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Rat Liver Function Induced By A High-Fat Diet After Giving Mahogany Seeds Ethanol Extract

Agave Chintya Gabriella Sijabat, Sri Isdadiyanto*, Agung Janika Sitasiwi

Graduate Program, Faculty of Science and Mathematics, Diponegoro University, Jalan Prof. Soedarto, S.H., Tembalang, Semarang, 50275, Indonesia

*Corresponding Author: isdadiyanto@yahoo.com

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Abstract. Intake of a high-fat diet can cause hyperlipidemia which increases the rate of triglycerides and cholesterol, and decrease the HDL levels. The increase of lipid levels in the blood can cause Non-Alcoholic Fatty Liver Disease (NAFLD) which triggers damage of liver function. Mahogany seeds have bioactive compounds that have the potential to be antioxidants in treating hyperlipidemia. This study aimed to analyze the liver function of rats by inducing a high-fat diet after giving mahogany seeds ethanol extract which was observed through the levels of SGOT and SGPT. Thirty male rats of the Sprague Dawley strain were divided into 6 groups of treatment, that were: P0 (only given commercial feed), P1 (given a high-fat diet), P2 (given a high-fat diet and simvastatin at a dose of 8 mg/200 g BW), P3, P4, and P5 (given a high-fat diet and mahogany seeds ethanol extract of 14, 28, and 56 mg/200gBW). The results showed that there was no significant difference between all treatment groups. Administration of mahogany seeds ethanol extract was able to prevent the increase of SGOT and SGPT levels. Mahogany seeds ethanol extract has the hepatoprotective effect against high-fat diet induced hepatotoxicity, thereby affirming its traditional therapeutic role in liver injury.

Keywords: High-fat diet; SGOT; SGPT; Swietenia mahagoni seeds

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INTRODUCTION

Hyperlipidemia represents several different disorders of lipid metabolism that are characterized by the increase of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and a decrease of high-density lipoprotein cholesterol (HDL-C) (Xiao et al., 2016). Hyperlipidemia is a significant risk factor for non-alcoholic fatty liver disease (NAFLD), which is the most common cause of chronic liver disease (Li et al., 2021).

NAFLD refers to the condition of the liver that contains excessive amounts of fat that is not related to alcohol consumption, which is still reversible but if there are no lifestyle changes it will increase to NASH (non-alcoholic steatohepatitis) which will eventually increase the occurrence of various degrees of fibrosis (Bedossa, 2016).

The liver is the main organ which responsible for biosynthesis, absorption and degradation of proteins and enzyme. Therefore, liver function can be reflected on certain limits on the levels or the activities of circulating biochemical compounds. Liver function disorder can be observed by measuring blood levels of biochemical markers such as SGOT and SGPT. The increase in the production of transaminase enzymes is a response to the condition of hepatic cell injury. Transaminase enzymes are released into the blood in large quantities when hepatocytes are damaged (Longo et al., 2013).

Administering medicine to reduce fatty liver is necessary to repair liver damage caused by hyperlipidemia. Statins are a group of medicine which used widely for first-line treatment to lower LDL cholesterol levels. Despite the benefits of statins in lowering cholesterol levels, the use of statins also has the potential to cause side effects, such as musculoskeletal pain (Mansi et al., 2013) and cause muscle damage (Fernandez et al., 2018). Alternative medicine to avoid the risk of these side effects can be done by utilizing herbal medicines which have relatively low side effects (Sukardiman & Ervina, 2020).

Medicinal plants and their derivatives are promisingly gaining wide usage worldwide as they are a potential source of bioactive agents for medication (Entredicho et al., 2019). One of the utilization of plant extracts used in medicine is mahogany seeds (*Swietenia mahagoni*). Phytochemical compounds of mahogany consist of the phenolics (flavonoids (catechins, epichatechins, and swietemacrophyllanin,) and tannins), triterpenoids and tetranortriterpenoids (limonoids: mahonin, secomahoganin, swietmanins, swiemahogins, swietenine and swietenolide), saponins and alkaloids (Sukardiman & Ervina, 2020). These compounds have the potential as antioxidants which act as prevention of oxidative stress due to hyperlipidemia. Flavonoid compounds will capture free radicals and will oxidize these free radical compounds. Flavanoids donate hydrogen from their aromatic rings to free radicals to reduce toxic free radicals and produce more stable and non-toxic flavonoid radicals. This reaction will produce radical products that are more stable or less reactive.

