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Administration of Holy Basil (*Ocimum Sanctum L.*) Leaves Extract Inhibiting the Enhancement of Malondialdehyde Level and Steatosis Number in Male Wistar Rats Given Used Cooking Oil

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ABSTRACT

Used cooking oil contains free radicals and free fatty acid (FFA) compounds which result in an increased risk of several degenerative diseases such as non-alcoholic fatty liver (NAFLD) which is characterized by steatosis thereby affecting a person's quality of life. The purpose of this study was to determine the effectiveness of holy basil leaf extract in inhibiting the increase in MDA levels and the amount of steatosis in male Wistar rats (*Rattus norvegicus*) given used cooking oil. This was experimental research using randomized post-test only control group design. The subjects were 30 male Wistar rats, aged 2-3 months, weighing 150-200 gram, randomly divided into 3 groups, namely the control group (P0), which was given 2ml of distilled water, treatment group 1 (P1), which was given 200mg/kg of holy basil leaves extract, and treatment group 2 (P2), which was given 400mg/kg of holy basil leaves extract. All groups were also administered with 0.42ml of used cooking oil each. The results showed that the MDA levels between both the treatment group 1 and the control group, and treatment group 2 and control group were significantly different ($p<0.001$). The results of the comparative test for the number of steatosis showed that the control group was not significantly different from treatment group 1 ($p=0.123$), but the control group was significantly different from treatment group 2 ($p=0.002$) with the lowest mean number of steatosis in treatment group 2 (46.87 ± 17.74). Administration of holy basil leaves extract (*Ocimum sanctum L.*) for 35 days at doses of 200mg/kg and 400/kg effectively inhibited the increase in MDA levels and at a dose of 400mg/kg effectively inhibited the amount of steatosis in male Wistar rats given used cooking oil.

Keywords: basil leaves extract, MDA, steatosis, used cooking oil, non-alcoholic fatty liver

INTRODUCTION

Chronic disease is a degenerative metabolic condition that accelerates the aging process and reduces the standard of living. Poor diet, excessive physical activity, and stress contribute to metabolic disorders and the formation of oxidative stress in the body.[1] Nonalcoholic Fatty Liver Disease (NAFLD) is now the most important cause of chronic liver disease worldwide, manifested by a spectrum of liver disorders in the absence of excessive alcohol consumption.[2] NAFLD is Early Aging which can be measured by one of the aging parameters, namely using the biomarker Malondialdehyde (MDA). One of the causes of NAFLD is consuming foods that are high in Free Fatty Acid (FFA) and free radicals such as used cooking oil.[3]

In previous studies, giving used cooking oil to rats caused inflammation and damage to liver cells. This is thought to be related to free radicals which cause lipid peroxidation which is characterized by increased levels of malondialdehyde (MDA).[4] Antioxidant compounds such as flavonoids have been frequently studied in NAFLD models and appear to show beneficial effects.[5] Antioxidant compounds are often found in types of plants in Indonesia, one of which is holy basil. Holy basil's natural antioxidant compounds are flavonoids, tocopherols, phenolic acids, ascorbic acid and tannins. Holy basil leaves with these various compounds are believed to be able to inhibit free radicals and prevent liver damage due to consumption of used cooking oil.[6]

In previous studies, such as that of Geetha and Vasudevan, it was stated that holy basil leaves at a dose of 200 mg/kg/day in albino rats had a hepatoprotective effect. Research by Galila stated that giving holy basil leaf extract at a dose of 200 mg/kg/day could inhibit the formation of lipid peroxidation thereby reducing levels of SGOT and SGPT.[7] Another study regarding the hepatoprotective activity test of holy basil leaf extract against SGPT and SGOT in male white rats induced by paracetamol, concluded that administration of holy basil leaf extract at a dose of 400 mg/kg showed a decrease in SGPT and SGOT levels.[8]

Based on the existing problems and previous studies, the author intends to prove the effectiveness of administration of holy basil leaf extract (*Ocimum sanctum L.*) in inhibiting the increase in malondialdehyde (MDA) levels and the amount of steatosis in male Wistar rats (*Rattus norvegicus*) given used cooking oil.

