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OXIDATIVE STRESS IN STREPTOZOTOCIN-INDUCED RATS AND THE ROLE OF *Uncaria gambir* (W.Hunter) Roxb. AS ANTIOXIDANTS

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Abstract

Hyperglycemia in diabetes mellitus (DM) increases the production of reactive oxygen species (ROS). That condition causes a decrease in antioxidant defense activity in the body so that the body cannot fight free radicals and triggers diabetic macroangiopathy complications. One source of natural antioxidants is the (*Uncaria gambir* (W.Hunter) Roxb. which has antioxidant and antihyperglycemic effects. so it is expected to inhibit oxidative damage in DM. This study aimed to determine the effect of the extract of *Uncaria gambir* (W.Hunter) Roxb. rod on antihyperglycemic activity. the antioxidant enzyme activity of SOD. and MDA levels in streptozotocin-induced rats. This study used an experimental method consisting of 5 treatments: two control groups (NC. PC) and three treatment groups (P1. P2. P3). and five replications for 21 days. Antioxidant activity was measured by scavenging the activity of hydroxyl radicals. Furthermore. testing of antihyperglycemic and antioxidant activity using test animals (rats). divided into three doses. namely 100. 200. and 400 mg/kg BW. This study used one-way ANOVA and Duncan's mean difference test. Results showed that the ethanol extract of the (*Uncaria gambir* (W.Hunter) Roxb. rod contained alkaloids. flavonoids. tannins. saponins. triterpenoids. and steroids. The antioxidant activity test of the pirated extract obtained IC50 55.646 ppm. and a dose of 100 mg/kg BW had the best activity as antihyperglycemic. increased SOD enzyme activity. and decreased MDA levels..

Keywords: antioxidants. diabetes. streptozotocin. *Uncaria gambir* (W.Hunter) Roxb.
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INTRODUCTION

Oxidative stress is implicated in many diseases, including diabetes mellitus, cancer, atherosclerosis, chronic fatigue syndrome, rheumatoid arthritis, and neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and Huntington's disease Bandaru et al., 2020; Nowotny et al., (2015) Hyperglycemia in diabetes increases the number of free radicals in the body. They are highly unstable and reactive, forming Reactive Oxygen Species (ROS) and hydroxyl radicals (Christiya & Radhika, 2020). Oxidative stress in diabetes mellitus is caused by an

imbalance of redox reactions due to carbohydrate and lipid metabolism changes. The number of free radicals that increase, as in diabetes, will oxidize and attack cell membrane lipid components resulting in lipid peroxidation (BP Muley et al., 2009; Saputera & Ayuchecaria, 2018). Oxidative stress and glycation are harmful to human health. However, oxidative stress can be prevented or inhibited by compounds called antioxidants. Antioxidants are compounds that can delay or inhibit the oxidation of lipids and other molecules, thereby inhibiting the initiation and propagation of oxidative chain reactions. Therefore, it has become important to use both natural and synthetic antioxidants to inhibit lipid peroxidation and prevent chronic disease (Hegde et al., 2010).

There has been significant interest in distilling essential oils and various plant extracts for natural antioxidants in recent years due to their excellent antioxidant properties. The island of Borneo has very high biodiversity compared to many other areas. On this island live about 15.000 species of flowering plants with 3.000 species of tree (Setyawan, 1970). Some of them are known to have medicinal effects, such as *Uncaria gambir* (W.Hunter) Roxb. *Uncaria gambir* (W.Hunter) Roxb. is included in the family Rubiaceae and the genus Uncaria.

Uncaria gambir (W.Hunter) Roxb. plants with tendrils can propagate to the top of the trees they are rambling. The tree is a shrub or medium tree with long vines. The rod of the *Uncaria gambir* (W.Hunter) Roxb. tree can spread or hang for more than 5 meters. and the Dayak community often uses these rod. *Uncaria gambir* (W.Hunter) Roxb. is empirically used in addition to the Dayak community to prevent and treat cancer. and it is also used for the treatment of diabetes mellitus. This is presumably due to the content of this *Uncaria gambir* (W.Hunter) Roxb. namely phenolics, flavonoids,tannins, and saponins which can be used as antioxidants to ward off free radical (Saputera & Ayuchecaria, 2018).

