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**EFFECT OF ETHANOL EXTRACT OF KARAMUNTING LEAF (*RHODOMYRTUS TOMENTOSA* (AITON) HASSK ) ON TESTOSTERONE HORMONE LEVELS OF DIABETES MELLITUS MODEL RATS****Husnul Khatimah<sup>1\*</sup>, Lena Rosida<sup>1</sup>, Siti Kaidah<sup>1</sup>, Ida Yuliana<sup>1,2</sup>**<sup>1</sup>Fakultas Kedokteran, Universitas Lambung Mangkurat<sup>2</sup>Wetland Research Centre, Fakultas Kedokteran, Universitas Lambung Mangkurat\*Correspondence email: hkhatimah@ulm.ac.id

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**ABSTRACT**

Karamunting leaf is a natural ingredient in South Kalimantan and is used for the treatment of diabetes mellitus. Diabetes mellitus (DM) can decrease hormone testosterone. Research on Karamunting leaves on testosterone levels had not been widely studied. The aim was to analyse the effect of administration ethanol extract Karamunting leaf on testosterone levels in diabetic rats induced by streptozotocin. The method was true experimental, Posttest Control Group. The research groups were divided into 4 groups. The control group giving streptozotocin induction at dosage 40 mg/kg bw rats intraperitoneally. The treatment group was a group of rats DM treated with ethanol extract of Karamunting leaf at dosage 100 mg/kg bw, 200 mg/kg bw, and 400 mg/kg bw. The treatment in the research group was carried out for 14 days. On day 15, blood was taken to measure testosterone levels by the ELISA method and glucose blood. The data were analysed with Kolmogorov Smirnov and Mann Whitney. Testosterone levels increased with administration of ethanol extract Karamunting leaves at dose 100 mg/kg bw ( $16.58 \pm 2.8$ ) and 400 mg/kg bw ( $16.17 \pm 2.07$ ) when compared with the control group ( $14.56 \pm 0.8$ ). Administration of ethanolic extract of Karamunting leaf in DM rats has an effect on testosterone levels.

**Keywords:** Diabetes mellitus; Karamunting Leaf; Testosterone

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**INTRODUCTION**

South Kalimantan is one of the regions that has rich forests natural ingredients that are efficacious as medicine. Exploration of natural materials that can be used as medicine is important to do in line with the high public interest in herbal medicines. Karamunting plant is one of the biological diversity found in South Kalimantan and must be developed because it has been reported as a potential plant as a phytomedicine.<sup>1</sup>

Karamunting plant (*Rhodomyrtus tomentosa* (Aiton) Hassk), which belongs to the Myrtaceae family, is traditionally used to treat burns, diarrhoea, abdominal pain, and diabetes.<sup>1</sup> The application of traditional medicine is generally considered secure than modern medicine. It's because traditional medicine has relatively fewer side effects than drugs from modern medicine.<sup>2</sup>

Karamunting leaves (*Rhodomyrtus tomentosa* (Aiton) Hassk)) can lower blood sugar levels because they contain three compounds, namely alkaloids, flavonoids and tannins.<sup>3</sup> Sulistyó's research (2007) obtained that the methanol extract of Karamunting leaves had a significant effect on reducing blood glucose levels of test animals at a dosage of 200 mg/kg bw.<sup>4</sup> Sinata and Arifin (2016) found that the water fraction of Karamunting leaves at a dosage of 10, 20, and 40 mg/kg bw can decrease blood glucose levels in diabetic mice.<sup>5</sup> Jamblang leaves have the same family as Karamunting leaves, namely the Myrtaceae family. Ethanol extract of jamblang leaves (*Syzygium cumini* L) at a dosage of 200 mg/kg bw can decrease blood glucose levels of white male rats with streptozotocin induction.<sup>6</sup>