Mahogany seeds (*Swietenia mahagoni*) have been mentioned in several studies to have various therapeutic effects. Sukardiman & Ervina, (2020) explained that mahogany seeds have the effect of reducing blood glucose levels in type II diabetes mellitus. Other research found the extract of mahogany seeds has the potential to be used as a herbal medicament to treat hypertension (Chng et al., 2018). Giving mahogany seed ethanol extract can reduce the occurrence of oxidative stress which is characterized by a decrease in blood MDA levels (Jawi et al., 2017). Entredicho et al. (2019) found a significant reduction in total cholesterol, triglycerides, and LDL-C and HDL-C which returned to normal after intake of mahogany seeds ethanol extract in Rats with hyperlipidemia. An acute toxicity test conducted by Balijepalli et al. (2015) on mahogany seeds with a single dose of 2 g/Kg BW did not show signs of toxicity in Sprague Dawley rats.

Research on the effect of giving mahogany seed ethanol extract (*Swietenia mahagoni*) on SGOT and SGPT levels in white rats (*Rattus norvegicus* L.) has never been done before. Mahogany seed ethanol extract would also be expected to play a protective role against hyperlipidemia. Therefore this study was conducted to evaluate the protective potential of mahogany seeds ethanol extract against liver disfunction which observed through levels of SGOT and SGPT in a hyperlipidemic rat model so that it can be used as a basic material for herbal therapy in cases of hyperlipidemia.

METHODS

This research has been approved by Health Research Ethics Committee, Faculty of Medicine, Diponegoro University with No. Etichal Clearance 26/EC/H/FK-UNDIP/IV/2022. This study used an experimental design posttest only control group with a completely randomized design. The animal models that have been used in this study were 30 male white rats (*Rattus norvegicus*) Sprague Dawley strain aged 2 months with an average weight of 200 grams in good health.

Preparation of Mahogany Seeds Extract

The mahogany seeds that have been used in this study were simplicia powder obtained from traditional plant sellers. Mahogany seeds powder is extracted using the maceration method and then diluted according to the dosage used. The dose of mahogany seed ethanol extract administration was made up to 14, 28, and 56 mg/200g BW.

Preparation of Animal Models

The animal models were divided into 6 groups, namely P0 (only given commercial feed), P1 (fed with a high-fat diet), P2 (fed with a high-fat diet and simvastatin at a dose of 8 mg/200 g BW), P3, P4, and P5 (fed with a high-fat diet and mahogany seeds ethanol extract 14, 28, and 56 mg/200g BW).

Making High-Fat Feed

The method for making high-fat feed as in Isdadiyanto *et al*. (2022). The High-fat feed consists of commercial feed and reused cooking oil obtained from one-liter cooking oil which was used to fry 450 g of tofu for 10 minutes at 150°-165°C using the deep fat frying technique in nine times frying. High-fat feed is made with a ratio of 10:1, that is, for every 30 g of commercial feed is added 3 ml of used cooking oil, then stirred until evenly distributed. In addition to providing high-fat feed, in this study rats were also given orally of organic duck egg yolk to obtain hyperlipidemia. The yolk dose of duck egg was given 2.5 ml/200 g BW using a stomach tube.

Treatment of Animal models

The acclimation process was carried out for 7 days in the cage used in the treatment. Each cage contained one male white rat. This research used cages in the size of 21cm x 15cm x 18cm filled with one rat. The cage is equipped with husks as a base, a place to feed, and a place to drink. The temperature and humidity of the rat cages were measured using a thermohygrometer. Temperature and humidity are measured every evening. The husks are replaced when it started to look damp.