Thus, this study aimed to investigate the dependent compound antioxidant activity and antihyperglycemic activity of the rod extract of *Uncaria gambir* (W.Hunter) Roxb. . In this study, we used a different approach. Primarily the antioxidant activity of plant extracts was measured by DPPH, ABTS, and FRAP methods (Madhvi et al., 2020). The parameter of hydroxyl radical movement measured antioxidant activity and antidiabetic testing using test animals (rats) divided into three doses. The results of this study will provide a deeper understanding of the health-promoting properties of *Uncaria gambir* (W.Hunter) Roxb. rods so that they will be identified for further investigation into food and drink value-added nutraceuticals for the benefit of humanity.

RESEARCH METHODS

This study used white male rats *(Rattus norvegicus)* as the models. This work was approved by the Animal Care and Experimentation Committee (Ethical Committee. Faculty of Medicine Palangka Raya University) No 32/UN24.9/LL/2021. The research was conducted in the Dry Laboratory of the Faculty of Medicine. University of Palangka Raya. and the Chemistry/Biochemistry Laboratory of the Faculty of Medicine. University of Lambung Mangkurat. using the pure experimental study with posttest-only with control group design. Experimental animals were 30 rats (Wistar) made into hyperglycemia by induced streptozotocin. The material used were extracts of *Uncaria gambir*. (W.Hunter) Roxb. rod. streptozotocin. aqua dest. phosphate buffer. glucose stick. KI 1.16 M. acetic acid.

Collection and Identification of Plant Material

Fresh rods of *Uncaria gambir* (W.Hunter) Roxb. were collected from Garung village. Pulang Pisau district. Central Kalimantan. Indonesia. Before use. ensure that the trunk is free from contamination. sand. and no microbial growth. The rods are then sun-dried and ground into a coarse powder in a commercial blender.

Extract Preparation

Four parts of 5 g of powdered fresh rods of dried *Uncaria gambir* (W.Hunter) Roxb. were weighed using an analytical balance. With the sample to solvent ratio fixed at 1:10. different concentrations of ethanol (v/v; 0%. 10%. 20%. and 30%) were prepared. The mixture was shaken for 60 min at 25°C and 150 rpm in a shaking incubator. After extraction. the extract was filtered using Whatman No.1 filter paper. The residual filtrate was collected and centrifuged at 4.500 rpm for 10 minutes. The supernatant was concentrated using a rotary evaporator at a temperature of 40°C. The concentrated extract was freeze-dried. wrapped in aluminum foil. and stored at -20°C until further analysis. The dose of extracting *Uncaria* s.p rod extract in the rat is divided into 100 mg/kg BW. 200 mg/kg BW. and 400 mg/kg BW. All experimental models and measurements were carried out in the Medical Chemistry/Biochemistry Laboratory. Faculty of Medicine. Lambung Mangkurat University. Banjarbaru. South Kalimantan.

Hydroxyl Radical Scavenging Activity Analysis

The scavenging activity for hydroxyl radicals was measured by the Fenton reaction. Reaction mixture contained 60 µL from 1.0 mM $FeCl_2$. 90 µl from 1 mM 1.10- phenanthroline. 2.4 ml buffer phosphate 0.2 M (pH 7.8). 150 µL from 0.17 M H_2O_2 and 1.0 ml extract. Adding H_2O_2 started the reaction. After incubation at room temperature for five minutes. the absorbance of the mixture at 560 nm was measured using a spectrophotometer. The percentage inhibition of hydroxyl scavenging activity was calculated using the following formula:

$$\% \text{ Hydroxyl Radical Scavenging Activity} = \left(1 - \frac{\text{Absorbance (test)}}{\text{Absorbance (blank)}}\right) \times 100$$

Acclimatization of Try Animals

All rats were put into separate cages for adaptation for one week. All rats were fed during the adaptation period. and drinking water was the same as their place of origin.

Streptozotocin Induction in Experimental Animals

The rats that had undergone acclimatization were weighed. after which they have fasted for 10 hours. After fasting for 10 hours. animal blood samples were taken from the tail vein at minute 0 to determine the initial blood glucose level. Furthermore. samples of rats were induced by streptozotocin at a dose of 45 mg/kg BW of rats intra-peritoneal. After being induced. rats are still given food and drink. waiting for four days. then measuring their blood glucose levels. Rats are considered hyperglycemia when blood glucose levels are more than 100 mg/dL.

