Effect of Mixture of Aqueous and Methanolic Extracts of *Momordica Balsamina* on Albino Rat Induced Diabetes

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Abstract

Amongst the major causes of deaths worldwide today, is diabetes mellitus. This ailment is frequently associated with a person's eating habits and heredity, and its recurrence rate is becoming seriously alarming. There are several anti-diabetic or hypoglycemic medications available for the treatment of the condition, but they are frequently expensive, out of reach for the poor, and frequently have unfavorable side effects. This highlights the need for scientific research to identify a different, safe, and efficient herbal drug that will be widely available, simple to use, and reasonably affordable for the treatment of the condition. In this study, the effects of *Momordica balsamina* aqueous methanolic extracts were investigated on albino rats induced with diabetes using alloxan. Albino rats weighing 150g and above and kept in the same condition with unrestricted access to food and water were used throughout the experiment. A solution of alloxan monohydrate was made by dissolving 0.9 mg in 6 mL of distilled water and the rats were given a single intraperitoneal injection of alloxan monohydrate (150 mg/kg). The blood glucose level of the rats was assessed both before and after alloxan diabetes induction and after treated with 50, 100 and 200 mg/kg of the aqueous methanolic extract of *M. balsamina* using a glucometer. The data obtained were subjected to statistical analysis at P-value (P < 0.05 and 0.01) using Pearson correlation analysis. The work conducted on albino rats indicated a substantial reduction in blood glucose levels after administering doses of 200 mg/kg body weight of *M. balsamin* aqueous methanolic extract. This effect was particularly notable between the third and thirty third days of the research period where a decreased in blood glucose level from 28.5±0.8 to 10.8±0.2, 8.3±1.1, 8.0±0.0, and 7.2±0.0 (mmol/L) in the third, sixth, ninth and twelfth days were observed respective, which also increased to 8.8±1.1 and 9.3±1.3 (mmol/L) in the fifteenth and eighteenth day, but continue decreasing throughout until become 7.4±1.5 (mmol/L) at thirty third day. It is worth mentioning that the test animals remained healthy and exhibited normal activity throughout the study. Furthermore, the LD50 test detected no indications of toxicity, suggesting the extract's relative safety. These findings strongly suggest that *M. balsamina* contains phytochemicals with potent anti-diabetic properties.

Key words: Phytochemicals, Aqueous methanolic extracts, Albino Rats, *Momordica balsamina*, Blood Glucose
Introduction

Diabetes mellitus (DM) is an endocrine condition characterized by abnormalities in lipid, carbohydrate, and protein metabolism (Prabhakar, 2016). In addition to causing hyperglycemia, DM is associated with various complex illnesses classified as acute, subacute, or chronic (Xu et al., 2020; Mbikay, 2012). Symptoms of DM include polydipsia, lack of energy, frequent urination, visual impairment, weight loss, and excessive eating (polyphagia) (Singla, 2015). Complications such as nephropathy, retinopathy, neuropathy, cardiovascular problems, hyperglycemia, diabetic ketoacidosis, and hyperosmolar nonketotic syndrome can also arise (Balaji et al., 2019). The incidence and prevalence of diabetes mellitus have been on the increase despite significant research and financing, particularly in developing nations (Nkechi et al., 2020). Other researchers have observed that, due to unchecked urbanization and the westernization of dietary practices, the disease will rapidly expand especially across Africa (Mbanya et al., 2010). For instance, Mauritania has 6%, Cameroon has 6.1%, and Congo has 7.1%. WHO stated according to report by Sunday et al. (2012) that, Nigeria has the highest incidence of diabetes in Africa, with a projected 1.7 million individuals suffering from the disease, which is expected to rise to 4.8 million by 2030 (Whiting et al., 2011).

Irrespective of flaw that causes the condition, there are several anti-diabetic or hypoglycemic medications for its treatment. However, due to poverty and economic crisis, these medications are frequently out of the reach of the poor. In addition, most often the medications have negative side effects (Lankatillake et al., 2019). Recently, the focus of the scientific community worldwide, is on how to control the disease without having any negative consequences (Sasidharan et al., 2010). Several researchers have revealed the potentials of many plants in Nigeria for treatment of
hyperglycemia (Yimer et al., 2019). Many communities utilize herbal medicines to treat various illnesses in recent times (Haruna et al., 2022a).

