Title: Comparison of the Hyperglycemic Control of *M. oleifera* Leaves Aqueous Extract and Glibenclamide tablets in Alloxan Monohydrate Induced Diabetic Rats.

Abstract

Introduction: Diabetes being one of the commonest non-communicable diseases worldwide has no cure. The available hypoglycemic drugs are costly, and have associated long-term side effects. *M.oleifera* leaves are used in many countries in Africa and Asia to treat diabetes. The study compared the hyperglycemic control of *M. oleifera* leaves aqueous extract and Glibenclamide tablet in alloxan monohydrate induced diabetic rats.

Methods: Twenty-four female Wister albino rats, made diabetic using alloxan monohydrate, received either *M. oleifera* extract, glibenclamide or distilled water were delivered intragastric. The mean body weight and mean fasting blood sugar were measured over a period of 28 days.

Results: Rats that received distilled water had a mean fasting blood sugar of 329.3±44.9mg/dl at the beginning, which increased to 448.0±189.9mg/dl on day14, all the rats were dead by day 21. The rats that received *M. oleifera* had blood sugar 443.4±134.7mg/dl at the beginning, dropped to 166.5±162.79mg/dl by day 14, and to 88.7±41.0 mg/dl by day 28. Rats that received glibenclamide had blood sugar 517.6±139.3mg/dl at the beginning, dropped to 209.0±201.9mg/dl on day14, and to 89.7±42.85mg/dl on day 28. The blood sugar of the *M.oleifera* and glibenclamide groups reached normal level by day 21 and remained within the normal range up to day 28. *Conclusion*: *Moringa oleifera* leaves aqueous extract has similar pattern to glibenclamide tablet in causing hypoglycemia to alloxan monohydrate induced diabetic rats.

Key words: *M. oleifera*, Alloxan induced diabetes; glibenclamide, hypoglycemia, Wister rats

Introduction

Diabetes mellitus is a serious disease with no cure, which is costlyis becoming increasingly common, especially in developing countries and disadvantaged minorities. It continues to be a global public health problem with affected individuals rising from 108 million in 1980 to 422 million in 2014, and with middle- and low-income countries most affected [1]. In Uganda, diabetes mellitus is at 2.8% prevalence [2], which closely compares with other countries in East Africa.

Diabetes mellitus (DM) is generally associated with metabolic disorders plus inflammation and oxidative stress. The disease is characterized by hyperglycemia and hyperlipidemia, which result from disturbances in carbohydrate, protein, and lipid metabolism. Type 1 diabetes (T1D), also termed juvenile-onset or insulin-dependent diabetes, is an autoimmune disease and a metabolic disorder characterized by T-cell-mediated destruction of pancreatic beta (β) cells, resulting in insulin deficiency and hyperglycaemia. Type 2 diabetes (T2D), (non–insulin-dependent) DM which occurs in adults, is caused by insulin resistance coupled with a failure of the β cell to compensate.

Chronic inflammation has been indicated as a risk factor for the development of type 2 diabetes with increasing evidence pointing toward a role of pro-inflammatory cytokines such as C-reactive protein (CRP), interleukin (IL)-6, and tumor necrosis factor (TNFα) in the pathogenesis of insulin resistance and type 2 diabetes[3,4,5].

Research evidence indicates that several trace elements are essential for normal glucose homeostasis that include : chromium, potassium, calcium, magnesium, copper, manganese and zinc[6]. Vitamin B1 as well as vitamins B6 and B12 support nervous system functions and helps prevent diabetic neuropathies. Targeted consumption of micronutrients can help to improve metabolic control, optimize treatment and reduce the risk of developing diabetic complications. As coenzymes, the B vitamins play a central role in carbohydrate, protein and lipid metabolism. Studies indicate that the majority of type 1 and type 2

diabetics have inadequate supplies of vitamin B1 and impaired thiamine metabolism [7]. Lack of folic acid and/or vitamin B12 leads to impaired metabolism of the amino acid methionine and is frequently accompanied by elevated plasma homocysteine concentration [8]. In diabetics, increased oxidative stress may be a result of decreased plasma concentration of the antioxidant vitamins C and E, coupled with the reduced postprandial intracellular ratio of ascorbic acid to its oxidized form (dehydroascorbic acid) [9].

