

Burdock Root (*Arctium lappa*) Reduces Steatosis and Serum Malondialdehyde in Wistar Rats Fed with Used Cooking Oil

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ABSTRACT

Utilization of used cooking oil may cause fat accumulation in the body leading to exceeding metabolic capacity of the liver and lipid peroxidation, subsequently triggering oxidative stress that will lead to non-alcoholic fatty liver disease (NAFLD). Burdock root (*Arctium lappa*) have hypolipidemic, antioxidant, and anti-inflammatory properties. This study aims to determine the effects of Burdock root to reduce steatosis and malondialdehyde (MDA) plasma levels in male Wistar rats fed with used cooking oil.

This study used a post-test only control group design. Thirty healthy male Wistar rats were randomized into three groups. All groups were given 0,42 mL of used cooking oil. Fifteen minutes after, the intervention group 0 (P0) was given 1 mL of distilled water 1x/day. The intervention group 1 (P1) was given 100 mg/kg BW of burdock roots ethanolic extract diluted in 1 mL of distilled water 1x/day. The intervention group 2 (P2) was given 200 mg/kg BW of burdock roots ethanolic extract diluted in 1 ml of distilled water 1x/day. After 28 days, histopathological examination of the liver tissue to measure steatosis and peripheral blood taken to measure serum MDA levels and compared between groups.

The results showed that the average steatosis in the P0 group was significantly higher than the P1 group ($15.51 \pm 3.22\%$ vs. $8.92 \pm 1.49\%$, respectively; $p = 0.00$). Similar results between the P1 group were also significantly higher compared to the P2 group ($5.18 \pm 1.31\%$; $p = 0.002$). The mean serum MDA level revealed a significantly higher results among the P0 group compared to P1 group as well as P1 group compared to P2 group (12.58 ± 1.92 nmol/mL vs. 9.76 ± 0.47 nmol/mL, respectively; $p=0.011$ and $(9.76 \pm 0.47$ nmol/mL vs. 8.69 ± 0.33 nmol/mL, respectively; $p=0.011$).

The conclusions of this study were that burdock roots could reduce steatosis and serum MDA in male Wistar rats that were given used cooking oil.

Keywords: Burdock roots, malondialdehyde, NAFLD, steatosis, used cooking oil.

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I. INTRODUCTION

An unhealthy lifestyle and diet are risk factors for metabolic syndrome. A poor diet can also come from the utilization of used cooking oil. Used cooking oil contains high levels of trans-isomer fatty acids, which have an adverse effect on lipid profiles and can induce lipid peroxidation and oxidative stress [1]. The presence of this buildup increases the likelihood of developing NAFLD [2].

Non-alcoholic fatty liver disease (NAFLD) is a condition of excessive accumulation of triglycerides (TG) in the hepatocyte cytoplasm. The prevalence of NAFLD in the adult population worldwide is 25%, with 13% in Africa, 23% in Europe, 32% in the Middle East, and 25% in Asia [3,4]. Recent reviews have found a strong association between NAFLD with obesity (51.3%), dyslipidemia (69.2%), type II diabetes (22.5%), and metabolic syndrome (42.5%) [3], [5].

These findings further raise awareness of NAFLD towards chronic liver disease [3], [6].

NAFLD develops when there is an imbalance between the accumulation and clearance of lipids in the liver [5]. Excess free fatty acid (FFA) buildup in the liver causes mitochondrial damage and increases reactive oxygen species (ROS) production [5], [6]. Increased ROS will easily react with lipids, causing lipid peroxidation and the formation of malondialdehyde (MDA), which can cause inflammation and injury to hepatocytes [7]. The increase of ROS causes liver lipotoxicity, produces additional free radicals, and promotes oxidative stress, all of which accelerate the course of NAFLD [7], [8].

Due to the oxidative stress involvement in the etiology, pro-inflammatory nature, and hepatocyte damage of NAFLD, oxidative stress can be targeted as one of the approaches for intervention. Burdock, a well-known plant, particularly in

China, provides high nutrients from the roots, leaves, and fruit, and it has a low harmful impact when consumed. The root is a part of the burdock commonly used as traditional medicine or consumed as food [9]. Burdock root is a rich source of polysaccharides, polyphenols, and flavonoids [10]. A study analyzing burdock root extract demonstrated high antioxidant activity with a mean IC_{50} value of 29.65 ± 4.03 $\mu\text{g/mL}$ [11]. Another study also showed that ethanol extract showed strong antioxidant activity with a mean EC_{50} value of 4.79 ± 0.15 $\mu\text{g/mL}$ [12].

