Anti-hyperglycemic activity of *Moringa oleifera* is partly mediated by carbohydrase inhibition and glucose-fiber binding

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Abstract

Background: Moringa oleifera has potential anti-hyperglycemic effects that is reported earlier by different scientific group using animal diabetic models. We aimed to explore the possible mechanism of action of *M. oleifera* extract through different method. *Methods*: Primarily we measured Fasting Blood Glucose and performed Glucose Tolerance Test, in type 2 diabetic rats. Further we studied the effect of extracts on pancreatic insulin concentration. Extracts effect on carbohydrate break down was assayed using α-amylase inhibition assays and assay of GI tracts six different segments. An *in situ* intestinal perfusion model and a glucose fiber assay was performed to see the potentiality of *M. oleifera* on glucose absorption. *Results: M. oleifera* showed no significant change in insulin secretion *in vivo*. Additionally, substantial effect of the extract was seen on retarded glucose absorption and in the *in situ* perfusion study of rat intestinal model. α-amylase action was inhibited by the extract, yet again, this findings was further confirmed via the Six Segment assay, where sucrose digestion was found to be inhibited throughout the length of the GI Tract. *Conclusion:* A combined *in vitro*, *in vivo* and *in situ* tests justified that the anti-hyperglycemic activity of *M. oleifera* is potential and its tissue level mechanism is also justified.

Keywords: *glucose tolerance, ELISA, hypoglycemia, type 2 diabetes*

Abbreviations

MO	*Moringa oleifera*
GI	Gastrointestinal
CAM	Complementary and Alternative Medicine
TCA	Trichloroacetic acid
STZ	Streptozocin
TG	Triglyceride
CMC	Carboxymethyl cellulose
WC	Water control
GC	Glibenclamide
ACB	Acarbose
DMP	Domperidone
LPM	Loperamide

Introduction

For the last few decades diabetes held its position as one of the world's predominant endocrine disorder **[1]**. By nature, it cannot be completely cured but it should be kept under tight control. Usually modified lifestyle, medications, diet, or a combination of all of these are prescribed to diabetic patients to control diabetes. Complementary and alternative medicines have become popular in peoples from developing countries for managing a multitude of disorders including, diabetes. However, unclear mechanism of action and lack of scientific evidences of efficacy of these therapies kept them far behind from important use. There is another drawback of these plants use in various ailments is no established safety profile; although it is thought that they are safe besides being economical, effective and their easy availability. Traditional practitioners globally emphasizes these advantages of medicinal plants in their day to day practice. Traditional use of medicines is recognized as a way to learn about potential future medicines. In 2001, researchers identified 122 compounds used in mainstream medicine which were derived from "ethno medical" plant sources; 80% of these compounds were used in the same or related manner as the traditional ethnomedical use **[2]**.

Moringa oleifera (*M. oleifera*) is also known as *Moringa pterygosperma* Gaertn, is a member of the Moringaceae family of perennial angiosperm plants, which includes 12 other species. This plant is commonly named in English as Moringa and drumstick tree **[3]**. *M. oleifera* is one of the most useful tropical trees **[4]**, it serves the following characteristics: high protein, vitamins, mineral and carbohydrate content of entire plants. This plant provides with high value of

nutrition for both humans and domestic animals; the seed contains high oil content (42%), which is edible, and with medicinal uses. Previous studies by Amaglo *et al.* **[5]**, Limon-Pacheco and Gonsebatt **[6]**, and Mahajan and Mehta **[7]** have reported different pharmacological potentiality including antioxidant, and anti-inflammatory property of *M. oleifera*. Moreover, Awodele *et al.*, **[8]** have studied to evaluate toxicological property of the aqueous extract of *Moringa oleifera* Lam (Moringaceae). Oyedepo *et al.*, **[9]** evaluated the anti-hyperlipidemic effect of aqueous leaves extract of *Moringa oleifera* while Gupta *et al.*, **[10]** worked on the evaluation of antidiabetic and antioxidant activity of *Moringa oleifera* in experimental diabetes model. Again, Jaiswal *et al.* **[11]** investigated for the role of *Moringa oleifera* in regulation of diabetes induced oxidative stress while Choudhary *et al.*, **[12]** assessed the antiulcer potential of *Moringa oleifera* root bark extract in rats. Although there have been several reports on the cholesterol and blood glucose reducing effect of different fraction of leaf extracts of *M. oleifera* in rats **[13-17]**, there is still paucity of information on the hypoglycemic activity of the extract at the doses investigated in the present study.