Recent studies indicate that an inadequate supply of vitamin D could be involved in the onset of numerous chronic diseases like diabetes mellitus types 1 and 2 [10, 11]. On the other hand, lack of vitamin D represents a risk factor for type 2diabetes and metabolic syndrome, since it increases insulin resistance and reduces insulin secretion from pancreatic beta cells [12]. There is evidence that vitamin D can help to prevent the destruction of insulin-producing pancreatic beta cells and thus combat the onset of type 1 diabetes [13]. The effects of vitamin D are assumed to be due primarily to the immunomodulatory action of the vitamin via T-helper cells and to the reduction of pro-inflammatory cytokines.

The therapeutic management of diabetes without any side effects remains a challenge. However, there is a growing interest in evaluating herbal remedies, which are seen to be less toxic with negligible side effects [14]. One such a plant is *Moringa oleifera* Lam.

M. oleifera leaves have for long time been used in folk medicine to treat diabetes in different communities of the world including Uganda [15, 16, 17]. M. *oleifera* is a rapid growing tree, native to the Sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. It was utilized by the ancient Romans, Greeks, Egyptians and Indians to treat several ailments 18].

The *M. oleifera* plant is drought tolerant , and is known to thrive best in tropical conditions. The plant also tolerates different soil types, and boasts as one of the few medicinal plants that is well documented.

The scientific classification of *Moringa oleifera* shows that it belongs to the Kingdom: Plantae; Division: Magnoliphyta; Class: Magnoliopsida; Order: Brassicales; Family: Moringaceae; Genus: Moringa; Species: *Moringa oleifera. Moringa oleifera* is the most widely cultivated variety of the genus Moringa in Asia and Africa [19, 20].

Micro-nutrients in *M. oleifera* leaves

M. oleifera leaves are said to provide 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk,9 times more protein than yoghurt, 15 times more potassium than bananas and 25 times more iron than spinach [21].

The leaves of *M. oleifera* are rich in minerals like calcium, potassium, zinc, magnesium, iron and copper [16, 22]. They contain around 25.5–31.03 mg of zinc/kg, which is the daily requirement of zinc in the diet [23]. The rich source of iron which was bioavailable in a rat model was found to be superior compared to ferric citrate, in overcoming iron deficiency [24].

Vitamins like beta-carotene of vitamin A, vitamin B such as folic acid, pyridoxine and nicotinic acid, vitamin C, D and E are also present in *M. oleifera* [25]. The leaves also have a low calorific value, and thus can be used in the diet of the obese [26].

Hypoglycemic phytochemicals

Medicinal plants are the main source of organic compounds such as polyphenols, tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. These organic compounds represent a source for the discovery and development of new types of antidiabetic molecules.

Phytochemicals like tannins, steroids and triterpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars were identified in *M. oleifera* leaves grown in Uganda [16]. *M. oleifera* leaves have

also been found to contain carotenoids, tocopherols [29], polyunsaturated fatty acids [30], folate [31], unique glucosinolates, flavonoids and phenolic acids in other studies [32, 33]. Among the flavonoids, flavonol glycosides (glucosides, rutinosides, and malonyl glucosides) of quercetin, kaempferol and isorhamnetin are predominantly found in *M. oleifera* leaves.

Phenols and flavonoids were previously found to have a hypoglycemic effect in alloxan monohydrate diabetes induced rats [27]. Alkaloids, tannins, steroids and quinines present in *C. papaya* leaf extracts caused hypoglycemia in streptozotocin-induced diabetic rats [28].