Diabetes mellitus is a chronic metabolic abnormality marked by hyperglycemia due to

failure in insulin secretion, insulin action, or both. Furthermore, diabetes mellitus is associated with severe interference in carbohydrates, fat, and protein metabolism.<sup>7</sup> Data by the international diabetes federation in 2014 showed that the number of people with DM in Indonesia increased to 9.116 million in 2014 and is expected to increase to 14.1 million in 2035.<sup>8</sup> The incidence of diabetes mellitus in South Kalimantan in 2018 was around 21,004 cases, with the highest incidence in Banjarbaru City as many as 6790 cases. The incidence of diabetes mellitus in South Kalimantan increased to 63,282 cases in 2019, with the highest incidence in the city of Banjarmasin, as many as 20,154 cases.<sup>9</sup>

Many studies reporting both in men with DM and animal models show that DM can cause infertility in men through various ways, including changes in spermatogenesis, degenerative and apoptotic changes in the testes, changes in sugar metabolism in the testicular blood barrier/Sertoli cells, decreased synthesis and secretion of testosterone, and erectile dysfunction.<sup>10</sup>

Decreased testosterone has been reported in a number of studies. Zhao et al. (2010) research on DM rats induced by streptozotocin for 4, 8 and 12 weeks found a decrease in serum testosterone content, follicle-stimulating hormone (FSH), and luteinising hormone (LH).<sup>11</sup> Research conducted by Mohassed et al. (2011) on DM rats induced by streptozotocin for eight weeks found a decrease in serum testosterone levels, and there was germ cell apoptosis and oxidative damage to the testes.<sup>12</sup> Research by Pontes et al. (2011) on streptozotocin-induced DM rats for three weeks found a significant decrease in serum testosterone levels and a significant reduction in the weight of the seminal vesicles and prostate gland.<sup>13</sup> Research conducted by Vikram et al. (2011) also showed a decrease in testosterone levels in rats induced by streptozotocin for six weeks.<sup>14</sup>

This study was conducted to determine the impact of ethanol extract of Karamunting leaves on testosterone levels in rats with hyperglycemia. This research has never been

done, and the existing research on Karamunting leaves only looks at blood sugar levels, not seeing complications of diabetes mellitus, one of which is a decrease in testosterone levels. Therefore, researchers are interested in conducting this research. This research is expected to be the basis for the development of herbal medicines for diabetes mellitus, especially in reproduction system complications.

## MATERIAL AND METHODS

This research has passed ethical approval from the Health Research Ethics Commission, Faculty of Medicine, Lambung Mangkurat University, with no 594/KEPK-FK ULM/EC/IV/2021. This research was conducted at the Banjarbaru Veterinary Center for the maintenance and treatment of rats. Testosterone examination was carried out in the Biochemical and Molecular Laboratory, Faculty of Medicine, Universitas Lambung Mangkurat.

The design of this research was a true experimental design with a posttest control group design. This research used 32 white male rats (*Rattus norvegicus*) Wistar strain, aged 2-6 months, and weight 200 – 300 g. Calculation of the number of test animals in the study used the Federer formula

The research group was divided into the control group (K) and the treatment group P1, P2, and P3. The division of research groups is as follows:

- a. The control group (K) is a group of experimental animals induced by Streptozotocin (STZ) at a dose of 40 mg/kgbw of test rats intra-peritoneally. Rats were considered diabetic if their blood glucose level was > 180 mg/dl. Hereinafter referred to as diabetic rats.<sup>15</sup>
- b. The treatment group (P) is a group of diabetic experimental animals treated with ethanol extract of Karamunting leaves:<sup>16</sup>
  - a) Group P1: Rat group given Streptozotocin and Ethanol Extract of Karamunting Leaf at a dosage of 100 mg/kg bw.

- b) Group P2: Rat group was given streptozotocin and Karamunting Leaf Ethanol Extract at a dosage of 200 mg/kg bw.
- c) Group P3: Rat group was given streptozotocin, and Karamunting Leaf Ethanol Extract at a dosage of 400 mg/kg bw.

