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The Protective Effect of Rambutan Honey on Macroscopic and Microscopic Examination of Pancreas *Rattus norvegicus* Induced by Traditional Alcoholic Beverages Ciu

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Abstract

The traditional ciu alcoholic beverages is a drink made from distilled sap and sticky tape. Ciu is a type of drink that has an alcohol content of 25%. Alcoholic beverages will be toxic if consumed in excess. The pancreas is one of the organs that undergoes a process of damage when toxic materials or metabolites accumulate in the organ. Rambutan honey is a natural ingredient that contains flavonoid compounds. Flavonoids act as antioxidants which can reduce damage to pancreatic cells due to toxic substances. The aim of this study was to determine the protective effect of rambutan honey on the macroscopic and microscopic examination of white rats (*Rattus norvegicus*) induced by traditional ciu alcohol. This research is experimental with 5 treatment groups including 1 control normal, 1 control negative and 4 ciu and honey treatments. Pancreatic tissue was observed macroscopically for color and texture. Microscopic observations were observed in the form of degeneration and necrosis. The microscopic results showed that there was a color difference between the control group and the alcohol-induced honey treatment group. The results of microscopic observations were known to show degeneration and necrosis in the positive group and the treatment group. The results of the study based on the ANOVA test obtained <0.05. Based on the Anova test and Duncan test it can be concluded that rambutan honey has a protective effect on the pancreatic histopathology of *Rattus norvegicus* in the form of normal cells and degenerated cells in groups K2 and K3.

Keywords: Ciu, Pancreas, Macroscopic, Microscopic

Introduction

Alcoholic beverages are drinks that are consumed and used by a limited group of people for certain reasons and purposes, either positive or negative. The use of alcoholic beverages is to warm the body and overcome pain. Alcoholic beverages also increase the economy in the beverage industry. Alcoholic beverages contain OH hydroxyl groups and hydroxyl groups bonded to tetrahedral carbons. The content of alcohol itself is ethanol which functions as an antiseptic and solvent. Based on the manufacturing process, alcohol is divided into several types, including modern, traditional and mixed. One example of traditional alcohol is ciu. Ciu is a type of alcoholic drink that has an alcohol content of 25- 30%. Ciu is one of an alcoholic beverages that widely consumed by Indonesian people. Ciu consumption could damage body tissues

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and organs, because the result of alcohol metabolism is a reactive molecule that forms Reactive Oxygen Species (ROS) (Yusuf et al.,2021).

According to the WHO (World Health Organization) the latest data (2024-2025) shows that global alcohol consumption has decreased slightly but remains deadly, causing 2.6 million deaths in 2019 and increasing to around 3.3 million people per year (old report), with the younger generation most affected, while cases in the Western Pacific region reach 500,000 deaths per year. WHO emphasizes that alcohol triggers more than 200 chronic diseases (cancer, liver cirrhosis), accidents, and violence, and recommends a safe limit of <2 drinks/day for men and <1 for women.

Indonesia is the province that has the highest prevalence of alcoholic beverage use compared to other provinces, namely East Nusa Tenggara (NTT) of 17.7%, (Edo, 2019). The consumption of traditionally produced alcoholic beverages is very worrying because the levels of alcohol they contain are not controlled. Consuming alcoholic beverages has claimed many lives and has increased. The GHO-WHO noted that alcohol use causes 4 million deaths each year and contributes to 60 types of diseases that arise due to alcohol abuse (Pribadi, 2017). A byproduct of alcohol metabolism that damages the structure and function of the kidneys is ROS, a reactive molecule that can form free radicals, thus triggering damage to cells and tissues (Kaushal et al., 2019).

Excessive consumption of alcoholic beverages can cause disease in organs (Fan et al, 2019), including the pancreas. Moderate consumption of alcoholic beverages, namely 20 g/day, is reported to cause an increased risk of developing the disease. Autopsies of alcohol abuse have shown that 75% have chronic pancreatitis. Alcoholic beverages will be toxic if consumed in excess. Alcohol toxicity is defined as its ability to damage organs in the body. According to Edo et al (2019), administration of alcohol to the pancreatic organs of white rats found edema, bleeding, leukocyte infiltrate, and acinar cell necrosis. Alcohol causes damage to necrotic cells in rats (Faustinawati, 2016) and alcohol metabolism also causes oxidative stress . Ciu is a type of alcoholic beverage with an alcohol content of 25-30%. It is made from distilled palm sap and sticky rice tape. Drinking this ciu has a bitter taste and can cause a burning sensation in the throat (Ferryrahmat et al., 2016).

