Comparison of the effectiveness of red rice syrup (oriza nivara) and acacia honey (acacia carpa) on lipid profiles in white rats (rattus norvegicus) and histopathological features of the liver induced by alloxan

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Comparison of the effectiveness of red rice syrup (oriza nivara) and acacia honey (acacia carpa) on lipid profiles in white rats (rattus norvegicus) and histopathological features of the liver induced by alloxan

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Abstract

Background: Rice is a staple food for almost all people across the Asian continent and contributes more than 22% of the world's energy intake. For Asian countries, rice is a primary staple food. Most of the food produced in rural Indonesia is a source of carbohydrates, such as corn, rice, and tubers. Indonesia as a tropical country is home to various types of plants with diverse nectar-producing potential. Various types of nectar from various plants have different nutritional content. Honey is an important natural product that has been used since ancient times for various medicinal purposes. In addition to its important role in traditional medicine, scientists also recognize honey as an effective new medicine for various diseases.
Purpose: To compare the effectiveness of brown rice syrup (Oryza Nivara) and Acacia honey (Acacia Carpa) on lipid profile in white rice (Rattus Norvegicus) and histopathological characteristics of the liver induced by alloxan.
Method: This type of research is a true experiment with a post-test only design in the control group and the intervention group. Animal care and handling were carried out at the Laboratory of the Faculty of Pharmacy, University of North Sumatra in March-June 2024. The samples used in this study were healthy male Wistar rats weighing 150-250 grams aged 8-12 weeks. The independent variables studied were brown rice syrup and acacia honey, while the dependent variable was the histopathological picture of the liver of male Wistar rats induced by alloxan.
Results: One-way ANOVA statistical test on days 0, 14, and 28 showed a p value <0.05. This shows that the brown rice syrup extract is effective against triglyceride levels. While acacia honey shows a p value > 0.05. This shows that acacia honey extract is not effective against Mandja Roenigk.
Conclusion: There is effectiveness of brown rice syrup extract on carbohydrates in Wistar rats induced with alloxan. However, there is no effectiveness of acacia honey extract on carbohydrates in Wistar rats induced with alloxan.

Keywords: Alloxan; Acacia Honey; Brown Rice Syrup; Histopathological Features of the Liver; Lipid Profiles; White Rats.

INTRODUCTION

Rice is a staple food for almost all people across the Asian continent and contributes more than 22% of the world's energy intake. For Asian countries, rice is a primary staple food. Most of the food produced in rural Indonesia is a source of carbohydrates, such as corn, rice, and tubers (Bandumula, 2018; Lin, Yu, Wen, & Liu, 2022). Brown rice, Oriza Nivara, is a plant rich in carbohydrates, fats, proteins, fibers, and minerals, and it contains flavonoid compounds that have the potential to be used as a diabetes treatment by lowering blood glucose levels through increased insulin secretion and preventing insulin resistance (Panjaitan, Dewi, & Khairani, 2023).

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Indonesia as a tropical country is home to various types of plants with diverse nectar-producing potential. Various types of nectar from various plants have different nutritional content. Nectar is the main ingredient used by bees to be converted into honey (Bugarova, Godocikova, Bucekova, Brodschneider, & Majtan, 2021). Honey is a sweet liquid produced by bees by processing flower nectar from flowers and other plant parts (extrafloral) and storing it in honeycomb cells. Nectar is a complex compound produced in the form of a sugar solution by plant glands (Kumar, Agrawal, & Hajam, 2021). Honey is widely used in everyday life, not only consumed directly but also used in the food, beverage, pharmaceutical, cosmetic, and herbal medicine industries because it contains various types of vitamins, minerals, and sugar (Nasharuddin, Sunaryo, & Puspitarini, 2022).

