Evaluation of the Hypoglycemic Activity and Safety of
Momordica charantia (Cucurbitaceae)

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ABSTRACT

Momordica charantia(MC) commonly called balsam pear or bitter melon has been implicated in hypoglycemia and other pharmacological activities. Aqueous extract of the leaves of Momordica charantia(MC) was tested for hypoglycemic activity by oral administration to both normal rats and alloxan-induced diabetic rats for 28 days. Wistar rats were divided into 4 groups A, B, C, D. Group A (control) was treated with intraperitoneal injection of the vehicle (saline alone), group B alloxan-induced diabetic rats administered orally equal volumes of vehicle (distilled water) alone, group C, normal rats and group D, alloxan-induced diabetic rats both administered 400mg/kg of the aqueous extract. On the 29th day, blood glucose, plasma insulin and the effect on Oral Glucose Tolerance Test (OGTT) were monitored at 3, 6 and 9 h, for 50% of the rats of each group, while the rest of the rats were sacrificed and the levels of certain biochemical parameters in the serum and examination of some visceral organs were investigated.

At the end of the experiment, the blood glucose level of group C rats was found to have reduced significantly (p<0.01) while there was no significant difference in the blood glucose level of group D rats. Significant reduction in blood glucose during OGTT was observed in group D rats at 3, 6 and 9 h, compared to non treated diabetic rats. No changes were observed in plasma insulin levels, indicating that the probable hypoglycemic mechanism involves improved glucose tolerance by preventing glucose from being reabsorbed into the intestines. Highly significant increase in alkaline phosphatase (p<0.001), AST and ALT (p<0.001) in alloxan-induced diabetic rats (B & D) and in normal rats administered the aqueous extract, was observed, indicating liver hyperactivity. Significant increases in creatinine clearance and a significant decrease in creatinine (p<0.01) in group C suggest proper functioning of the kidneys.

Macroscopic examination of some visceral organs revealed profound pathological differences in diabetic rats and normal rats administered the aqueous extract.
The observed results indicate that *Momordica charantia* (MC) has strong hypoglycemic activity and is safe for use as a therapeutic agent.

**KEYWORDS:** *Momordica charantia, MC, Oral Glucose Tolerance Test, OGTT, hypoglycemic activity, toxicity.*
INTRODUCTION

The use of herbs in therapy originated in antiquity and most of its history is associated with folklore. In as much as these herbs serve as a potential source of food for human consumption, a large number of their preparations or remedies have been shown to be particularly effective and standardized proportions or extracts from them have been incorporated into the practice of orthodox medicine (1,2).

There is no doubt that modern therapeutics owes a debt to traditional herbal remedies from different parts of the world for the gift of effective agents, e.g. atropine, coumarin anticoagulants, glycosides, the ergot alkaloids, the vinca alkaloids and quinine, just to mention a few (3,4).

One of such important plants is Momordica charantia (MC), commonly known as balsam pear or bitter melon. Over the years, it has been used in the preparation of various remedies for numerous therapeutic purposes (5,6). It is a climbing annual herb, of the Cucurbitaceae family, usually found in moist and moderately dry tropics such as the rain forest in West Africa (7). It possesses undivided tendrils formed from modified shoots, opposite leaves which are circular and kidney-shaped and have five to seven lobes, ovate to oblong in shape and sometimes toothed along the margin. The plant produces small irregular five lobed male and female flowers of an orange yellow colour. The inferior of the female flower develops into oblong fruit with a bumpy surface. The fruit, a berry hangs down on a slender stalk and is green at first but later turns bright yellow. Inside are pale gray to brown slightly flattened seeds with a raised pattern on both sides. They are surrounded by a blood red pulp, which forms a striking contrast with the yellow coloured skin of the fruit (8,9).

Investigations to evaluate the effect of MC on the glucose tolerance showed that 75% of the patients investigated responded (10,11). A possible synergistic interaction in patients, between a drug, chlorpropamide and a curry made from MC and garlic, has been reported (12). An improvement on the tolerance of external load of glucose by non-insulin dependent diabetics was reported by Leatherdale et al., after oral administration of 50ml of MC fruit juice (13). A similar observation was made by Athar et al., in patients seven days after oral ingestion of 50mg/kg body weight of dried MC powder (14). Furthermore, an insulin-like protein called “plant insulin”, isolated from this plant, has been shown to possess hypoglycemic properties, when injected subcutaneously (15,16).

