



## **THE EFFECT OF GALOBA (*HORNSTEDTIA SP.*) FRUIT EXTRACT ON MALONDIALDEHYDE SERUM LEVEL OF HYPERGLYCEMIC MICE (*MUS MUSCULUS*) STREPTOZOTOCIN-INDUCED**

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### **ABSTRACT**

**Background:** Hyperglycemia or increased blood sugar levels is a sign of diabetes mellitus. In hyperglycemia, there will be an increase in Reactive Oxygen Species (ROS) in the body so that it will lead to a state of oxidative stress characterized by an increase in malondialdehyde (MDA) levels. Galoba fruit is an endemic fruit in Maluku Province that contains antioxidants. Antioxidants contained in galoba fruit can help endogenous antioxidants to overcome oxidative stress.

**Objective:** This study aims to determine the effect of galoba fruit extract on serum MDA levels of hyperglycemia mice induced by streptozotocin. **Methods:** This study is an experimental study with post-test only control group design and random sampling. The sample consisted of 24 mice divided into 6 groups, namely normal control (KN), negative control (K-), positive control (K+), treatment 1 (P1), treatment 2 (P2), and treatment 3 (P3). The KN group was only given a standard diet, the K- group was only induced streptozotocin, the K+ group was induced streptozotocin and treated with metformin for 21 days, and the P1, P2, and P3 groups were induced streptozotocin and given galoba fruit extract concentrations of 100%, 75%, and 50% for 21 days. After treatment, the mice were dissected to collect blood serum from the heart of the mice to measure MDA levels. Serum MDA levels were measured by the TBARS method. **Results:** The average serum MDA levels of the KN, K-, K+, P1, P2, and P3 groups were 358.75 nmol/mg, 1278.75 nmol/mg, 522.08 nmol/mg, 526.16 nmol/mg, 442.66 nmol/mg, and 432.41 nmol/mg. MDA data obtained was then tested with one way ANOVA and obtained a value of  $p=0.00$  ( $<0.05$ ). **Conclusion:** Based on the Tukey test, both P1, P2, and P3 have the same effect on reducing serum MDA levels of mice after being induced by streptozotocin with P3 providing the greatest decrease.

**Keywords:** Hyperglycemia, galoba fruit, mice, streptozotocin (STZ), malondialdehyde (MDA)

### **BACKGROUND**

Hyperglycemia is a condition of increased blood sugar levels.<sup>1</sup> According to the American Diabetes Association (ADA), normal fasting blood sugar levels are  $<100\text{mg/dL}$ , and a person is said to be experiencing hyperglycemia if their fasting blood sugar levels exceed this value. If the fasting blood sugar level is  $>100\text{mg/dL}$  then a person is categorized as prediabetes. If the fasting blood sugar level is  $\geq 126\text{mg/dL}$  then a person is said to be suffering from diabetes mellitus.<sup>2</sup>

The number of hyperglycemia sufferers continues to increase. According to data from the International Diabetes Federation (IDF), in 2019 the prevalence of the global population aged 20-79 years with hyperglycemia prediabetes was 7.5% or around 373.9 million people. This number is expected to increase in 2030 to 8% or 453.8 million and in 2045 the prevalence is estimated to be 8.6% or 548.5 million people.<sup>3</sup>

Hyperglycemia cannot be separated from diabetes mellitus. Diabetes mellitus is found in every world population and the number of sufferers is increasing. The World Health Organization (WHO) estimates that there were 442 million adults with diabetes worldwide in 2014.<sup>4</sup> According to the IDF, the prevalence of diabetes mellitus sufferers aged 20-79 years in 2019 was 9.3% or 463 million people. The number is estimated to increase to 10.2% or 478.4 million people in 2030 and 10.9% or 700.2 million people in 2045.<sup>3</sup>

According to the results of basic health research in 2018 by the Ministry of Health of the Republic of Indonesia, based on doctors' diagnoses, the prevalence of diabetes in Indonesia among those aged  $>15$  years was 2%, an increase from the results of basic health research in 2013 of 1.5%. According to the 2018 Maluku Province basic health research report, the prevalence of diabetes mellitus is based on the results of a doctor's diagnosis in residents  $>15$  years old, 1.12% of Maluku residents suffer from



diabetes mellitus.<sup>5</sup> In 2019 and 2020, diabetes mellitus was included in the top 10 diseases most commonly suffered in Maluku Province, namely 6212 sufferers in 2019 and 9012 sufferers in 2020.<sup>6</sup>