However, basic mechanism of action of *M. oleifera* still remain unclear to till today as an anti-hyperglycemic herb. The aim of the current study is to paint a comprehensive picture of effects *M. oleifera* on sucrose breakdown and glucose absorption, insulin release, and intestinal enzyme functions. Our study will help to identify the particular organ or organ system responsible for the previously seen hypoglycemic activity of this plant.

Materials and Methods:

Plant Collection and Processing

M. oleifera leaves were collected from Jahangirnagar University, University Ayurvedic Research Centre (UARC), Dhaka, Bangladesh. The plant was identified by a taxonomist prior to further processing and a voucher specimen was deposited at the National Herbarium at Mirpur, Dhaka, Bangladesh with accession number DACB-12290. Then leaves were cleaned off of dirt and other debris and then thoroughly washed under running tap water followed by air drying for granules preparation.

The solvent (ethanol 80%) was collected from organic chemistry laboratory of North South University, Dhaka. About 400 g of grinded powder was taken in flat-bottomed container, with which 2000 ml of solvent was added gradually and with regular stirring. The container kept sealed and let in mechanical shaker for 6 days average 2-3hour daily. The mixture is filtrated in stepwise processes. A clean, white cotton cloth used with double folded and the mixture is passed through it. It was the primary filtration where larger fibers are separated from the mixture. Then cotton was used later to filter the mixture to separate larger materials. Finally filter paper was used for the separation of clear filtrate from the mixture. The amount of filtrate obtained was 150 ml. The filtrate (Ethanol extract) obtained was evaporated by Rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 5 to 6 rpm and at 57^0C temperature. It rendered a gummy concentrate of greenish color that was designated as crude extract or ethanolic extract. This crude ethanolic extract was then dried by freeze drier and preserved at $+4^0$C until further use.

Animal Handling

Long Evan type normal healthy and Type 2 diabetic rats of both sex were farmed in the animal house of the Department of Pharmaceutical Sciences, North South University, Dhaka, and the weight of rats was about 180-220 gm. All test animals were kept at an ambient temperature of 22 ± 5°C and humidity was maintained at 50-70%. 12 hrs day-night cycle was maintained to avoid fluctuations in the circadian rhythm, and the rats were kept in translucent plastic cages with wood shavings provided as bedding; while grilled cages was replaced with bedding prior to fasted rat testing, to prevent corpophagy. Standard rat pellets and filtered drinking water was provided to the test animals *ad libitum* throughout the experiment apart from certain test required to fasting, during that time only water was given to them. The designed experimental protocol was designed and subsequently approved by the Ethics Committee on Animal Research, North South University, following the "Revised guide for the care and use of laboratory animals by American Physiological Society" **[18]**.

Diabetes Induction

Intraperitoneal streptozotocin (STZ) was given to normal and healthy rats to induce Type 2 diabetes in citrate buffer solution at a dose of 90 mg/kg. New born rat aged less than 48 hrs and weighing 7 gm were chosen for this procedure. Three months later, fasting blood glucose levels of 8–12 mmol/L were selected for the experiments and an oral glucose tolerance test (OGTT) was performed for further confirmation of type 2 diabetes **[19]**.

