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Antidiabetic Activity of Ethanol Extract of Red Castor Roots (*Jatropha gossypiifolia* L.) in White *Wistar* Rats Induced by Alloxan

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Abstract

Diabetes mellitus is a metabolic disorder indicated by high blood glucose levels. Red castor is a plant that has potential in the treatment of diabetes mellitus. This study aimed to measure the impact of ethanol extract from the roots of the red castor plant (Jatropha gossypiifolia L.) in reducing blood glucose levels in male white Wistar rats that have been induced with alloxan at a dose of 150 mg/kgBW. The study used 25 male rats divided into 5 treatment groups. The negative control group was given distilled water as a substitute for the substance being tested, while the positive control group was given a dose of glibenclamide of 5 mg/kg BW. Furthermore, three different treatment groups were administered red castor root extract at varying doses of 25, 50, and 100 mg/kgBW. Treatment was given for 14 days. Alloxan 150 mg/kgBW was used so that rats experienced an increase in blood glucose levels exceeding 200 mg/dL. The results of decreasing blood glucose levels respectively at doses of 25, 50, and 100 mg/kgBW were 101.0 mg/dL, 81.6 mg/dL, and 79.4 mg/dL. The findings of the conducted research and data analysis lead to the conclusion that the ethanol extract derived from the red castor roots exhibits the ability to reduce blood glucose levels in male Wistar rats. Statistical analysis revealed that the impact of using ethanol extract of red castor roots at doses of 25 mg/kgBW, 50 mg/kgBW, and 100 mg/kgBW is equal to the effect of using 5 mg/kgBW glibenclamide.

***Keywords**: Alloxan, diabetes mellitus, red castor, Wistar strain*

INTRODUCTION

Jatropha gossypiifolia L. or also called red castor is a plant originating from Brazil and has spread widely throughout the world, especially in tropical plains. Jatropha gossypiifolia leaves are thought to have anti-inflammatory, antimicrobial, and insecticidal properties. The roots and stems have anti-inflammatory and antimicrobial properties; The seeds and fruit can be used to fight influenza and as a sedative, analgesic, or anti-diarrhea (Wu et al., 2019). Red castor decoction is also used as an antidiabetic by people in Colombia and the Dominican Republic (Granados et al., 2015). Red castor can be considered an alternative in the treatment of Type II Diabetes Mellitus because it exhibits antihyperglycemic effects (Kumar et al., 2022). Administration of ethanol extract of red castor leaves has shown a significant antidiabetic effect (Wibisono et al., 2022). In this study, red castor roots (RCR) were used to see its effect as an antidiabetic agent.

The roots and stems of red castor oil contain several alkaloids, flavonoids, steroids, and mineral salts such as nitrogen, phosphate, and potassium (Qonita and Ramadhan, 2019). One potential alternative treatment for diabetes sufferers is to use flavonoid compounds (Cahyana and Adiyanti, 2021). Solvents that can be used for the extraction of flavonoid compounds are polar solvents such as ethanol, methanol, ethyl acetate, acetone, water, and isopropanol (Riwanti et al., 2018). In this research, ethanol solvent was used to extract flavonoid compounds from RCR which have the potential to be antidiabetic agents. Based on this explanation, researchers wish to study the potential of ethanol extract of RCR as an antidiabetic at the preclinical stage.

RESEARCH METHODOLOGY

Research Tools

The tools used in this research were analytical scales, maceration vessels, Buchner funnels, rotary evaporators, water baths,

visible spectrophotometers (Star Dust MC 15), centrifugators, sonicators, and glassware.

Research Materials

The materials used in the research were ethanol extract of RCR from Medang Island, NTB, 96% ethanol, alloxan 150 mg/kgBW (Sigma Aldrich), glibenclamide 5 mg/kgBW (Indofarma), distilled water, GOD FS glucose kit reagent (Dyasis), *Wistar* male white rats aged 2-3 months with body weight an average of 150-200 grams from Pharmacology Laboratory, Muhammadiyah University of Surakarta.

Preparation of Ethanol Extract of RCR

In this research, red castor plants were determined. The purpose of plant determination is to find out the true identity of the plant by looking at the key to determination. Determination was carried out at the Setia Budi University Laboratory with certificate number 77E/DET/UPT-LAB/21.08/2023. Based on the results of this determination, it can be concluded that the plant used is the species *Jatropha gossypiifolia* L. with a description of the plant in the form of roots including tap root type; The stem is woody with many branches and brown in color; Long-stemmed single leaf, elliptical shoot, transparent spots; Flat-stemmed panicle compound flowers; The fruit has three veins and is green when young and then black when ripe; and round brown seeds with black spots containing oil.