All treatment groups were given 30 grams of commercial feed and 60 mL of drinking water during the acclimation period. Commercial feeding will be carried out in group P0 from the first day of acclimation to the end of the treatment, and in groups P1 – P5, commercial feeding will only be carried out during the acclimation period. High-fat feed was given 30 grams per day to groups P1 – P5 for 32 days after the acclimation period, while duck egg yolks were given once every two days in the morning orally as much as 2.5 ml/200g BW. Mahoni seeds ethanol extract and simvastatin were given orally every evening for 32 days. Measurement of daily feed and water consumption was taken every day. The Rats were weighed every week to determine weight gain rats body.

Collecting Blood Sample

Animal models were put into a closed container and anesthetized using cotton that had been moistened with chloroform until they fainted, then abdominal dissection was taken until the rat's heart was visible. Rat blood was taken from the heart using a syringe with a size of 3 ml, then immediately put in the EDTA tube and homogenized by rotating the EDTA tube slowly and carefully. Measurement of SGOT-SGPT levels using the Kinetic UV International Federation of Clinical Chemistry (IFCC) method.

Data Analysis

Data from each parameter were analyzed using Kruskal-Wallis and followed by the Mann-Whitney test with a 95% confidence level. The Significance value is if the variables analyzed have P <0.05. Statistical analysis using the IBM SPSS Statistics 26 program.

RESULT AND DISCUSSION

The high-fat diet used in this study was a mixture of commercial feed with cooking oil and duck egg yolk that administered by an oral tube. Reheated cooking oil will break the double bonds and making reused cooking oil contained a high free fatty acid which form the free radicals and an increase in trans fatty acids (Leong, 2021). The average amount of trans fatty acids increased with increasing frequency of frying, while duck eggs contained 9.30-11.80% protein, 11.40-13.52% fat, 1.50-1.74% sugar, and ash levels 1.10-1.17%. (Ganesan et al., 2014). Egg yolk lipids consist of 62% triglycerides, 33% phospholipids, and 5% cholesterol (Cao et al., 2021).

Data on daily feed and water consumption was obtained by calculating the difference between the amount of feed and water given to the waste. Data collection for daily feed and water consumption was taken every day during the treatment.

Table 1. The mean of daily feed and water consumption

Groups	Variable	
	Feed Consumption	Water Consumption
P0	18.12^{a}±1.07	37.24^{a}±7.91
P1	11.34^{a}±0.72	34.34^{a}±9.44
P2	11.20^{a}±1.68	33.07^{a}±9.91
P3	12.95^{a}±4.44	37.35^{a}±9.64
P4	13.57^{a}±3.59	36.59^{a}±6.38
P5	11.93^{a}±1.98	33.18^{a}±6.69

[a] Numbers that have the same superscript in the same column show no significant difference (P>0.05)

Feed consumption for each animal is different, it depends on body weight, production level, stress level, animal activity, mortality, energy content in feed, and ambient temperature (Sirotkin et al., 2021). The results of the Kruskal Wallis test on daily feed and water consumption obtained a value of P> 0.05 which indicated that there was no significant difference in giving mahogany seeds ethanol extract on the daily consumption of feed and water of male white rats induced by high feed fat.

Administration of the material treated with the amount given did not have a significant difference in the average daily consumption of feed and water. This shows that there was no change in the appetite and drinking of the rats when given the ethanol extract of mahogany seeds.