Acute effects of ethanolic extracts of *M. oleifera* on glucose homeostasis

M. oleifera extract was suspended in distilled water and orally administered to 12 h fasted rats, and control group animals received only an equal volume of distilled water; to test blood glucose change in fasting condition. Effects on glucose tolerance were similarly evaluated by administration of *M. oleifera* extracts together with glucose (2.5 g/10 ml per kg body weight) after a fasting period of 12 h, control group received only glucose solution. In either cases blood sample was collected from the tail vein of rats, serum separated by centrifugation and stored at -22°C until further analysis. Blood glucose was analyzed by GOD-PAP method **[20]** (glucose kit, Randox™, UK).

Effects of *M. oleifera* on plasma Insulin

Blood was drawn out from Type 2 diabetic rats, 1 hr after administration of *M. oleifera*. The amount of insulin released from the pancreas *in vivo*, was determined using Rat Insulin ELISA Kit (Crystal Chem™, USA).

Effects of *M. oleifera* on intestinal glucose absorption

An *in situ* intestinal perfusion technique **[21]** was used to determine the effect of *M. oleifera* intestinal absorption of glucose in 36 hr fasted non-diabetic rats anaesthetized using Ketamine (80 mg/kg). Ethanol extract of *M. oleifera* (5 mg/mL, 10 mg/ml, 20 mg/mL equivalent to 0.25mg/kg, 0.5 g/kg, 1 g/kg) was suspended in Krebs Ringer buffer, along with glucose (54 g/l). These were passed through rat pyloris via a butterfly cannula and the perfusate collected by means of a tube inserted at the end of ileum. The control group was perfused with Krebs Ringer buffer along with glucose only. Perfusion was carried out at a rate of 0.5 ml/min for 30 min at

37°C. The results were presented as percentage of absorbed glucose, calculated from the percentage change in the amount of glucose in solution before and after the perfusion.

Effects of *M. oleifera* on sucrose absorption from the gut

The effect of *M. oleifera* on sucrose absorption from gastrointestinal was assayed by determining the unabsorbed sucrose content following oral sucrose load by Six-Segment Study as described by Hannan *et al* **[22]**. 12 h fasted, type 2 diabetic rats were administered 50% sucrose solution per oral (2.5 g/kg body mass) along with three doses of *M. oleifera* 500 mg/kg dose and equal volume of water for control. Blood was sampled at the following time intervals, 30, 60, 120 and 240 min, after sucrose load for the quantification of blood glucose. At these time intervals, some of the rats were sacrificed for determining unabsorbed sucrose contents of the GI tract. The GI tract was excised and separated into six segments: the stomach, the upper 20 cm, middle and lower 20 cm of the small intestine, the caecum and the large intestine. Each segment was rinsed with acidified ice-cold saline followed by centrifugation at 3000 rpm (1000 g) for 10 min. The supernatant was pipette out and boiled for 2 h, in sulphuric acid, to hydrolyse the sucrose. The sulfuric acid was later neutralized by NaOH solution. Both plasma glucose concentration, and the amount of glucose released from residual sucrose in the GI tract was determined. The GI sucrose content was calculated from the amount of liberated glucose **[23]**.

Effects of *M. oleifera* on gut motility

GI motility was determined by means of $BaSO_4$ milk following the previously described method of Chattarjee **[24]**. $BaSO_4$ milk was prepared by mixing $BaSO_4$ as 10 % (w/v) in 0·5 % carboxy methyl cellulose to form a suspension. The ethanol extract was administered per oral, 1hr before the oral administration of $BaSO_4$ milk. Control group was administered distilled water only (10 ml/kg). Rats belonging to all groups were sacrificed 15 mins after $BaSO_4$ administration. The distance travelled by $BaSO_4$ milk was measured, and represented as a percentage of total length of the small intestine (from pylorus to ileocaecal junction).