Hypoglycemic potential of *M. Oleifera* leaves

Moringa oleifera leaf extract possess potent hypoglycemic effects through the normalization of elevated hepatic pyruvate carboxylase enzyme and regeneration of damaged hepatocytes and pancreatic cells in rats, and also via its antioxidant properties on the liver and pancreas, plus an increase in β-cell mass and insulin production by the β-cells [34].

105 The phytochemicals that exist in *M. oleifera* are capable of acting on animal cells and tissues to inhibit
106 membrane bound enzymes, which affect DNA formation and destroy cell membranes [35, 36, 37]. The
107 leaves have antioxidants which can combine with reactive oxygen species to prevent cell damage that is
108 believed to occur in diabetes mellitus [38, 39, 40]. Additionally, the methanol extract of *M. oleifera* was
109 found to have immunomodulatory activity in rats, which could be useful in treating type 1 diabetes [41].
110 The therapeutic potential and medicinal properties of *M. oleifera* leaves has been evaluated in a number
111 of studies using animal models and they have been proved to have hypoglycemic activities [42, 43].
112 Extracts of *M. oleifera* leaves also have anti-inflammatory activities which are reported to contribute to
113 the hypogylycemic activities [44, 45, 46].
114 Safety evaluation of *Moringa oleifera* leaves done by researchers found them safe for human and
115 animal consumption by WHO standards [47, 48, 49, 50, 51]. There are few studies that have compared
116 the hyperglycemic control of herbal medicines with orthodox medicines on the market [66]. The aim of
117 this was to compare the hyperglycemic control of *Moringa oleifera* leaves aqueous extract and
118 glibenclamide tablets on alloxan monohydrate induced hyperglycemia in Wister albino rats. Ethical
119 approval was obtained from the Institution Review Board (IRB) of the Makerere University College of
120 Health Sciences, School of Biomedical Sciences, SBS-HDREC 565. The animals were treated humanely
121 according to international standard OECD guidelines (2001).

122 **Materials and Methods**

123 **Plant handling and extraction**

124 Fresh mature green leaves of *Moringa oleifera* were collected from Wakiso district, Uganda's central
125 region, growing on the hillside loam soil, harvested during the rainy season between 9.00 and 11.00 a.m.
126 Plant species and family were confirmed by a Makerere University plant taxonomist, and a specimen
127 voucher number (41302) deposited at the Makerere University herbarium. It was air-dried in a shade for

about 3 weeks until constant weight was attained, away from direct sun shine to protect the active compounds. The leaves were pulverized into coarse powder using a mortar and pestle to ease the extraction.

***Moringa oleifera* phytochemical extraction.**

Serial extraction which followed the established method starting with ether, then ethanol and lastly water was done [52, 53]. Briefly, one liter of ether was mixed with 500gm of *M.oleifera* leaf powder and shaken at intervals for two days. The mixture was decanted and filtered. The residue was air-dried for 3 days and 700ml of ethanol (98% V/V) added, and left to evaporate until it dried. The dry residue was then soaked in 700ml of warm water at 40^0C to facilitate the extraction. The ether and ethanol solvents were recovered using a rotary evaporator (BUCHI Rotavapor R-205) while the water extract was freeze dried into powder. The powder was dissolved in distilled water to make a stock solution from which the rats were dosed.

Study animals

This study used 24 female Wister rats aged 8-10 weeks reared in the Makerere University, College of Veterinary Medicine, Animal Resources and Biosafety's animal house. The experiment was carried out in the animal house at the department of Physiology, Makerere University College of Health Sciences. The animals received 12hrs of light and 12hrs of darkness, fed on commercial rat pellets and allowed to take food and tap water *ad libitum*. The rat housing was kept at room temperature. The rats were of normal body temperature, active and feeding well; and weighed 90-110gm each. Pregnant or Nursing rats were excluded from the study.