Oxidative stress that can cause pancreatic histopathological changes can be relieved with natural ingredients, such as honey. One of the compounds contained in honey is an anti-oxidant. In a study by Adityarini et al (2020) honey was used to improve oxidative stress in rats induced by toxic substances. A honey solution concentration of 0.4 mL/20 g was better at providing a protective role than 0.2 mL/20 g because the concentration of antioxidant compounds was higher so that it could balance the production of free radicals in toxic substances. Indonesia itself experiences almost 1 in 3 patients experiencing acute pancreatitis due to alcohol (Xiao, 2016).

Honey shows a protective effect on the pancreas against oxidative stress as evidenced by a decrease in lipid prooxidation levels. Honey also restores the activity of SOD (Superdioxide dismutase) and pancreatic catalase. This study aims to determine the protective effect of rambutan honey on macroscopic and microscopic examination of the pancreas of white rats (*Rattus norvegicus*) induced by traditional ciu alcoholic beverages. This research has passed an ethical review with letter no. KEPK/UMP/26/XII/2022.

Research Methods

Treatment of experimental animals

This research uses the type of experimental research. This study used 5 groups, 4 of which were treated with ciu traditional alcoholic drinks. Group 1 (K0) as a normal control, namely rats were not given alcohol and without natural ingredients. Group 2 (K1), namely the negative control group, was given 4 mL/200 g BB of alcohol to rats without giving natural ingredients. Group 3 (K2), namely the group that was given alcohol as much as 4 mL/200 g BB and honey with a concentration of 25% as much as 0.5 mL/200 g BB. Group 4 (K3), namely the group given 4 mL/200 g BB of alcohol and 0.5 mL/200 g BB of honey with a 50% concentration. Group 5 (K4), namely the group given alcohol as much as 4 mL/200 g BB, then honey with a concentration of 75% as much as 0.5 mL/200 g BB. The treatment was given for 21 days. The sampling technique for the rat pancreas was simple random sampling. The selection of white mice as experimental animals is due to the closeness of the human organ system to various types of white mice so that the research results obtained can describe the possible results in humans .

Preparation

The test animals were adapted for 7 days and treated for 21 days. Sampling was carried out on the 21st day. Pancreatic organ tissue was taken from alcohol-induced white rats (*Rattus norvegicus*) and then given honey. The preparation is carried out according to Didik (2021) the first step is washing the tissue with NaCl. This washing aims to remove blood, after that it is put into a tissue cassette then fixed with 10% NBF for 10 hours. The second step, namely Dehydration, is the preparation stage soaked in graded alcohol, namely 50% for 1.5 hours, 70% for 1.5 hours, and 96% (2 times) for 2 hours which aims to remove water from the tissue so that the tissue can be cut thin. The third step is Clearing where this process is carried out to remove the water-drawing agent and replace it with chemicals, in this process a solution of xylol I is used for 1 hour and xylol II for 2 hours. The next step is embedding. If the embedding has been done, the next step is cutting, namely slicing which begins with adjusting the thickness of the slices. For the pancreas cut to the size of um. The selected slices are taken with an object glass that has been coated and then dried. The next step was staining the preparations by incorporating the preparations into xylol (2 times). The preparations were then put into the alcohol for 5 minutes. The preparations were soaked in distilled water for 10 minutes. The preparation is dripped with hematoxylin for 5 minutes or until the best color results are obtained. The preparations are washed in running water. If it is finished, then it is then put in eosin alcohol dye for 2 minutes then the preparation is rinsed with xylol. The last step is mounting, mounting is the final stage where the entul process covers the tissue between the glass object and the cover glass by the entelan after the mounting stage is complete, carry out microscopic observations then analyze the damage to the pancreas.

