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Research Article

The Effectiveness of Walnuts Extract and Metformin on Blood Sugar Level Reduction in Hyperglycemic Induced Alloxan Rats

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ABSTRACT

This study aims to determine the effectiveness of walnut extract (*Canarium indica*) and metformin in reducing blood sugar level of Wistar rats (*Rattus norvegicus*) induced by alloxan. Experimental research was conducted with pre-post test control group design. The sample in this study were 28 rats induced by alloxan to become hyperglycemic. The test animals were divided into four groups, each consisting of seven: negative control (Na CMC 1%), positive control (metformin 150 mg/kg BW), walnut extract group 300 mg/kg BW (60mg/200gr BW), and extract 600 mg/kg BW (120mg/200gr BW). The intervention was given walnut extract for 21 days. Blood sugar level was analyzed using Autocheck Multi-Monitoring System on day 0, day 3, day 6, day 14, and day 27 and analyzed using spss with paired t-test and ANOVA test. This research was conducted with the permission of the Ethics Committee of the Hasanuddin University Faculty of Public Health with number: 2584 / UN.4.14.1 / TP 072 / 2020. The results showed a significant reduction in fasting blood sugar level in all groups ($p < 0.05$). There was a significant decrease in blood sugar level between the four groups ($p = 0.006$). The highest reduction in blood sugar level was in the extract group 600mg/kg BW (301.0 mg/dl), then positive control(KP) group (metformin 150mg/kg BW) (288.5 mg/dl), extract 300 mg/kg BW (247.71 mg/dl), and the lowest decrease in thenegative control(KN) group (Na CMC 1%) (41.55 mg/dl). In this study, found no significant differences between walnut extract and metformin in order decrease blood fasting glucosa level. In conclusion walnut extract the same effectiveness as metformin. The intervention of walnut extract for 21 days was able to reduce blood sugar level in alloxan-induced hyperglycemic rats, starting with a dose of 300mg/kg BW to 600mg/kg BW.

Keywords: Alloxan, Walnut Extract (*Canarium indica*), Hyperglycemic, Rat

INTRODUCTION

Diabetes mellitus is a health problem in the 21st century. Globally, the prevalence of diabetes in 2019 is around 9.3%, and the latest estimate will increase by around 0.9% in 2035 to 10.2%. Hyperglycemic is an indicator of diabetes mellitus^{1,2}. Hyperglycemic is a sign of diabetes mellitus. Hyperglycemic is a medical situation due to an increase in blood glucose level beyond normal limits. The American Diabetes Association (ADA) strives to overcome diabetes problems by means of pharmacology (drugs) and non pharmacology (nutrition, and physical activity). New evidence shows that foods containing unsaturated fatty acids and antioxidants such as nuts can reduce fasting blood sugar level³. In some Asian countries walnuts are one of the largest commodities. Plant genus walnuts are used as anti-inflammatory, antibacterial, and

antidiabetic⁴. Walnuts contain antioxidant compounds and contain unsaturated fatty acids. Walnuts contain flavonoids, polyphenols, and phenolics which play a role in reducing oxidative stress in hyperglycemia^{5,6}. Research related to walnuts has not been widely reported, especially those related to metformin (biguanide group), although there have been many in vitro studies showing that walnuts contain bioactive compounds that act as antioxidants, especially nuts from Selayar Islands, Indonesia.

This study aims to determine the effectiveness of walnut extract and metformin in reducing blood sugar level of alloxan-induced rats.

METHODS

Walnut Extract Preparation

Fresh walnuts (*Canarium indica*) are obtained from Selayar Regency, South Sulawesi Province.

Fresh walnuts are still soaked in 96% ethanol, stirred every 4 hours for 15 minutes, then soaked for 1 x 24 hours (one day). The results of the first soaking are filtered using Whatman filter paper number one, so that the walnut pulp are obtained. After that the dregs from the first immersion are re-soaked using 96% ethanol or remaceration. The remaseration process was carried out 2 times. The results of the filtrate from all immersion are combined then evaporated using an evaporator at a temperature of 55°C, then in a water bath with a temperature of 50°C in order to obtain a thick reddish yellow extract.