Induction of hyperglycemia

Alloxan monohydrate (Sigma, St. Louis, MI, USA) was used to induce hyperglycemia in the rats. Alloxan monohydrate was dissolved in 0.9% normal saline and injected intraperitoneally in a single Dose of 100 mg/kg body weight to overnight-fasted rats [34]. Interventions were introduced when the rats showed fasting blood glucose levels >250 mg/dL, as well as a reduction in body weight with signs of polyphagia, polyuria, and polydipsia.

Dosing of animals

The animals were randomly allocated to 3 groups of 8 rats each. The rats in each group were made diabetic using alloxan monohydrate. Each group received the intervention intragastric once a day for 28 days. Food was withdrawn from the rats at 10.00p.m, but they were allowed to take tap water *ad libitum.* Food was reintroduced after weighing and measuring blood sugar. The rats were allocated to different groups as follows:

Group I: Diabetic rats received 1ml distilled water once daily for 28 days (negative control) .

Group II. Diabetic rats received 500mg/kg of *Morings oleifera* aqueous extract once daily for 28 days.

Group III: Diabetic rats received 0.04mg/kg tablet Glibenclamide (positive control) once daily for 28 days.

On a weekly basis, body weight and fasting blood sugar for each rat was measured between 8.00 and 9.00 a.m, using "On Call plus Blood Glucose Meter" glucometer purchased from Acon Laboratories, Inc. 10125 Mesa Rim Road , San Diego, CA92121, USA from an ear lobe prick.

Data analysis

The data was analyzed using Prism 7 (GraphPad) software (SanDiego, CA, USA) where the means and standard deviations were compared. The data from each intervention group were compared on days 7,

14, 21 and 28 against the results of day 1 using ordinary one way ANOVA with Dunnets adjustment for multiple comparisons. The level of significance was fixed at $p \leq 0.05$.

Results

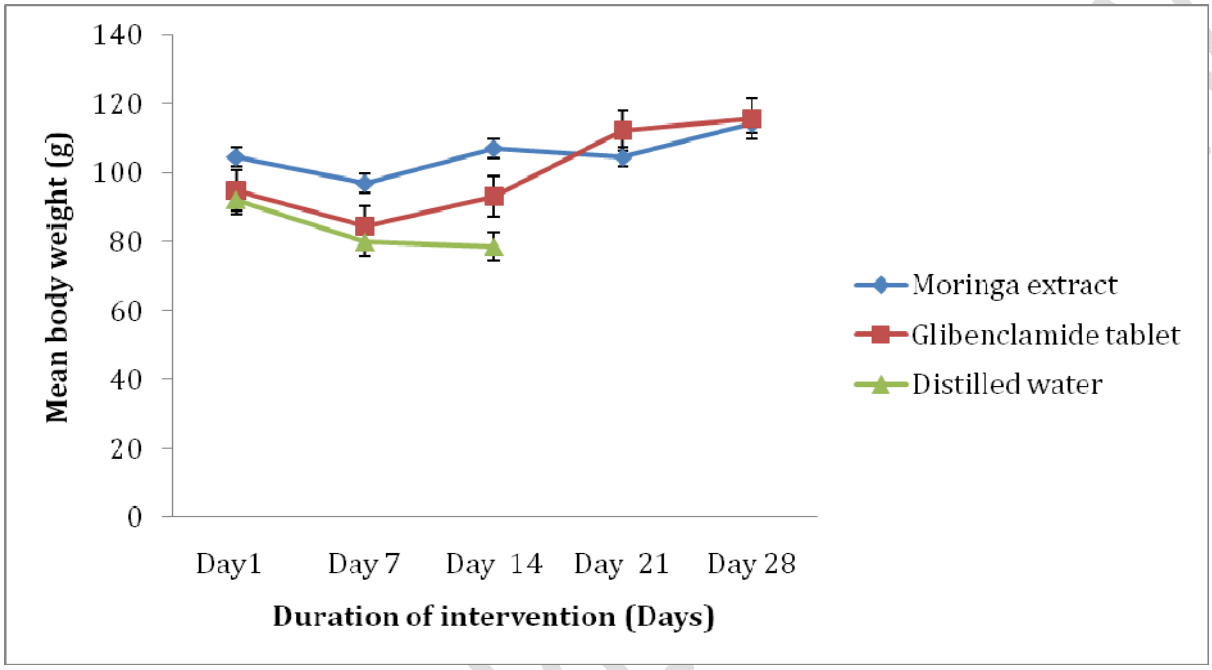

Figure 1. Mean body weight (g) against days during intervention for *M. oleifera* leaves aqueous extract, glibenclamide tablets and distilled water