The data source used in this study was primary data obtained by researchers directly from microscopic observation data on rats. The results of macroscopic and microscopic observations were then analyzed using SPSS

Research Results and Discussion

This study aims to determine whether or not there is an effect on the histopathological organ of the pancreas of white rats (*Rattus norvegicus*) Wistar strain (Komang *et al*., 2014) that have been given rambutan honey with concentrations of 25%, 50%, and 75% after being induced by traditional alcohol ciu and to find out the damage that occurs if administered in a predetermined dose and time with hematoxylin-eosin dye. The effect of honey was seen based on the macroscopic and microscopic appearance of the pancreatic histopathology of rats (*Rattus norvegicus*). The results obtained based on macroscopic and microscopic examinations were processed using the SPSS (Statistical Product and Service Solution) test.

Figure 1 Macroscopic Observation of Rat (Rattus norvegicus) Pancreas. Pancreas Macroscopic Appearance of White Rat (Rattus norvegicus) K0 = Normal control group, K1 =negative control group (Ciu), K2 = Ciu + 25% Honey, K3 = Ciu + 50% Honey,K4 = Ciu + Honey75%. Source: Primary Data

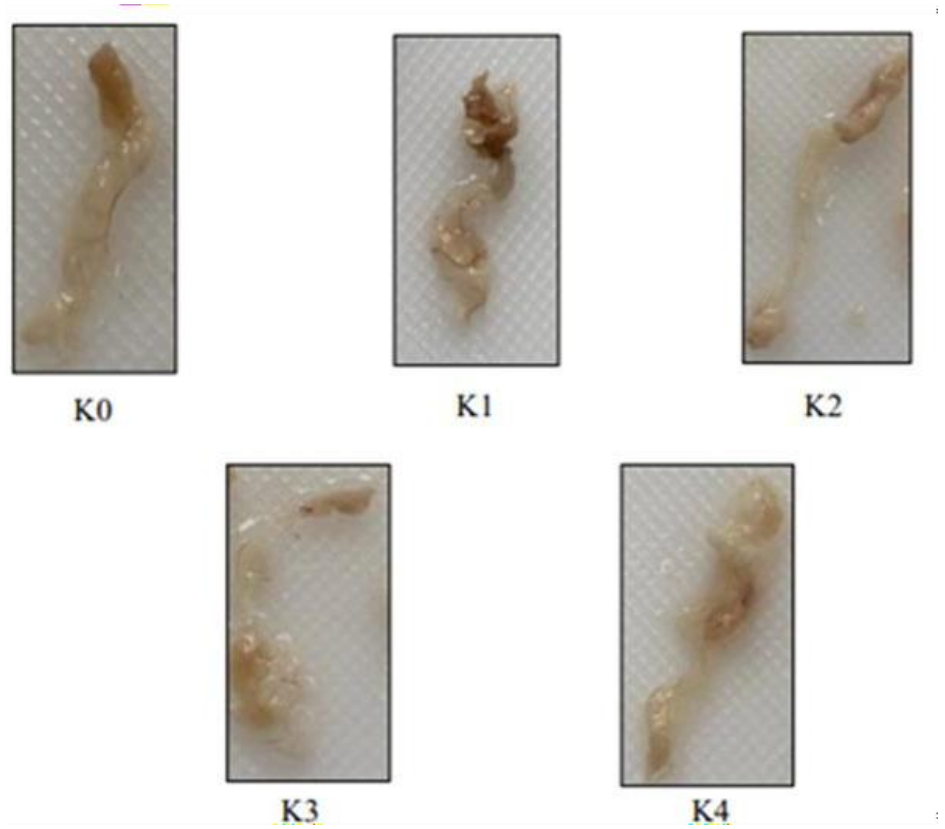

Table 1 Macroscopic Observation of Rat (*Rattus norvegicus*) Pancreas

Group	Color	Macroscopic		Microscopic
		Texture	Size	avarage
Normal Control (K0)	Yellowish White	Springy	1,5 x 0,5 cm	1,0
Negative Control (Ciu) (K1)	Yellowish White	Springy	1,3 x 0,3 cm	2,6
Ciu + Honey 25% (K2)	Yellowish White	Springy	1,3 x 0,5 cm	2,4
Ciu + Honey 50% (K3)	Yellowish White	Springy	1,2 x 0,6 cm	2,4
Ciu + Honey 75% (K4)	Yellowish White	Springy	1,2 x 0,5 cm	1,9

