journal homepage: https://ojs3.unpatti.ac.id/index.php/biopendix

B I O P E N D I X P-ISSN:2407-4969
Volume 12, Number 1, March 2025 E-ISSN:2684-8341
Pages: 1-6 DOI:https://doi.org/10.30598/biopendixvol12issue1page1-6

Effect of Giving Ethanol Extract of Palm Fruit (*Arenga pinnata* Merr.) on The Lipid Profile of White Rats (*Rattus norvegicus* L.) Induced by Carbon Tetrachloride (CCl_4)

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Submitted: March 12, 2025; Revised: March 15, 2025; Accepted: March 18, 2025; Published: March 31, 2025

Abstract. Carbon tetrachloride (CCl_4) is a chemical substance in the form of a clear liquid, volatile and toxic with a sweet odor. Palm fruit (*Arenga pinnata* Merr.) is a plant that has high antioxidants and is able to reduce the effects of free radicals in the body. The purpose of this study was to evaluate the impact of ethanol administration of palm fruit (*Arenga pinnata* Merr.) on total cholesterol, triglyceride, HDL and LDL levels of white rats (*Rattus norvegicus* L.) which were induced by carbon tetrachloride (CCl_4). This study used a completely randomized design with 20 mice divided into 5 treatments and 4 replications. Negative controls were given food and drink, positive controls were given carbon tetrachloride 1 mL/kg BW + olive oil ratio 1:3, Treatments 1,2, and 3 were given carbon tetrachloride 1 mL/kg BW + olive oil with a ratio 1:3 + palm fruit ethanol extract in graded doses (P1=90, P2=180, and P3=360 mg/kg BW) for 15 days. Data analysis used one way ANOVA and continued with the Duncan test. The results showed that 1 mL/kg BW of carbon tetrachloride could damage the lipid profile of white rats. Giving ethanol extract of palm fruit (*Arenga pinnata* Merr.) to P1 and P2 at a dose of 90 mg/kg BW and 180 mg/kg BW had no significant effect. Meanwhile, P3 with a dose of 360 mg/kg BW had a significant effect on reducing total cholesterol, triglyceride and LDL levels and could significantly increase HDL levels at a significant level ($p<0.05$).

Keywords: *Arenga pinnata* Merr; Carbon tetrachloride (CCl_4); lipid profile

INTRODUCTION

Carbon tetrachloride (CCl_4) is a chemical substance in the form of a clear liquid that easily evaporates and has a sweet smell. Carbon tetrachloride is toxic and is widely used, especially in the refrigeration industry, fire extinguishers, as a pesticide, and as a stain remover (Elsawy et al., 2019). Carbon tetrachloride (CCl_4) has been extensively used to study hepatotoxicity in animal models. In the body, carbon tetrachloride (CCl_4) is metabolized by cytochrome enzymes, leading to the formation of trichloromethyl radicals (CCl_3). These highly reactive radicals can interact with cellular components such as nucleic acids, proteins, and lipids, interfering with crucial cellular functions, including lipid metabolism (Elgawish et al., 2015). They also induce an increase in the concentration of highly reactive lipid peroxyl and free peroxide radicals. The rise in lipid peroxidation of unsaturated fatty acids and the concentration of free peroxide radicals can lead to changes in lipid profiles and a decrease in antioxidant enzyme activity (Weber et al., 2003).

A lipid profile is an overview of lipid levels in the blood, serving as an indicator for detecting cardiovascular disorders (Rosiana & Widhiantara, 2020). Lipids play an essential role in the body as energy storage but can cause various health issues if present in excessive amounts. In the blood, lipids circulate as lipoproteins, which consist of cholesterol, triglycerides, phospholipids, and proteins (Domínguez et al., 2019). Lipid metabolism disorders, characterized by an increase or decrease in lipid fractions in the blood plasma, are known as dyslipidemia. This condition is caused by elevated levels of total cholesterol, LDL (*Low-Density Lipoprotein*), Triglycerides, and decreased HDL (*High-Density Lipoprotein)* levels in the blood (Senduk et al., 2016). Changes in metabolism and lipid profile damage can be caused by lipid and lipoprotein modifications, which are correlated with the production of free radicals induced by heavy metal contamination (Naru et al., 2023).

The sugar palm (*Arenga pinnata* Merr.) is a versatile plant that thrives in tropical and dry forest areas. This palm is an angiosperm species, meaning its seeds are enclosed within the fruit's flesh. Originating from Southeast Asia, it is well known for its year-round food production, especially during the dry season when other food sources are scarce. One of its most valuable products is sweet sap, commonly known as saguer, which can be consumed as a beverage or used as a raw material for sugar production. One particularly popular part of the sugar palm is its semi-mature endosperm, commonly referred to as buah enau or kolang-kaling. This fruit is widely consumed and holds high economic and cultural significance. However, its empirical use as an analgesic, antioxidant, and anti-inflammatory agent remains relatively low. Therefore, this study aims to analyze the phytochemical screening and antioxidant potential of sugar palm fruit extract as a novel therapeutic agent against free radicals in the body (Sovia & Anggraeny, 2019).