Effect of *M. oleifera* on jejunal nutrient absorption by glucose dialysis-tube retardation assay

Dry, precut dialysis sacs (inflated diam. approx. 16 mm, length = 30 cm, Sigma Aldrich™, USA) were soaked in 1 g sodium azide/L. The bag was loaded with 6 mL sodium azide (1 gm/L) and 36 mg glucose alone (the control sac) or after addition of fine powder of *M. oleifera*. The dry fibrous powder was wetted by an aqueous solution of sodium azide (1 g /L) for 14 h prior to the experiment. The sacs were closed at the ends and hung in a solution of 100 mL of sodium azide (1 g /L) and then placed in a stirred bath at 37°C for 1 hr. At 30 and 60 mins time interval, 2 mL of the dialysate was analyzed for glucose by the GOD-PAP method as previously described.

The effect of fiber on nutrient absorption was indicated by the glucose dialysis retardation index

$$-\left(\frac{Total\ glucose\ diffused\ from\ sac\ containing\ fiber \times 100}{Total\ glucose\ diffused\ from\ sac\ containing\ no\ fiber\ present}\right)$$

Effect of *M. oleifera* on α-amylase activity

The effects of *M. oleifera* powder on starch digestibility was determined as a function of time in a fiber-enzyme-starch mixture system using a dialysis membrane with a cut-off molecular weight of 12,000da (inflated diam. approx. 16 mm, length = 30 cm, Sigma Aldrich™, USA) as previously described with minor modifications **[25]**. A solution was prepared by mixing 0.2 g of powdered *M. oleifera* and 0.04 g α-amylase (obtained from human saliva, Sigma Aldrich™ USA) in 10 ml of potato starch solution (4 g/100 ml) was dialyzed in 200 ml deionised water at 37°C. Following the incubation period, 10, 30, 60, and 120 min, glucose concentration in the dialysate solution was assayed using the GOD-PAP method as described previously. The control was run without the addition of powder.

Determination of glucose-adsorption capacity

The assay was conducted following the procedure by Ou *et al* **[26]**, where the glucose-adsorption ability (mM/mol/gm) was measured by mixing 1g of insoluble plant powder or Carboxymethyl cellulose (CMC) with 100mL of glucose solution at a constant temperature of 37°C for 6 hrs. This was then followed by centrifugation at 3500 rpm for 15 min. Glucose concentration in the supernatant was assayed using GOD-PAP method as previously described.

Statistical analysis

Statistical tests were conducted using GraphPad Prism 6. Results are presented as means ± SEM. Experiments with data being collected at several time intervals, were analyzed using repeated measures ANOVA followed by Bonferroni adjustment ensuring an error margin within ≤ 5%. One-way ANOVA was carried out and pair-wise comparisons were made with the control group using Dunnett's test to maintain an acceptable error margin of 5%. A two-tailed P value of <0.05 was considered statistically significant.

Results

Acute effects of *M. oleifera* on glucose homeostasis

Oral administration of *M. oleifera*, at any doses, did not alter the hyperglycaemic condition of fasted type 2 diabetic rats (Figure 1). The extract also did not show any effect on glucose tolerance and plasma insulin level after an acute glucose insult (Figure 2) & (Figure 3).