In hyperglycemia conditions, glucose autooxidation will occur. This process will increase the production of Reactive Oxygen Species (ROS) and cause oxidative stress.<sup>7</sup> Oxidative stress is a condition resulting from increased production of free radicals reduced activity of antioxidant defenses, or both.<sup>8</sup> Oxidative stress can occur due to an imbalance between ROS and antioxidant levels in the body.<sup>7</sup> Oxidative stress in hyperglycemia sufferers can be determined one way by measuring malondialdehyde (MDA), which is a marker of oxidative stress in blood serum. The high concentration of MDA indicates an oxidation process in the cell membrane.<sup>8</sup> Chemically, MDA is more stable than other compounds, so MDA is more often used as a marker of oxidative stress.<sup>7</sup>

Galoba is one of the endemic plants in Maluku Province which is consumed by the people as a traditional medicine.<sup>9</sup> Galoba can be found in forest areas, especially humid areas.<sup>10</sup> This plant contains flavonoid compounds such as flavones, flavanones, flavonols, flavanonols, anthocyanidins, aurones, flavonoids, furan chromones, biflavones, isoflavones, isoflavanones, chalcones, xanthenes and dihydrochalcones which can act as antioxidants.<sup>8</sup>

In research conducted by Cheng, *et al* in 2019 regarding the administration of *Sargassum fusiforme* to hyperglycemic mice (mice were categorized as hyperglycemic if their blood sugar levels were >97mg/dL) after being induced by streptozotocin (STZ), it was found that the MDA levels of mice induced by STZ and given *Sargassum fusiforme* extract lower than mice induced by STZ and not given therapy. *Sargassum fusiforme* is a type of brown algae that contains antioxidants and is used as traditional medicine in China.<sup>11</sup> Research by Husfa RK, *et al* in 2020 regarding the effect of green tea containing secondary metabolite compounds on the serum MDA levels of diabetic mice found that giving green tea affected reducing the serum MDA levels of mice.<sup>12</sup> Research by Gustaman, *et al* in 2020 to look at the antioxidant activity of Galoba fruit found that Galoba fruit contains secondary metabolite compounds including flavonoids, quinones, and terpenoids. Based on the description of several research results, the author conducted this research to

determine whether giving Galoba fruit extract which contains secondary metabolite compounds as an exogenous antioxidant can affect MDA levels in the serum of hyperglycemic mice.<sup>13</sup>

## METHODS

This research is experimental research with a post-test-only control group design, namely by comparing the control group with the experimental group. The samples used were Swiss Webster mice weighing 20-30 grams. Samples were selected randomly and divided into 6 groups, namely the normal control group (KN) received no treatment, the negative control group (K-) only received STZ induction treatment, the positive control group (K+) received STZ induction treatment and therapy with metformin, while the experimental group received STZ induction treatment and therapy with Galoba fruit extract at a concentration of 100% (P1), concentration of 75% (P2), and concentration of 50% (P3).

Galoba fruit extract is made using the maceration method. The solvent used is 96% ethanol. After the extract is obtained, a phytochemical test is carried out on the extract to ensure the secondary metabolite content in the extract. The phytochemical tests carried out were the flavonoid test, alkaloid test, and terpenoid test. Before being given to mice, the extract was made in 3 different concentrations, namely 100%, 75%, and 50% concentration with the addition of distilled water. Galoba fruit extract was given to mice every day for 21 days after induction with STZ for 5 days. Blood sugar levels were measured in mice to ensure that the mice had experienced hyperglycemia by cutting the tip of the mice's tails. Blood sampling for MDA measurements was carried out on day 22. Blood was taken from the mice's intracardiac. Previously, mice were anesthetized with ketamine-xylazine. MDA levels are measured using the Thiobarbituric Acid Reactive Substance (TBARS) method, where TBA will react with MDA and produce a color that can be read with a UV-Vis spectrophotometer.