According to a study, burdock root possesses hypolipidemic, antioxidant, anti-inflammatory, and anti-diabetic effects [13]. Burdock root extract can enhance lipid metabolism, boost antioxidants, and lower inflammatory indicators. This study aims to investigate the effects of burdock root extract in reducing steatosis and MDA levels through its decreasing excessive lipid metabolism and ROS properties.

II. MATERIALS AND METHODS

A. Burdock Roots and Extract Preparation

Fresh burdock roots were imported from China and the extraction methodology herein was similar to previous studies. Roots were washed, cut into pieces, then air dried and protected from light. Briefly, 2 kg of the dried roots were then milled to a size of 60 mesh. Then, the burdock powder was macerated with 70% ethanol with a root/solvent ratio of 1:5 for 72 hours at room temperature. This mixture was then filtered using Whatman paper and the remaining ethanol was removed using a rotary evaporator under 50°C with reduced pressure to obtain a concentrated extract. The extract obtained was $\sim 800\text{g}$ and then stored in the refrigerator at 4°C [12], [14].

B. Used Cooking Oil Preparation

Standard food-grade palm oil, local brand Bimoli, was purchased from the local market. Palm cooking oil is used for frying tofu at a temperature of 180°C for eight minutes and then repeated six times.

C. Animals

30 eight to twelve weeks old male Wistar rats (weighing 180 to 200 g) were kept in cages with 12 hours light / 12 hours dark cycle and free access to tap water and commercial chow. All animals were carefully monitored during the experiment.

D. Experimental Design

This study was a true experimental study with a randomized post-test only control group design and was conducted for 28 days in Integrated Biomedical Laboratory Unit at Udayana University. After seven days of adaptation, the rats were randomized into P0, P1, and P2 ($n = 10$, each group). All groups were administered 0.42 mL of used cooking oil orally with the help of a gastric tube once a day at 9 AM. In the P0 group, rats were given intervention with distilled water 1 mL, once a day, 15 minutes after receiving oil. In the P1 and P2 group, rats received burdock roots ethanolic extract (BREE), 100 mg/kg body weight (BW) and 200 mg/kg BW orally by gastric tube, respectively, 15 minutes after the oil was administered. Before administering

burdock extract to the P1 and P2 group, it will be diluted with distilled water up to 1 mL.

After 28 days, rats were euthanized by administering ketamine/xylazine. All groups had their peripheral blood taken to measure the serum level of MDA. Blood samples were collected from the medial canthus orbitalis, centrifuged for 5 minutes and serum was stored at -20°C . For histopathological assessment of steatosis, pieces of the liver were fixed in buffered formalin and then stained with hematoxylin and eosin (H&E). After all procedures have been completed, the rats will be properly incinerated.

E. Examination of Steatosis and MDA Level

Steatosis in H&E staining can be observed by the number of hepatocytes characterized by structures resembling vacuoles. The observation process was carried out with 100x magnification to determine the five high-power fields (HPF) with the highest number of steatosis (hot spots) by shifting the preparation from the left end to the right end followed with 400x lens magnification to calculate the number of steatosis and the number of hepatocytes in the five areas. All total steatosis is divided by the total number of hepatocytes from each area and multiplied by 100% to determine the percentage.

The levels of serum MDA were determined by using QuantiChrom thiobarbituric acid reactive substances (TBARS) assay kits (BioAssay Systems, USA) in accordance with the manufacturer's instructions.

F. Statistical Analysis

All statistical analyses were performed using IBM SPSS statistics for Mac version 26 (IBM Corp., New York, 2019). All data were expressed as the mean $\pm$ standard deviation (SD). The significant differences were evaluated using one-way ANOVA if the data is normally distributed and the variance of the data is homogeneous. If the data does not fulfill the parametric test conditions, the Kruskal-Wallis test should be used. Statistical significance was set at $p < 0.05$.

III. RESULT

A phytochemical study was done with spectrophotometry to evaluate the natural compounds in burdock root. The result showed that BREE in this study has a low antioxidant property. The result of phytochemical study is presented in Table I.