MATERIALS AND METHODS

This study was conducted from July to August 2024. The maintenance and treatment of experimental animals were carried out at the Zoology Laboratory, Faculty of Science and Technology, UINSU. Plant identification was conducted at the Herbarium Medanese Laboratory, FMIPA USU. The phytochemical screening of sugar palm fruit extract was performed at the Chemistry Laboratory, FMIPA USU. Blood sample collection took place at the Zoology Laboratory, Faculty of Science and Technology, UINSU, while the examination of total cholesterol, triglycerides, LDL, and HDL levels was conducted at the UPT. Health Laboratory, Jl. Williem Iskandar Pasar V Barat 1.

Materials

The equipment used in this study included an oven, blender, gastric probe, syringes, analytical balance, serum cups, hematocrit pipettes, surgical instrument set, micropipettes, spectrophotometer, rat cages, water bottles, feeding containers, jars, pins, knives, baking trays, and a steamer. The materials used included 20 male white rats (Rattus norvegicus L.), standard rat feed, ethanol extract of sugar palm fruit (Arenga pinnata Merr.), carbon tetrachloride (CCl_4), 96% ethanol, olive oil, distilled water, microtubes, and filter paper.

Methods

This study was an experimental research utilizing a Completely Randomized Design (CRD) with five treatment groups, each replicated five times.

K(-) : Standard rat feed only.
K(+) : Given carbon tetrachloride (1 mL/kg i.p) dissolved in olive oil at a 1:3 ratio and injected on days one, four, seven, ten, and thirteen for 16 days.
P1 : Given carbon tetrachloride & olive oil (1 mL/kg i.p) on days one, four, seven, ten, and thirteen for 16 days. Additionally, given sugar palm fruit extract (90 mg/kg p.o) daily from days 1 to 16.
P2 : Given carbon tetrachloride & olive oil (1 mL/kg i.p) on days one, four, seven, ten, and thirteen for 16 days. Additionally, given sugar palm fruit extract (180 mg/kg p.o) daily from days 1 to 16.
P3 : Given carbon tetrachloride & olive oil (1 mL/kg i.p) on days one, four, seven, ten, and thirteen for 16 days. Additionally, given sugar palm fruit extract (360 mg/kg p.o) daily from days 1 to 16.

Data analysis

The observed data on total cholesterol levels, triglycerides, HDL, and the calculated LDL values will be analyzed using a one-way ANOVA test with SPSS 29 (Statistical Product and Service Solutions). If a significant difference is found ($P<0.05$), the analysis will be followed by the Duncan Multiple Range Test (DMRT) to compare differences between groups.

RESULTS AND DISCUSSION

Results of Total Cholesterol Levels

The observation results showed differences in the average total cholesterol levels for each treatment group. The total cholesterol level observation the results are displayed in the Table 1.

Table 1. Results of Total Cholesterol

Group	Cholesterol (mg/dl) ± SD	P = Value
Negative Control	52.75 ± 2.06	
Positive Control	67.00 ± 9.69	
P1	61.00 ± 2.58	0.012
P2	65.75 ± 4.57	
P3	59.00 ± 3.55	

Based on Table 1, the one-way ANOVA test results for total cholesterol levels showed a significance level of ($p = 0.012$), indicating that the administration of ethanol extract of sugar palm fruit and carbon tetrachloride

(CCl_4) had a significant effect ($p < 0.05$) on the total cholesterol levels in white rats. The observations revealed a highly significant difference between the negative control group (52.75 ± 2.06), which received standard feed, and the positive control group (67.00 ± 9.69), which was administered carbon tetrachloride (CCl_4) at a dose of 1 mL/kg BW for 15 days. This result suggests that administering carbon tetrachloride (CCl_4) at this dose for 15 days significantly increases total cholesterol levels in the blood. The increase in total cholesterol levels in the negative control group after carbon tetrachloride induction was markedly different from the normal control. This is because carbon tetrachloride induces free radical formation, leading to oxidative stress or an imbalance between free radicals and antioxidants in the test animals' bodies. According to Table 1, P1 (61.00 ± 2.58) and P2 (65.75 ± 4.57) showed a significant difference compared to the negative control (52.75 ± 2.06), whereas P3 (59.00 ± 3.55) did not show a significant difference and had values close to the negative control (52.75 ± 2.06). This finding suggests that P3, with a dose of 360 mg/kg BW of ethanol extract of sugar palm fruit, is the most effective dose for reducing total cholesterol levels in white rats induced with carbon tetrachloride (CCl_4). The presence of secondary metabolite flavonoids in the ethanol extract of sugar palm fruit acts as an antioxidant, which contributes to reducing the free radicals produced by carbon tetrachloride (CCl_4) (Nadhira et al., 2023). These antioxidants are essential for shielding the body from free radicals and oxidative stress. Flavonoid compounds in sugar palm fruit extract function as antioxidants by regulating the equilibrium between oxidants and antioxidants, neutralizing free radicals by donating hydrogen ions, thus stabilizing the ions. This stable ion state reduces oxidative stress in tissues, helping to repair damaged cells (Fadillah & Febriani, 2024).