Treatment of diabetes aims at maintaining blood glucose homeostasis, prevention of ketosis and secondary complications. Major mode of control is through diet and exercise, insulin replacement therapy and by the use of oral hypoglycemic agents (17,18). Most oral hypoglycemic agents are Western drugs. Hypoglycemic herbs are widely used as non-prescription treatment for diabetes, though very few have been standardized and their efficacy demonstrated in systematic clinical trials as those of Western drugs (19,20).

In supplementing the treatment of regimes of orthodox medicine with herbal medicines, effort should, however, be made to determine all the active principles of the herbal product being taken. It is a general belief held by most people that herbal preparations are harmless and ineffective but unfortunately, herbal products could be exceedingly toxic e.g. Podophyllum peltatum plant is neurotoxic and teratogenic (2,21) Issues of standardization, characterization, preparation, efficacy and toxicity therefore, remain paramount and necessitate careful supervision and monitoring (22).
This study aims at evaluating the hypoglycemic activity and toxicity of the aqueous extract of MC, with the view to ascertain its safety profile and have some information on the mechanism involved in the hypoglycemic action.

**MATERIALS AND METHODS**

**Sample Collection and Extraction:** Plant material (MC) was purchased from the Mushin Herbs market, Lagos-Nigeria after identification by an herbalist and authentification by Dr. Tsabang Nole, a botanist at IMPM. The leaves were then air dried, pulverized by use of a mechanic grinder and passed through 40-mesh sieve to get the fine powder. 500g of the ground material was then moistened appropriately with distilled water and allowed to stand for approximately 4h in a tightly closed container at room temperature. The mass was then packed into a percolator and distilled water added to form a shallow layer just above the mass and the percolator was closed. The mixture was allowed to stand for 24h, after which the outlet of the percolator was opened and the liquid contained therein allowed to drip out completely. The marc was then pressed and the liquid added to the percolate, which was then freeze-dried, weighed and kept in air-tight containers at 4°C for further use. A weighed amount of the dried aqueous extract was then dissolved in distilled water to make a concentration of 400mg/kg body weight (BW) for experimentation.

**Animals:** Healthy adult male albino rats of Wistar Strain (180-200g) were divided into four groups (A, B, C, and D) of 10 each and housed in clean cages and maintained in a well ventilated animal house. The animals were fed with standard rat chaw and given clean drinking water.

**Experimental Design**

**Induction of Diabetes to Group B and D Rats:** After an overnight fast, rats were administered intraperitoneal(i.p) injection of 120mg/kg BW of alloxan freshly dissolved in saline (100mg/ml), to induce diabetic state with a blood glucose level of more than 250mg/dl. Blood glucose was monitored after alloxan treatment to confirm the diabetic state and diabetic rats were included in the experiment ten days after treatment. Rats of all groups were weighed prior to the experiment and daily treatment was as follows for 28 days:

**Group A:** Control. Single i.p. injection of vehicle (Saline alone).

**Group B:** Alloxan Diabetic. Oral administration with equal volume of water alone.

**Group C:** Normal. 2ml 400mg/kg BW of aqueous extract of MC.

**Group D:** Alloxan Diabetic. 2ml 400mg/kg BW of aqueous extract of MC.

**Biochemical and Histopathological Analysis:** At the end of the 28th day, the animals are fasted overnight and then 50% of the animals in each group were sacrificed by cervical dislocation and the serum collected for the analysis of some biochemical parameters. Safety endpoints included effects on serum glucose (GLU), cholesterol (CHOL), triglycerides (TGY), urea nitrogen (BUN), uric acid (URIC), creatinine clearance (CCT), creatinine (CRE), alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST). Some visceral organs are also collected for histopathological examination.

**Oral Glucose Tolerance Test (OGTT):** On the 29th day after an overnight fast, blood was collected from each of the remaining animals for fasting blood glucose examination. An oral dose of glucose (10ml/kg BW; 50%w/v) was then administered to all the animals alongside 2ml 400mg/kg BW of the aq. extract of MC. Blood glucose levels were then estimated with time using a glucose kit (Menarini Diagnostics, Italy), in which glucose oxidase and peroxidase
enzymes are used along with chromogen 4-aminophenazone and phenol, resulting in the formation of a coloured compound that can be measured at 500nm (Life Scan, USA) (23,24).