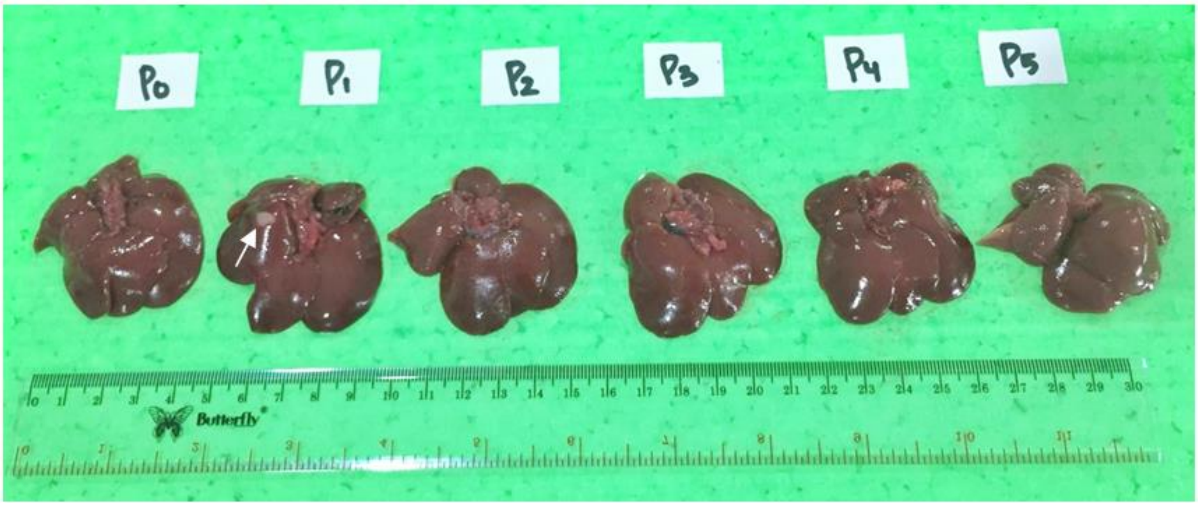

Figure 1. Macroscopic appearance of rat livers after treatment for 32 days. White arrows indicate fat lumps.

Macroscopic observations of rat livers in all treatment groups showed a reddish-brown color that looked the same. The liver surfaces of P0, P2, P3, P4 and P5 groups looked fairly smooth which showed the characteristics of a normal liver but the liver at P1 was found to have a white lump on the upper surface of the liver which indicated the condition of fatty liver (Figure 1). This is caused by the continuous feeding of high-fat feed. The result of this study was in line with research that conducted by Fan et al. (2020) who got the liver in the group that was fed a high-fat feed had granular lumps on the surface which indicated high fat deposition in the liver.

One of the indicators of liver function disorder is increased levels of liver enzymes in serum. The test used to detect an increase in transaminase enzymes is by examining Aspartate Aminotransferase (AST) or Serum Glutamic Oxaloacetate Transaminase (SGOT) and Alanine Aminotransferase (ALT) or Serum Glutamic Pyruvate Transaminase (SGPT), which in normal conditions SGOT levels in the blood are 39-92 U/L and SGPT levels are 17-50 U/L (Hidayat & Wulandari, 2021).

SGOT is a mitochondrial enzyme released from the heart, liver, muscles, and kidneys. This enzyme catalyzes the transfer of an amino group from aspartate to α-ketoglutarate (Kumar & Gill, 2018). Another enzyme, SGPT, is a cytosolic enzyme in the body, especially in the liver. Both of these serum increases according to inflammation or hepatic cell necrosis. Examination of transaminase enzymes is helpful in the early diagnosis of liver cell damage.

The mechanism for increasing the SGOT and SGPT enzymes is caused by the entry of excessive toxic substances into the body which will then be metabolized by cytochrome P-450 enzymes in the liver into free radicals (Mitra et al., 2020). These free radicals then bind to hepatocyte cells so that the hepatic membrane changes its permeability, these changes trigger damage to the hepatocyte membrane which will cause enzymes released by hepatocytes to be more easily transferred into the bloodstream (Chen et al., 2020).

Table 2. SGOT and SGPT levels in male white rats after administration of ethanol extract of mahogany seeds

Group	Variable	
	SGOT (IU/L)	SGPT (IU/L)
P0	$75.40^a \pm 11.46$	$34.40^a \pm 1.51$
P1	$93.80^a \pm 28.82$	$45.60^a \pm 15.71$
P2	$91^a \pm 36.24$	$34.80^a \pm 6.72$
P3	$81.40^a \pm 24.50$	$40.60^a \pm 5.32$
P4	$86.20^a \pm 26.16$	$39.20^a \pm 8.26$
P5	$81.80^a \pm 12.79$	$35^a \pm 3.24$

[a] Numbers that have the same superscript in the same column show no significant difference (P>0.05)

The results of the Kruskal Wallis test for SGOT and SGPT obtained P> 0.05 which showed that there was no significant difference in giving mahogany seeds ethanol extract on SGOT and SGPT levels in male white rats induced by high-fat diet. The highest average SGOT and SGPT contained in rat livers were in the P1 group, this indicated that the P1 group had the highest effect on liver function disorders compared to the other treatment groups. SGOT levels in P1 obtained values higher than normal levels, while SGPT levels in all groups showed values that were within the normal range.