Preparation of Experimental Animals

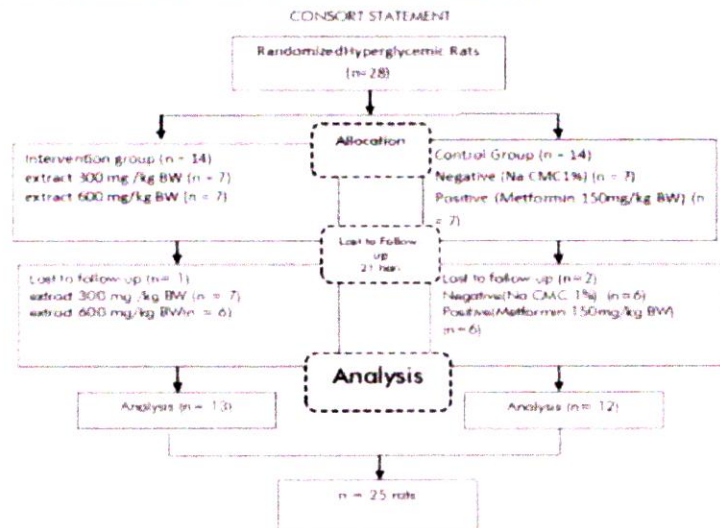
22 test animals used in this study were male wistar rat (*Rattus norvegicus*) 3-4 months old and weighing 180-270 grams. The test animals were obtained from the animal house of the Faculty of Pharmacy, Hasanuddin University, which were adapted for 7 days before intervention, and were given standard feed and drinking water ad libitum. The rats were fasted for 8 hours before checking blood sugar. The implementation of this research was carried out with the permission of the Ethics Committee of the Hasanuddin University Faculty of Public Health with number: 2584 / UN.4.14.1 / TP 01.02 / 2020

Experimental Design

Twenty-eight male rats aged 3-4 months were acclimatized for one week and divided into four

groups. The test animals were made in hyperglycemic conditions using alloxan-induced intraperitoneally at 130 mg/kg BW, with the previous condition fasting for 8 hours. Fasting blood sugar level 15 were measured 3-6 days after alloxan injection. rats with fasting blood sugar level > 200 mg/dl were declared hyperglycemic 22 and used for further research. Walnut extract (300 and 600 mg/kg BW) was administered daily for 21 days in the intervention group.

The test animals were 3 randomly divided into four groups as follows: negative control (KN) (Na CMC-1%), positive control (KP) (metformin 150mg/kg BW), walnut extract 300mg/kg BW (EK1), and extract 600 mg/kg BW (EK2). During the intervention process at week 3 or on day 15, and 18 interventions there were 3 rat from the KN group (Na CMC 1%), KP (metformin 150 mg/kg BW) and EK2 group (walnut extract 600 mg/kg BW) Each rat was included in the exclusion criteria (died during the intervention) so that the number analyzed at the end of the study was twenty-five rat. Fasting blood sugar level were measured 8 using the Multi-Monitoring System Autocheck, on day 0, day 3, day 6, day 24 (for hyperglycemic rats on the 3 days after alloxan induction), and day 27 (for hyperglycemic rats on the 6 days after alloxan induction). Before the autocheck is used, the tool calibration is done first using a special chip on the tool.