The extraction process in this research used 96% ethanol filter with the maceration method. The maceration process was carried out for 24 hours. The maceration results were filtered using a Buchner funnel and the macerate and filtrate were obtained. The macerate was again macerated with 96% ethanol solvent, while the filtrate was concentrated using a rotary evaporator at a temperature of ± 60^{o}C with a rotation speed of 80 rpm. Once the evaporation process is complete, the extract undergoes further concentration by subjecting it to a water bath with a temperature range of 40-60^{o}C for a duration of 12 hours. This step effectively eliminates any residual solvent, resulting in the formation of a dense and concentrated extract (Haryoto and Devi, 2018).

Phytochemical Test

This phytochemical content analysis was carried out to find out what compounds are contained in the ethanol extract of RCR, and as further support that RCR has the potential to be an antidiabetic agent.

Test for alkaloid content

One hundred mg of thick extract was taken in a test tube and 1 mL of 2N HCl was added then divide it into two parts. In tube 1, 3 drops of Mayer's reagent were added, in tube 2, 3 drops of Dragendroff's reagent were added. The results showed a yellow color in tube 1, and an orange color in tube 2 indicating the presence of alkaloid compounds contained (Rahayu *et al.*, 2015).

Test for flavonoid content

One hundred mg of thick extract was taken in a test tube and three drops of concentrated HCl were included. The extract mixture is heated over a bath until the color changes to red indicating the presence of flavonoids contained (Rahayu *et al.*, 2015).

Test the phenol and tannin content

One hundred mg of the thick extract was taken in a test tube and three drops of $FeCl_3$. The presence of phenolic compounds is indicated if the color changes to bluish-black (Segara and Kurniawan, 2023).

Test the Saponin content

One hundred mg of thick extract was taken in a test tube and one mL of distilled water for one minute. The formation of one-centimeter layer of foam indicates the presence of saponins. (Rahayu *et al.*, 2015).

Test for terpenoid content

One hundred mg of thick extract was taken in a test tube and two mL of chloroform and three mL of concentrated H_2SO_4. The results obtained will show a separation between the two mixtures and indicate the presence of terpenoids if there is a reddish

brown color in the middle of the separation (Rahayu *et al.*, 2015).

Preparation and Care of Test Animals

All test animals were acclimatized for 7 days by providing sufficient food and drink to adapt the test animals to the experimental environment and avoid stress in the rats which could cause disruption to the research (Harahap *et al.*, 2015). The test animals chosen in this study were male *Wistar* rats that were healthy or without defects, weighing between 150-200 grams, and aged 2-3 months. Care of test animals in research must uphold animal welfare. During the research, rats were given pellet food of approximately 10-20 grams/day. Rats were also given distilled water ad libitum. The rats housing is made of a plastic box with a maximum height of 22-24 cm which allows the rats to stretch completely upright. Rat cages must have a filter or ventilation at the top, the temperature ranges between 20-26^{o}C, air humidity ranges from 40-70% and the lighting system in the cage is dark at night and bright during the day (Putra, 2016). This research has been implemented with ethical rules to state that the planned research activities meet ethical feasibility so that it is feasible to carry out. Information on ethical rules is shown in Letter number 5046/A.1/KEPK-FKUMS/VIII/2023 from the UMS Medical Faculty Ethics Commission.

Animal Modeling of Hyperglycemic Tests

Rats were induced with 150 mg/kgBW alloxan dissolved in NaCl and administered intraperitoneally. Blood glucose levels were monitored on day 0 as initial data and on day 5 of induction to check whether the test animals had experienced a hyperglycemic condition. Blood collection is carried out through the conjunctival vein using a capillary tube in the eye. Test animals are used as diabetic rats models if they show blood glucose levels > 200 mg/dL. Determination of blood glucose levels is carried out using the spectrophotometric method with the Glucose Oxsidase – Peroxidase Aminoantypyrine (GOD-PAP) reagent (Wahyuni *et al.*, 2020).

Antidiabetic Activities Test

Antidiabetic activity research was carried out using 25 male *Wistar* rats divided into 5 different groups. The hyperglycemic test animals were subsequently administered antidiabetic therapy for a duration of 14 days, based on their individual group divisions: Group I (negative control), Group II (positive control with glibenclamide 5 mg/Kg BW), Group III-V were administered RCR ethanol extract at dosages of 25, 50, and 100 mg/Kg BW, respectively. Blood glucose levels were measured and rats were weighed on days 0, 7, and 14.