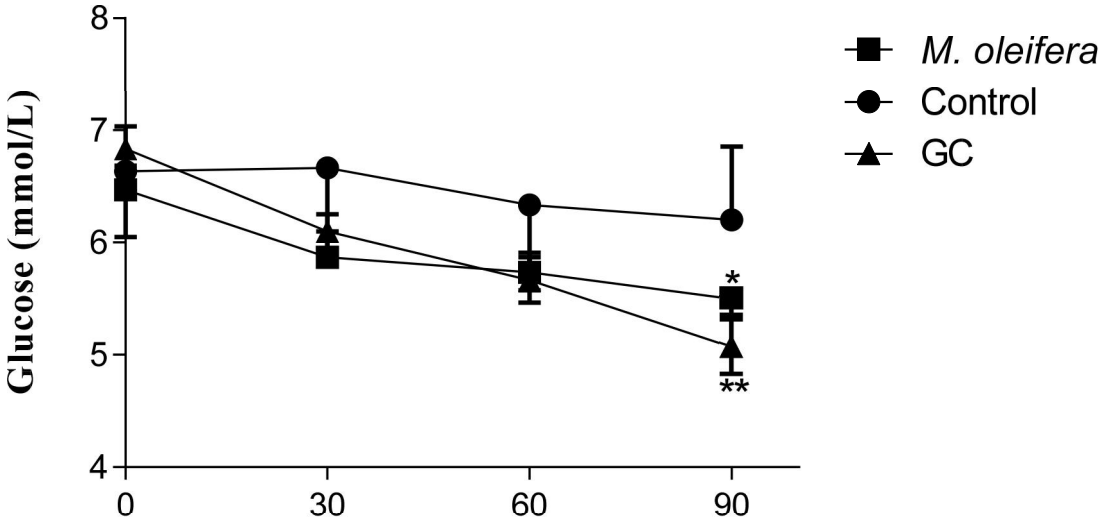

Figure 1: Effects of ethanol extract of *M. oleifera* on fasting blood glucose level in type 2 diabetic rats. Values are means and standard deviations represented by vertical bars (n=10). Fasted rats were given ethanol extract of *M. oleifera* (500 mg/kg) or Glibenclamide (GC) (0.5 mg/Kg) or only water (control) by oral administration. Mean values marked with an asterisk (*) were significantly different from those of respective control rats ($p<0.05$) (derived from repeated-measures ANOVA and adjusted using Bonferroni correction).

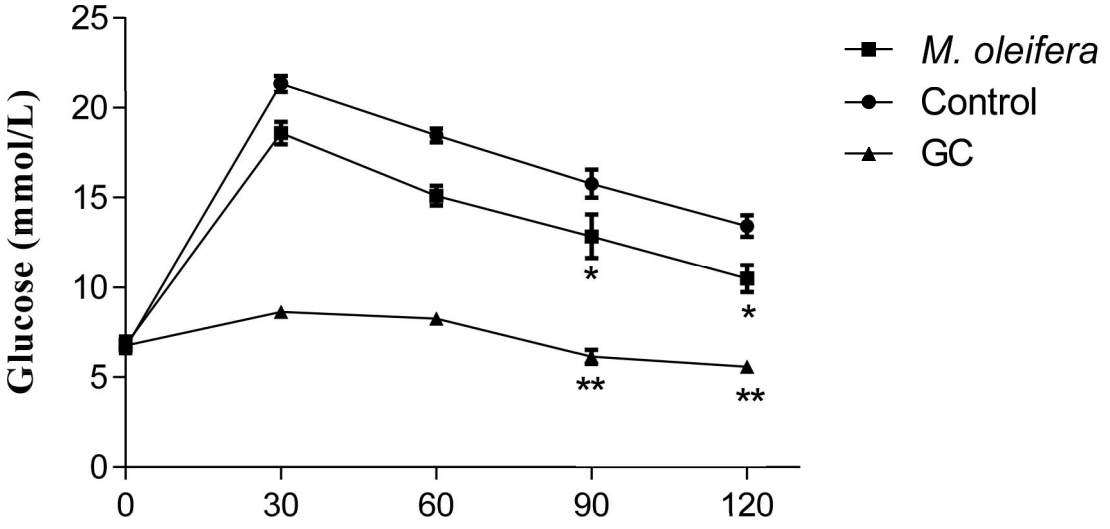

Figure 2: Effects of ethanol extract of *M. oleifera* (MO) on glucose tolerance in type 2 diabetic rats. Values are means and standard deviations represented by vertical bars (n=10). Fasted rats were given ethanol extract of *M. oleifera* (500 mg/kg body weight) or Glibenclamide (GC) (0.5 mg/Kg) or only water (control) by oral administration with glucose (2.5 g/kg body weight). Mean values marked with an asterisk (*) were significantly different from those of respective

control rats ($p<0.05$) (derived from repeated-measures ANOVA and adjusted using Bonferroni correction).