METHODS

This research was experimental, with posttest only control group design. Preparation and analysis of basil leaf extract was carried out at the Postharvest Engineering Laboratory, Faculty of Agricultural Technology, Udayana University. The research was conducted at the Integrated Biomedical Laboratory, Drug and Experimental Animal Development Division of Udayana University. Histopathological examination was carried out using HE staining at the Histology Laboratory, Faculty of Veterinary Medicine, Udayana University. Examination of MDA levels was carried out using RAT MDA ELISA KIT at the Biochemistry Laboratory, Faculty of Medicine, Udayana University. The sample needed in this experiment was 27 male Wistar rats (n=9), 2-3 months old, weighing 150-200 grams. To anticipate drop out, 10% of total sample were added, with the total amount to 30 rats divided into 3 groups: control and treatment group 1 and 2 (n=10). This research has been approved by the ethics commission of Udayana University, Bali, (B/252/UN14.2.9/PT.01.04/2022).

Normality test was assessed using Shapiro-Wilk test and homogeneity test was assessed with Levene's test. Comparability test for MDA was assessed using Mann Whitney test because the data was non-normally distributed, while steatosis number was assessed using One Way Anova test because it was normally distributed and homogeny, and proceeded with Least Significant Difference (LSD) test.[9]

RESULTS

Normality test on MDA level and steatosis number for each group was done using Shapiro-wilk test, presented on Table 1. Based on Table 1, it was concluded that the data on MDA level in control group was non-normally distributed ($p<0.05$), while the steatosis number was normally distributed ($p>0.05$). The data on MDA level and steatosis number in the treatment groups was normally distributed ($p>0.05$)

Table 1. Normality Test

Variables	n	*P*	Description
MDA Level in Control	9	0,009	Non-normally distributed
MDA Level in Treatment 1	10	0,820	Normally distributed
MDA Level in Treatment 2	10	0,267	Normally distributed
Steatosis Number in Control	9	0,447	Normally distributed
Steatosis Number in Treatment 1	10	0,689	Normally distributed
Steatosis Number in Treatment 2	10	0,587	Normally distributed

Because MDA level in the control group was non-normally distributed, the data were then transformed using the Log(10) and square root (SQRT) methods. The data resulting from the transformation were then tested for normality again, and still showed an abnormal distribution of data ($p <0.05$). The test results on the MDA content data were the basis for the non-parametric test.

Homogeneity test in this study was only applied to the data on steatosis number. MDA content data was not tested because the MDA data was tested using a non-parametric test that did not require data homogeneity. Data on the steatosis number between group was tested using Levene's test and concluded to be homogeny and can be seen in table 2.

Table 2. Homogeneity Test on Steatosis Number

Variabel	n	*P*
Steatosis Number	29	0,860

Comparability test aimed to compare MDA levels between groups after treatment. Significance analysis was tested with the Kruskal Wallis test because the distribution of the control data was not normal. The results of the comparative analysis are presented in Table 3 and a p value of <0.001 was obtained, meaning that the comparison of MDA levels in the groups differed significantly ($p <0.05$). To find out which group was different from the control group, a follow-up test with the Mann Whitney test was carried out, with a significantly different interpretation when $p <0.05$. The test results can be seen in table 4

Table 3. Comparability Test of MDA Level

Subject Group	n	Median	Minim	Max	Interquartile Range	*p*
Control	9	0.45	0.39	0.94	0.32	<0.001
Treatment 1	10	0.33	0.30	0.38	0.04	
Treatment 2	10	0.25	0.16	0.30	0.09	

Table 4. Comparability Analysis of MDA Level

Subject Group	N	Median	Minim	Max	Interquartile Range	*p*
Control	9	0.45	0.39	0.94	0.32	<0.001
Treatment 1	10	0.33	0.30	0.38	0.04	
Control	9	0.45	0.39	0.94	0.32	<0.001
Treatment 2	10	0.25	0.16	0.30	0.10	

Table 4 shows the analysis of significance with the Mann Whitney test was p<0.001, meaning that the comparison of MDA levels between groups was significantly different (p<0.05).