**Radioimmunoassay of Insulin (RIA):** Plasma insulin was measured by the radioimmunoassay Berthold Models LB2111 and LB2104 Multi-Crystal Gamma Counter with Sodium Iodide 1-1/8” × 1-1/4” Crystals as performed by the Department of Child Health, University of Missouri-Columbia, using Pharmacia Insulin RIA kit (Pharmacia Diagnostics AB, Uppsala, Sweden). RIA is a double-antibody batch method. Insulin in the specimen competes with a fixed amount of 125I-labelled insulin for the binding sites of the specific insulin antibodies. Bound and free insulin are separated by adding a second antibody, centrifuging and decanting. The radioactivity in the pellet is then measured, which is inversely proportional to the quantity of insulin in the specimen (25,26,27). The test is used to measure insulin levels in the bloodstream and is also useful in determining pancreatic β-cell activity.
RESULTS

Table 1:
Results of Biochemical Tests on rats after treatment with aq. Extract of Momordica charantia for 28 days

<table>
<thead>
<tr>
<th>Dose</th>
<th>G</th>
<th>GLU mmol/l</th>
<th>CHOL mmol/l</th>
<th>TGY mmol/l</th>
<th>BUN mmol/l</th>
<th>URIC mmol/l</th>
<th>CCT mmol/l</th>
<th>CRE Umol/l</th>
<th>ALP U/l</th>
<th>ALT U/l</th>
<th>AST U/l</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>18.88 ± 0.28</td>
<td>18.12 ± 0.08</td>
<td>6.60 ± 0.52</td>
<td>18.40 ± 2.36</td>
<td>1.16 ± 0.08</td>
<td>142.80 ± 1.44</td>
<td>170.24 ± 4.08</td>
<td>326.48 ± 6.72</td>
<td>68.68 ± 3.04</td>
<td>90.52 ± 1.68</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>26.32 ± 1.64</td>
<td>17.34 ± 2.20</td>
<td>6.62 ± 1.24</td>
<td>16.24 ± 1.24</td>
<td>1.02 ± 0.02</td>
<td>102.80 ± 3.24</td>
<td>196.74 ± 2.84</td>
<td>426.96 ± 3.41</td>
<td>144.20 ± 7.41</td>
<td>164.96 ± 5.40</td>
</tr>
<tr>
<td>400</td>
<td>C</td>
<td>5.56 ± 0.72</td>
<td>25.04 ± 5.80</td>
<td>5.76 ± 0.04</td>
<td>38.72 ± 0.84</td>
<td>1.92 ± 0.04</td>
<td>284.80 ± 1.76</td>
<td>127.74 ± 3.16</td>
<td>329.56 ± 6.92</td>
<td>74.44 ± 4.72</td>
<td>102.88 ± 6.29</td>
</tr>
<tr>
<td>400</td>
<td>D</td>
<td>19.42 ± 3.32</td>
<td>21.49 ± 2.17</td>
<td>6.71 ± 1.88</td>
<td>40.26 ± 2.49</td>
<td>2.01 ± 0.84</td>
<td>296.60 ± 8.94</td>
<td>128.62 ± 7.32</td>
<td>356.34 ± 4.20</td>
<td>98.64 ± 8.80</td>
<td>128.70 ± 7.60</td>
</tr>
</tbody>
</table>

Results are means ± SD, n=4
Difference from control: Highly significant : a = p<0.001; Significant : b = p<0.01

Key: GLU=Glucose, CHOL=Cholesterol, TGY=Triglycerides, BUN=Blood Urea Nitrogen, URIC=Uric Acid, CCT=Creatinine Clearance Test, CRE=Creatinine, ALP=Alkaline Phosphatase, ALT=Alanine Transaminase, AST=Aspartate Transaminase.
G = Group; A = Group A (Control); B = Group B (Diabetic); C = Group C (Normal); D = Group D (Diabetic).

After 28 days of oral administration of the aqueous extract, there was a highly significant (p<0.001) decrease in blood glucose in normal rats (group C) compared to the control as shown in Table 1. At the same time there was a significant (p<0.01) increase in non treated diabetic rats (group B) whereas treated diabetic rats showed no significant difference in blood glucose level. A summary of the renal function tests in Table 1 shows decreased values in creatinine which correlates with increased values in creatinine clearance test, as well as blood urea nitrogen and uric acid in normal rats compared to the control. This shows proper functioning of the kidneys. Table 1 also shows a highly significant (p<0.001) increase in ALP, ALT and AST in diabetic rats, reflecting hyperactivity of the liver and the cardiac muscles, which might be due to the diabetic condition.
**Figure 1:**
Effects of long term treatment of the aq. extract of *Momordica charantia* on Blood glucose levels of male albino rats. Each histogram is mean of 5 rats.

