

PRECLINICAL TESTING OF ETHANOL EXTRACT OF FOREST BITTER MELON (*MOMORDICA BALSAMINA*) LEAVES AS ANTIHYPERGLYCAEMIA AND ACUTE TOXICITY

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ABSTRACT

*Diabetes mellitus is a metabolic disease characterised by hyperglycaemia due to impaired insulin secretion, insulin action, or both. The use of medicinal plants, such as forest bitter melon (*Momordica balsamina*) leaves, as an alternative treatment for diabetes is increasingly attracting attention. This study aimed to test the antihyperglycaemia effect of ethanol extract of forest bitter melon leaves and assess its acute toxicity. The research method was conducted by observing the blood KGD of mice induced by dexamethasone and sucrose. The mice were then treated with bitter melon leaf ethanol extract at concentrations of 10%; 15% and 20%. Acute toxicity test was conducted by Brine Shrimp Lethality Test (BSLT) method. Data were analysed with ANOVA test. The results of the antihyperglycaemia test showed a p -value = 0.035 (H_0 rejected) which means there is a significant difference in the pre and post test KGD values with the most effective concentration reducing the KGD of mice is 20% with an average of 75 mg/dl. ⁵⁰ Acute toxicity test showed LC value = 203.19 ppm (< 1000 ppm) indicating relatively high toxicity. In conclusion, ethanol extract of bitter melon leaves has antihyperglycaemia activity with a very toxic classification value.*

Keywords: Antihyperglycaemia; *Momordica_balsamina*; acute toxicity.

INTRODUCTION

Diabetes mellitus is a serious medical condition characterised by elevated blood glucose levels over a long period of time. This condition can lead to various serious complications such as heart disease, stroke, and kidney damage. According to data released by the International Diabetes Federation, in 2021 there were approximately 537 million adults worldwide living with diabetes, and this number is projected to increase to 643 million by 2030. Diabetes mellitus itself is divided into several types, but the most common is type 2 diabetes. Type 2 diabetes is often linked to lifestyle factors, such as unhealthy diet and lack of physical activity. This shows the important role a healthy lifestyle plays in preventing and managing this condition. Therefore, it is important for us to familiarise ourselves with a healthy diet and exercise routine to reduce our risk of developing type 2 diabetes. With its

increasing prevalence, it is important for the public to be more aware of the importance of maintaining health and preventing diabetes through a healthy lifestyle. Education and socialisation about the dangers of diabetes and how to prevent it need to be improved so that the number of people with diabetes can be reduced and people can live healthier and better quality lives.

Momordica balsamina, also known as forest bitter melon, is a plant rich in phytochemical compounds that have pharmacological potential. Previous studies have shown that bitter melon leaf extract contains flavonoids, alkaloids, and saponins that contribute to its antihyperglycaemia activity (Azizah et al., 2018). Flavonoids, for example, are known to have antihyperglycaemic effects by increasing insulin sensitivity and reducing insulin resistance in body cells (Jemadu et al., 2024). In addition, these compounds also have antioxidant properties that can help protect body cells from damage due to oxidative stress.

The importance of acute toxicity studies especially in the use of herbal extracts as a treatment cannot be underestimated. By conducting acute toxicity assessment, we can ensure that the use of herbal extracts is safe for consumers and does not cause harmful adverse effects. The Brine Shrimp Lethality Test (BSLT) method is one of the most commonly used methods in acute toxicity research. By using *Artemia salina* larvae as a model, we can assess how toxic a compound is to living organisms. Thus, we can get very valuable information about the safety level of an herbal extract to be used in therapy. In research related to the toxicity of ethanol extract of forest bitter melon leaves, important information will be obtained about the safe dose that can be used in therapy. Thus, health experts and practitioners can design effective treatments without causing health risks to patients. Therefore, this study is needed to ensure the quality and safety of using herbal extracts as alternative medicine.

