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Effect of Oral Administration of *Mytragina Speciosa* on Blood Ketone Level and Glomerular Histology in Streptozotocin Induced Diabetic Mice

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ABSTRACT

Introduction: Diabetes mellitus is a chronic metabolic disease characterized by increased sugar levels that cause serious damage to various organs. Diabetic ketoacidosis is one of the complications of diabetes mellitus. It is characterized by a decrease in the patient's consciousness, increased blood sugar levels, and the presence of acidic ketone bodies. It is a serious condition that can cause a coma or even death. *Mytragina Speciosa*, known as kratom, is a plant often found in Southeast Asia, including Indonesia. This herb is frequently used as a stimulant to treat fatigue and help improve productivity. Based on previous research, *Mytragina Speciosa* is rich in alkaloids, flavonoids, and phenols. These compounds are very useful for inhibiting the activity of α-glucosidase and lipase enzymes in the pancreas, so they have a potential effect on diabetes mellitus. However, there has been no research related to the administration of *Mytragina Speciosa* its effect on blood ketone levels and glomerular diameter.
Objective: to determine whether there is an effect of *Mytragina Speciosa* on blood ketone levels and glomerular diameter in mice with a diabetes mellitus model.
Methods: Hyperglycemia is induced by Streptozotocin. On the 14th day, the mice will be checked for hyperglycemia and blood ketone levels. Administration of Mytragina Speciosa begins on day 15 for 2 weeks. At the end of the second week, the mice will be checked for blood ketone levels using a special kit to detect ketone bodies. Furthermore, the glomerular diameter will also be assessed.
Results: In this study, treatment for 2 weeks only caused an increase in blood sugar levels in mice, but did not cause an increase in blood ketone levels in the diabetes group or other treatment groups ($p > 0.05$). Histological preparation of the Glomerulus showed a decline in glomerular diameter.
Conclusions: The administration of *Mitragyna speciosa* extract did not result in a significant increase in blood ketone levels in diabetic mice. Many factors are involved in increasing blood ketone. It is necessary to monitor ketone levels during treatment as well as have a longer treatment time to see the effect of *Mitragyna speciosa* on ketones.

Introduction

Diabetes mellitus is a chronic metabolic disease characterized by increased sugar levels that cause serious damage to various organs. It is estimated that around 422 million people in the world suffer from

diabetes and 1.5 million die from diabetes (WHO, 2023). Diabetes mellitus has several categories, type 1, type 2, maturity-onset diabetes of the young (MODY), and gestational diabetes (Sapra, 2023). The chronic course of the disease includes acute and chronic complications. One of the acute complications of diabetes mellitus is diabetic ketoacidosis, which is characterized by a decrease in the patient's consciousness, increased blood sugar levels, and the presence of acidic ketone bodies. It is a serious condition that can lead leads to coma or event death (CDC, 2022).

Mytragina Speciosa, known as kratom, is a plant often found in Southeast Asia, including Indonesia. This herb is used by the community as a stimulant to treat fatigue and improve work productivity (BPPK, 2022). According to previous research, *Mytragina Speciosa* is rich in alkaloids, flavonoids, and phenols. These compounds are very useful in inhibiting the activity of α-glucosidase and lipase enzymes in the pancreas, so they have a potential effect on diabetes mellitus (Limcharoen, 2022). However, there has been no research related to the administration of *Mytragina Speciosa* to reduce ketone levels when acute complications occur.

The presence of various compounds contained in *Mytragina Speciosa* extract requires an in-depth study of the health potential, reducing ketone bodies in conditions of diabetes mellitus with an acute increase in blood sugar levels.

Methods

This research is purely experimental and bioinformatics using a database. The research design used was a design with a Post Test Only Control Group Design. The study will be conducted in the integrated research laboratory of the Faculty of Medicine, Unusa.

Mytragina speciosa

The extraction method used in this study was maceration. A total of 3 kg of dried Mitragyna speciosa Korth simplicia powder was extracted using a 96% methanol solvent. Change the solvent every 1x24 hours and macerate for 7x24 hours. The macerate is concentrated using a rotary evaporator and a water bath to obtain a thick extract.

Streptozotocine

N-(Methylnitrosocarbamoyl)-α-D glucosamine with formula C8H15N3O7 with serial number A610130-0001.

Subject

Before testing, mice were acclimatized for one week in the UNUSA Faculty of Medicine Research Laboratory cage. Before treatment, animals were weighed, marked, and recorded. Mice used are those

that meet inclusion criteria and have passed the ethical review. Then, they were grouped according to the intervention. The analyses carried out in this research included: blood sugar levels in mice, ketone levels, and histological preparation of kidneys.