Figure 2. Microscopic Observation of Normal Rat (Rattus norvegicus) Pancreas Table 1. Macroscopic Observation Results of Rat (Rattus norvegicus) Pancreas. Description IL = Langerhans Islet, N = Normal Cell, M = Mucous acini, S = Serous acini. Hematoxylin-Eosinstaining at 400x magnification (Personal Documentation, 2023).

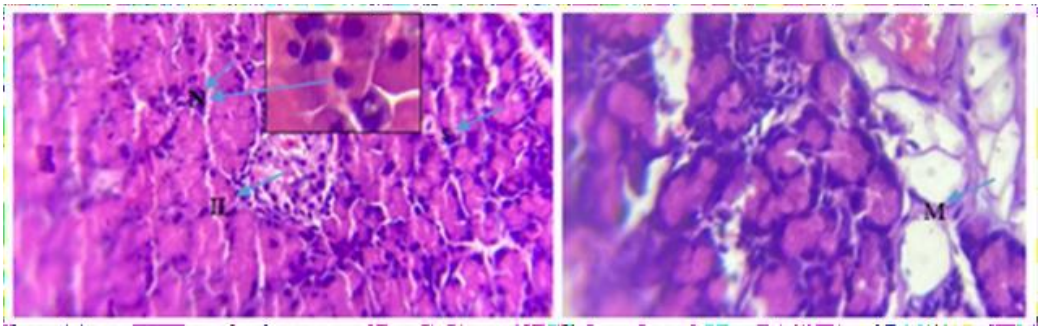

Figure 3. Microscopic Observation of the Rat (Rattus norvegicus) Pancreas Damage exists. K0 = normal control group, K1 = negative control group, K2 = ciugroup + 25% honey, K3 = ciu group + 50% honey, K4 = ciu group + 75% honey.

Description: IL = Ilset Langerhans, NK = Necrosis, D = Degeneration(Documentation Personal, 2023)