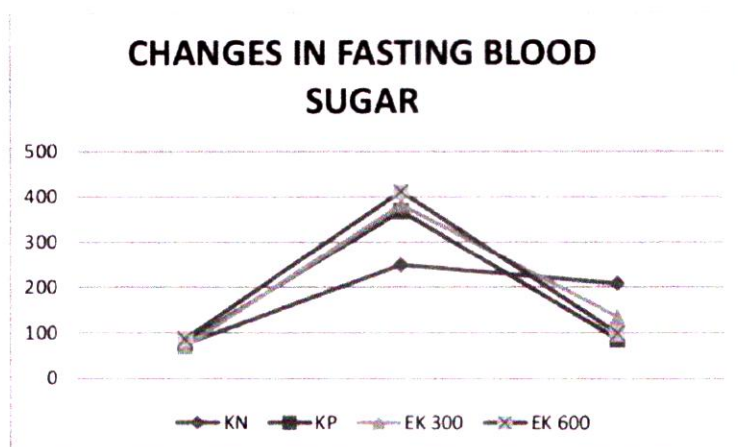


Data Analysis

Data were analyzed using the SPSS program. The data analysis technique used was paired t test 10 and ANOVA then followed by the LSD method to determine the differences between groups. The

significance was set at p <0.05. Data presentation is done in tables and graphs.

RESULTS



Graph 1: Changes in Fasting Blood Sugar (FBS)

Information: KN (negative control (Na CMC 1%)), KP (positive control (metformin 150 mg/kg BW)) EK 1 (walnut extract 300 mg /kg BW), EK 2 (walnut extract 600 mg /kg BW)

In graph 1 shows , an increase in fasting blood sugar level induced by alloxan on days 3 and 6. The highest increase was in the EK 2 group (extra 600 mg/kg BW) (86 mg/dl to 399.83 mg/dl). Giving EK2, can reduce fasting blood sugar level (399.83 mg /dl to 98.83 mg/dl normal). In the EK 1 group (extract 300mg/kg BW) (69.71 mg/dlto 382.42mg/dl), and after the intervention for 21 days there was a decrease in fasting blood sugar level to (134.71 mg/dl). Change of reduction in the KP group (metformin 150 mg/kg BW) did not difference muchfrom the extract

group. KP fasting blood sugar level (77.66mg/dl), post alloxan induction increased 4 times from early fasting blood sugar (368.33mg/dl), and there was a decrease in fasting blood sugar to normal (85.33mg/dl) for 21 days of intervention. The KN group (Na CMC 1%) also experienced an increase in fasting blood sugar (174mg/dl) where the average initial blood sugar (74.83 mg/dl, became 248.83 mg/dl), after treatment with Na CMC 1 % KN group experienced a change in decreasing fasting blood sugar level (41.55to 207.33 mg/dl).

Table 1: Average Fasting Blood Sugar Level (FBS) Pre and Post Before and After Intervention between groups

Group	FBS Pre (Hyperglycemic) Mean ± SD	FBSPost (3 Weeks) Mean ± SD	P value*	Δ	P Value **
KN	248,83 ± 42,31	207,33 ± 14,43	0,016	↓41,55	0,006
KP	368,33 ± 137,58	85,83 ± 6,36	0,004	↓288,50	
EK1	382,42 ± 148,62	134,71 ± 16,88	0,003	↓247,71	
EK 2	399,83 ± 145,62	98,83 ± 8,24	0,003	↓301,00	

Note: KN (Negative control (Na CMC 1%)), KP (positive control (metformin 150 mg/kg BW)) EK 1 (walnut extract 300 mg/kg BW), EK 2 (walnut extract 600 mg/kg BW), * Paired Test ** One Way Anova

Based on table 1, the average fasting blood sugar of all groups before treatment experienced hyperglycemic. After 21 days of treatment, all groups experienced a decrease in fasting blood sugar, where the highest decrease occurred in the KP group (metformin 150mg/kg BW) (368.33 mg/dl decreased to 85.83 mg/dl). Changes in the decrease in fasting blood sugar level in the EK 2 group (extract 600 mg/kg BW) (399.83 mg/dl decreased to 98.83mg/dl), then changes in the decrease in the EK1 group (extract 300mg/kgBW) (382.48 mg/dldecreased to 134.71 mg/dl), and

the KN group (Na CMC 1%) (248.83 mg/dl) and only decreased to 207.33mg /dl). The Paired T-Test in all groups experienced a significant reduction in blood sugar, and Anova test showed that all groups experienced a significant decrease (p = 0.006). It was found that there was a difference in fasting blood sugar level between the KN group (Na CMC 1%) and the KP group (metformin 150 mg/kg BW) (41.55mg/dl) and (288.50mg/dl), and average change in decreasing fasting blood sugar level in the intervention EK1 (257.0 mg/dl) and EK2 600