Honey is an important natural product that has been used since ancient times for various medicinal purposes. In addition to its important role in traditional medicine, scientists also recognize honey as an effective new medicine for various diseases. Iron can synthesize heme production and increase hemoglobin levels. Acacia honey increases red blood cells in the body, increases blood flow, and improves circulation. Honey contains carbohydrates, proteins, vitamins A, B1, B3, B12, B5, C, D, E, K, beta carotene, minerals, salts, iron, sulfur, magnesium, calcium, potassium, sodium, phosphorus, and antibiotics. Honey also contains antioxidants and digestive enzymes. Vitamin C in honey supports iron absorption, while vitamins B12 and folate support the formation of new cells (Wahyuni, Widowati, & Dahlan, 2023). Honey has high levels of antioxidants and plays an important role in lowering cholesterol because antioxidants can inhibit the absorption of cholesterol deeper into the blood. These antioxidants can also convert cholesterol into bile salts in the liver and release them into the intestines, which are then excreted through feces (Ranneh, Akim, Hamid, Khazaai, Fadel, Zakaria, & Bakar, 2021).

Health problems have shifted from infectious diseases to degenerative diseases. This is thought to be caused by changes in lifestyle, diet, environmental factors, lack of physical activity, and stress factors. A sedentary lifestyle, excessive intake of fatty and cholesterol-rich foods, and lack of fiber can trigger degenerative diseases. In people with hypercholesterolemia, this condition is usually found in adults. In men, cholesterol levels tend to increase between the ages of 35-50 years. Total cholesterol levels can be influenced by food intake, especially foods that are sources of fat. An increase in fat intake by 100 mg/day can increase total cholesterol by 2 to 3 mg/dL, which can have a significant impact (Yani, 2015; Bennett, Reeves, Billman, & Sturmberg, 2018).

Hypercholesterolemia is a disorder of cholesterol metabolism characterized by blood cholesterol levels above normal limits (Sniderman, Tsimikas, & Fazio, 2014). Characterized by an increase in total cholesterol levels accompanied by an increase in plasma low-density lipoprotein (LDL) levels in the blood. High cholesterol levels in the body can be caused by increased cholesterol synthesis and absorption (Schade, Shey, & Eaton, 2020). If cholesterol levels in the blood are higher than normal, it can trigger various diseases. High serum cholesterol (hypercholesterolemia) is known to increase the risk of atherosclerosis and coronary heart disease (Yusuf, Paramata, & Rahma, 2021).

Cholesterol is a neutral fat used by the liver to synthesize hormones and folate. Cholesterol is found in tissues and plasma in the form of plaque or free cholesterol, lipoproteins transport both forms into the plasma (Mo, Chen, Liu, Tang, & Yi, 2016). The four main groups of lipoproteins are chylomicrons, very low-density lipoproteins (VLDL), LDL, and high-density lipoproteins (HDL). Each lipoprotein has a different function and is broken down and processed differently (Hidayati, Kumalasari, Kusumawati, & Andyarini, 2020).

RESEARCH METHOD

This type of research is a true experiment with a post-test only design in the control group and the intervention group. The care and handling of experimental animals were carried out at the Laboratory of the Faculty of Pharmacy, University of North Sumatra in March-June 2024. The samples used in this study were healthy male Wistar rats weighing 150-250 grams aged 8-12 weeks. Male Wistar rats were used because their body size is relatively stable and have more consistent body metabolic reactivity to various chemical exposures or drug exposures and help avoid variations caused by

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hormonal cycles that occur in female rats that can affect drug responses, metabolism, and behavior that can inhibit research results. The independent variables studied were brown rice syrup and acacia honey, while the dependent variable was the histopathological picture of the liver of male Wistar rats induced by alloxan.

The research equipment used were cages, food and drink containers, water, syringes, oral probes, droppers, scissors, cotton, ether, analytical scales, rat scales, filter paper, micropipettes (Sacorex), tissues, petri dishes, beakers, glass funnels, blenders, rotary evaporators, bactosonics, centrifuges, Ethylene Diamine Tetra Acetic Acid (EDTA) tubes, glucometers, and lipid pro meters. The materials used were Oriza Nivara brown rice syrup extract, Acacia Carpa acacia honey, gloves, male Wistar rats, standard feed, drinking water, alloxan, 70% alcohol, sample pots and chloroform.