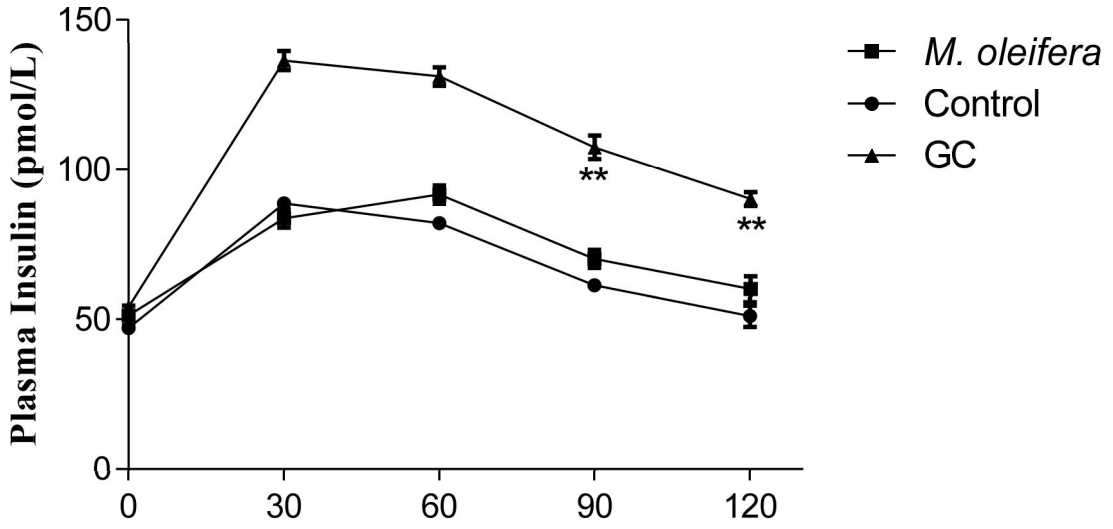

Figure 3: Effects of ethanol extract of *M. oleifera* (MO) on plasma insulin level in type 2 diabetic rats. Values are means and standard deviations represented by vertical bars (n=8). Rats were given ethanol extract of *M. oleifera* (500 mg/kg) or Glibenclamide (GC) (0.5 mg/Kg) or only water (control) by oral administration. Mean values marked with an asterisk (*) were significantly different from those of respective control rats ($p<0.05$) (derived from repeated-measures ANOVA and adjusted using Bonferroni correction).

Effect of *M. oleifera* on serum glucose after sucrose load

M. oleifera showed a significant ($p<0.05$) suppression of serum glucose level at 60 min compared to control, where peak serum glucose was observed after administration of sucrose load. 500 mg/Kg dose of *M. oleifera* maintained this trend of suppression of glucose level at 120 min too (Figure 4).