(301.1 mg/dl). The results indicates that the EK1 (extract 300 mg/kg BW) and EK 2 (extract 600 mg/kg BW) intervention groups also experienced a decrease and were not much different from the KP group given metformin (150 mg/kg BW).

Table 2: Comparison between groups of LSD (Least Significant Difference) Advanced Test

Intergroup Comparisons	P Value
EK 1 vs KN	0,006
EK 1 vs KP	0,720
EK 2 vs KN	0,002
EK 2 vs KP	0,802

Information: KN (Negative control (Na CMC 1%)), KP (Positive control (metformin 150 mg /kg BW)), EK 1 (walnut extract 300 mg/kg BW), EK 2 (walnut extract 600 mg/kg BW)

In table 2, the results of further analysis of the LSD (Least Significant Difference) test between EK1 (extract 300 mg/kg BW) and EK2 (extract 600 mg/kg BW) with the KP group (metformin 150 mg/kg BW) were not significantly different ($p > 0.05$), however, there was a significant difference ($p < 0.05$) with the KN group (Na CMC 1%).

DISCUSSION

Diabetes mellitus is a metabolic syndrome disorder caused by hyperglycemia⁷. In this study, it was found that a significant effect of walnut extract administration on reducing fasting blood sugar in hyperglycemic rats.

Some literature explains that functional food can improve metabolic disorders including hyperglycemic, Walnut¹¹ which contain antioxidant compounds and unsaturated fatty acids. Walnuts contain flavonoids, polyphenols¹¹ and phenolics which acts as antioxidants to reduce oxidative stress in hyperglycemia^{5,6}. The content of bioactive compounds such as antioxidants in some extracts can reduce blood sugar level through the regeneration process of pancreatic beta, decrease gluconeogenesis, and inhibit alpha-glucosidase enzyme activity, and oxidative stress^{8,9}.

Graph 1 in this study shows an increase in early fasting blood sugar level after alloxan induction and a decrease in fasting blood sugar level after 21 days of intervention in all groups of tested animals. The average initial blood sugar level in the group giving EK2 (extract 600 mg/kg BW) (86.00 mg/dl) after alloxan induction increased to (399.83 mg/dl), and after giving walnut extract decreased to normal (98.83mg/dl). In the intervention group EK1 (extract 300mg/kg BW) the average initial fasting blood sugar level (69.71 mg/dl), after alloxan induction increased (382.42 mg/dl), and after intervention decreased to (134,71 mg/dl), the KP group (metformin 150mg/kg BW) had an average baseline blood sugar level (77.66 mg/dl), post alloxan induction

has quadrupled (368.33 mg/dl), and After 21 days of intervention, fasting blood sugar level changed to normal (85.33 mg/dl), and the KN group (Na CMC 1%) had an average fasting blood sugar level before induction (74.83 mg/dl), after induction (248.83 mg/dl) after intervention (207.33 mg/dl).

Several previous studies using test animals have shown intervention with legume extracts can reduce fasting blood sugar level. According to a study using walnuts can lower blood glucose level in streptozocin-induced rats⁷.