### **Plant Material and Preparation of Extract**

The determination of the Karamunting plant was carried out at the Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University. The leaf herbarium samples of the Karamunting plant were obtained from the Martapura area, Banjar Regency, South Kalimantan. The ethanol extract of Karamunting leaves was made at the Pharmacology and Therapeutics Laboratory, Faculty of Medicine, Lambung Mangkurat University.

The ethanol extract of Karamunting leaves was prepared as follows: 3 kg of Karamunting leaves were first cleaned under running water to clean from dirt. Then, the leaves were dried in the sun for four days until the moisture content was reduced to the remaining 10%; in this case, can use the oven as a tool, with a temperature of 40° C for 15 minutes. After the Karamunting leaves dried, the Karamunting leaves were reduced in size using a knife. Prior to maceration extraction, 1.2 kg of Karamunting leaf was ground in a blender to reduce the size of the Karamunting leaf to expand the surface that would come into contact with the solvent. The solvent used is 96% ethanol. Then the Karamunting leaves were macerated with 96% ethanol. Filtering was done every day, and the pulp was soaked again with a new solvent. The macerator was closed, left in a cool place, protected from light. Concentrated extracts were obtained using a rotary cavity evaporator at a temperature of 40-50°C. The extract solution was made in 3 different doses of 100, 200, and 400 mg/kgbw. The treatment solution was made using a 1% CMC solution by dissolving 1 gram of CMC in 759 distilled water until it swelled and the solution was homogenous.

### **Animal Experiment**

The test animals were weighed and then grouped into four groups and placed separately. Prior to the experiment, the test animals were adapted to laboratory conditions for one week before the experiment. The test animals have fasted for 18 hours. Blood sampling was carried out on day 8 to determine the initial blood glucose level using a glucometer by cutting the tip of the rat's tail 1 mm using scissors. The rat's blood was touched to the glucometer stick, and the numbers that appeared on the glucometer screen were recorded. Blood glucose observations were carried out on day 0 and day 3 after STZ induction.<sup>17</sup>

Furthermore, groups of white rats K, P1, P2, P3 were given streptozotocin at a dosage of 40 mg/kg bw intra-peritoneally on day 0 with one dose. After being induced, the rats were still given food and drink ad libitum. On the 3rd day blood, glucose levels were measured with a glucometer. Rats are considered diabetic if blood glucose levels are >180 mg/dl.<sup>15</sup> On the 4th day, experimental animals were treated by giving a test solution of ethanol extract of Karamunting leaves at a dose of 100, 200, 400 mg/kg bw for 14 days. Giving ethanol extract of Karamunting leaves was carried out using a gastric probe of about ± 2 ml. Rats were given food and drink ad libitum. Measurement of blood sugar and testosterone levels were measured on the 15th day. Before the blood was taken from the heart using a three cc syringe on the 15th day, the rats were anaesthetised, then 2 ml of blood that came out was accommodated in a sterile test tube (Eppendorf tube) which had been previously sterilised. The blood was centrifuged at 1000 rpm (rounds per minute) for 10 minutes. After being separated, 250 microliters of serum were taken. Measurement of testosterone levels was carried out using the ELISA (Immulite Total Testosterone) method.<sup>18</sup> Blood glucose observations were carried out with a glucometer.

The data obtained were tested for normality with the Kolmogorov-Smirnov test and for homogeneity with the Levene test. If

the  $p$ -value  $> 0.05$ , then the data is normally distributed and homogeneous to be analysed using parametric tests. The results of the measurement of testosterone levels will obtain numerical data.

Suppose it is known that the data is normally distributed and homogeneous. In that case, the research data will be analysed using parametric test analysis with ANOVA test to determine the difference in testosterone levels between control data and treatment data. This study uses a 95% confidence interval and a significance level of  $p < 0.05$ . If it is not normally distributed and homogeneous, data transformation is performed. If successful, the ANOVA test is performed. If not successful, Kruskal Wallis non-parametric test and Mann Whitney post hoc test were performed.