Blood Glucose Sampling

Blood sampling was carried out before induction (baseline), the 5th day after induction (post alloxan), and twice during treatment, namely on the 7th day and the 14th day. 1 mL of blood was taken from the conjunctival vein located in the eye using a capillary tube, then collected in an eppendroph and centrifuged. The serum formed was determined by blood glucose levels using the Glucose GOD PAP method with the GOD FS glucose kit reagent. Samples, blanks and standards were incubated for 10 minutes at 37^{o}C. Then it was analyzed using a visible spectrophotometer (Star Dust) with a wave of 546 nm (Muntafiah *et al.*, 2019).

Data analysis

The data obtained from this research are blood glucose levels processed using the SPSS program. The data was analyzed using the one way anova test with a confidence level of 95% (P=0.05). The one way anova test was used to determine whether there was an effect of ethanol extract of RCR on reducing blood glucose levels in rats. So there are two possibilities in the ANOVA test, whether there is a significant difference or not a significant difference between treatment groups. If the results show a significant difference, you can

continue using the LSD (Least Significant Difference) test.

RESULT AND DISCUSSION

RCR powder originating from Medang Island, NTB was macerated with 96% ethanol as a solvent. The maceration method was chosen because there are several advantages, namely the method is easy to do and suitable for use for simplicia which is likely to easily evaporate when exposed to heat or cannot withstand heating. Extraction is carried out using an ethanol filter because it can increase cell wall permeability. Ethanol also has good absorption capacity so it can extract as many compounds as possible (Haryoto and Devi, 2018). The principle of the maceration method is that the solvent will penetrate the cell wall and the active substance will dissolve due to the difference in concentration between the active substance solution inside the cell and outside the cell, so that the solution with a high concentration will be pushed out of the cell (Handoyo, 2020). The filtrate was concentrated using a rotary evaporator to separate the sample extract from the solvent. The yield calculation was carried out and the yield of the extract was 2.1% w/w.

The resulting extract was used for detection chemical compounds. Detection of chemical compounds in this study was carried out qualitative with phytochemical screening methods. Phytochemical screening was carried out using the tube method and seen by the color change. According to the information presented in **Table 1**, it is evident that the ethanol extract derived from RCR, which was utilized as a test sample, comprises terpenoid, flavonoid, alkaloid, tannin, phenolic, and saponin compounds (Priyadi *et al.*, 2014; Segara and Kurniawan, 2023). The results obtained showed that the ethanol extract of RCR contained terpenoids, flavonoids, alkaloids, tannins, phenolics, and did not contain saponin compounds. Compounds that are thought to have an effect as antidiabetic agents are flavonoid compounds. According to research by Cahyana and Adiyanti (2021) flavonoid compounds can be used as a potential alternative treatment for diabetes sufferers.

Table 1. Results of phytochemical screening of ethanol extract of RCR

Secondary Metabolites	Indicator of Positive Results	Results of RCR Ethanol Extract
Flavonoids	Forms a dark red color	+
Saponin	Layer of foam is formed	-
Terpenoids	A reddish-brown color form	++
Alkaloids	Orange color change	+
Tannin	Forms a dark blue/greenish black color	+
Phenolic	Forms a dark blue/greenish black color	+

Note : Description: (++) more intens, (+) intens, (-) none

The Wistar rats test animals were subjected to hyperglycemia through the induction of alloxan. The mechanism of action of Alloxan is to degrade β cells in the islets of Langerhans, that occurs because reactive oxygen compounds form superoxide radicals through the redox cycle. This redox cycle will form highly reactive hydroxyl, causing damage to pancreatic β cells. Damage to pancreatic β cells causes the production of the hormone insulin to be inhibited so that blood glucose cannot be converted into energy, resulting in blood glucose levels rising (Setiadi, 2020). Based on monitoring results on the 5th day, it was discovered that the rats experienced an increase in blood glucose levels, resulting in hyperglycemia as evidenced by the rats's blood glucose levels above 200 mg/dL (**Table 2**). The rats were divided into 5 groups and given treatment into negative control by administering distilled water, positive control by administering glibenclamide 5 mg/kgBB, and administering ethanol extract of RCR with 3 dose levels 25; 50; and 100 mg/kgBB.