Data on blood sugar levels and ketone levels were calculated as the average (mean) and standard deviation (SD). Data processing was then continued with normality analysis using the Shapiro-Wilk test and Levene's homogeneity test. If the data obtained is normally distributed and homogeneous, the One Way ANOVA test will be carried out ($p<0.05$), followed by with the LSD test to compare between groups. If the data does not meet one of the requirements for parametric analysis, a non-parametric statistical test can be carried out using the Kruskal-Wallis test, then continued with the Mann-Whittney test with a confidence level of 95% ($\alpha = 0.05$) to see the differences between treatment groups. Furthermore, kidney histology will be carried out descriptively, with a focus on glomerular diameter.

Results and Discussion

The research used 24 mice divided into four groups. After being treated for two weeks, the mice were euthanized, and blood ketones were checked by taking blood from the tail. The blood sugar results in mice can be seen in the graph (Figure 1).

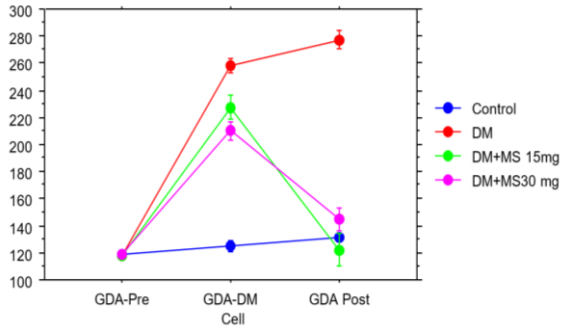

Figure 1. Mytaragina speciosa extract down-regulate blood glucoce level in diabetic mice. Blood glucose was measured by glucometer in each group (n = 6) other than control group.

Figure 1 shows the blood glucose level changes in the diabetic and experimental animals of each group. STZ-induced diabetic mice showed increases of blood glucose level after STZ injection and at the end of study (red line). Blue line represent blood glucose level in the normal limit. Whereas DM+MS 15mg and DM+MS 30 mg groups increased in blood glucose and decleaned after each doses of *Mytaragina speciosa extract*.

It was found that blood sugar levels decreased after administration of mitaraginine extract, which was close to the control group or normal group. Meanwhile, blood ketone results can be seen in the following table. It shows that there is no significant difference in the ketone levels between diabetic mice and other groups (Table 1).

Table 1. Blood Ketone Level (mmol/L)

Groups	1	2	3	4	5	6
Normal	0,2	0,3	0,3	0,2	0,3	0,4
Diabetes	0,4	0,3	0,3	0,3	0,3	0,2
Extract 15 mg/kgBB	0,3	0,3	0,3	0,2	0,2	0,3
Extract 30 mg/kgBB	0,3	0,3	0,3	0,2	0,3	0,3

The average ketone level obtained from this study was 0.3 mmol/L with the highest level being 0.4 mmol/L and the lowest level being 0.2 mmol/L (Table 2). Furthermore, Figure 2 shows that blood ketone levels are the same as lower than the normal group (KN) in the 15 mg/kgbb extract group and the same as KN in the 30 mg/kgbb extract group.

Table 2. Mean, minimum and maximum of blood ketone Level (mmol/L)

	N	Min	Max	Mean	Std. Dev
Ketone	24	.20	.40	.2833	.05647

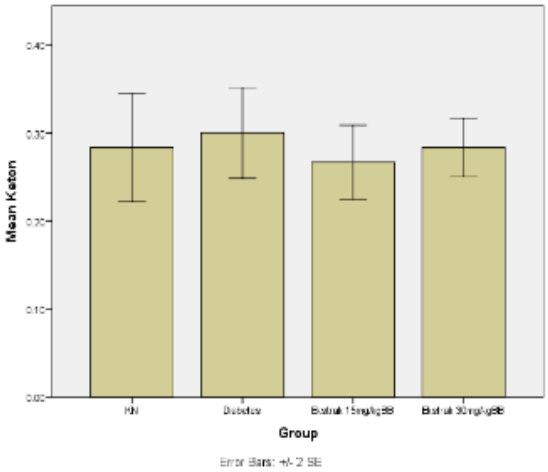

Figure 2. Mean and standard deviation of blood ketone

The normality test using Shapiro-Wilk produced a p-value <0.05, so further analysis used the Kruskal-Wallis method. After carrying out the Kruskal-Wallis test, a p-value > 0.05 was obtained, indicating that there were no significant differences between the normal group and the treatment group.

Meanwhile, in the histology of the kidneys, it appears that there is a widening of the Bowman space in the diabetes group, 15 mg/kgbb extract and 30 mg/kgbb extract, which indicates damage to the kidneys. This can be compared with the histology in the normal group (Figure 3).