## RESULT

**Table 1.** Average Fasting Blood Sugar Levels in DM Model Rats before and after Treatment

Group	Before Treatment (mg/dl)	After Treatment (mg/dl)
Control	344,8±42	338,1±40
Treatment P1	360,2±102,4	154,8±89,6
Treatment P2	370,1±47,3	148,3±50,4
Treatment P3	421±61,1	92,75±62,1

**Table 2.** Average Testosterone Levels in DM Model rats

Groups	Mean ± SD (nMol/L)	p
Control	14.56 ± 0.8	
Treatment P1	16.58 ± 2.8	
Treatment P2	13.15 ± 2.33	0.007
Treatment P3	16.17 ± 2.07	

$p$ -value tested with Kruskal Wallis test

Then the normality test and homogeneity test were carried out; the results were not normally distributed and homogeneous in treatment groups P2 and P3 ( $p = 0.013$  and  $p = 0.0024$ ). After the data transformation was carried out, it was not successful, so that the data analysis used a non-parametric test, the Kruskal Wallis test; the results were  $p = 0.007$ , which means that there was a significant difference in the average testosterone levels of the DM rat

The study was conducted on 32 white male rats (*Rattus norvegicus*) Wistar strain. The induction of Streptozotocin 40 mg/kg bw was administered by intraperitoneal injection to the group of rats on day 0, and on day three after administration of STZ 40 mg/kg bw, fasting blood sugar was checked on the rats. Rats with blood sugar above 180 mg/dl were considered rats with diabetes mellitus. Rats with diabetes mellitus in treatment group P1 were given ethanol extract of Karamunting leaves 100 mg/kg bw, treatment group P2 was given ethanol extract of Karamunting leaves 200 mg/kg bw and treatment group P3 was given ethanol extract of Karamunting leaves 400 mg/kg bw. The treatment was carried out for 14 days; on the 15th day, the rats were drawn blood to examine testosterone and blood sugar levels, and the results obtained can be seen in tables 1 and 2.

group. Followed by the post hoc test, Mann Whitney test, the results showed that there were significant differences between the control group and treatment group P3 ( $p = 0.029$ ), treatment groups P1 and P2 ( $p = 0.014$ ), treatment group P2 and treatment P3 ( $p = 0.02$ ).

## DISCUSSION

In table 1, it can be seen that on the 3rd day of STZ administration, it was found that

the fasting blood sugar levels of rats increased in both the control and treatment groups P1, P2, and P3. This was because STZ administration in rats caused damage to pancreatic cells selectively. The toxic effect of STZ begins with the uptake of STZ into the cell through the low-affinity glucose-2 transporter (GLUT2) in the cell plasma membrane. Its toxic effect is more selective on pancreatic cells because it is based on the chemical structure of STZ, which has a glucose group making it easier for STZ to enter cells because pancreatic cells are more active in taking up glucose than other cells.<sup>19</sup> Cell death caused by STZ administration was due to the STZ methylnitrosourea group causing DNA methylation, particularly at the O6 position of guanine. This triggers DNA disruption, which ultimately leads to the death of pancreatic cells through the depletion of cellular energy stores. In addition, efforts to repair defective DNA through activation of poly ADP ribose polymerase (PARP) will further reduce cellular NAD<sup>+</sup>.<sup>20</sup> STZ-induced cell death involves three pathways:<sup>20</sup>

a. DNA methylation via the formation of carbonium ions (CH<sub>3</sub><sup>+</sup>) causes the nuclear enzyme poly ADP-ribose synthetase to be activated, which plays a role in cell repair mechanisms.

b. Nitric oxide production

c. Formation of free radicals such as hydrogen peroxide

In table 2, it can be seen that the average testosterone level in a group of control was decreased than in a group of treatment P1 and P2.