Data processing was carried out using the One Way Analysis of Variance (One Way ANOVA) test after ensuring that the data was normally distributed and homogeneous. After that, it was continued with the Post Hoc Test and Tukey test.



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## RESULTS

### Phytochemical Test

The phytochemical tests carried out on galoba fruit extract were the flavonoid test, terpenoid test and alkaloid test. The following are the results of the phytochemical test.

**Table 1.** Result of Phytochemical Test

Compound Group	Reagent	Test Result	Information
Flavonoid	NaOH, HCl	+	A yellow color forms
Alkaloid	Cloroform, NH <sub>3</sub> , Wagner Reagent	-	No precipitate formed
Terpenoid	Cloroform, H <sub>2</sub> SO <sub>4</sub>	+	A brownish ring forms

### Blood Sugar Levels

When measuring the blood sugar levels of mice after treatment, the following results were obtained.

**Table 2.** Result of Blood Sugar Levels

Group	Mean
KN	92 mg/dL
K-	244,25 mg/dL
K+	110 mg/dL
P1	107 mg/dL
P2	101 mg/dL
P3	100 mg/dL

### MDA Levels

**Table 3.** Result of MDA Levels

Group	Mean ± SD
KN	358,75 ± 166,23 nmol/mg
K-	1278,75 ± 153,54 nmol/mg
K+	522,08 ± 119,76 nmol/mg
P1	526,16 ± 118,76 nmol/mg
P2	442,66 ± 159,73 nmol/mg
P3	432,41 ± 132,93 nmol/mg

## DISCUSSION

Phytochemical testing is a testing method to determine the content of secondary metabolite compounds in plants.<sup>14</sup> From the results of phytochemical tests on galoba fruit extract, it is known that galoba fruit samples taken in Waiheru, Ambon City contain secondary metabolite compounds in the form of flavonoids and terpenoids. In accordance with the results of research conducted

by Gustaman, *et al* in 2020, it was found that galoba fruit also contains secondary metabolite compounds, flavonoids and terpenoids. However, apart from flavonoids and terpenoids, this research also found other secondary metabolite compounds, namely alkaloids and quinones.<sup>13</sup> Differences in plant secondary metabolite content can be influenced by differences in growing areas, altitude, rainfall and soil nutrient conditions.<sup>15</sup>

Flavonoids contain aromatic groups which, when reacted with bases, will conjugate and form a yellow color. The aromatic groups in the flavonoids in galoba fruit extract will break down into molecules such as acetophenone which is yellow when it reacts with the base NaOH.<sup>16</sup> A positive terpenoid test is indicated by the formation of a brownish ring. The positive results are based on the ability of the terpenoid compounds contained in galoba fruit extract to produce color by adding concentrated H<sub>2</sub>SO<sub>4</sub> after previously dissolving it in chloroform.<sup>17</sup>

Fasting blood sugar levels in this study were measured before STZ injection, after STZ injection, and after therapy was given. Before STZ injection, blood sugar levels in all treatment groups were within normal limits. After STZ injection, fasting blood sugar levels increased. In groups P1, P2, and P3, blood sugar levels decreased after therapy with galoba fruit extract.

The increase in fasting blood sugar levels after STZ injection is caused by STZ containing glucose groups which are specifically cytotoxic to pancreatic beta cells. Because of its activity in taking glucose from other cells, the pancreatic beta cells will be invaded by STZ and the STZ will begin to damage the pancreatic beta cell.<sup>18</sup> After being given therapy with galoba fruit extract, blood sugar levels in groups P1, P2, and P3 decreased due to the content of secondary metabolites or antioxidants contained in galoba fruit.

