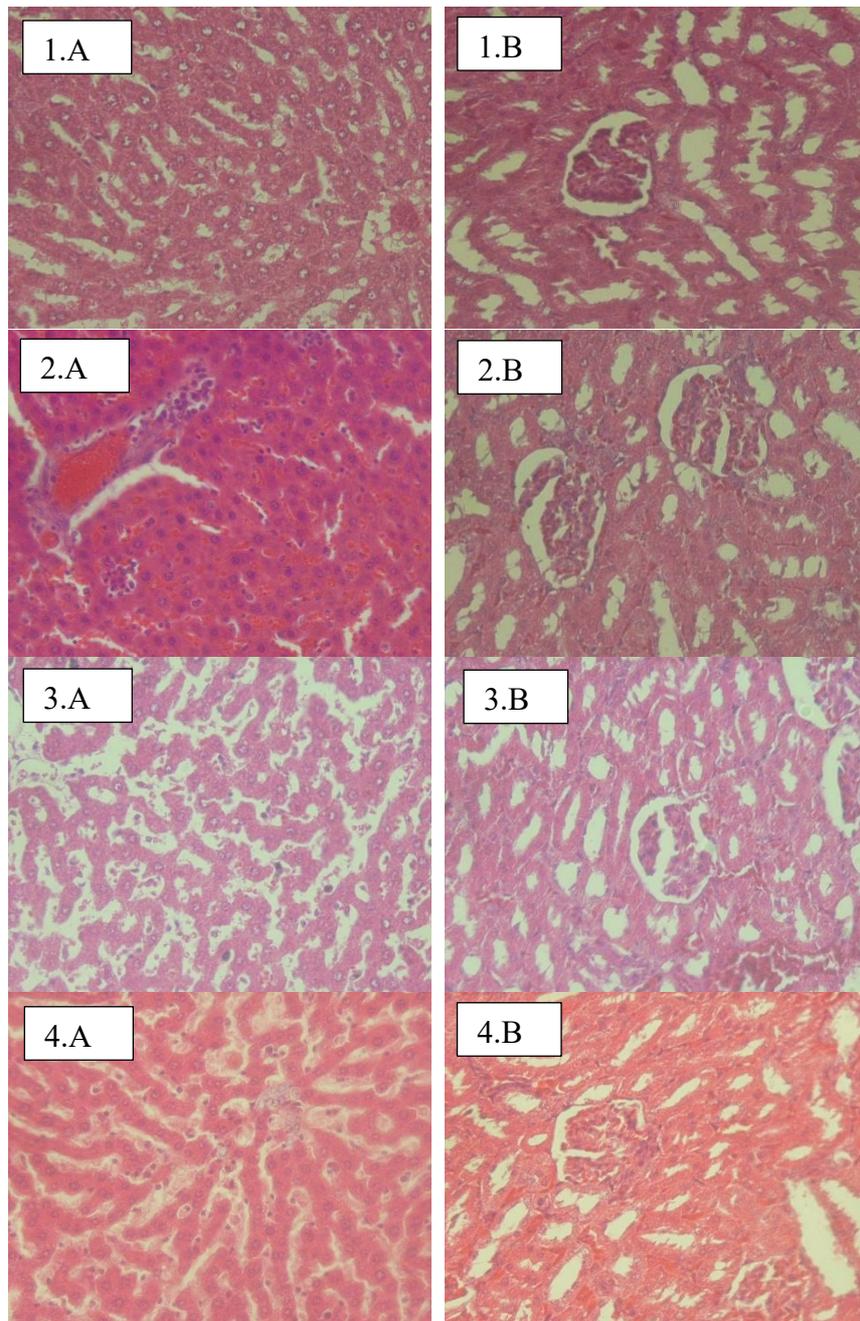


Figure 6 Histopathological photomicrograph of the liver (A) and kidney (B).

Note: day 14 with Hematoxylin Eosin staining method in 1: normal control group of rats with standard feed (G1), 2: negative control group with standard feed without supplementation (G2), 3: positive control group with standard feed and vitamin E supplementation (G3), and 4: group with standard feed with BSD supplementation (G4); A liver tissue and B kidney tissue.

a. Figure 6.1 shows a photomicrograph of liver tissue (A) and kidney (B) in the normal rat group (G1). It is shown in Figure 6.1.A normal hepatocyte cell, with no visible damage, and similarly in the kidney tissue (Figure 6.1.B), the glomerulus, distal tubules, and proximal tubules appear to be in normal condition. The observation results showed that the number of inflammatory cells was 33.22 ± 15.84 units, indicating a very low degree of inflammation (Table 6).

b. Figure 6.2 shows photomicrographs of liver tissue (A) and kidney (B) in the negative control group of rats (G2), which is the group induced with LPS and fed standard feed without supplementation. It appears that hemorrhage and necrosis have occurred in the liver and kidney tissues. The hemorrhagic condition resulted in inflammatory cells being neither clearly visible nor damaged, leading to a low inflammatory cell detection of 58.22 ± 19.11 , and the degree of inflammation was also relatively low.

c. Figure 6.3 shows that vitamin E intake can prevent necrosis in liver cells; the degree of inflammation and the number of inflammatory cells in the liver are not significantly different ($P > 0.05$) from those of normal rats

(G2). Still, in the kidneys, the degree of inflammation and the number of inflammatory cells are higher compared to normal rats. It is suspected that vitamin E supplementation at specific doses over a relatively long period may cause kidney inflammation.

d. Figure 6.4 shows that the number of inflammatory cells in the liver tissue (87.44 ± 31.30 units) and kidneys (7.78 ± 3.35) is higher compared to normal rats. Still, the degree of inflammation in the liver and kidneys is not significantly different from that of normal rats ($P > 0.05$). This is suspected to be due to the slower inflammatory healing process induced by LPS with BSD supplementation, and no necrosis was detected in the liver and kidney tissues.