STZ injection significantly reduces testosterone levels. Induced diabetes by STZ is accompanied by hypogonadism due to a decrease in Leydig cells in the testes and disruption of androgen biosynthesis in a cell of Leydig. In addition, STZ causes a decrease in insulin due to the destruction of pancreatic B cells. Insulin is expressed in the testes and regulates the normal function of Leydig cells by supporting the synthesis of DNA and steroidogenesis during puberty. Also, insulin plays an important role in the Sertoli cell job, where insulin help mediate the transport of

glucose and the synthesis of lactate, an important substance for germ cells.<sup>21</sup>

The presence of high glucose levels or hyperglycemia in DM model rats causes an increase in oxidative reactivity (ROS) and can trigger oxidative stress in the body. The provenance source of ROS in mammalian cells is the “dropping” of electrons from the mitochondria of the respiratory chain and their subsequent transfer to molecular oxygen, resulting in superoxide anion (O<sub>2</sub><sup>•-</sup>) formation. In conjunction with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and nitric oxide (NO), superoxide is considered to be one of the major “primary” ROS, forming most of the variety of other ROS found in cells in further reactions.<sup>22</sup> In conditions of diabetes, there are several sources of ROS described; one of them is advanced glycation end products (AGEs). AGEs are a heterogeneous class of compounds formed by non-enzymatic glycation of proteins, which is accelerated in diabetes as a result of hyperglycemia and oxidative stress. Receptors for AGEs (RAGE) are present in testes, epididymis and sperm.<sup>19,20</sup> N<sup>ε</sup>-carboxymethyl lysine, a prominent AGE, accumulates in the reproductive tract of patients with diabetes, as well as in animal models of diabetes and metabolic syndrome. The degeneration of testosterone production was induced by AGEs through the induction of oxidative stress and endoplasmic reticulum stress and decreased levels of StAR, 3β-HSD and P450<sub>scc</sub> in Leydig cells.<sup>23</sup>

The presence of excessive lipid peroxidation reactions at the level of gonadal cell tissue (testes) can cause several changes in sexual function, including decreased levels of the hormone testosterone in the serum due to abnormal functioning of Leydig cells.<sup>24</sup> Damage to the germinal epithelial cell membrane of the seminiferous tubules and degeneration of testicular cells are the main consequences of the presence of ROS. Reactive oxygen compounds such as O<sub>2</sub><sup>•-</sup>, H<sub>2</sub>O<sub>2</sub>, and OH<sup>-</sup>, are mediators that play an important role in oxidative damage to cells, including Sertoli cells.<sup>25</sup> This decrease in the number of Sertoli cells and Leydig cells is

also thought to occur due to free radicals resulting in disruption of the hypothalamus, which secretes GnRH (Gonadotrophin Releasing Hormone), to stimulate the pituitary to secrete LH and FSH hormones that act on Leydig cells and Sertoli cells, this disorder causes cell activity decreases.<sup>26</sup>

The presence of ethanol extract of Karamunting leaves was proven to increase. The average level of testosterone in DM model rats can be seen in the post hoc results between the control group and treatment group 3 who were given ethanolic extract of karamunting leaves at a dosage of 400 mg/kg bw got a significant value ( $p = 0.029$ ). This is possible because Karamunting leaves (*Rhodymyrtus tomentosa* (Aiton) Hassk) contain phenolic compounds, saponins, tannins, steroids, terpenoids and flavonoids.<sup>5</sup> Karamunting leaves (*Rhodymyrtus tomentosa* (Aiton) Hassk) can lower blood sugar levels because they contain three compounds, namely alkaloids, flavonoids and tannins.<sup>3</sup> Research by Luthfiah, Pertiwi (2020) found that the ethanol extract of Karamunting leaves at a dose of 100 mg/kg bw, 200 mg/kg bw, and 400 mg/kg bw decreased blood sugar levels compared to positive controls, and there was a significant difference to positive controls.<sup>16</sup>