The result of significance analysis on the average number of steatosis between groups was done using One Way Anova and was found to be significantly different (p<0.05). The analysis is presented in Table 5.

Table 5. Average Number of Steatosis Between Groups

Group	**n**	**Mean Steatosis ±SD**	***F***	***p***
Control	9	74.70±16.49	6,078	0,007
Treatment 1	10	61.92±17.92		
Treatment 2	10	46.87±17.74		

To find out which group was different from the control group, a follow-up test with the LSD test was carried out, with a significantly different interpretation if p <0.05. The test results can be seen in Table 6.

Table 6. Comparison of Steatosis Number Between Groups

Group	Average Number of Steatosis	*P*	Interpretation
Control and Treatment 1	12,78	0,123	Not significant
Control and Treatment 2	27,84	0,002	Significant

In Table 6, the control group and treatment group 1 showed a value of p = 0.123, meaning that the average number of steatosis in the control group was not significantly different from the treatment group 1. The control group and treatment group 2 showed a value of p = 0.002. This means that the mean number of steatosis in the control group was significantly different from the treatment group 2.

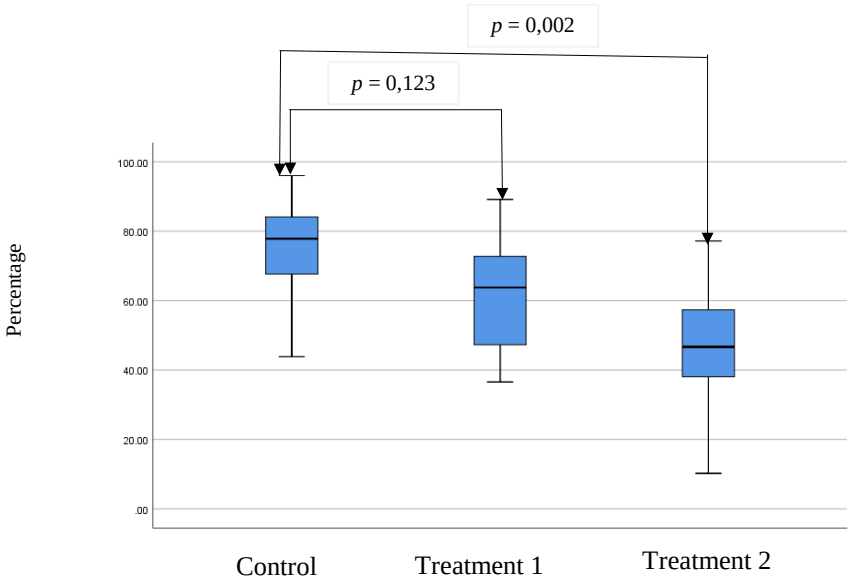
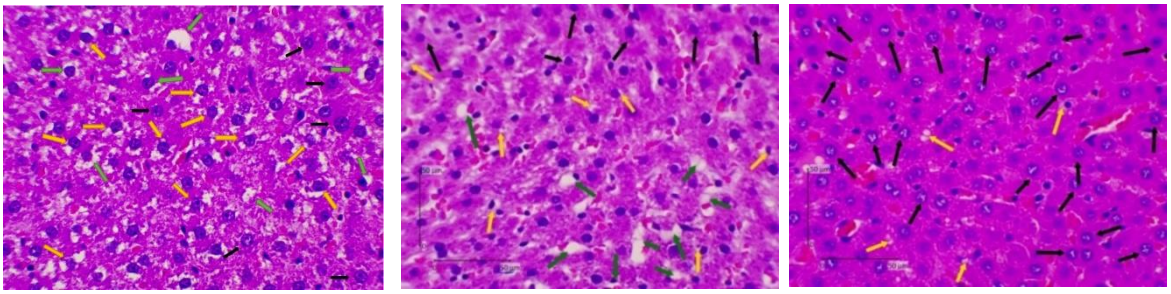