Based on the data in Figure 6 and Table 6, the inflammation scores in the liver and kidneys for the BSD-supplemented rat group showed no significant difference compared to the normal rat group, with low liver inflammation scores (1) and no kidney inflammation (0). In contrast, the G3 rat group with vitamin E intake had medium inflammation scores (2). This is consistent with the MDA, SOD, and CAT data in the liver and kidneys, indicating that the BSD supplement is more effective at improving oxidative stress induced by LPS. Additionally, when viewed at the levels of MDA, IL6, IL10, and $TNF\alpha$, it is evident that BSD intake can improve oxidative stress in blood serum and prevent $TNF\alpha$ production, a tumor necrosis factor. The condition is significantly different from the levels of MDA, IL6, IL10, and $TNF\alpha$ in the G2 rat group. Although the degree of inflammation and the number of inflammatory cells are lower, Figure 6.2 shows that hemorrhage has occurred in the liver tissue, suggesting that the level of cell damage is very high, rendering inflammation no longer visible.

Table 4 Degree of inflammation and number of inflammatory cells in the liver and kidneys of 2-month-old male Wistar rats treated with LPS on day 14.

Group	Derajat inflamasi		Total of inflammatory cells	
	Liver*	Kidney	Liver	Kidney
G1: Normal	0.33±0.50	0.00±0.00a	33.22±15.84a	0.00 ± 0.00a
G2: Control negatif	0.33±0.50	0.00±0.00a	58.22±19.11ab	0.00 ± 0.00a
G3: Vitamin E	0.67±1.00	1.11±1.05b	52.67±48.42a	44.67±14.60c
G4: Bforliefe	0.44±0.73	0.22±0.44a	87.44±31.30b	7.78±3.35b

Note: Data from 3 treatment replicates and 3 analysis replicates, numbers followed by different letters indicate significant differences ($P < 0.05$). Derajat peradangan (tidak terdeteksi = 0, ringan = 1, sedang = 2, dan berat = 3). *Nonsignificativo ($P > 0.05$)

Sustained oxidative stress in group G2 will lead to sepsis. Sepsis is an excessive response of an organism to stimulation due to the influence of Lipopolysaccharides (LPS) from Gram-negative bacteria. The intensity of this inflammatory response is related to the interaction of immune cells. During this response, several inflammatory mediators, such as cytokines and chemokines, increase in the circulation [56]. Cytokine production activates leukocytes and increases the production of free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Furthermore, toll-like receptors (TLRs) activate nuclear factor kappa B (NF- κ B) and stimulate pro-inflammatory cytokines such as tumor necrosis factor-alpha ($TNF-\alpha$), interleukin-1 (IL-1), and interleukin-6 (IL-6) [57]. This triggers the activation of the complement pathway, which stimulates procoagulation. Cytokines stimulate immune, endothelial, and epithelial cells to increase ROS, such as superoxide (O_2^-), nitric oxide (NO), and peroxynitrite (ONOO $^-$). The intake of BSD supplements will prevent the production of IL6 and $TNF-\alpha$ and stimulate the production of IL10, SOD, GSH, and CAT, thereby improving oxidative stress in serum and in liver and kidney tissues. Superoxide dismutase (SOD) is an endogenous antioxidant found in all organisms that use oxygen as a source of life [58]. This enzyme catalyzes the conversion of superoxide into H_2O , a reaction considered the primary antioxidant defense against ROS. Another important enzyme is Glutathione peroxidase (GPx), which uses GSH as a cofactor within the cell. Contains selenium, which is important in the disposal of H_2O_2 . The GPx enzyme converts hydrogen peroxide into water, and the role of catalase (CAT) is the same as that found in the peroxisome. Catalase becomes essential when the concentration of hydrogen peroxide increases because its reaction is faster than that of glutathione peroxidase [49], and [59].

CONCLUSION

Based on the research results and discussion, it can be concluded that the BSD supplement, which has a protein content of 3.41% bb, ash 0.22%, total sugar 20.80%, reducing sugar 2.21%, and total energy 3.95 KCal, and contains high levels of dietary fiber, phenolic compounds, flavonoids, and total chlorophyll, has a DPPH radical scavenging ability and IC50 equivalent to commercial vitamin E. In addition, the intake of the supplement for 14 days in male Wistar rats induced with LPS can also improve oxidative stress conditions, as evidenced by its ability to reduce MDA levels in blood serum, liver, and kidneys, decrease IL6 and TNF- α production, and increase SOD, GSH, and CAT levels in the kidneys and liver. Therefore, BSD supplements are highly potential as a functional drink source of antioxidants.

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AI tools were not used.

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