Giving Extract of *Uncaria gambir* (W.Hunter) Roxb.

Uncaria gambir (W.Hunter) Roxb. extract is made into a solution and given daily using the gastric sonde. 1 hour after a meal using a dose of 100 mg/kg BW. 200 mg/kg BW. and 400 mg/kg BW. Glucose levels were measured one week after administering the test solution using a glucometer.

Phytochemical Test Quantitative Analysis

Quantitative analysis to determine the total levels of secondary metabolites (alkaloids. flavonoids. saponins. and tannins) contained in the ethanol extract of *Uncaria gambir* (W.Hunter) Roxb. uses UV-Vis Spectrophotometer.

Supernatant preparation

After being treated with the extract for 21 days. Each group of rats will take blood for superoxide dismutase and malondialdehyde activity. Blood sampling was done by anesthetizing mice using a combination of ketamine and xylazine and then dissected. Blood was taken from the heart using a 5 mL disposable needle and stored in a 3 mL plain vaculab. Then centrifuged at 300 rpm for 10 minutes. The resulting supernatant was used for measurement of antioxidant enzyme activity. including SOD activity and MDA levels

Measurement of Superoxide Dismutase Activity

Measurement of SOD activity in the supernatant was measured by the Misra and Fridovich method. A total of 500 mL supernatant was added to 0.800 mL of carbonate buffer (100 mM. pH 10.2) and 100 mL of epinephrine (3mM). Changes in the absorbance of each sample were then recorded at a wavelength of 480 nm with a spectrophotometer for 2 minutes at an interval of 15 seconds. Similarly. for the blank and standard solutions. one SOD unit was defined as the amount of enzyme required to inhibit 50% of epinephrine autoxidation. Mixture diluted 1/10. Then read the absorbance using a spectrophotometer.

Data analysis

Each experiment was performed in triplicate. and the results were recorded as the mean % antioxidant and antiglycation activity ± SD. The IC50 value is calculated from the graph plotted of each activity against the sample concentration. IC50 was defined as the required extract concentration that caused 50 percent of each activity measured in this study. Ascorbic acid was used to compare (positive control) with the same concentration as plant extracts. IC50 is calculated using regression analysis on MS excel 2019 from windows 10. The study results of glucose data were determined for homogeneity and normality to determine the statistical analysis used. Data were analyzed using a one-way ANOVA test to determine the mean difference between treatments using the SPSS program. If there is a difference. it is continued by using Duncan's Post Hoc test to determine the difference between the treatment groups. Based on the significance value.

RESULT AND DISCUSSION

This study used a sample of *Uncaria gambir* (W.Hunter) Roxb. rods obtained from Garung village. Pulang Pisau Regency. Central Kalimantan. *Uncaria gambir* (W.Hunter) Roxb. rods have previously been identified to ensure that the correct plant is used *Uncaria gambir* (W.Hunter) Roxb. The extraction method used is the maceration method using 96% ethanol as a solvent. The solvent was chosen as the filtered fluid because it is non-toxic. neutral. and has good absorption.

a. Phytochemical Screening

UV-Vis Spectrophotometry was used to determine levels of secondary metabolites from the ethanol extract of *Uncaria gambir (*W.Hunter) Roxb. The complete content was determined by selecting the range of alkaloids, flavonoids, saponins, triterpenoids, steroids, and tannins contained in the ethanol extract of the rod of *Uncaria gambir* (W.Hunter) Roxb. based on the standard curve of the

typical solution of each compound. Phytochemical screening of the extract revealed the presence of various bioactive components, of which triterpenoids and flavonoids were the most prominent. The summarized result of the phytochemical test is in Table 1.