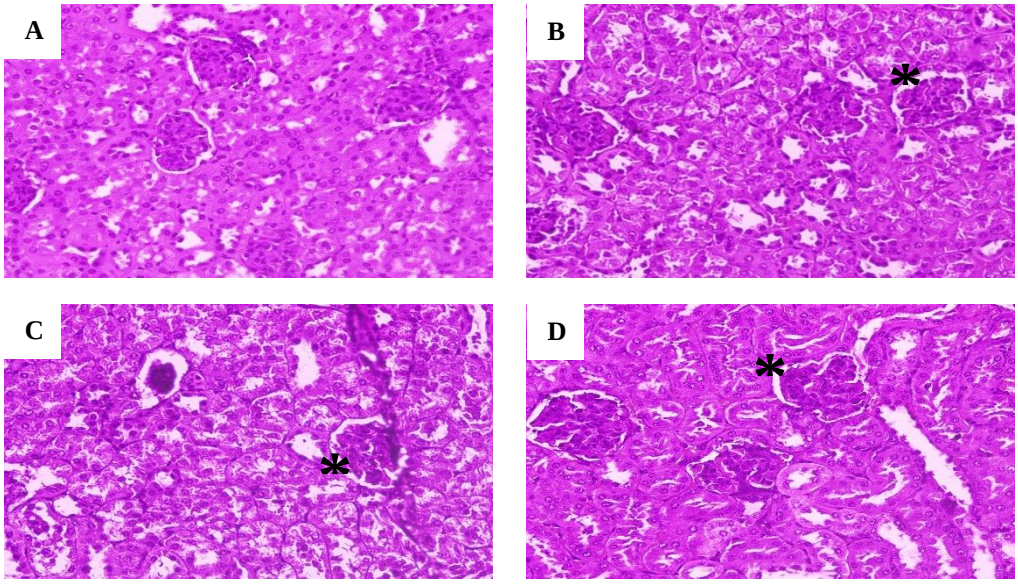

Figure 3. A shows the glomerular condition of the control/normal group, B shows the glomerulus of the diabetes group, C is the 15 mg/kg BW extract treatment group and D is the 30 mg/kg BW extract group. It appears that the condition of the glomerulus in images B C and D is almost the same, namely there is widening of the Bowman space

Discussion

Ketone bodies are a source of energy used by the body when glucose is not available in sufficient quantities. Ketones are always present in the blood and levels will increase when fasting and exercising for a long time. Diabetes is the most common cause of high blood ketone levels (Laffel, 1999).

Normal blood ketone levels are <0.5 mmol/L, and can reach up to 6-7.5 mmol/L during prolonged fasting up to 25 mmol/L in uncontrolled diabetes patients. Excessive accumulation of ketones in the blood can cause dangerous effects on the body because it can induce oxidative stress, play an important role in inducing insulin resistance by downregulating insulin receptors and phosphorylation of insulin receptor substrate-1, and induce pro-inflammatory factors to cause systemic inflammation (Kanirkala, 2016). In addition, an increase in ketones, or ketosis, can develop into ketoacidosis, which can reduce blood pH and cause problems with the body's organs (Laffel, 1999).

Several studies state that Mitragyna speciosa, or Kratom, has antioxidant activity and is a potent α-glucosidase inhibitor. Administration of Mitragyna speciosa to diabetic mice significantly showed improvements in body weight, changes in blood sugar, dyslipidemia, and also improved pancreatic damage histologically and immunohistochemically (Zhang, 2023). Furthermore, Zailan et al. stated that Mitragyna speciosa showed antioxidant scavenging activity because it

has a higher flavonoid content. Apart from that, it was also found that Mitragyna speciosa can inhibit the α-amylase enzyme and has the potential to act as an antidiabetic (Zailan, 2022).

In this study, treatment for 2 weeks only caused an increase in blood sugar levels in mice but did not significant increase in blood ketone levels in the diabetic mice following the administration of *Mytaragina speciosa* extract. In diabetes mellitus, there is an increase in the production of ketone bodies and a decrease in their clearance. The accumulation of ketones in the blood will then cause damage to cells. Therefore, controlling blood sugar and monitoring ketones is the best way to prevent complications and even death in diabetes patients (Kanirkala, 2016).

Conclusion

This study did not show an increase in blood ketone levels in both DM mice and after administration of Mitragyna speciosa extract. Multiple factors influence the levels of ketone bodies in the blood, it is essential to monitor ketone levels throughout the treatment and consider longer treatment durations to fully evaluate the effect of Mitragyna speciosa, it is necessary to monitor ketone levels during treatment as well as a longer treatment time to see the effect of Mitragyna speciosa on ketones.

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