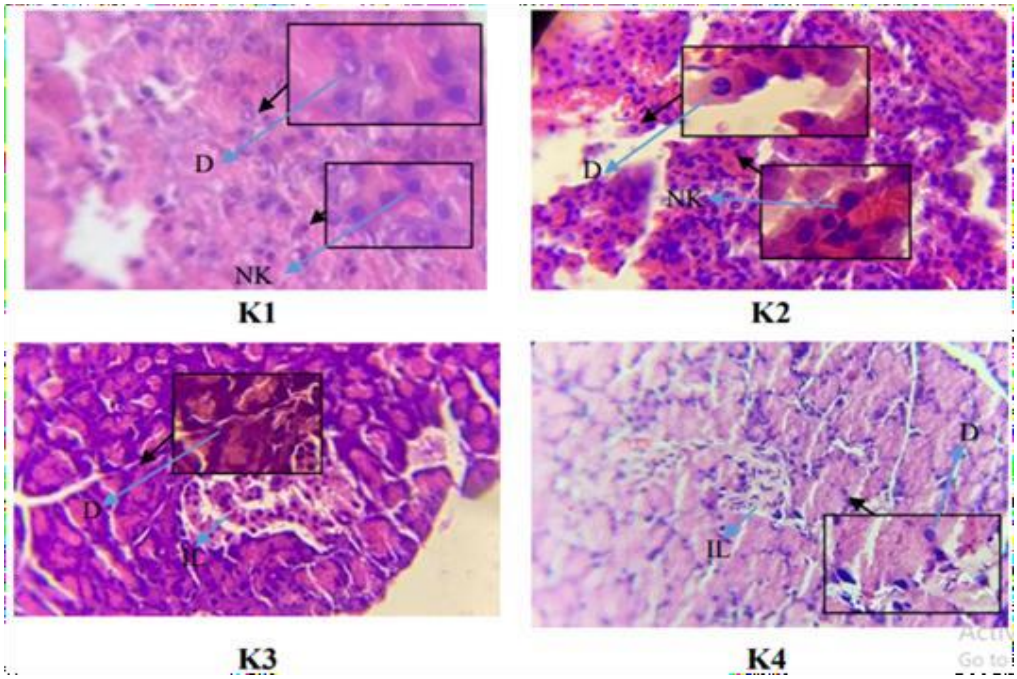

Table 3 Results of Anova Test Analysis

Treatment Test Results

	Sum of Squares	df	MeanSquare	F	Sig.
Between Groups	9,270	4	2,317	7,428	002
Within Groups	6,240	20	3,120		
Total	15,510	24			

Table 4 Post Hoc Analysis Results

	N	1	2	3	4
Normal Group (K0)	25	10.80			
Group Ciu + Honey 75% (K4)	25		19.60		
Group Ciu + Honey 50% (K3)	25			24.00	
Group Ciu + Honey 25% (K2)	25				26.40
Negative Group (Kiu) (K1)	25				27.60
Sig.		1,000	1,000	1,000	.296

The effect of giving rambutan honey to white rats that have been induced by ciu traditional alcohol is seen macroscopically and microscopically. Macroscopic observations include color and texture. There are changes in texture and size. In the normal control group (K0) the results were 1.5 x 0.5 cm. In the negative control group (K1) and the treatment group (K2, K3, K4) the results showed a change in size. Based on Guyton (1984) states that this is influenced by feed and drinking water because these are extrinsic factors. In groups (K0), (K1), (K2), (K3), (K4) the pancreas is yellowish white. Morphological observations showed no significant differences in each treatment. This happens because the possibility of damage that occurs is not visible macroscopically. In microscopic observation, the field of view was seen on each preparation and there were 5 preparations in each treatment. The results of microscopy observations were tested using the SPSS (Statistical Product and Service Solutions) test.

At the end of the study, the mice were terminated using cotton that had been treated with ether and left for a few minutes to ensure that the mice died. The dead rats were then operated on to get the pancreas organ. The pancreatic organs that have been taken are then made preparations and observed under a microscope for microscopic observation with a magnification of 400x and viewed in several fields of view (based on figures 2 and 3). The normal control preparations (K0) which have been observed showed no damage. In normal mice without being given treatment, the islands of Langerhans show a uniform shape and do not experience changes, besides that the condition of the islands of Langerhans is intact and tight. In preparations given the traditional ciu alcohol drink as much as 4 ml/200 g BW of rats for 21 days, degeneration and necrosis were found. This is in line with Edo's study (2019) which gave alcohol drinks for 10 days at a dose of 8 ml/kg BW to rats and found necrosis and edema in the rat pancreas. According to Yesi's research (2021) histopathological damage to the pancreas is characterized by changes in the shape of the pancreas in the form of shrinkage and reduction in the size of the islets of Langerhans.

The pancreas is a double gland consisting of an exocrine part that secretes pancreatic enzymes namely amylase, lipase, trypsin enzyme and an endocrine part that produces hormones. Pancreatic secretions are used to see the activity of the protease enzyme. The pancreas consists of two main types of tissue, namely acini and islets of Langerhans. The presence of ethanol substances that enter the body in excess can cause the cells in the islets of Langerhans to experience damage. Degeneration is an endocrine cell disorder that affects the structures in the cell causing a reduction in cell mass and the endocrine arrangement becomes disorganized, becomes smaller, and some even disappears. Cell degeneration occurs due to disturbances that affect the structure in the cell and disrupt the cell's metabolic processes. Degeneration is reversible (temporary). Based on figures 2 and 3, degeneration occurred in groups K1, K2, K3, and K4. Necrosis is one of the basic patterns of cell death which is characterized by the presence of empty spaces in the islets of Langerhans. Necrosis is also characterized by the loss of the cell nucleus in pancreatic cells. In normal cells, the islets of Langerhans are intact and tight. Normal cells in the pancreas are round and have a nucleus in the middle(Nuralifah, 2022). The physical effects experienced from consuming alcoholic beverages include damage to the liver, kidneys, pancreas, lungs, heart, stomach and body metabolism (Lestari, 2016).