The BSLT (Brine Shrimp Lethality Test) method has proven to be one of the simple yet effective techniques in evaluating the potential toxicity of compounds. Through this study, *Artemia salina* larvae will be incubated with ethanol extract of forest bitter melon leaves and then observed and determined the LC50. LC50 is the concentration level of a compound that causes death in 50% of the larval population within a certain time (Anisa et al., 2022). The use of the BSLT method in this study is expected to provide valid data on the safety of ethanol extracts of bitter melon leaves before further preclinical tests are carried out on experimental animals. Thus, the results of this study can be a strong basis in determining the potential toxicity of compounds contained in the ethanol extract of bitter melon leaves

and as a first step in evaluating the safety of its use in the health and pharmaceutical fields. In addition, the BSLT method also allows researchers to predict the potential toxicity of these compounds to other organisms, so that it can provide broad benefits in the development of safe and effective medicines.

METHOD

a. Maceration extraction

The extraction process was carried out by maceration method using ethanol solvent. Forest bitter melon leaves that had been collected from Deliserdang Regency were dried then powdered and soaked in ethanol for 72 hours. After the soaking process, the extract was filtered using a filter cloth and evaporation was carried out to remove the solvent, thus obtaining a thick extract that was ready to be tested (Chairunnisa et al., 2019). This process is important to ensure that the bioactive compounds contained in the leaves can be extracted optimally.

b. Momordica balsamina antihyperglycaemia test in mice

Antihyperglycaemia test was conducted by giving ethanol extract of forest bitter melon leaves to mice with concentrations of 10%; 15%; and 20% which had been induced using dexamethasone and sucrose for 7 days. In this test, glibenclamide was used as a comparator. Blood glucose levels were measured before and after treatment to evaluate the effect of the extract on glucose control (Dewi et al., 2017a). This study is expected to show that bitter melon

leaf extract has the ability to significantly reduce blood glucose levels, which can be an alternative treatment for diabetes.

c. Toxicity test with BSLT

To assess acute toxicity, *Artemia salina* larvae were placed in concentrations of 50 ppm; 100 ppm; 200 ppm; 300 ppm; 400 ppm and 500 ppm of ethanol extract of forest bitter melon leaves. After 24 hours, the number of surviving larvae was counted using Probit analysis to determine the LC_{50} . This method gives an idea of how safe the extract is to be used in medicine (Fadli et al., 2019). The data obtained from this test will be the basis for determining the safe dose for further tests.

RESULTS AND DISCUSSION

The reduction of blood glucose level (BGL) in mice was seen from the effect of treatments on the reduction of blood glucose level after administration of several interventions, including CMC (control), glibenclamide, and ethanol extract of forest bitter melon leaves (EEDPH) at concentrations of 10%, 15%, and 20%. The data showed that the control group (CMC) had an insignificant reduction in glucose levels compared to the other treatments, indicating that CMC had no relevant antidiabetic effect. The trend graph of the mean decrease in each group can be seen in Figure 1.

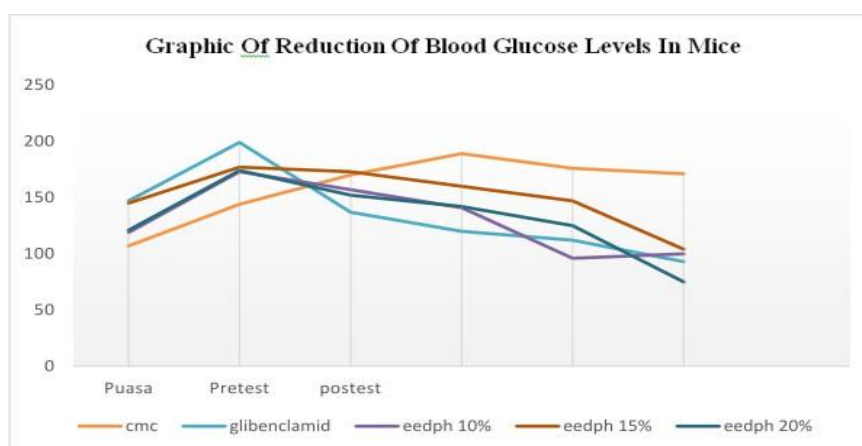


Figure 1. Graphic of Reduction Of Blood Glucose Levels In Mice

In Figure 1, glibenclamide, which is a standard antidiabetic drug, showed a consistent and significant reduction in blood glucose levels, which was more effective than all other treatments, especially in the post-test phase. This illustrates the effectiveness of glibenclamide