Figure 1. Comparison of Mean Number of Steatosis

From the results of statistical analysis tests, it could be concluded that treatment group 2 effectively inhibited the increase in the number of steatosis. Histopathological picture of control group, treatment group 1 and treatment group 2 can be seen in figure 2.



(P0) **(P1)** **(P2)**

Figure 2. Histopathology of Rat Liver Tissue in Control Group (P0), Treatment 1 (P1), and Treatment 2 (P2)

Description: Yellow arrows indicated micro-vesicular steatosis, green arrows indicated macro-vesicular steatosis and black arrows indicated normal hepatocytes (400x magnification).

In Figure 2, the control group had the most micro-vesicular and macro-vesicular steatosis compared to the other groups. In the picture of the treatment group 1 (P1) there was less micro-vesicular and macro-vesicular steatosis than the control group. It can be seen that the treatment group 2 had the most normal hepatocyte cells with the least micro-vesicular steatosis compared to the other groups and no macro-vesicular steatosis was seen.

DISCUSSION

Administration of Holy Basil Leaves Extract on MDA Level

Based on this study, it can be concluded that administration of 200mg/kg BW mg and 400mg/kg BW mg of holy basil leaves extract inhibited the increase in MDA levels in Wistar rats fed with used cooking oil. After an analysis test of used cooking oil in this study, a peroxide value of 11.40 meqO2/1000 was obtained, which was more than that the National Standard (2012) allows (maximum 10 MeqO2/1000). Used cooking oil which has a high peroxide value can be a source of free radicals.[10] When free radicals in used cooking oil react with PUFA (Poly Unsaturated Fatty Acids) compounds, they will cause oxidative damage to lipid compounds. Lipid peroxidation products, such as MDA (Malondialdehyde) can be detected.[11]

The positive effect of holy basil leaves extract in preventing oxidative stress is due to the presence of high content of flavonoid bioactive compounds. The phytochemical tests at the Laboratory of the Faculty of Agricultural Technology, Udayana University showed that holy basil leaves contain IC 50 26.17 ppm, flavonoids 14734.85 mg/100g QE, phenol 3060.24 mg/100g GAE, and tannins 5039.31 mg/100g TAE. Flavonoids can reduce the harmful effects of free radicals directly by inhibiting lipid peroxidation through peroxidase activation. Flavonoids can also protect the body from further ROS reactions by capturing ROS, blocking propagation reactions, and stimulating the formation of endogenous antioxidants such as superoxide dismutase and reducing MDA levels and reducing 8-OHdG levels due to HO* which normally react with DNA and cause cell death.[12] Apart from flavonoids, holy basil leaves extract contains tannin compounds which also capture hydroxy free radicals and tocopherol compounds (Vitamin E) which are lipophilic so that they can prevent lipid peroxidation in cell membranes thereby reducing MDA levels.[13]

Administration of Basil Leaves Extract on the Number of Steatosis

After analysis of used cooking oil in this study, an FFA value of 0.44% was obtained, which is more than the National Standard (2012) allows (maximum 0.3%). Free Fatty Acid (FFA) from used cooking oil and lipolysis of adipose tissue causes inflammation, increases the accumulation of ectopic fat, and further increases insulin resistance.[14] Insulin resistance stimulates gluconeogenesis in hepatocytes and reduces glycogen formation. Increased levels of glucose and insulin stimulate Hepatic de novo lipogenesis (DNL), namely the formation of new lipids from carbon-carbohydrate bonds or amino acids. DNL together with excess FFA uptake causes the liver to store fatty acids in lipid droplets and become steatotic.[15]