Results of Triglyceride Levels

The observation results showed differences in the average triglyceride levels for each treatment group. The triglyceride level observation results are presented in the following Table 2.

Table 2. Results of Triglyceride Levels

Group	Triglyceride (mg/dl) ± SD)	P = Value
Negative Control	66.25 ± 14.15	
Positive Control	118.75 ± 16.74	
P1	101.75 ± 26.70	0.008
P2	98.75 ± 11.44	
P3	88.25 ± 10.81	

Based on Table 2, the one-way ANOVA test results for triglyceride levels showed a significance level of ($p = 0.008$), indicating that the administration of ethanol extract of sugar palm fruit had a significant effect ($p < 0.05$) on reducing triglyceride levels in white rats induced with carbon tetrachloride (CCl_4). Carbon tetrachloride enters the body through body cavities and then diffuses into the bloodstream via capillaries. Inside the body, carbon tetrachloride is activated by cytochrome enzymes to form trichloromethyl free radicals, which create covalent bonds between reactive substances and cellular components. The formation of these free radicals disrupts essential cellular processes such as lipid metabolism and damages polyunsaturated fatty acids bound to phospholipids. The observation results for triglyceride levels showed a highly significant difference between the negative control group (66.25 ± 14.15) and the positive control group (118.75 ± 16.74). This finding suggests that The results indicate that the administration of carbon tetrachloride significantly increased triglyceride levels in the blood after induction at a dose of 1 mL/kg BW for 15 days. Based on Table 2, P1 (101.75 ± 26.70) and P2 (98.75 ± 11.44) showed significantly different values compared to the negative control (66.25 ± 14.15), whereas P3 (88.25 ± 10.81) was not significantly different from the negative control (66.25 ± 14.15). This suggests that P3 (88.25 ± 10.81) had values close to the negative control (66.25 ± 14.15). These results indicate that administering ethanol extract of sugar palm fruit at a dose of 360 mg/kg BW is effective in reducing triglyceride levels in white rats induced with carbon tetrachloride (CCl_4). The decrease in triglyceride levels may be attributed to the presence of secondary metabolite compounds that act as antioxidants. These antioxidants donate their electrons and hydrogen to free radical molecules, converting them into more stable substances. This process disrupts the free radical chain reaction and inhibits oxidative stress, thereby reducing the free radicals generated by toxic substances (Vishnoi et al., 2018).

Results of HDL Levels

The observation results showed differences in the average HDL levels for each treatment group. The HDL level observation results are presented in the following Table 3.

Table 3. Results of HDL Levels

Group	HDL (mg/dl) ± SD)	P = Value
Negative Control	40.75 ± 0.95	0.001
Positive Control	35.50 ± 0.57	
P1	37.25 ± 1.70	
P2	36.50 ± 1.29	
P3	37.75 ± 1.70	

Based on Table 3, the results of the one-way ANOVA test for HDL (High-Density Lipoprotein) levels showed a significance level of ($p = 0.001$), demonstrating that the administration of ethanol extract of sugar palm fruit had a significant effect ($p < 0.05$) on increasing HDL levels in white rats induced with carbon tetrachloride (CCl_4). The observation results showed a highly significant difference between the negative control group (40.75 ± 0.95) and the positive control group (35.50 ± 0.57). This indicates that carbon tetrachloride (CCl_4) significantly reduced HDL levels in white rats after induction at a dose of 1 mL/kg BW for 15 days. Based on Table 3, P1 (37.25 ± 1.70) and P2 (36.50 ± 1.29) showed significantly different values compared to the negative control (40.75 ± 0.95), whereas P3 (37.75 ± 1.70) was not significantly different from the negative control (40.75 ± 0.95). This suggests that P3 (37.75 ± 1.70) had values close to the negative control (40.75 ± 0.95). These findings indicate that administering ethanol extract of sugar palm fruit at a dose of 360 mg/kg BW is effective in increasing HDL levels in white rats induced with carbon tetrachloride (CCl_4). The increase in HDL levels is attributed to the presence of secondary metabolite compounds in the ethanol extract of sugar palm fruit, which function as antioxidants (Ogbuehi et al., 2014). Secondary metabolite testing confirmed the presence of flavonoid compounds in the sugar palm fruit extract, which act as antioxidants and enhance immunomodulatory responses by promoting cytokine production in response to toxic substance induction. Additionally, these compounds contribute to cell regeneration, reducing the activity of free radicals generated by toxic substances (Ichsan, 2022).