TABLE I: PHYTOCHEMICAL STUDY OF BREE

Compound	Unit	Result
Polyphenol	mg GAE/100 g	3045,05
Flavonoid	mg QE/100 g	647,95
IC_{50}	ppm	912,28

The normality and homogeneity tests were performed on steatosis' percentage and MDA levels. The results showed the percentage of steatosis data is normally distributed and homogeneous. On the other hand, MDA levels is normally distributed but variances not homogeneous. The comparative analysis is presented in Table II, Fig. 1, and Fig. 2.

By H&E staining section of liver, we observed steatosis. Because of the histological fixation, lipid droplets in cytoplasm are usually dissolved by the solvents used to

prepare the samples. As a result, the samples will seem to contain vacuoles within the hepatocytes as seen in Fig. 3.

TABLE II: COMPARATIVE ANALYSIS OF STEATOSIS PERCENTAGE AND MDA LEVELS BETWEEN GROUPS

Group	Steatosis (%)	MDA (nmol/ml)
P0	15,51±3,22	12,58±1,92
P1	8,92±1,49*	9,76±0,47*
P2	5,18±1,31*	8,69±0,33*

Data are expressed as mean ± SD; P0=placebo group; P1=intervention group 1 with BREE 100 mg/kg BW; P2=intervention group 2 BREE 200 mg/kg BW.

*Values are significantly different ($p < 0.05$) when compared to the placebo group.

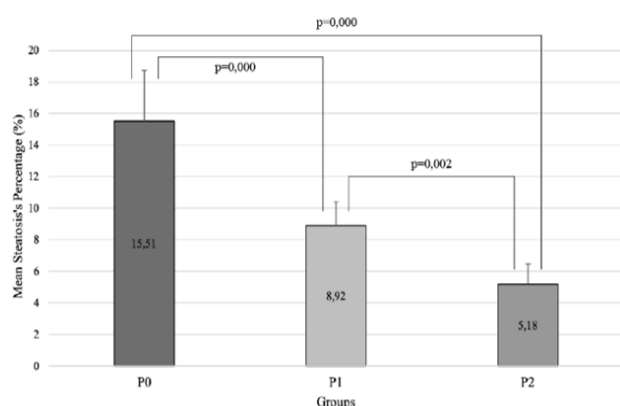


Fig. 1 Comparison of steatosis' percentage between groups.

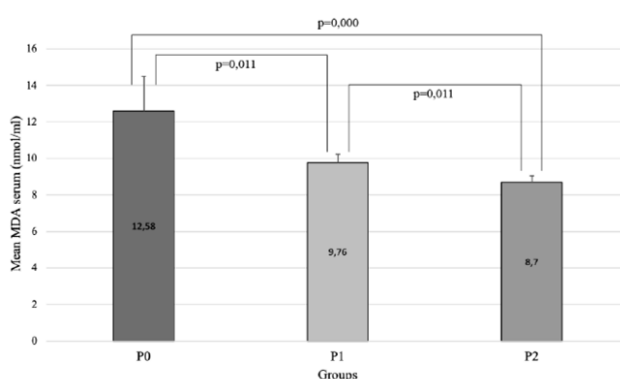


Fig. 2 Comparison of MDA levels between groups.

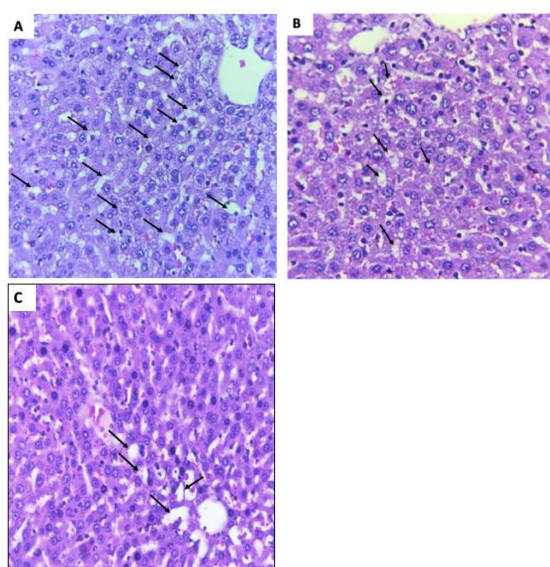


Fig. 3 Comparison of Hepatocytes Between Groups 400x Magnification, Black arrow=Steatosis: A. P0 group; B. P1 group; C. P2 group.