Figure 1 shows that the mean body weight of alloxan diabetic rats during the 28 days of treatment. The rats that received 1ml distilled water all died by the third week of the study, while those that received 500mg/kg *M.oleifera* leaves aqueous extract and those that received 0.4mg/kg glibenclamide tablets survived up to day 28. Generally, the mean body weight of the diabetic rats that received *M.oleifera* aqueous extract and glibenclamide increased slightly, although the increase was not significant. In contrast, the mean body weight of the diabetic rats that received 1ml distilled water had reduced by day 14, although the decrease was not significant.

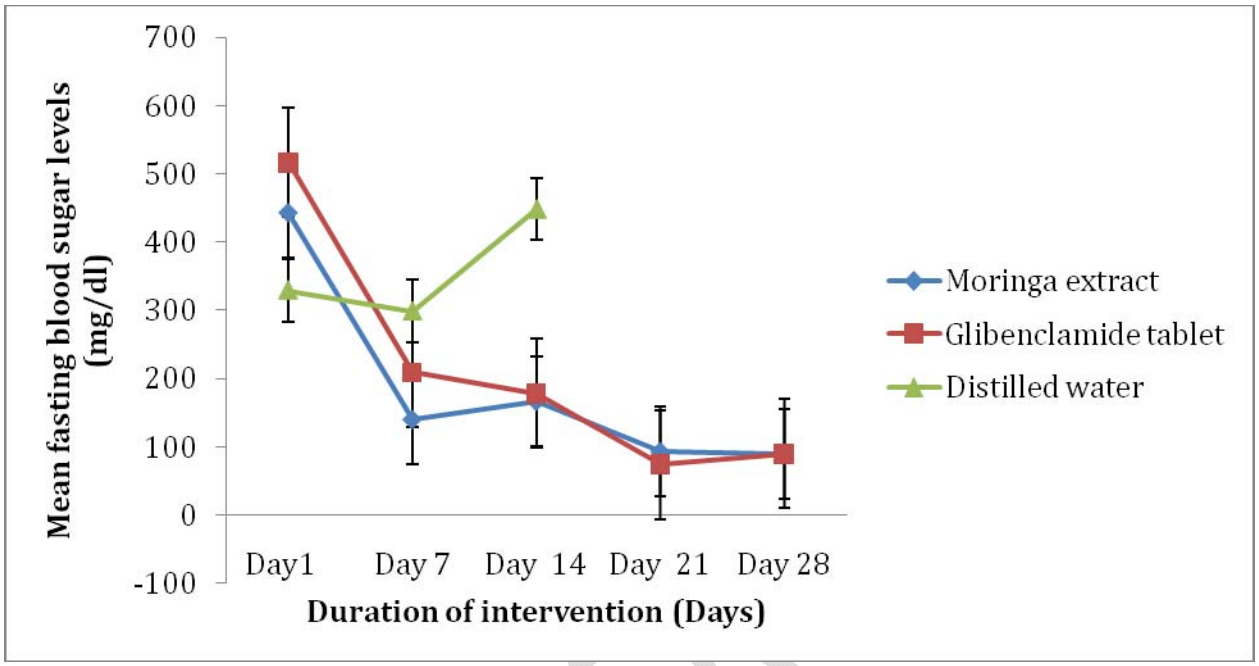

Figure 2. Mean fasting blood sugar against days during intervention for *M. oleifera* aqueous extract Glibenclamide tablet and Distilled water.