Based on the data obtained, the treatment group that was given ethanol extract of mahogany seeds descriptively showed a decrease in SGOT and SGPT levels but statistically, the decrease was not significantly different. This indicated that mahogany seeds ethanol extract was not toxic and had hepatoprotective properties. The Statin class used in this study was simvastatin as the cholesterol-lowering agent in the P2 group. From the results, we can observe that mahogany seeds ethanol extract can balance the ability of statins as standardized medicine in maintaining liver function.

P2 as a group of rats given a high-fat feed and simvastatin 8 mg/200 gBW showed SGOT level was 91 ± 36.24 IU/L and SGPT level was 34.80 ± 6.72 IU/L. Simvastatin is a standardized drug commonly consumed for treating hyperlipidemia to reduce total and LDL cholesterol (Rahman et al., 2022). The mode of action of simvastatin is to inhibit the HMG-CoA reductase enzyme activity, which is the precursor of cholesterol synthesis, where it inhibits the first step in the mevalonate pathway in cholesterol synthesis, increases the affinity of the LDL receptor and the speed of LDL catabolism as well as the extraction of hepatic LDL precursors so that plasma LDL levels decrease (Miyajima et al., 2022). Therefore the result of SGOT and SGPT levels in this group still within the normal range.

The ethanol extract of mahogany seeds contains flavonoids, tannins, triterpenoids, saponins, and alkaloids (Sukardiman & Ervina, 2020) which can act as antioxidants. Antioxidant compounds are able to capture and neutralize free radicals by breaking free radical chain reactions by donating hydrogen atoms to form radical-antioxidant bonds with more stable properties to prevent further damage (Suwignyo et al., 2023).

Flavonoid can inhibit the activity of Acyl-CoAcholesterol acyltransferase (ACAT) and HMG-CoA reductase which function to catalyze the formation of mevalonate in cholesterol biosynthesis, when its work is inhibited, the formation of cholesterol by the liver is also hampered so that cholesterol levels in the blood decrease and LDL levels as cholesterol carriers also decrease (Zeka et al., 2017). Alkaloids can inhibit the work of the pancreatic lipase enzyme resulting in increased fat secretion through the feces (Rajan et al., 2020). The absorption of cholesterol in the intestine can also be inhibited by the activity of tannin compounds by reacting with mucosal proteins and intestinal epithelial cells. Tannin in the body will bind to the body's protein and will coat the intestinal wall so that the absorption of fat is hampered. This causes the formation of cholesterol in the liver to be inhibited and the absorption of cholesterol in the intestine is also inhibited, thus causing a decrease in total cholesterol and triglyceride levels in the blood (Ge et al., 2016)

The mahogany seeds ethanol extract might have inhibited the oxidative stress reaction in the animal models. The ethanol extract of mahogany seeds has an effect as an anti-free radical. The antioxidant compounds present in the ethanol extract of mahogany seeds have a protective effect against hepatotoxicity. Flavonoids work by suppressing the cytochrome P-450 enzyme system so that they inhibit the formation of free radicals (Sunil & Xu, 2019). Flavonoids are antioxidants because they have a phenolic hydroxy group in their molecular structure which has the ability to catch free radicals by removing hydrogen atoms and forming radical compounds that are more stable and non-reactive (Suwignyo et al., 2023). In this study, mahogany seed was tested to validate its use as hepatoprotective agent. The presence of antioxidant compounds that contained in mahogany seeds ethanol extract was able to maintain the levels of SGOT and SGPT in rats which fed by a high-fat diet in order to prevent increased levels of liver enzyme marker in blood serum and remain in normal amounts.

CONCLUSION

Based on the research that has been done, it can be concluded that mahogany seeds ethanol extract depicted its hepatoprotective effect in maintaining normal levels of liver enzymes marker as measured by SGOT and SGPT levels. Thus this study provides a plausible explanation that this plant showed hepatoprotective activity attributed to its phytochemical constituents. Suggestion for further research is to analyze the antioxidant potential of the ethanol extract of mahogany seeds on the activity of the superoxide dismutase enzyme in counteracting free radicals.

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