This research includes the manufacture of brown rice syrup, the formation of research groups, preparation and treatment of experimental animals, alloxan induction in experimental animals, lipid profile testing (HDL, LDL, triglycerides, total cholesterol), the manufacture of histopathology preparations of male white rat livers, liver damage assessment, and analysis of research data using SPSS software.

The work procedure starts from day 0, namely selecting male Wistar rats aged 8-12 months, weighing 150-250 grams and in good health. In addition, initial blood glucose levels were measured, then 28 rats with normal blood sugar levels were taken and adapted for 7 days in a cage that had been given basic and standard feed and drinking water was available. On the 7th day, the samples were grouped into 4 treatment groups, with the number of samples according to the Federer formula, namely 6 Wistar rats for each group. Then 1 experimental animal was added to each group to avoid the drop out criteria, namely the experimental animal died.

Group 1 (K-) is a negative control group that is given standard feed, drinking water, and 30 mg/dl alloxan injection. Group 2 (K+) is a positive control group that is given standard feed, drinking water, 30 mg/dl alloxan, and 0.5 gr/kgBW acacia honey. Group 3 (P1) is intervention group 1 which was given a small dose of brown rice syrup 5 mg/KgBW, drinking water, and alloxan injection 30 mg/dl. Group 4 (P2) is intervention group 2 which was given a large dose of brown rice syrup 15 mg/KgBW, drinking water, and alloxan injection 30 mg/dl.

The experiment was conducted for 28 days in each group by checking blood glucose levels after 3 days of alloxan induction and lipid profiles using pro lipids (HDL, LDL, Triglycerides, Total Cholesterol) on day 0, day 7, day 14, day 21, and day 28. On day 28, surgery was performed and the liver was removed for preparation.

Data analysis using statistical software with normality test, Shapiro-Wilk test is used to determine whether the data is normally distributed. Furthermore, homogeneity test is Levene's test to check whether there is a similarity of variance or not. Data that is normally distributed and homogeneous is continued with One-Way ANOVA parametric test with a confidence level of 95% with a value (p> 0.05. Furthermore, it is analyzed using the Post Hoc test to see the differences between groups. This research has received permission from the Health Research Ethics Committee (KEPK) University of Prima Indonesia with the number: 057/KEPK/UNPRI/V/2023.

RESEARCH RESULTS

(a) (b) (c)

Figure 1. (a) Process of cooking brown rice, (b) Mixing barley malt powder, (c) Fermentation results.

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Figure 1. The process of cooking brown rice by adding about 500 ml of water to the rice and stirring it until smooth. Heat to a temperature of 140°F, add about 250 ml of barley malt powder and stir well. Cook the rice for 1 hour, reduce the temperature to 120-130°F. Stir the rice occasionally for 2-3 minutes until the temperature drops to 135-145°F. Turn off the heat, cover the pot, and let it sit for 1 hour. Repeat this process after 4 hours with a fermentation time of 6 hours. Furthermore, the rice mixture will taste sweet and have a consistency similar to wet paper because the starch has been drained.

(a) (b)

Figure 2. (a) Filtering the rice mixture, (b) Result of cooking brown rice syrup.

Figure 2. shows the filtration process to obtain brown rice syrup. The filtered mixture is boiled for 80 minutes and stirred occasionally. Continue boiling for 10-20 minutes until bubbles form and the liquid thickens. Then remove the syrup and let it cool to room temperature.

Table 1. Results of Phytochemical Screening of Brown Rice Syrup

Type of Test	Reagent	Result (+/-)
Flavonoid	Mg dan HCl	(+)
Saponin	Aquadest	(+)
Tanin	$FeCl_3$ 1%	(-)
Alkaloid	Wagner	(-)
	Mayer	(-)
	Dragendorf	(-)
Triterpenoid	H_2SO_4	(+)
Glikosida	Molisch's Reagent Test	(+)

Based on Table 1. phytochemical testing shows that brown rice syrup contains flavonoids, saponins, triterpenoids, and glycosides, but does not contain tannins or alkaloids. Based on the results, brown rice syrup extract (Oriza Nivara) has an effect on triglycerides at day-0, day-14, and day-28 in alloxan-induced Wistar rats, while Acacia Honey (Acacia Carpa) also affects triglycerides in alloxan-induced Wistar rats.