Figure 2, shows that all the animals had blood sugar of more than 300mg/dl on day one before interventions. In the group that received distilled water, fasting blood sugar significantly rose during the intervention period, leading to all rats dying before the end of the third week. However, the fasting blood sugar levels of the animals that received *M.oleifera* leaves aqueous extract and glibenclamide tablets dropped significantly between day 1 and day 7, and then again between day 14 and day 21. Between day 7 and day 14, the reduction in fasting blood glucose was not significant in the rats that received an aqueous extract of *M. oleifera* leaves, and glibenclamide. By day 21, the fasting blood sugar levels in both groups of rats treated with an aqueous extract of *M. oleifera* leaves, and oral hypoglycemic agent glibenclamide had returned to normal, with mean blood glucose levels of

92.7±28.38 mg/dl, and 74.0±10.15mg/dl in the aqueous *M.oleifera* and glibenclamide treatment groups respectively.

During the 4th week of the study (days 22 to 28) the change in fasting blood sugar in the rats was minimal and it remained in the normal range, with the mean+SD of the *M.oleifera* extract and glibenclamide treatment groups being 88.7±41.00 mg/dl, and 89.7±42.85mg/dl respectively. In general the graphs of *M.oleifera* extract and tablet glibenclamide interventions had a similar pattern.

Discussion

The hypoglycemic effect of *M. oleifera* leaves aqueous extract and glibenclamide tablets on alloxan monohydrate induced diabetic rats were investigated. Study results show that the aqueous extract of *M. oleifera* leaves grown on Ugandan soil have hypoglycemic effects in Wister albino rats. They also show that there was insignificant increase in the mean body weight for *M.oleifera* extract and the glibenclamide group which is an indicator of diabetes control.

The mean body weight of the diabetic rats that received *M.oleifera* aqueous extract and glibenclamide increased slightly, although the increase was not significant. Glibenclamide is taken in dose of 5 mg daily and is clinically effective in lowering blood glucose and cause weight gain. Most type 2 diabetic patients experience weight loss if diabetes is not well controlled. Normal weight is an indicator of good clinical diabetic control. Although there is limited documentation of controlled studies that confirm *M. oleifera* leaves to cause weight loss, studies in human have shown that it reduces weight through its inhibition of α-amylase enzyme [26, 65]. However animals studies have shown increase in body weight when diabetic rats are treated with *M.oleifera* aqueous extract [34].

The leaves of *M.oleifera* are used in Ugandan rural communities to treat diabetes mellitus [16]. However, there is currently no recommended standard method of preparation and use in the

communities. Previous preparations have involved maceration of fresh leaves of *M.oleifera* or powder in different quantities of water. This scenario encouraged us to carry out an experiment to compare the hyperglycemic control of *M. oleifera* leaves aqueous extract and Glibenclamide tablets in alloxan monohydrate induced diabetic rats. The results show that the aqueous leaf extract of *Moringa oleifera* compares well with the oral hypoglycemic drug glibenclamide in causing hypoglycemia in alloxan monohydrate induced diabetic rats

The study results agreed with those from earlier studies around the world which found out that *M.oleifera* leaves extracts reduced blood sugar in laboratory animals or has a hypoglycemic effect [34-46]. The fact that the quantity and quality of phytochemicals (non-nutritive secondary metabolites), which have medicinal principles, greatly depend on the soil, has encouraged many researchers from different regions and countries to explore the hypoglycemic effects of *M.oleifera* extracts grown locally in diabetic rat models[34-46]. Tannins, steroids and triterpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars are phytochemicals that may have hypoglycemic effect also identified in *M.oleifera extract* [16, 47]. Each of thee phytocemicals or in combination may act together to reduce blood sugar in vitro.