The research focused on investigating the potential antidiabetic properties of a combination of aqueous and methanolic extracts of *M. balsamina* on albino rats with induced diabetes using alloxan monohydrate. The aim of the study is to explore the use of *M. balsamina* extracts as a treatment for diabetes, with albino rats serving as a case study.

One important aspect of this research is the use of alloxan to induce diabetes in the experimental albino mice. Alloxan has the ability to specifically destroy the beta-cells responsible for insulin production. A study by Danilova et al. (2014) revealed that alloxan effectively inhibits glucokinase, an SH-containing protein necessary for insulin release triggered by glucose. This inhibition is likely due to the compound's structural similarity to glucose and its efficient uptake into the beta-cells through the GLUT2 uptake mechanism. Furthermore, alloxan is known to decrease glutathione levels due to its strong bonding with SH-containing biological molecules, as highlighted by Gupta et al. (2016).

![Structures of Alloxan and Alloxan hydrate](image)

*Fig 1. Structures of Alloxan and Alloxan hydrate (Lenzen, 2007).*

**Study Area**
The study area is Kazaure Dam of Kazaure local government which fall between $12^\circ 39' 10''$ N, $8^\circ 24' 43''$E and cover a land mass of about 690sq mi or 1,780km$^2$ of land (Danyaya et al., 2022).

(Kazaure Dam water by Google earth.com)

**Materials and Reagents**

The materials used throughout the investigation are of high analytical quality. Similarly, all the reagents used were also of high analytical grade and they were used without further purification.
Sample Collection

The leaves of *Momordica balsamina* also commonly known as "Garahuni," in many Hausa communities, were randomly sampled from the banks of Dam in Kazaure, where they grow in abundance. The samples were conveyed to Hussaini Adamu Federal Polytechnic Herbarium, where a certified Botanist identified them before they were brought to the lab for further processing, extraction, and analysis.

Sample Processing

The bench and surrounding working environment in the laboratory were properly washed with detergent and copiously rinsed with distilled water. Furthermore, cotton wool soaked in a hydrogen hypochlorite (bleach) was used to sterilize the bench to avoid contamination.

After rinsing the leaves of *M. balsamina* with running tap water and then with distilled water, they were left to dry for seven days at room temperature. Following this, the dried leaves were pulverized using a sterilized pestle and mortar, sieved, and placed into plastic bags before extraction (Haruna et al., 2022b).

Extraction

Approximately 20 g of Momordica balsamina powdered leaves were immersed in deionized water for 48 hours. The mixture was then transferred to a 500 mL beaker, allowed to settle, and filtered to obtain an aqueous extract. This extract was concentrated using a vacuum rotary evaporator. Additionally, another 20 g of powdered leaves were extracted using methanol in a Soxhlet extractor followed by concentration using a rotavapour. The resulting methanolic extract was combined with the aqueous extract to form aqueous-methanolic extracts. These extracts were further concentrated and dried for future use (Maina et al., 2018).
Experiment with diabetes induced albino rats

For this investigation, albino rats weighing 150g and above were used throughout the experiment. They were kept in the same condition with unrestricted access to food and water.

A solution of alloxan monohydrate was prepared by dissolving 0.9 mg of alloxan monohydrate in 6 mL of distilled water. The rats were then administered a single intraperitoneal injection of alloxan monohydrate at a dose of 150 mg per kilogram of body weight. The following relationship was utilized.

\[
V \ (\text{ml}) = \frac{W \times 150 \ (\text{mg/kg})}{S} - 1
\]

Where, \( V \) represents the volume of alloxan administered

\( W \) = weight of the albino rats (kg)

\( S \) = Concentration of the stock alloxan solution (mg/ml)

The diabetic albino rats were chosen for the investigation after receiving the intraperitoneal injection alloxan solution in groups 2, 3, 4, and 5. The blood glucose level of the rats was assessed both before and after alloxan diabetes induction using a glucometer in accordance with the report of Radenković et al., (2016).