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Table 2. Average Triglycerides with of Brown Rice Syrup Extract

Days	Extract Concentration	N	Trigliserida
D0	K-	3	71.66
	K+	3	117.00
	P1	3	313.66
	P2	3	237.66
D14	K-	3	112.66
	K+	3	239.33
	P1	3	196.33
	P2	3	179.33
D28	K-	3	106.33
	K+	3	206.00
	P1	3	172.33
	P2	3	154.00

In Table 2. it can be observed that on D1 the highest average triglyceride value was found in group P1 which received 5 mg of brown rice syrup, which was 313.66. On D14, the highest average value was also found in group P1, which was 196.33, and on D28, the highest average value was again found in group P1, which was 172.33.

Table 3. Average of Mandja Roenigk with Acacia Honey Extract

Extract Concentration	N	Trigliserida
K-	4	142.50
K+	4	167.25
P1	4	137.25
P2	4	158.50

In Table 3. it can be seen that the best administration of Acacia Honey Extract (Acacia Carpa) was in group P2, namely the administration of 15 mg of honey with an average Mandja Roenigk score of 158.50, while the positive control group obtained a score of 167.25.

Table 4. Normality Test of Brown Rice Syrup

Days	Shapiro-wilk		
	Statistic	Df	P-value
D0	0.886	12	0.104
D14	0.974	12	0.946
D28	0.894	12	0.134

Based on Table 4. the normality test in the brown rice syrup group on days 0, 14 and 28 obtained a p value > 0.05. So that the data distribution was proven to be normal, then data testing can be continued using the one way anova statistical test.

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Table 5. Normality Test of Acacia Honey Extract Groups

Group	Shapiro-wilk		
	Statistic	Df	P-value
K-	0.967	4	0.824
K+	0.846	4	0.213
P1	0.875	4	0.316
P2	0.742	4	0.053

Based on Table 5, the normality test in the acacia honey group obtained a p value > 0.05, thus proving the normal distribution of data. Furthermore, data testing can be continued using the one-way ANOVA statistical test.

Table 6. One-Way ANOVA Test of the Effectiveness of Brown Rice Syrup Extract

Days	Group	N	P-value
D0	K-	3	0.001
	K+	3	
	P1	3	
	P2	3	
D14	K-	3	0.011
	K+	3	
	P1	3	
	P2	3	
D28	K-	3	0.03
	K+	3	
	P1	3	
	P2	3	

Based on Table 6. The results of the one-way ANOVA statistical test on days 0, 14, and 28 showed a p value <0.05. This shows that the brown rice syrup extract is effective against triglyceride levels.

Table 7. One-Way ANOVA Test of the Effectiveness of Acacia Honey Extract

Group	*N*	*P-value*
K-	4	0.284
K+	4	
P1	4	
P2	4	

Based on Table 7. the administration of acacia honey shows a p value > 0.05. This shows that acacia honey extract is not effective against Mandja Roenigk.

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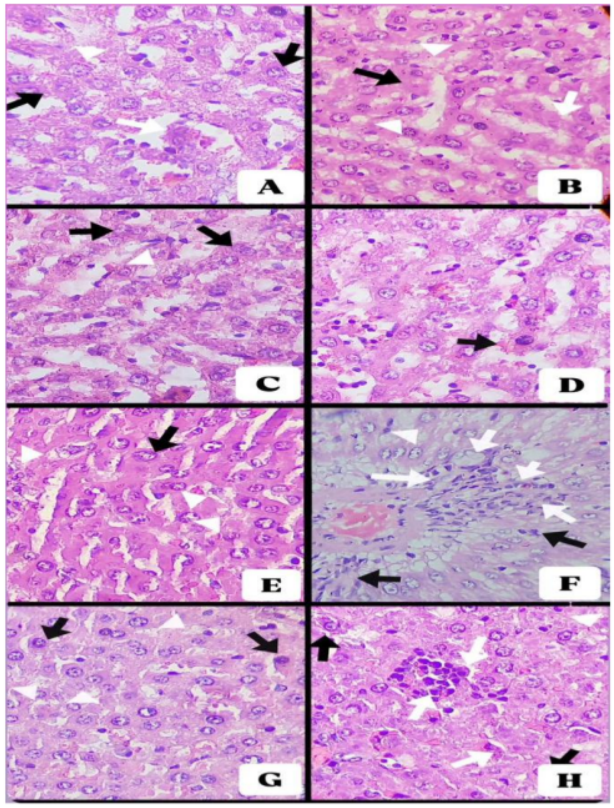