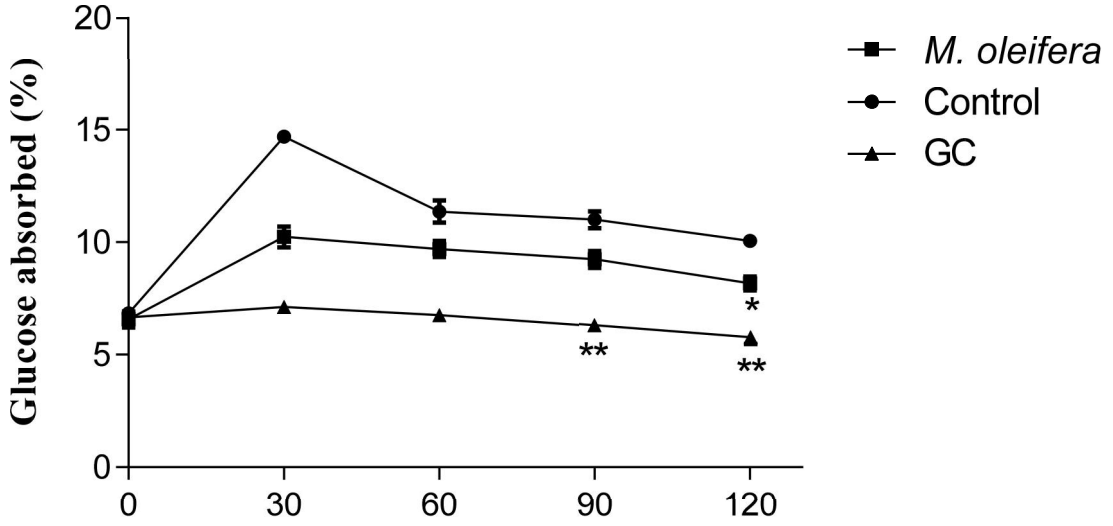

Figure 4: Effects of ethanol extract of *M. oleifera* (MA) on serum glucose after the sucrose load in type 2 diabetic rats. Rats were fasted for 20 h and administered orally with a sucrose solution (2.5 g/kg body weight) with or without ethanol extract of *M. oleifera* (500 mg/kg body weight) or Acarbose (ACB) (200 mg/Kg) or only water (control). Values are means and standard deviations represented by vertical bars (n=8). Mean values marked with an asterisk (*) were significantly different from those of respective control rats ($p<0.05$) (derived from repeated-measures ANOVA and adjusted using Bonferroni correction).

Effect of *M. oleifera* on intestinal glucose absorption

1000 mg/Kg doses of *M. oleifera* extract, when perfusated with glucose, showed significant ($p<0.05$) reduction in the percentage of glucose absorption during most of the perfusion period (Figure 5).

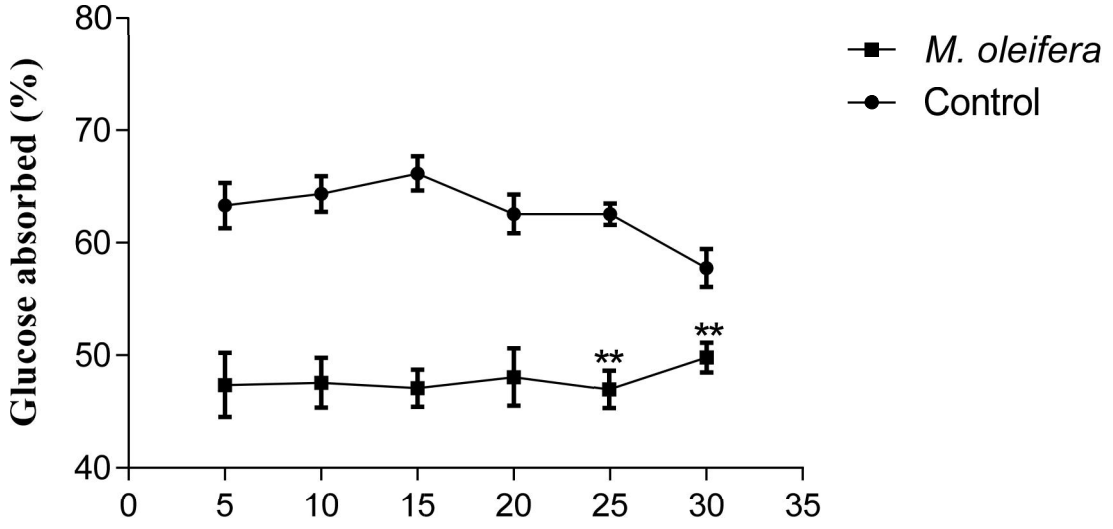

Figure 5: Effects of ethanol extract of *M. oleifera* (MA) on intestinal glucose absorption in type 2 diabetic rats. Rats were fasted for 36 h and the intestine was perfused with glucose (54 g/l) with (treated group) or without (control group) ethanol extract of *M. oleifera* (5mg/ml, 10mg/ml, and 20mg/ml; each subject received 15 ml of perfusion). Values are means and standard deviations represented by vertical bars (n=8). Mean values marked with an asterisk (*) were significantly different from those of respective control rats ($p<0.05$) (derived from repeated-measures ANOVA and adjusted using Bonferroni correction).