Table 1.Phytochemical Test Results of *Uncaria gambir* (W.Hunter) Roxb. Rod Ethanol Extract

No	Paramater	Level	Description
1.	Saponin (%)	7.000 ± 0.300	Triplo
2.	Alkaloid (%)	14.567 ± 0.404	Triplo
3.	Flavonoid (mg/g)	48.500 ± 0.250	Triplo
4.	Tanin (mg/ g GAE)	0.257 ± 0.008	Triplo
5.	Triterpenoid (mg/g)	86.467 ± 0.577	Triplo
6.	Steroid (mg/g)	41.133 ± 0.156	Triplo

b. Hydroxyl Radical Scavenging Activity

Activity of the trunk extract on hydroxyl radical has been shown in Figure 1. *Uncaria gambir* (W.Hunter) Roxb. extract exhibited concentration dependent scavenging activity against hydroxyl radical generated in a Fenton reaction syrod. Figure 1 represented the mean values ± standard error (Mean ± SE) of hydroxyl radical scavenging activity of ethanol extract of *Uncaria gambir* (W.Hunter) Roxb. and ascorbic acid. The result shows that extract can scavenge hydroxyl radical. Ascorbic acid is found to have a better activity in all concentration compare to plant extract. Furthermore, the value of R2 , r, and IC50 for plant extract and ascorbic acid were evaluated. The IC50 value was found to be 79.283 ppm while IC50 value for ascorbic acid was 56.646 ppm. Both plant extract and ascorbic acid shows a strong correlation with scavenging activity (table 2). The IC50 of plant extract is found lower than ascorbic acid.

Table 2. Regression coeffiecient (R^2), correlation coefficient (r), and IC50 value of ascorbic acid and *Uncaria gambir* (W.Hunter) Roxb.

Parameters	Hydrogen Peroxide	
	Ascorbic Acid	Uncaria. sp
R^2	0.918	0.999
R	0.958	0.999
IC50	56.646	79.283

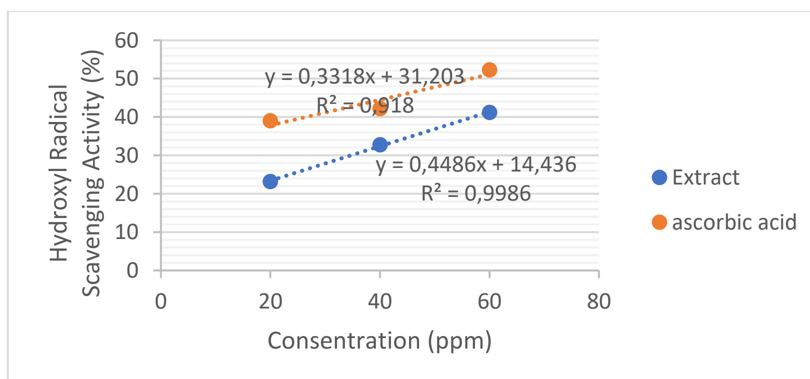

Figure 1. Hidroxyl radical scavenging activity of *Uncaria gambir* (W.Hunter) Roxb. extract and ascorbic acid

c. Blood Glucose Level

STZ-induced rats at a dose of 40 mg / kgBW intraperitoneally, after the third day obtained an increase in glucose levels of 327.20 mg /dL. Then followed by giving *Uncaria gambir* (W.Hunter) Roxb. extract at a dose of 100 mg / kgBW, 200 mg / kg BW and 400 mg / kgBW for 14 days (Figure 2).

Table 3. Blood glucose level of rats before and after Streptozotocin-induced.

Streptozotocin-induced	Glucose level	*p* value
Before	109.73	0.000
after	327.20	

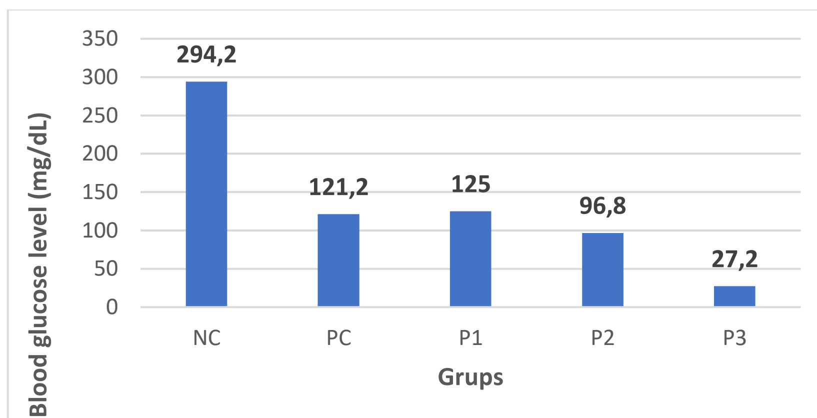

Figure 2. Mean of blood glucose level after *Uncaria gambir* (W.Hunter) Roxb. extract treatment (p<0.05) NC= Negative Control (aquadest); Positive Control (glibenclamid); P1=100 mg/kgBW; P2=200 mg/kgBW; P3 = 400 mg/kgBW.