Flavonoids in holy basil leaves can stimulate PPARα which is believed to reduce steatosis by regulating FFA transport, stimulating enzymes that play a role in β-oxidation and reducing inflammation by inhibiting NF-Kb. In addition, flavonoids work by reducing the expression of the SREBP-1c gene and/or protein, improving insulin sensitivity and normalizing insulin levels. The SREBP-1c protein is a transcription factor in controlling DNL through the induction of lipogenic enzymes, resulting in steatosis.[15]

The results from this study showed that the dose of 400mg/kg of holy basil leaves extract could inhibit the amount of steatosis, which is in line with previous research regarding the hepatoprotective activity test of holy basil leaf extract (*Ocimum sanctum L.*) where the dose of 400mg/kg showed a decrease in SGPT and SGOT levels in male Wistar rats given paracetamol.[8] Another research by Festi *et al*. showed that administration of *Ocimum sanctum* leaf extract at a dose of 400 mg/kg BW can reduce the degree of inflammation by decreasing the average number of infiltrating PMD inflammatory cells in the stomach of gastritis rat models.[16] This strengthens the theory that a dose of 400 mg/kg BW is an effective dose in inhibiting steatosis because it also inhibits inflammatory pathways.

In the results of this study, a dose of 200 mg/kg did not significantly inhibit steatosis, which is not in line with the theory of previous researchers, such as Touiss *et al*., which showed that administration of phenolic acid to rats for 5 weeks at a dose of 200 mg/kg given a high-fat diet can significantly reduce triglyceride levels in plasma lipid profiles by up to 42%.[7,17] This could be because in previous studies the control group was given a high-fat diet, while this study used cooking oil was used instead, so the concentrations of each FFA content and peroxide compounds were different.

The positive effect of the ethanol extract of holy basil leaves (*Ocimum sanctum L.*) in this study is expected to be carried forward to the human research stage. The doses of 200 mg/kg and 400 mg/kg were converted from rats to humans using the conversion table, the following results were obtained:[18]

HED (mg/kg) = Animal dose (mg/kg) x animal Km/Human Km

200mg/kgBB x 6/37 = 32mg/kg

400mg/kgBB x 6/37 = 64mg/kg

Human weight 50kg = 32mg/kg x 50kg = 1.600 mg

= 64mg/kg x 50kg = 3.200 mg

The dose of holy basil leaves ethanol extract that can be consumed in 50kg human is around 1.6-3.2 grams per day.

Effect of Holy Basil Leaves in Anti-Aging Medicine

The aging process can be prevented and slowed down in many ways. The basic principle is to combat free radicals and the emergence of oxidative stress, which are known to be the main causes of the aging process itself.[1] The compound of the ethanol extract of holy basil leaves can reduce ROS and prevent oxidative stress through a direct mechanism, namely by capturing or neutralizing free radicals induced by used cooking oil in experimental animals. The antioxidant effect of holy basil leaves ethanol extract plays a role in decomposing peroxides, so that peroxy radicals cannot bind to lipids and prevent lipid peroxidation, thereby inhibiting liver damage. The positive effect of holy basil leaves ethanol extract (*Ocimum sanctum L.*) on various diseases including NAFLD is expected to be useful as a prevention of diseases related to the aging process so that life expectancy is not only longer but also has a good quality of life at old age.

Conclusions and Recommendations

Administration of both 200mg/kg and 400mg/kg dose of holy basil (*Ocimum sanctum L.*) leaves extract inhibited the increase of MDA level but only the dose of 400mg/kg of holy basil leaves ethanol extract was effective in inhibiting the increase of steatosis number in male Wistar rats given used cooking oil.

Further research is needed to determine the effect of inhibition through other pathways such as inflammatory pathways or metabolic pathways and also to determine the effectiveness of oral administration of holy basil leaves extract in non-alcoholic fatty liver disease.

Conflict of Interest

All researchers declare that there is no conflict of interest related to this article

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All authors contribute equally in compiling this research article

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