Alkaloids reduce blood sugar levels by hindering the absorption of glucose in the intestine, escalating the transport of glucose and stimulating glucose synthesis in the blood, stimulating the synthesis of glycogen and detaining the enzymes glucose 6-phosphate, fructose 1,6-bisphosphatase and escalating glucose oxidation 6-phosphate dehydrogenase.<sup>27</sup> Flavonoids are a group of polyphenols that have the ability to inhibit lipid peroxidation, metal chelation-oxidation reduction activity and inhibit processes related to Reactive Oxygen Species (ROS).<sup>28</sup> Flavonoids can prevent damage to pancreatic cells because of their ability to protect pancreatic islet cells from oxidative damage.<sup>29</sup> In addition, flavonoids can hinder glucose reabsorption from the kidneys and can escalate the solubility of blood glucose so that it is easily excreted in the urine.<sup>3</sup> The results of Isdamayani and Panunggal (2015) research

show that flavonoids have antidiabetic activity through their role as secondary antioxidants that work by cutting free radical chain oxidation reactions, capturing free radicals, and inhibiting gluconeogenesis in type 2 diabetes mellitus patients. Flavonoids can prevent oxidative stress in streptozotocin-induced diabetic rats.<sup>30</sup>

The mechanism of tannins in reducing blood glucose content is by hindering the absorption of glucose in the intestine and rendering the regeneration of pancreatic cells, which have an effect on adipose cells, thereby strengthening insulin activity. Tannins can reduce the free radicals in the body and increase glucose uptake in the blood through the activity of insulin mediators, thereby reducing blood glucose.<sup>30</sup> Tannins are known to trigger glucose and fat metabolism so that the accumulation of these two sources of calories in the blood can be prevented. Tannins have antioxidant activity and inhibit the growth of a tumour. Tannins also have hypoglycemic activity by escalating glycogenesis.<sup>31</sup>

Flavonoids and tannins have antioxidant activity so that they can capture free radicals that cause repair of damage to pancreatic beta cells that cause DM. There is an improvement in the pancreatic tissue. There will be an increase in the amount of insulin in the body so that blood glucose will enter the cells, resulting in a decrease in blood glucose in the body.<sup>26</sup> Increased insulin will increase the effect of gonadotropins, luteinising hormone (LH) and follicle-stimulating hormone (FSH) on Sertoli cells and Leydig cells in the testes. The insulin hormone will stimulate Leydig cells so that it will improve Leydig cell function and cause an increase in FSH receptors. Increased FSH receptors will increase FSH levels which also affect the increase in LH levels.<sup>32</sup> This increase will trigger an increase in the production of the hormone testosterone and androgen binding protein (ABP) which are used in the process of spermatogenesis.<sup>33</sup> Reduced oxidative stress can reduce insulin resistance and prevent the development of pancreatic cell dysfunction and damage.<sup>5</sup> This

explains the increase in testosterone levels in treatment groups P1 and P3 compared to the control group. Although treatment group P1 experienced an increase in testosterone levels, it was not significantly different when compared to the control group ( $p = 1.43$ ).

The treatment groups P2 had lower mean testosterone levels than control groups, treatment groups P1 and P3. This may be because, in the P2 treatment group, one rat had a testosterone level of 8.61, thus causing the average testosterone level in the P2 treatment group to be lower than the P1 and P3 treatment groups. Visual observations made on rats with low testosterone levels, rats drink more often, urinate frequently, rat fur is also a bit dry, indicating that the rats are dehydrated compared to other rats. This indicates that these rats experienced physical stress, which was probably due to oxidative stress related to uncompensated hyperglycemia conditions despite being given Karamunting leaves. In the future, it is necessary to conduct research on the expression of enzymes involved in the process of steroidogenesis, expression of AGEs in testis, specifically in Leydig cell, and oxidative stress parameters.

## CONCLUSION

Treatment of ethanol extract of Karamunting leaves at a dosage of 400 mg/kg bw can increase testosterone content in rats with the diabetes mellitus model.

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