Experimental Design

Lethal Dose (LD50) and The Antidiabetic effect of the aqueous methanolic extract of \( M. \) balsamina leaves were both tested on a total of 27 rats following a method reported by Ubhenin et al., (2019). Following inducing the rats with diabetes using alloxan, 15 of the 27 albino rats were separated into five clusters of three reach as in below:

Rats in cluster number 1 (3): are healthy.
Cluster number 2 (3): Once every day, 0.1 ml (50 mg/kg body weight) of *M. balsamina* extract were given orally to diabetes rats.

Cluster number 3 (3): *M. balsamina* extract were given orally to diabetes rats once a day in a dose of 0.22 ml (100 mg/kg body weight).

Cluster number 4 (3): *M. balsamina* leaves extract were given orally to diabetes rats once a day in a dose of 0.48 ml (200 mg/kg body weight).

Cluster number 5 (3): Control rats for diabetes who were not treated (Siboto et al., 2018).

**Monitoring the Glucose Level of the Induced Rats in Relation to *M. balsamina***

The glucose level were monitored using MGD-1002A model meter at intervals of three days after treating the albino rats with different concentrations (50, 100, and 200 mg/kg) leaves extract of *M. balsamina*.

**LD$\text{50}$ Test on the Extract**

In an acute toxicity test to determine the LD50, 12 rats were divided into two stages. The first stage involved three groups of two rats each, receiving 500, 700, and 900 mg/kg of the aqueous methanolic leaf extract of *M. balsamina* orally. After 24 hours, the rats were observed for signs of mortality and general behavior.

In the second phase, three groups of two rats each were administered 1000, 2000, and 3000 mg/kg of the same leaf extract. Rats were monitored for 24 hours to detect any indications of toxicity or death.

**Data Analysis**
Table 2 indicates the statistical analysis of the data at P-value (P 0.05 and 0.01) using Pearson correlation analysis.

**Results and Discussion**

The properties of leaves extract of *M. balsamina* on alloxan monohydrate-induced diabetes albino rats is presented as mean and standard deviation in Tables 1.

### Table 1: Results of the extract of *momordica balsamina* (Garahuni) on alloxan induced diabetes Albino Rats

<table>
<thead>
<tr>
<th>Extract Conc. (mg/kg)</th>
<th>Groups</th>
<th>Glucose level before induction</th>
<th>Blood glucose levels in (mmol/L) during the Sampling time in days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Nor</td>
<td>8.4±1.0</td>
<td>8.6±0.7</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>7.8±1.1</td>
<td>26.3±0.5</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>7.7±1.2</td>
<td>27.5±0.8</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>9.7±1.6</td>
<td>28.5±0.8</td>
</tr>
<tr>
<td></td>
<td>Pos</td>
<td>7.0±1.5</td>
<td>27.2±0.5</td>
</tr>
</tbody>
</table>

Key: Nor = (Normal), Pos. = Positive albino rats, respectively.

### Table 2: Result of the Pearson correlation analysis of leave extract of *M. balsamina* on alloxan induced diabetes albino rats

<p>| Correlations analysis of the extract on the alloxan diabetes albino Rat |
|--------------------------|---------------------|
| day 0                    | 0                   |
| day 3                    | .582                | 1                  |
| day 6                    | .485                | .991**             | 1                  |</p>
<table>
<thead>
<tr>
<th>Day</th>
<th>Correlation</th>
<th>Insulin Utilization</th>
<th>Beta-Cells Annihilation</th>
<th>Annulation Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>.471</td>
<td>.989</td>
<td>1.000**</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>.467</td>
<td>.984</td>
<td>.980* .980*</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>.496</td>
<td>.993**</td>
<td>.991** .990** .997**</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>.442</td>
<td>.869</td>
<td>.907 .908 .806 .843</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>.490</td>
<td>.971*</td>
<td>.956* .954* .994** .986* .744</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>.480</td>
<td>.993**</td>
<td>.996** .996** .994**</td>
<td>.999** .866 .978*</td>
</tr>
<tr>
<td>27</td>
<td>.508</td>
<td>.989*</td>
<td>.980* .979* .999** .998** .808 .995** .994**</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>.419</td>
<td>.925</td>
<td>.959* .961* .886 .913 .987* .834 .931 .885</td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>.501</td>
<td>.995**</td>
<td>.999** .998** .988**</td>
<td>.996** .886 .969* .999** .989* .944</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed) and *. Correlation is significant at the 0.05 level (2-tailed).