One way ANOVA test obtained p <0.05, then continued by post hoc test by using Duncan's test. The results of the Duncan's test showed differences between treatment groups and negative control groups. This proves that the ethanol extract of *Uncaria gambir* (W.Hunter) Roxb. can lower blood glucose levels in STZ-induced rat. Between dose of 100 mg/kgBW vs. 200 mg/kgBW did not significant difference, meaning dose of 100 mg/kgBW had the same ability with dose of 200 mg/kgBW in lowering blood glucose level. Between dose of 100 mg/kgBW vs. control positive (glibeclamid) did not significant difference, meaning dose of 100 mg/kgBW had the same ability with dose of glibenclamid in lowering blood glucose level. Between doses of 100 mg/kg BW vs. 400 mg/kg BW was significantly different, meaning that the dose of 100 mg/kg BW had a different (lower) ability than the dose of 400 mg/kg BW to lower blood glucose levels. Between dose of 200 mg/kgBW vs. control positive (glibenklamid) did not significant difference, meaning dose of 200 mg/kgBW had the same ability with dose of glibenklamid in lowering blood glucose level. The dose of 400 mg/kg BW showed differences between groups control (glibenclamide and aqua dest), meaning that the dose of 400 mg/kg BW had a different (lower) ability than the group control (glibenclamide and aqua dest) to lower blood glucose levels.

d. Superoxide Dismutase (SOD) level

Measurement of the SOD activity of the *Uncaria gambir* (W.Hunter) Roxb. extract is shown in Figure 3, it was found that the positive control group of diabetic rats or glibenclamide (PC) for 2 weeks showed the highest value of 0.012 while the lowest value of negative control (NC) was 0.002.

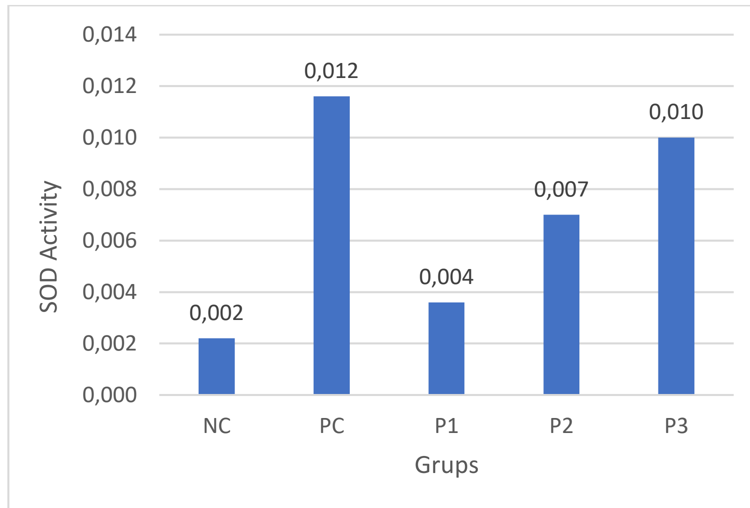

Figure 3 . Differences in Superoxide Dismutase (SOD) activity in the blood of white rats (*Rattus norvegicus*) after administration of *Uncaria gambir* (W.Hunter) Roxb. rod extract