Free radicals are one source of oxidative stress. In the oxidative cycle, ADH and Cytocrome P450 work to catalyze alcohol into acetyldehida. When acetyldehyde is produced continuously it will cause an increase in ROS and depletion of glutathione ROS, increased ROS will cause DNA damage and also damage lipoproteins so that the pancreas will experience oxidative stress because the result of alcohol metabolism is a reactive molecule that forms Reactive Oxygen Species (ROS) (Yusuf et al.,2021). Oxidative stress is thought to have an impact on cell damage, causing cells to experience degeneration and necrosis.

The necrosis that occurred in the K1 group was different from the other test groups because the K1 group was only given the traditional alcoholic drink, ciu, which triggered the damage. The protective effect that occurred in groups K2, K3, and K4 had differences due to the administration of ciu traditional alcohol and rambutan honey with different concentrations. This situation indicates that the varying concentrations of honey make the effects obtained also vary.

In the ANOVA test based on table 2, the results obtained were p <0.05, which means that the data distribution had a significant difference between the control group and the treatment group so that it was continued with Duncan's Post Hoc test. Duncan's test results obtained normal control (K0) which was 10.80, Treatment group 3 (K4) obtained a value of 19.60, Treatment group 2 (K3) obtained a value of 24.00, Group 1 (K2) obtained a value of 26.40, and Negative control group (K1) obtained a value of 27.60. Based on Duncan's Post Hoc test, it produced different values between the normal control group (K0), the treatment group (K3) and (K4). The results also have the same value between the negative control group (K1) and treatment group 2 (K2). The value obtained can be concluded that the negative control group (K1) and treatment group 1 (K2) have the same damage value, indicating that giving 25% honey does not provide a protective effect. In the treatment group (K3) and (K4) have different values. Based on the results of the study it was known that giving honey with a concentration of 50% and 75% had a protective effect on the histopathological appearance of the pancreas of white rats (*Rattus norvegicus*) which had been induced by traditional ciu alcohol.

The rambutan honey given has a protective effect on cells. The protective effect on the pancreatic histopathology that has been induced by the traditional ciu drink is due to the administration of rambutan honey. Rambutan honey itself has various antioxidant compounds, for example, flavonoids. Flavonoid content in the study was tested using a 10% NaoH test. The 10% NaOH test was carried out by putting 1 mL of rambutan honey in a test tube, then adding a few drops of 10% NaOH. The presence of flavonoids in rambutan honey is indicated by a color change from yellow to brown Flavonoids are a group of plant polyphenolic compounds that are widely distributed in foodstuffs. in vitro, Flavonoids are proven to have strong biological effects because their antioxidant properties act against hydroxyl radicals, superoxide anions, and peroxyl radicals (Fadmi et al.,2017). Antioxidants protect the body from free radicals by neutralizing free radicals by donating electrons so that they stabilize before they can cause cell necrosis / damage(Nurmalasari, 2021). The antioxidant activity of flavonoids protects pancreatic beta cells through various mechanisms, one of which is by scavenging free radical cells (Arsyad et al., 2018). Honey has various compounds that can be beneficial for the body, honey contains flavonoids and polyphenols (Rakhmat et al., 2021).

Conclusion

Based on the results of observations and discussion in this study it can be concluded Administration of traditional ciu alcohol drink to white rats (*Rattus norvegicus*) as much as 4 ml/200 g BW of white rats for 21 days can affect the macroscopic and microscopic examination of the pancreas of white rats (*Rattus norvegicus*) in the form of pancreatic cell degeneration and necrosis. Giving honey with a concentration of 50% and 75% for 21 days can provide a protective effect on the macroscopic and microscopic examination of the pancreas of rats (*Rattus norvegicus*) which have been induced by traditional ciu alcohol drink as much as 4 ml/200 g BW of rats. Based on the results of observations, the higher the concentration of honey, the better it is in providing a protective effect on the pancreas.

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