The toxic glucose analogue alloxan selectively destroys insulin-producing cells in the pancreas (Madhariya et al., 2023), and subsequently results in an insulin-dependent diabetes mellitus, a condition known as "alloxan diabetes" which is characterized as type 1 diabetes in humans (Macdonald et al., 2017). As depicted in Table 1, after being treated with alloxan to induce diabetes, the test animals developed the disease within three days as their blood glucose levels rose. A decline in insulin secretion as well as in ability of the body to utilized glucose are both caused by the annihilation of beta-cells in the pancreatic islets by alloxan monohydrate (Umar, 2015). The findings from the current study revealed a reduction in glucose utilization as observed. The findings further reveal that, aqueous methanolic extract appeared to have a significant anti diabetic property, especially at higher concentration levels (200 mg/kg), as depicted in Table 1 where the effect was particularly notable between the third and thirty third days of the research period where a decreased in blood glucose level from 28.5±0.8 to 10.8±0.2, 8.3±1.1, 8.0±0.0, and 7.2±0.0 (mmol/L) in the third, sixth, ninth and twelfths days were observed respective, which also
increased to 8.8±1.1 and 9.3±1.3 (mmol/L) in the fifteenth and eighteenth day, but continue decreasing throughout until become 7.4±1.5 (mmol/L) at thirty third day. This further suggests that the extracts possesses synergistic interactions with a number of secondary metabolites as reported by other researchers (Kennedy and Wightman, 2011). Tran et al. (2020) and Zheng et al. (2012), have demonstrated the effectiveness of phytochemicals as bioactive anti-diabetic agents. Similarly, Saponins was found to be the next secondary metabolite after alkaloid in leaf extract of *M. balsamina* (Karumi, 2004). This metabolite may significantly lower type 2 diabetics' hyperglycemia and oxidative damages brought by hyperglycemia (Zheng et al., 2012). Similarly, Alli et al. (2012) observed that in rats with alloxan-induced diabetes, saponin extract displayed exceptional antidiabetic action, close to a typical medicine metformin. Furthermore, the result revealed that, *M. balsamina* leaves could possess active ingredients as an anti-diabetic drug. Further supporting the efficacy of the extracts is the fact that the blood glucose levels of the untreated animals were higher than those of the treated, particularly cluster IV, where the Rats received daily treatments of 200 mg/kg of extract. The statistical analysis result showed that the treatment and control groups, vary significantly. The efficacy of the leaves extracts on the test animals was particularly observed at dosages of 200 mg/kg.

**The Lethal Dose (LD50)**

The findings of this study showed that the aqueous methanolic extract of *M. balsamina* was not harmful between 50 and 2000 mg/kg, according to the LD50 value, and no toxic effects were seen in the experimental animals after 24 hours of providing such quantities. On the other hand, a dosage of 2000 mg/kg body weight may be lethal given that experimental rats experienced mortality and
lightheadedness at a dose of 3000 mg/kg extract. Under 2000 mg/kg, the LD50 of *M. balsamina's* aqueous methanolic extract could therefore be regarded as safe for consumption. The study also shows that the liver and other essential organs, including blood, may become more toxic at the greatest dosage of *M. balsamina* specie. According to OECD Guideline 423 (Priyadarshini *et al.*, 2014), the LD50 of *M. Momordica* may fall under class 5 as no death occurred up to 2000 mg/kg. The toxicity assessment of the FAO/WHO Expert Committee on Food Additives, reported that a compound might be deemed non-toxic if there is no death seen at 2g/kg of body weight Mbaka *et al.*, (2010). In this situation, 2000 mg/kg and less of the aqueous methanolic extract of *M. balsamina* are regarded as safe for use in any applications.

**Conclusion**

It was concluded that, *M. balsamina* extracts possess dose-dependent effect. It stimulates the remaining pancreatic cell to secrete more insulin. The plant's leaves has Antidiabetic properties, which can be harnessed to treat human diabetes as the results suggests. The present findings also buttress the traditional usage of the plant for the aforementioned uses. The knowledge obtained from the study could also encourage further research with view to commercialization of product for wider usage to reduce the menace of diabetes.

**Reference**