It is believed that the hypoglycemic effect of *M. oleifera* is probably due to presence of phytochemicals that have anti-inflammatory [44, 45, 46], antioxidant [38, 39, 40], and immunomodulatory [41] effects. Intake of flavonoids has been shown to protect against chronic diseases associated with oxidative stress, including cardiovascular disease, cancer and *M.oleifera* leaves are a good source of flavonoids [55]. Phenolic acids and flavonoids affect glucose homeostasis by influencing β-cell mass and function, plus increasing insulin sensitivity in peripheral tissues, which are good for diabetes prevention and management [57, 57]. These compounds have also been shown to benefit patients with other chronic conditions such as, hypercholesterolemia, high blood pressure, non-alcoholic liver disease, and cancer

[58]. Condensed tannin extracts showed promising antidiabetic effects with potential α-amylase and α-glucosidase inhibition activities [59] and the tannin-rich extract from plant material could be an interesting candidate for the treatment of several health disorders associated with oxidative stress such as hepatocellular injury and diabetes [60]. The hypoglycemic activity of *M.oleifera* leaves could also be due to presence of terpenoids, which appears to be involved in the stimulation of β cells and the subsequent secretion of preformed insulin [61]. Quercetin is found in dried *M.Oleifera* leaves, at high concentrations, as quercetin-3-O-β-d-glucoside (iso-quercetin or isotrifolin). Quercetin is a strong antioxidant, with multiple therapeutic properties including hypoglycemia [62]. It can protect insulin-producing pancreatic β cells from Streptozotocin (STZ) induced oxidative stress and apoptosis in rats [63]. In studies where diabetes is induced in rats doses as low as 250mg/kg of *M.oleifera* leaves extract have caused hypoglycemia [64].

Despite the wealth of knowledge on the hypoglycemic effect of *M.oleifera* leaves in animal models, there is yet no drug developed from the plant phytochemicals to manage diabetes.

Conclusion

From this study we can conclude that *M.oleifera* leaves aqueous extract has similar pattern to glibenclamide tablet in causing hypoglycemia to alloxan monohydrate induced diabetic rats.

Recommendation

More studies are needed to develop hypoglycemic drugs from *M.oleifera* leaves in a bid to effectively, safely and cheaply treat diabetes mellitus. Clinical trials in normal human volunteers to determine the safety of *M.oleifera* leaves extract.

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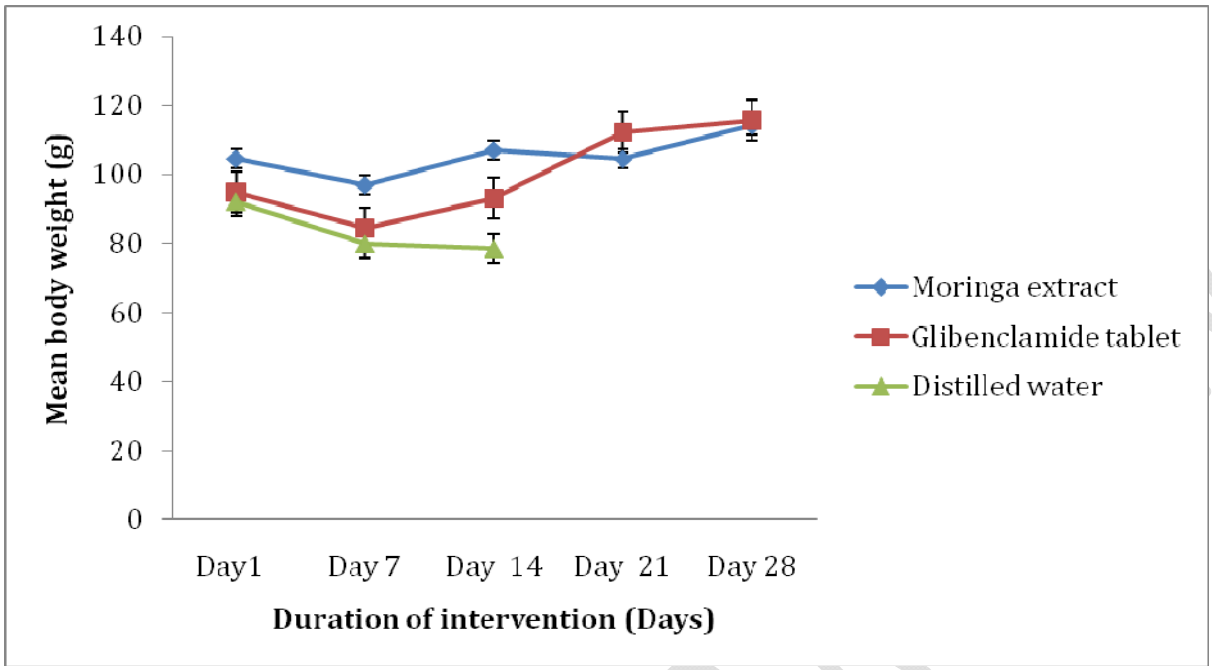