This research is in line with research conducted by Wahyuwardani, *et al* in 2020 which examined the potential of kepundung leaves which contain antioxidants as anti-diabetics. It was found that the antioxidant content was able to work as an anti-diabetic. Antioxidants are able to protect pancreatic beta cells from the toxicity of free radicals produced during hyperglycemia so that glucose levels are maintained within normal limits because insulin can still be produced.<sup>19</sup>



Free radical levels in the body can be measured using the oxidative stress biomarker, namely MDA.<sup>20</sup> MDA in this study was measured using the TBARS method. The TBARS method is a method for measuring MDA where one MDA molecule will react with two TBA molecules and produce a pink color. TBA is needed to provide color to the sample to be measured using UV-Vis spectrophotometry.<sup>21</sup>

STZ is a glucose analogue that selectively accumulates in pancreatic beta cells via the GLUT-2 transporter in the plasma membrane. The nitrosourea methyl group from STZ will then cause DNA alkylation which will result in DNA fragmentation. The fragmented DNA will then activate poly-ADP-ribose which will result in dephosphorylation resulting in an increase in ROS and triggering oxidative stress.<sup>22</sup>

The decrease in MDA levels in the treatment group was caused by the flavonoid and terpenoid content found in galoba fruit. These two types of secondary metabolites are able to neutralize the effects of ROS so that oxidative stress conditions can be overcome. This activity is caused by the presence of hydroxyl groups in the structure of antioxidant molecules which can bind to ROS and convert ROS into more stable compounds.<sup>23</sup>

The very reactive nature of ROS due to the presence of unpaired electrons causes ROS to bind with surrounding cell molecules to stabilize itself. However, these cell molecules will then become unstable and turn into new free radicals, and this process will continue continuously. The presence of antioxidant molecules that bind to free radical molecules will stabilize the reaction of these free radicals so that ROS will be reduced and oxidative stress will be resolved so that MDA levels will also be reduced.<sup>24</sup>

This research is in accordance with research conducted by Cheng, *et al* in 2019 that MDA levels in STZ-induced mice increased when compared with normal mice that were only treated with a standard diet. It was also found that giving Sargassum Fusiforme Fucoic acid which contains antioxidants to mice that had been induced by STZ had lower MDA levels compared to the group of mice that were only induced by STZ.<sup>11</sup> Likewise, research conducted by Husfa, *et al* in 2020. This research found that giving green tea containing antioxidants had an effect on reducing MDA levels in diabetic mice.<sup>12</sup>

The ANOVA test results showed a significance of 0.00 ( $\text{sig} < 0.05$ ). These results indicate a significant difference between the average MDA levels of the test groups. In the three treatment groups with galoba therapy (P1, P2, and P3), based on the Tukey test they were in the same group, and different from the K-group. This means that at concentrations of 100%, 75%, and 50% galoba fruit extract both have an effect on reducing MDA levels.

### **Correlation of MDA with Fasting Blood Sugar Levels**

STZ can cause damage to pancreatic beta cells resulting in impaired insulin production which leads to hyperglycemia. An increase in blood sugar levels will stimulate excessive production of NO and superoxide in mitochondria. This overproduction will favor NADPH overexpression which can produce large amounts of superoxide. The production of large amounts of ROS such as NO and superoxide can cause oxidative stress conditions which can be characterized by an increase in MDA. Therefore, if blood sugar levels increase, MDA levels will also increase.<sup>7</sup>

In this study, blood sugar levels were in line with MDA levels. Blood sugar levels and MDA levels were highest in the negative control group and lowest in the treatment group. In accordance with research conducted by Puspitasari, *et al* in 2013 regarding the effect of Mahogany seed extract which contains antioxidants on blood sugar levels and MDA in diabetic mice. In this study, increases and decreases in blood sugar levels were in line with increases and decreases in MDA levels.<sup>25</sup>

### **CONCLUSION**

In this study, a significant difference was found between the MDA levels of mice treated with galoba fruit extract, both concentrations of 100%, 75%, and 50% compared to mice that were only induced by STZ. From this it can be concluded that galoba fruit extract has a significant effect on the serum MDA levels of STZ-induced hyperglycemia mice.

### **ETHICAL APPROVAL**

Ethical approval was issued by the Ethics Committee of the Faculty of Medicine, Universitas Pattimura Number: 081/FK-KOM.ETIK/VIII/2023.





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### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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### AUTHOR CONTRIBUTIONS

Conceptualization, YIR, RDA, IK; methodology, YIR, RDA; data analysis, YIR, RDA; data collection, YIR; wrote the original draft, YIR; review and edit: RDA, IK; supervision, RDA.

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