Data analysis using the Kruskal-Wallis test of SOD activity in control mice with a group that was given treatment for 14 days showed that SOD activity increased significantly, obtained at $p < 0.05$, then continued by post hoc test by using the Mann-Whitney test. The results of the Mann-Whitney test showed differences between treatment groups and negative control groups. This proves that the extract of *Uncaria gambir* (W.Hunter) Roxb. . can increase SOD activity in STZ-induced mice. The results of the Mann-Whitney test found differences between all treatment groups and positive control groups, P1 and P2 ($p = 0.008$), P1 and P3 ($p = 0.002$), and P2 and P3 ($p = 0.016$). This proves that the administration of Uncaria ethanol extract can increase SOD activity in STZ-induced rat pancreas. The higher the dose of *Uncaria gambir* (W.Hunter) Roxb. extract, the higher the activity SOD is formed.

e. Malonaldehyde (MDA) level

Measurement of MDA level is shown in Figure 4, it was found that the positive control group of diabetic rats or glibenclamide (PC) for 2 weeks showed the highest value of 298,2 mmol/L while the lowest value of diabetic rats given *Uncaria gambir* (W.Hunter) Roxb. 400 mg/kgBW (P3) was 282,8 mmol/L.

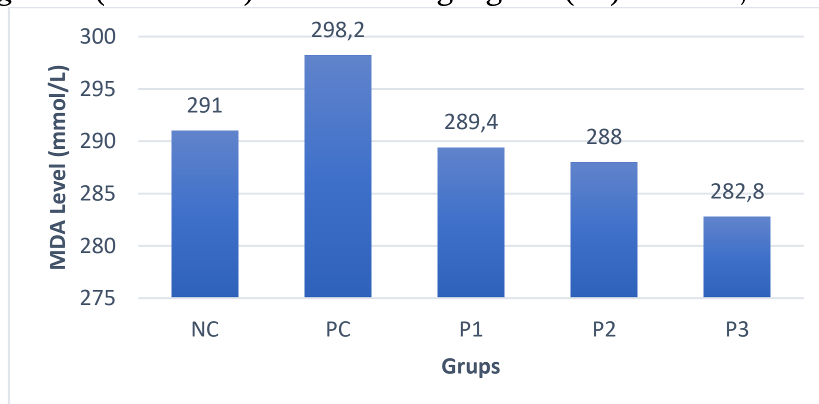

Figure 4. Differences in Malonaldehyde (MDA) Levels in the Blood of White Rats (Rattus norvegicus) after giving the extract of *Uncaria gambir* (W.Hunter) Roxb rods extract

This results of of the statistical with nonparametric analysis, namely the Kruskal Wallis test showed that p =0.001 (p<0,05), with means that there are minimal differences in the treatment groups. Then followed by post hoc test using the Mann Whitney test. The results of the Mann Whitney test found that there were differences between all treatment groups and negative control groups and all treatment groups, and positive control groups but there was no difference between P1, P2, and P3. This proves that the administration of *Uncaria gambir* (W.Hunter) Roxb. extract can reduce serum MDA levels of hyperglycemic mice at a dose of 100 mg / kgBW.

The antioxidant activity of the ethanol extract of the *Uncaria gambir* (W.Hunter) Roxb. rod was expressed in the percentage of inhibition of the extract against hydroxyl free radicals. The difference in absorption between hydroxyl absorbance and sample absorbance measured by UV-Vis spectrophotometer is a way to get the percent inhibition of the ethanol extract of *Uncaria gambir* (W.Hunter) Roxb. The amount of antioxidant activity is indicated by the IC50 value, which is the concentration of sample solution needed to inhibit 50% of hydroxyl free radicals. The hydroxyl radical is a highly reactive oxygen center radical formed by the reaction of various hydroperoxides with transition metal ions. Hydroxyl radicals directly cause lipid peroxidation and are the most dangerous among ROS to damage cellular components. It also participates in DNA damage and induces carcinogenesis, mutagenesis, and cytotoxicity (Pizzino et al., 2017). The results of the antioxidant activity test of the ethanol extract of *Uncaria gambir* (W.Hunter) Roxb. compared with the comparison solution, namely vitamin C, showed that the ethanol extract of *Uncaria gambir* (W.Hunter) Roxb. had lower antioxidant activity than the comparison.

The results showed that *Uncaria gambir* (W.Hunter) Roxb. extract was able to reduce fasting blood glucose levels in hyperglycemic male Wistar rats induced STZ (Chopra et al., 2018), and the best treatment that could reduce fasting blood glucose levels was P3 (a group of rats induced by streptozotocin + *Uncaria gambir* (W.Hunter) Roxb. extract at a dose of 400mg/kg BW) with an average The average decrease in blood glucose levels was 42 mg/dL.