IV. DISCUSSION

A. Effect of BREE on Steatosis

Non-alcoholic fatty liver disease is an imbalance between lipid synthesis and degradation that exceeds the metabolic capacity of the liver [5]. In this study, used cooking oil was utilized to induce NAFLD in rats. The cooking oil was used to fry tofu till 180°C and repeated six times. For 28 days, 0.42 mL of used cooking oil was administered orally through a gastric tube. Results showed the average steatosis in the P0 group was 15.51±3.22%, the P1 group was 8.92±1.49%, and the P2 group was 5.18±1.31%. These findings suggest that administering used cooking oil for 28 days led to an increase in steatosis.

Cooking oil used repeatedly has a high content of trans fatty acids [15]. Furthermore, used cooking oil is high in saturated fatty acids (SFA) and FFA [16]. Trans fatty acid intake is often associated with metabolic syndrome [15]. According to studies, trans fatty acids result in a significant increase in total cholesterol, low-density lipoprotein (LDL), TG, very-low-density lipoprotein cholesterol (VLDLC), and a decrease in high-density lipoprotein (HDL) [17]. Additionally, it will also induce *de novo* lipogenesis (DNL), which will lead to FFA production [18]. Excess FFA buildup induces β -oxidation and esterification to form TG, which are stored in lipid droplets and form steatosis. Excessive β -oxidation will cause mitochondrial dysfunction, eventually leading to a decrease in β -oxidation [8].

FFA accumulation may induce stress in the endoplasmic reticulum, which leads to an increase in the production of ROS, causing oxidative stress and insulin resistance [8]. Excessive production of ROS will damage mitochondrial DNA. Furthermore, ROS can easily react with PUFA in lipid-rich environments, causing lipid peroxidation and producing more ROS [7].

In this study, statistical analysis revealed that both intervention groups were significantly lower than the placebo group in the percentage of steatosis ($p < 0.05$). Both dosages of BREE were significant in lowering steatosis but administering a dosage of 200 mg/kg BW was better than 100 mg/kg BW ($p < 0.05$). The following findings are the outcome of administering BREE, which is rich in polysaccharides, polyphenols, lignans and flavonoids.

Arctigenin, one of the lignans found in burdock root, has an effect on lipid metabolism. According to Song *et al.* [19], arctigenin effectively inhibits lipogenesis and lipolysis while enhancing fatty acids oxidation (FAO) and glucose absorption. Administration of arctigenin can induce phosphorylation of AMPK α . When AMPK α is activated, it causes the inactivation of acetyl-CoA carboxylase (ACC), an enzyme that synthesizes malonyl-CoA, an FAO inhibitor. Inactivation of ACC reduces the synthesis of malonyl-CoA and increases levels of carnitine palmitoyltransferase 1 (CPT1) and FAO. CPT1 is a rate-limiting enzyme for mitochondrial FAO. Furthermore, activation of AMPK suppresses lipogenesis by downregulating the peroxisome proliferator activated receptors γ (PPAR γ) and sterol Regulatory Element Binding Protein 1c (SREBP1c) pathways [19].

PPAR γ is expressed in adipose tissue and its activation promotes adipocyte differentiation and expression of proteins

involved in fatty acid uptake, transport and synthesis [20]. Furthermore, PPAR γ increases the expression of lipoprotein lipase, an enzyme that splits fat into adipocytes, limiting the flow of fatty acids to the liver. Ligands of PPAR γ and PPAR α modulate the activity of stearoyl-CoA desaturase-1 (SCD1), which enhances VLDL production [21].

Arctigenin can also inhibit SREBP-1c, a transcription factor that regulates DNL by inducing lipogenic enzymes that promote steatosis. AMPK activation inhibits SREBP-1c. Activation of AMPK also inhibits liver X receptor (LXR)- α , which regulates SREBP-1c transcription [20]. According to research, flavonoids can also stimulate AMPK activation and inhibit LXR- α [21]. Polyphenols can also increase FAO and modulate insulin resistance. Furthermore, polyphenols can also modulate DNL by affecting the activity of lipogenic enzymes and increasing the expression of lipolytic proteins [22].