In table 1, it can be seen that changes in fasting blood sugar level before and after treatment show that there are significant differences between groups on fasting blood sugar level with p value (0.006). The KN group (Na CMC 1%) experienced changes in blood sugar level with a decrease of only (41.55 mg/dl). Although alloxan is toxic to pancreatic beta cells by reducing the number of insulin-carrying granules and increasing blood sugar levels. However, this study shows that in a few days after alloxan induction, blood sugar levels will decrease, this is because pancreatic beta cells can increase the expression of beta Langerhans cells which are immunoreactive to insulin^{10,11}. The KP group that was given metformin 150 mg/ kg BW experienced a very significant decrease, which was around 288.50 mg/ dl, and was not much different from the decrease in the 300 and 600 extract groups with decreases of (247.71 and 301.00 mg/ dl).

The results of the analysis using the post hoc LSD (Least Significant Difference) follow-up test in Tabel 2 showed that the intervention between the EK1 group (extract 300 mg/kg BW) and the EK2 group (extract 600 mg/kg BW) with the KP group (metformin 150 mg/kg BW) has p-value (0.720) and (0.802). The results of the analysis indicated that the EK1 (extract 300mg/kg BW) and EK2 (extract 600mg/kg BW) extract groups had an effective effect on reducing fasting blood sugar level and were not significantly different from the

metformin group. Decreased blood glucose in the positive control group with metformin was due to the ability to inhibit liver gluconeogenesis and increase insulin sensitivity¹². The mechanism of action of metformin has the same mechanism of action of bioactive flavonoids and terpenoids derived from walnuts.

As for the mechanism of action of flavonoids in terms of lowering blood sugar to inhibit the formation of ROS (Reaxy Oxidative Stress), these bioactive compounds can increase the superoxide dismutase enzyme and glutathione level¹³. Superoxide dismutase and glutathione are natural antioxidant compounds found in the human body. Increased level of glutathione in the body can increase insulin sensitivity¹⁴. Terpenoids can also reduce blood glucose level and can help regenerate pancreatic beta cells and increase insulin sensitivity¹⁵. Thus these two bioactive compounds have the same mechanism as metformin in reducing blood sugar level by increasing insulin sensitivity.

Apart from the content of flavonoids and tannins, it turns out that other bioactive compounds such as alkaloids, tannins and saponins are also able to reduce fasting blood sugar level through different mechanisms. Alkaloids inhibit glucose absorption in the intestine, increase glucose transport in the blood, are able to stimulate glycogen to inhibit glucose synthesis by inhibiting glucose 6-phosphatase enzymes, and increasing glucose 6-phosphate dehydrogenase oxidation¹⁵⁻¹⁷, then the mechanism of saponins and tannins has a role in lowering blood sugar by activating glycogen synthesis, suppressing disaccharide activity, modulating insulin response, regenerating insulin activity, and suppressing the occurrence of gluconeogenesis and inhibiting alpha-glucosidase activity^{18,19}.

The decrease in blood sugar level in hyperglycemic rats induced by alloxan, which were given walnut extract intervention was not only due to antioxidants, but it turned out that walnuts also contain unsaturated fatty acids which can reduce blood sugar level with an anti-inflammatory pathway. Based on several studies showing that walnuts¹⁴ also contain fatty acids, namely unsaturated fatty acids such as oleic acid, linoleic acid, linoleic acid, palmitic acid²⁰. However, in this study, the analysis of unsaturated fatty acids in walnut extract from Selayar was not carried out on changes in fasting blood sugar level, so it is necessary to study this.

CONCLUSION

In this study it was concluded that all groups of KN (Na CMC 1%), KP (metformin 150 mg/kg BW), EK 1 (walnut extract 300 mg/kg BW), and

EK 2 (extract 600 mg/kg BW), can reduce blood sugar level of alloxan-induced rats after 21 days of intervention. The highest reduction blood glucose level was in the 600 mg/kg BW extract group and the lowest was in the Na CMC 1% group. The decrease in fasting blood sugar level in the extract group 300 mg/kg BW and extract 600 mg/kg BW experienced a significant change with the KN group (Na CMC 1%) and was not significant in the KP group (metformin 150 mg/kg BW). The extracts of 300 and 600 mg/kg BW were as effective as metformin in reducing blood sugar in rats against changes in fasting blood sugar level.

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