The decrease in fasting blood glucose levels in male Wistar rats is due to the active compounds contained in *Uncaria gambir* (W.Hunter) Roxb. The chemical content may also influence the effects of lowering blood glucose levels in the three treatment groups in each dose. The range of *Uncaria gambir* (W.Hunter) Roxb. is alkaloids, flavonoids, saponins, triterpenoids, steroids, and tannins. According to the research results, compounds with antidiabetic activity are flavonoids, steroids/triterpenoids, and tannins (Karandikar et al., 2014; Nurulita et al., 2012).

Flavonoids can lower blood glucose levels with their ability as antioxidants. Flavonoids are protective against damage to cells as insulin producers and can increase insulin sensitivity (Russo et al., 2019). Antioxidants can suppress beta cell apoptosis without altering the proliferation of pancreatic beta cells (Kaneto et al., 1999). Antioxidants can bind free radicals, as proven in research by Asmat *et al.*, (Asmat et al., 2016) thereby reducing insulin resistance. Antioxidants can reduce Reactive Oxygen Species (ROS). In ROS formation, oxygen will bind to the free electrons that come out due to leakage of the electron chain. It is this reaction between oxygen and free electrons that produces ROS in the mitochondria (Zhao et al., 2019). Antioxidants in flavonoids can donate hydrogen atoms. Flavonoids will

be oxidized and bind to free radicals so that free radicals become more stable compounds (Munteanu & Apetrei, 2021). Another mechanism is the ability of flavonoids. especially quercetin, to inhibit GLUT 2 of the intestinal mucosa to reduce glucose absorption. This causes a reduction in the absorption of glucose and fructose from the intestines, so that blood glucose levels fall. GLUT 2 is thought to be a major glucose transporter in the gut under normal conditions. In Song's research. it was found that flavonoids can inhibit glucose absorption. When quercetin is ingested with glucose, hyperglycemia is significantly decreased. This shows that quercetin can inhibit glucose absorption through GLUT 2 (Alkhalidy et al., 2018). Flavonoids can also inhibit phosphodiesterase thereby increasing cAMP in pancreatic beta cells (Hossain et al., 2016). Increased cAMP will stimulate the release of protein kinase A (PKA) which stimulates insulin secretion to increase (Tengholm & Gylfe, 2017).

In addition to flavonoids, ingredients that play an essential role in lowering blood glucose levels are saponins, steroids/triterpenoids, and tannins. According to research results by Sinulingga *et al.*,(Sinulingga et al., 2020) that the content contained in the ethanol fraction of parasite leaf water can inhibit the alpha-glucosidase enzyme that causes diabetes mellitus type 2, with the IC50 value being found to be potent in inhibiting the activity of the alpha-glucosidase enzyme competitively or non-competitively.

Increased levels of MDA in this study indicate the high oxidation process by free radicals on cell membranes. Based on the measurement of serum MDA levels, the positive control rats had the highest MDA levels compared to the other treatment groups, which was 298.2 mmol/L (figure 4). The high level of MDA is to the research conducted by Obi et al., Chukwunonso Obi et al., (2016) which states that glibenclamide is a drug that can lower blood glucose levels but is unable to reduce lipid peroxidation levels because it does not contain antioxidants. This shows that the ability of glibenclamide to make normal MDA levels is not as good as when using *Uncaria gambir* (W.Hunter) Roxb.

The results of the Mann-Whitney test showed that the average ratio of MDA levels between the negative control mice and the positive control mice was significantly different, namely $p = 0.008$. This proves that the role of STZ as an inducer of DM has been successfully carried out. The extract group with a 400 mg/kg BW dose had a minor MDA ratio compared to the other treatment groups, which was 282.8 mmol/L. This indicates a decrease in MDA levels in hyperglycemic rats treated with *Uncaria gambir* (W.Hunter) Roxb. The increase in extract levels was accompanied by a decrease in body free radicals (MDA).