Figure 3. Histopathology Results of Rat Liver with Hematoxylin-eosin Staining at 400x Magnification

In Figure 3. (A) negative control day 0, (B) administration of acacia honey day 0, (C) brown rice syrup 5 mg day 0, (D) brown rice syrup 15 mg day 0, (E) negative control day 28, (F) administration of acacia honey day 28, (G) brown rice syrup 5 mg day 28, (H) brown rice syrup 15 mg day 28. White arrows are necrotic cells, white triangles are hydropic degeneration, and black arrows are parenchymal degeneration.

DISCUSSION

The results showed that the highest effectiveness of brown rice syrup extract (Oriza Nivara) was in group P1 with a dose of 5 mg brown rice syrup, where the triglyceride level was 313.66 on day 0. On day 14, the highest level was also found in group P1, which was 196.33 and on day 28, the highest average was again found in group P1, which was 172.33. Based on the one-way ANOVA statistical test of giving brown rice syrup extract on days 0, 14, and 28, a p value of <0.05 was obtained, so there was a significant effectiveness of brown rice syrup extract on triglycerides.

Similar research on the use of brown rice on total cholesterol levels in hypercholesteremia patients showed a significant difference in total cholesterol levels before and after administration of brown rice (Legi, Kawulusan, & Gedoan, 2019). This study is in line with a study comparing the effectiveness of black rice extract and brown rice extract on changes in the lipid profile of Wistar rats (Rattus norvegicus). The results of the study showed that the effect of black rice extract was no different from the effect of brown rice extract in lowering total cholesterol and triglycerides (Herlambang, Kapantow, & Kawengian, 2015).

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This is in accordance with the theory that the diet of people with high blood cholesterol levels includes a high intake of fiber, especially soluble fiber such as apples, brown rice, oatmeal, and nuts. Regular consumption of brown rice is very beneficial for health because it can lower LDL levels, prevent coronary heart disease, prevent cancer, and reduce the risk of diabetes (Ravichanthiran, Ma, Zhang, Cao, Wang, Muhammad, & Pan, 2018).

Based on the results of the study, the administration of acacia honey extract in group P2 was the most effective. This means that 15 mg honey has an average mangyalonic value of 158.50, compared to 167.25 in the positive control. The p-value for acacia honey> 0.05, indicating that acacia honey extract does not have a significant effect on Mangja Roenigk. Previous research examining the effects of honey on total cholesterol and triglyceride levels in male Wistar rats (Rattus norvegicus L.) induced with palm wine found a decrease in average total cholesterol and triglyceride levels after administration of honey with a p-value of 0.004 (Anwarul, Asfur, Irma, & Andina, 2019).

In contrast, research on the effects of Dorsata honey on plasma total cholesterol and LDL levels in hypercholesterolemic patients showed that Dorsata honey had a significant effect on plasma total cholesterol and LDL levels in hypercholesterolemic patients (Yusuf et al., 2021). This is in accordance with research showing that honey contains antioxidant compounds that can inhibit the lipid oxidation process. Inhibition of lipid oxidation causes suppression of the formation of acetyl-CoA, which plays a role in triglyceride biosynthesis, resulting in lower serum triglyceride levels (Daeli & Ardiaria, 2018).

CONCLUSION

There is effectiveness of brown rice syrup extract on carbohydrates in Wistar rats induced with alloxan. However, there is no effectiveness of acacia honey extract on carbohydrates in Wistar rats induced with alloxan.

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