Significant difference: $a=p<0.01$, $b=p<0.05$

**Figure 2:**
Effects of long term treatment of the aq. extract of *Momordica charantia* on plasma insulin of male albino rats. Each histogram is mean of 5 rats.

No significant difference noticed.
Figure 3:
Effects of long term treatment of the aq. extract of Momordica charantia on blood glucose levels of male albino rats.

![Blood Glucose Levels Graph](image)

Diabetic animals administered the extract behaved in the same manner as the control.

After long term administration of the aqueous extract, examination of fasting blood glucose revealed a significant decrease (p<0.05 and p<0.01 respectively) in blood glucose in diabetic rats (groups B and D) after 6 h of observation whereas no significant difference was observed in blood insulin even after 9 h as shown in figures 1 and 2 respectively, in correlation with the results obtained from the biochemical assays.

Figure 3 shows that during Oral Glucose Tolerance Test (OGTT), diabetic rats (Group D) administered the aqueous extract after a glucose load behaved in the same manner as the control. There was a two fold increase in blood glucose after 30 min of oral glucose load and at 60 through 90 min. There was a decrease up to 180 min when the blood glucose level came back to normal. The blood glucose level in non-treated diabetic rats (Group B) was, however, significantly higher than the other groups and the control after long term administration of the aqueous extract. Upon administration of a glucose load and then the aqueous extract during OGTT, there was a threefold increase in blood glucose level after 30 min, which decreased to normal after 180 minutes.

Macroscopic examination of some visceral organs revealed profound pathological differences in diabetic rats and non diabetic rats after administration of the aqueous extract of MC as shown on the pathological plates of the liver, kidney and heart of diabetic rats in figures 4, 5 and 6.
DISCUSSION

MC had been implicated in hypoglycemia (28,29,30), and this was again confirmed in this
study as shown in Table 1. This hypoglycemic activity could be due to an increase in insulin response or probably the transport of blood glucose to peripheral tissues. Other causes of hypoglycemia may include pancreatic lesions, endocrine disease or some forms of liver or kidney disorder (31). The renal function tests indicated hyper function of the kidneys; creatinine is removed from the plasma by glomerular filtration and excreted in urine without being reabsorbed by the tubules making it a relatively accurate and useful measure of the glomerular filtration rate (32). This correlates with the decreased serum creatinine levels due to high clearance rate and increased blood urea nitrogen as a result of pre-renal causes such as cardiac compensation, water depletion due to increased intake or excessive loss (as observed in excessive passing out of watery stool by the experimental animals) and increased protein catabolism (33). Apart from lowering the blood sugar level in diabetic rats, there was no difference in the level of ALP, ALT and AST in diabetic rats administered MC extract from the control, which indicates a potential of this plant in protecting vital organs like the liver, hence a probable strong antioxidant effect (34,35).

Whereas the aqueous extract of MC had a significant hypoglycemic effect on blood glucose levels, it had no significant effect on the plasma insulin levels of diabetic rats. This non significant change in plasma insulin levels does not suggest the absence of any appreciable stimulatory effect of the extract on the existing β-cells of the endocrine pancreas in the diabetic rats as earlier reported (36). Hafizur et al. recently reported an increase in β-cell area and number of β-cells in diabetic rats treated with MC fruit extract as compared to the untreated diabetic rats, thus confirming the pancreatic modulatory effect of MC (37). This indicates that the probable hypoglycemic mechanism involves improved glucose tolerance by preventing glucose from being re-absorbed into the intestines and/or the existence of insulin-like compounds in the extract (38). Biochemical studies indicate that bitter melon regulates cell signaling pathways in pancreatic β-cells adipocytes and muscles (39). Clinical data regarding the antidiabetic potentials calls for better designed trials to further elucidate therapeutic effects (40,41).

The tissue damage revealed by histopathology in the diabetic rats as opposed to normal rats reveals that administration of aq. Extract of MC does not have any effect on the visceral organs as shown on figures 4, 5 and 6. Drawing from the results of the biochemical and the histopathological analysis of this study, as well as earlier studies on toxicity (42), we can confirm the safety of the aqueous extract of MC.

In conclusion, MC possesses a modest hypoglycemic and antioxidant activity and this study provides evidence for a biochemical mechanism which carries blood glucose lowering effect of MC. Since earlier studies have demonstrated its dietary quality and safety, it can be recommended for the management of diabetes. Further studies on the nature of active principles involved would be of immense importance.
REFERENCES