Decreased levels of free radicals will reduce the incidence of damage to pancreatic cells so that pancreatic cells can secrete insulin again. In hyperglycemia, there is an indirect increase in free radicals such as superoxide anion (O_2*). Superoxide anion free radicals circulating in the blood can increase molecular modifications in various tissues that cause oxidative stress. The reaction of free radicals with unsaturated fatty acids in cell membranes and plasma lipoproteins produces lipid peroxidase, which forms MDA (Jové et al., 2020). The decrease in MDA levels in rats given extracts of *Uncaria gambir* (W.Hunter) Roxb. because there are phenolic compounds that can scavenge free radicals, thereby reducing the formation of singlet oxygen and metal chelates. The compounds contained in the rod can help the synthesis of enzymatic antioxidants and increase the concentration of

antioxidants in the tissue and minimize the occurrence of oxidative stress (Olszowy, 2019).

The measurement of SOD activity is seen from the results of the absorption of SOD activity and is calculated in percent of SOD activity (inhibition rate %). The results of the measurement of blood plasma SOD activity in the negative control group and positive control and treatment groups can be seen in table 6. The data obtained were then analyzed statistically using the Kruskal Wallis test with a significance value of $p < 0.05$. Statistical analysis was performed by comparing the SOD activity of the negative control group. positive control. and treatment groups. Statistical analysis using the Kruskal-Wallis test obtained a p-value = 0.000 ($p < 0.05$). indicating significant differences between the treatment groups.

Further analysis with Mann-Whitney ($p<0.05$). Based on the Mann-Whitney statistical test. it was concluded that the negative control group (P1) and the P2. P3. and P4 groups tended to experience an increase in SOD activity and showed a significant difference between all treatment groups in blood plasma with a p-value = 0.002 ($p<0.05$). Assessment of antioxidant activity was seen from the percentage of SOD enzyme activity. SOD is an enzyme that catalyzes superoxide radicals into stable products. namely hydrogen peroxide and oxygen (Khare et al., 2019). SOD enzymes are included in endogenous antioxidants or can be referred to as enzymatic antioxidants found in the body (Ighodaro & Akinloye, 2018).

Oxidative stress that occurs in diabetes mellitus causes an increase in the rate of lipid peroxidation which contributes to the production of free radicals, including the formation of superoxide anions, thereby causing oxidative modifications that result in the inactivation of SOD (Radi, 2018). Lipid peroxidation that occurs in diabetes or hyperglycemia causes an increase in the permeability of the pancreatic cell membrane so that it interferes with the vital function of cells as a provider of the hormone insulin. Production of the hormone insulin is reduced, as well as its function, so it is not able to direct the entry of glucose into the tissues. This condition causes blood glucose levels to be high, which contributes to a decrease in SOD activity (Ito et al., 2019). The decrease in SOD activity in the negative control group indicates excessive utilization in attenuating free radicals. In contrast, in the glibenclamide group and extracts, their increased activity indicated their ability to scavenge ROS, thereby contributing to a protective effect against oxidative stress and preventing further damage to membrane lipids. The increase in SOD activity observed in glibenclamide-treated diabetic rats agrees with previous reports(Erejuwa et al., 2011; Jiby Elias et al., 2010).

SOD activity showed no significant difference between the *Uncaria gambir* (W.Hunter) Roxb. dose of 400 mg/kg BW with glibenclamide, proving that the extract significantly increased SOD activity and positive control, although the mechanism was different. Glibenclamide can lower blood glucose levels so that hyperglycemia can be controlled. The mechanism of reducing blood glucose levels is carried out by stimulating insulin hormone secretion, increasing glucose uptake from the blood to tissues, glucose oxidation, and activating glycogen synthesis in the liver and adipose tissue (Pandarekandy et al., 2017). The mechanism of action of *Uncaria gambir* (W.Hunter) Roxb. rod extract on increasing SOD enzyme activity is thought to be due to the content of flavonoid that act as antioxidants. Furthermore. this enzyme can neutralize the body's superoxide radicals and make them in a physiological state (Engwa, 2018).

CONCLUSION

This study concludes that the ethanolic extract of the rod of *Uncaria gambir* (W.Hunter) Roxb. exhibits antioxidant and anti-hyperglycemic activity. which can help prevent various human oxidative stress and glycation-related diseases

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