Table 2. Average blood glucose level (BGL) data of ethanol extract of RCR

Group	Average Blood Glucose Levels (mg/dL)				%Decrease BGL
	Baselines	Post Alloxan	Day 7	Day 14	
Negative Control	100.8 ± 13.3	321 ± 103.2	285 ± 104.7	276.2 ± 33	13.96*
Positive Control	118.2 ± 13.4	210 ± 13.5	119.4 ± 18.7	89.2 ± 21.4	57.52
RCR 25 mg/kgBB	89 ± 8.9	383 ± 115.72	136.4 ± 4.4	101.0 ± 9.2	73.63
RCR 50 mg/kgBW	109 ± 12.23	369± 179	106.6 ± 3.1	81.6 ± 14.2	77.89
RCR 100 mg/kgBW	101.2 ± 7.16	399 ± 160.7	100 ± 8.2	79.4± 18	80.10

Note: *indicates there is a significant difference in reduction in BGL with the others group ($p < 0.05$)

The positive control group was given glibenclamide 5 mg/kgBW once a day orally for 14 days (Biringan *et al.*, 2021). Glibenclamide is included in the sulfonylurea group whose primary way of working is by stimulates insulin secretion from the granules of pancreatic beta cells, stimulates through its interaction with the ATP sensitive K channel on the membrane and this situation will open the Ca^{2+} ion channel. By opening the Ca^{2+} ion channel, Ca^{2+} ions will enter the beta cells thereby stimulating the insulin cells (Widyastuti *et al.*, 2022). Positive control treatment for 14 days resulted in a reduction in blood glucose levels of 57.52% with the final result being that the average blood glucose level on day 14 was 89.2 mg/dL (**Table 2**). So it's known that on the 14th day the rats no longer experienced hyperglycemic conditions. With the final results the average blood glucose level on day 14 was 276.2 mg/dL (**Table 2**). This proves that on the 14th day of negative control treatment, the rats still experienced a hyperglycemic condition. So it can be interpreted that the effect of alloxan was still working until the 14th day in maintaining the rats's hyperglycemic condition.

Based on the profile rats body weight before and after treatment (**Figure 1**), the group treated with ethanol extract of RCR at a dose of 100 mg/kgBB experienced an improvement in body weight as seen from post alloxan until the 14th day. Meanwhile, the group treated with ethanol extract of RCR with doses of 25 and 50 mg/kgBW actually experienced a decrease in body weight from post alloxan to day 14. Data on the body weight of the glibenclamide positive control increased from baseline to day 7, but decreased again on day 14. In the negative control group, data showed that the rats's body weight increased and decreased which tended to be constant. In the weight change profile, it was found that the average weight change was not yet included in the category of vulnerability to insulin resistance because the change in body weight did not exceed 20% (Trisviana, 2012). Three different dose levels of RCR extract, specifically 25; 50; and 100 mg/kgBW, were orally administered once a day for a duration of 14 days. Following this administration, blood glucose levels were monitored on the 14th day. The results obtained from monitoring blood glucose levels of the ethanol extract of RCR at a dose of 25 mg/kgBW were 101.0 mg/dL, a dose of 50 mg/kgBW was 81.6 mg/dL, and a dose of 100 mg/kgBW was 79.4 mg/dL. So it is known that rats no longer experience hyperglycemic conditions. A dose of ethanol extract of RCR 25 mg/kgBW was found to experience a percentage reduction in blood glucose levels of 73.63%, a dose of ethanol extract of RCR 50 mg/kgBW was 77.89%, and a dose of ethanol extract of RCR 100 mg/kgBW of 80.10%. The percentage reduction in blood glucose was obtained by calculating blood glucose levels alloxan induction compared to blood glucose levels on the 14th day treatment.