Figure 1. Mean body weight (g) against days during intervention for *M. oleifera* leaves aqueous extract, glibenclamide tablets and distilled water

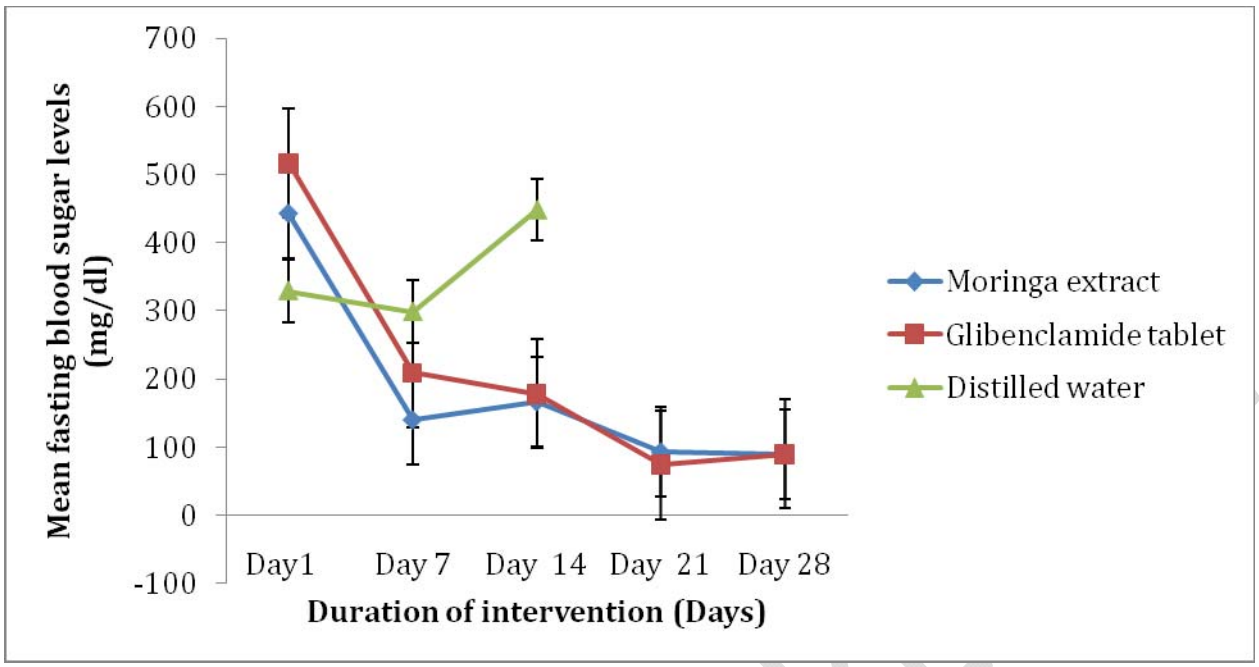

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Figure 2. Mean fasting blood sugar against days during intervention for *M. oleifera* aqueous extract Glibenclamide tablet and Distilled water.

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