Results of LDL Levels

The observation results showed differences in the average LDL levels for each treatment group. The LDL level observation results are presented in the following Table 4.

Table 4. Results of LDL Levels

Group	LDL (mg/dl) ± SD)	P = Value
Negative Control	2.25 ± 1.50	0.003
Positive Control	7.25 ± 1.25	
P1	6.75 ± 1.70	
P2	5.50 ± 2.08	
P3	4.75 ± 0.95	

Based on Table 4, the results of the one-way ANOVA test on LDL levels showed a significance level of ($p=0.003$), indicating that the administration of ethanol extract of sugar palm fruit had a significant effect ($p<0.05$) on reducing LDL levels in white rats induced with carbon tetrachloride (CCl_4). The observation results of LDL levels showed a significant difference between the negative control group (2.25 ± 1.50) and the positive control group (7.25 ± 1.25). This finding suggests that administering carbon tetrachloride at a dose of 1 mL/kg BW every three days for 15 days can significantly increase LDL levels in white rats. Based on Table 4, LDL levels in P1 (6.75 ± 1.70) and P2 (5.50 ± 2.08) were significantly different from the negative control (2.25 ± 1.50). Meanwhile, P3 (4.75 ± 0.95) showed no significant difference from the negative control (2.25 ± 1.50), indicating that P3 (4.75 ± 0.95) had values close to those of the negative control (2.25 ± 1.50). This effect is attributed to the presence of secondary metabolites in the ethanol extract of sugar palm fruit, specifically alkaloids and flavonoids. Flavonoids function as inhibitors of the HMG-CoA reductase enzyme, which plays a role in cholesterol synthesis. When cholesterol is transported from the intestines to the liver, HMG-CoA reductase converts acetyl-CoA into mevalonate during cholesterol synthesis. Inhibiting this process reduces cholesterol synthesis and increases LDL receptors in extrahepatic tissues and liver cell membranes. Reduced cholesterol synthesis leads to a decrease in total cholesterol levels and LDL levels, which transport lipids in the blood (Krishnaveni & Gowda, 2015). Alkaloids in sugar palm fruit extract act as antioxidants by donating hydrogen ions. Additionally, alkaloids can inhibit the activity of pancreatic lipase enzymes, leading to increased fat excretion through feces. As a result, fat absorption in the liver is inhibited, preventing its conversion into cholesterol. The reduced cholesterol absorption subsequently lowers LDL levels (Kusumawardani et al., 2024).

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my beloved parents, my father Amir Husein Rambe and my mother Erlina Ritonga, for their unwavering support and immense love, which have enabled me to complete my final project successfully. I would also like to extend my heartfelt thanks to all the lecturers and teaching staff of the Faculty of Science and Technology, Biology Study Program, at the State Islamic University of North Sumatra, Medan, for equipping me with the knowledge that has helped me complete my final project well.

CONCLUSION

Based on the research conducted on the effect of ethanol extract of sugar palm fruit (*Arenga pinnata* Merr.) on the lipid profile of white rats (*Rattus norvegicus* L.) induced with carbon tetrachloride, the following conclusions were drawn:

1. The delivery of ethanol extract of sugar palm fruit (*Arenga pinnata* Merr.) significantly reduced total cholesterol levels in white rats (*Rattus norvegicus* L.) induced with carbon tetrachloride, with a dose of 360 mg/kg BW being the most optimal.
2. The delivery of ethanol extract of sugar palm fruit (*Arenga pinnata* Merr.) significantly reduced triglyceride levels in white rats (*Rattus norvegicus* L.) induced with carbon tetrachloride, with a dose of 360 mg/kg BW being the most optimal.
3. The delivery of ethanol extract of sugar palm fruit (*Arenga pinnata* Merr.) significantly increased HDL (*High-Density Lipoprotein*) levels in white rats (*Rattus norvegicus* L.) induced with carbon tetrachloride, with a dose of 360 mg/kg BW being the most optimal.
4. The delivery of ethanol extract of sugar palm fruit (*Arenga pinnata* Merr.) significantly reduced LDL (*Low-Density Lipoprotein*) levels in white rats (*Rattus norvegicus* L.) induced with carbon tetrachloride, with a dose of 360 mg/kg BW being the most optimal.

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