in reducing blood glucose levels, in accordance with its pharmacological mechanism as a stimulator of insulin secretion. The group given bitter melon leaf extract, especially at 20% concentration, showed a trend of decreasing glucose levels close to the results of glibenclamide, indicating the potential hypoglycaemic effect of the bioactive compounds in the extract. Figure 1 also shows preliminary evidence that the ethanol extract of bitter melon leaves has potential as a natural antidiabetic agent, although its effectiveness is still below that of glibenclamide at the tested concentrations. However, the need for further research to determine the mechanism of action, toxicity, and dose optimisation of the forest bitter melon leaf extract is crucial. These findings may provide a basis for the development of alternative plant-based antidiabetic therapies that are safer and more affordable.

Based on the results of the toxicity test using the BSLT method with the calculation of the LC₅₀ value, it can be seen that the higher concentration (500 ppm) resulted in a greater percentage of mortality of *Artemia salina* larvae (86.6%) than the lower concentration (50 ppm, 13.3%). This shows a direct relationship between the concentration of the sample and its toxicity. These results indicate that the active compounds in the test samples have toxic activity that can be measured quantitatively through the percentage of mortality. From this BSLT test, the average probit value was 4.85, which indicates that this test is able to provide relevant data for toxicity calculations. The logarithmic relationship between concentration and probit to predict mortality at a certain concentration can be seen in Figure 2.

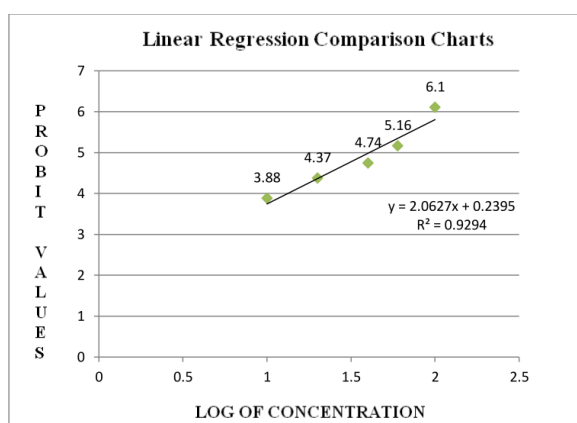


Figure 2. Probit Linear Regression Comparison Charts

The LC₅₀ value of the tested sample provides an indication of the compound's potential toxicity to the test organism, which can be interpreted for pharmaceutical or environmental safety applications. If the LC₅₀ value is at a low concentration (e.g., below 100 ppm), then the

compound is classified as having high toxicity, according to the toxicity classification criteria. Based on these data, it appears that the compounds in the test have significant toxicity, as at the medium concentration (200 ppm), the mortality rate reached 40%.

Overall, the results of this study provide important insights into the potential toxicity of the tested compounds. However, it is important to continue this research with further toxicity tests in more complex animal models or in vitro studies in human cells to ensure the relevance and safety of the compounds in pharmaceutical applications.

CONCLUSION

Based on the results of this study, it was concluded that the group given forest bitter melon leaf extract, especially at 20% concentration, showed a trend of decreasing glucose levels close to the results of glibenclamide, indicating the potential hypoglycaemic effect of the bioactive compounds in the extract. Acute toxicity test showed LC₅₀ value = 203.19 ppm (<1000 ppm) indicating a relatively high toxicity, which indicates a highly toxic classification value.

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