The pathogenesis of NAFLD involves oxidative stress, resulting in inflammation and hepatocyte damage. Polyphenols and flavonoids are antioxidant compounds that reduce free radicals and inhibit pro-oxidant enzymes. Flavonoids have been found to be very effective scavengers [23].

Burdock shows anti-inflammatory effects along with hypolipidemic and antioxidant effects. Arctigenin has been shown in studies to decrease the production of nitric oxide (NO), tumor necrosis factor (TNF)- α , and interleukin (IL)-6. Arctigenin inhibits NO production by downregulating the expression of inducible nitric oxide synthase (iNOS). Excess NO has been shown to reduce the effectiveness of LXR- α in removing lipids, thereby increasing lipid accumulation [24].

Burdock root has anti-inflammatory effects by inhibiting the NF- κ B pathway. Arctigenin and polyphenols can directly decrease NF- κ B activation by blocking NF- κ B DNA binding and decreasing iNOS expression, which inhibits NO production. When oxidative stress occurs, NF- κ B is activated, and antioxidants affect and suppressing the NF- κ B pathway [23], [25].

Burdock root exhibited high antioxidant activity, with a mean IC₅₀ of 29.65 $\pm$ 4.03 μ g/mL [11]. Meanwhile, in this study, the antioxidant capacity of burdock root was 209.07 mg/L, with an IC₅₀ of 912.28 ppm.

B. Effect of BREE on MDA

MDA is an indicator of lipid peroxidation and is used as a biomarker of oxidative stress [26]. Fat accumulation in hepatocytes increases ROS production, which leads to increased lipid peroxidation and, eventually, more production of ROS. The increase in ROS is linked to mitochondrial dysfunction in NAFLD patients [27].

In this study, the administration of used cooking oil is expected to induce NAFLD by increasing lipid peroxidation increasing MDA levels. The results showed the MDA level in the P0 group was 12.58 $\pm$ 1.92 nmol/mL, the P1 group was 9.76 $\pm$ 0.47 nmol/mL, and the P2 group was 8.69 $\pm$ 0.33 nmol/mL. Comparability tests between groups showed that the administration of BREE was significantly lower compared to the group that was only given a placebo (p <0.05). According to the results, both dosages of BREE were significant but administering a dosage of 200 mg/kg BW was better than 100 mg/kg BW (p <0.05).

A decrease in MDA levels suggests an increase in glutathione peroxidase (GPx) function and activity, which causes the lipid peroxidation reaction to be inactivated. *In vitro* studies also demonstrate that phenolic acid can prevent lipid peroxidation [28]. Furthermore, burdock root has been shown to prevent lipid peroxidation via its antioxidant activity and hydroxyl radical scavenging capacities [29].

The MDA levels results are consistent with prior research that indicates administering BREE can lower plasma MDA levels. The administration of BREE to diabetic rats reduced MDA levels in the liver and kidneys [30]. Because of the hypolipidemic impact of Burdock root, the lipid peroxidation reaction was inactivated, and ROS production was reduced. According to research, burdock root contains phenolic acids, flavonoids, and arctigenin. The primary components are caffeoylquinic acid and quercetin, which have been proven to have considerable antioxidant activity [29].

C. Limitations of the Study

The antioxidant capacity in this study showed that BREE had a very weak antioxidant capacity. The results differ significantly compared to the study conducted by Skowronska et al. This discrepancy may be related to the source of the burdock root, which is fresh root obtained from a burdock farm, whereas in this study, the source was an imported product. Therefore, the freshness was reduced, which may have influenced the antioxidant capacity. This study also lacked negative control, resulting in no information on the normal state when comparing steatosis outcomes with MDA levels. Moreover, because there was no positive control in this study, there was no comparison of the effects of steatosis outcomes and MDA levels.

V. CONCLUSION

The current study's findings strongly suggest the effect of burdock root in reducing steatosis and MDA level. The efficacy of BREE was demonstrated by significant improvement in the amelioration of steatosis's percentage and MDA levels. This effect must be attributed to hypolipidemic and antioxidant compounds found in the extract. Therefore, burdock root could represent a potential treatment option for NAFLD. More study is needed to acquire a greater antioxidant effect and to discover how Burdock affects other oxidative stress measures.

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