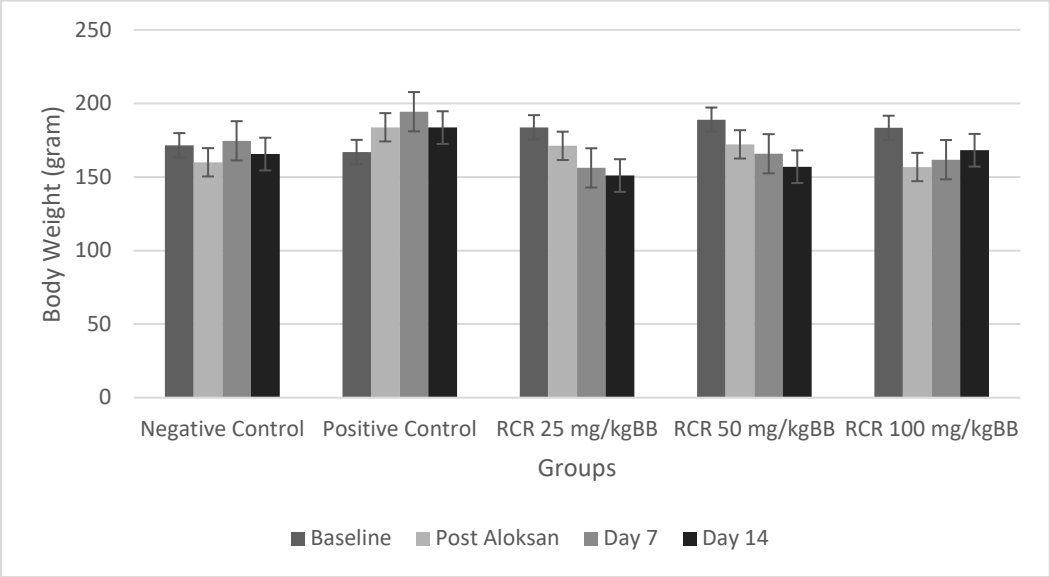

Figure 1. Profile of rats body weight before and after treatment

The greatest decrease in BGL (Blood Glucose Level) occurred between post-alloxan and day 7 (**Table 2**). There were significant differences between the negative control treatment group and the positive control group and the three dose levels (**Table 2**). The results of decreasing blood glucose levels respectively at doses of 25, 50, and 100 mg/kgBW were 101.0 mg/dL, 81.6 mg/dL, and 79.4 mg/d. This shows that administration of the extract for 14 days was able to reduce blood glucose levels in test animals. Test results between extract doses of 25; 50; and 100 mg/kgBW compared with the positive control ($p > 0.05$). This can be interpreted that the extract dose of 25 mg/kgBW, 50 mg/kgBW, and 100 mg/kgBW of RCR has a equal effect to glibenclamide dose of 5 mg/kgBW in reducing blood glucose levels. The decrease in blood glucose is thought to be caused by the presence of one or more compounds contained in the extract. In this study, the extract contained flavonoids, terpenoids, alkaloids, tannins and phenolics. The flavonoid compound that is abundant in human food nutrition is quercetin that involved in several biological actions such as: glucose homeostasis; insulin sensitivity and secretion; glucose utilization in peripheral tissues; inhibition of intestinal glucose absorption (Al-Ishaq *et al.*, 2019). The regulatory effect of quercetin on nuclear factor kappa-light-chain-enhancer of the activated B cells (NF-κB) also helps in increasing glucose-stimulated insulin secretion (Al-Ishaq *et al.*, 2019). The other component that tought to play a role in this extract is terpenoid that can stimulates pancreatic beta cells to secrete insulin and stimulating the regeneration of Langerhans cells so that damage to Langerhans cells, especially beta cells, can be reduced gradually and their number returns to normal (Akuba *et al.*, 2022; Nur alfaeni *et al.*, 2022). Other ingredients that have the potential to lower blood glucose levels are alkaloid and tannin. Alkaloids possess the ability to reduce blood glucose levels by inhibiting the α-glucosidase enzyme in the duodenal mucosa. This inhibition hinders the breakdown of polysaccharides into monosaccharides, leading to a slower release of glucose and a decreased rate of absorption into the bloodstream. Consequently, this results in the avoidance of elevated peak blood glucose levels. Tannin also exhibits blood sugar-lowering activity by inhibiting the function of α-glucosidase. This inhibition prevents excessive sugar absorption and

controls the rate at which sugar increases in the digestive system, ensuring it remains within a moderate range (Akuba *et al*., 2022).

CONCLUSIONS

The findings of the conducted research and data analysis lead to the conclusion that the ethanol extract derived from the RCR (*Jatropha gossypiifolia* L) exhibits the ability to reduce blood glucose levels in male *Wistar* rats. This effect is observed when administering doses of 25, 50, and 100 mg/kgBW. Furthermore, the impact of the extract is found to be similar to that of glibenclamide, a medication commonly prescribed for managing diabetes, at a dose of 5 mg/kgBW. It was concluded that red distance can be considered in future research and projects designed to produce biologically active compounds and molecules of this type.

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