Effect of *M. oleifera* on unabsorbed sucrose content in the gastrointestinal tract

Upon oral administration of sucrose along with *M. oleifera* (500mg/Kg), significant amount of unabsorbed sucrose was remained in the stomach, upper, middle, and lower intestine at 30 min and 1h. This amount of residual sucrose remained significant in caecum and large intestine till 4h ($p<0.05$; Figure 6).

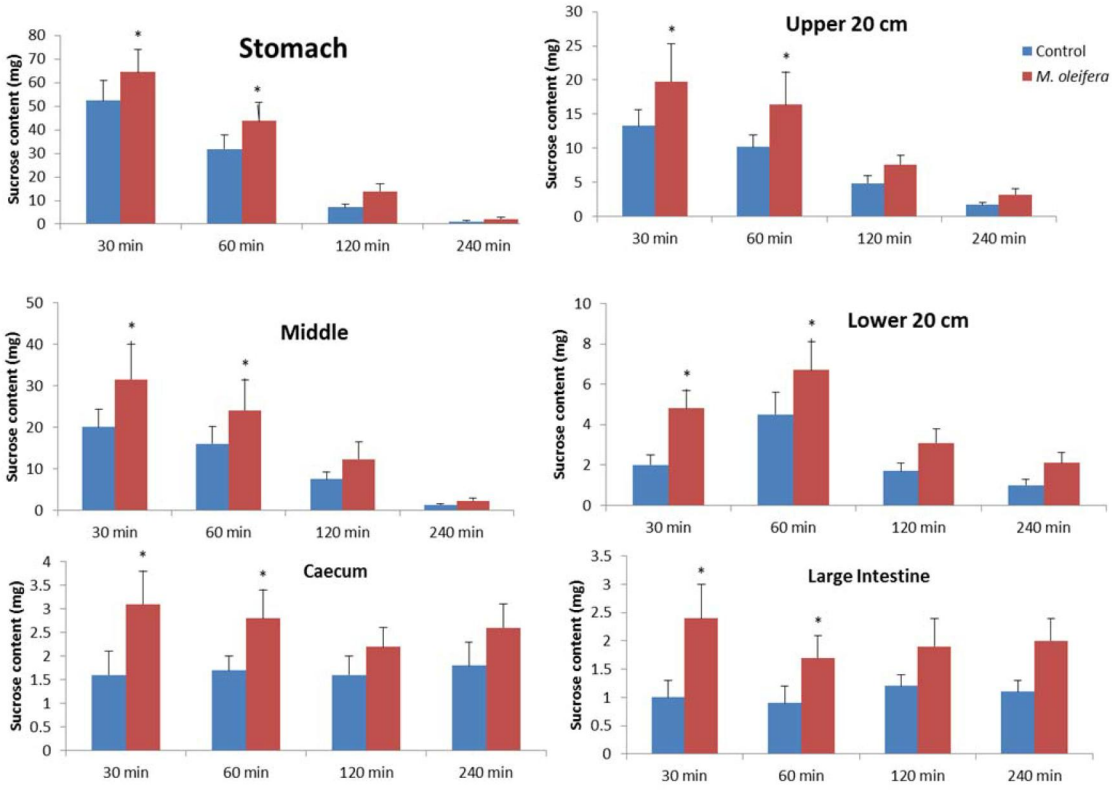

Figure 6: Effects of ethanol extract of *M. oleifera* (MA) on gastrointestinal sucrose content after oral sucrose loading in type 2 diabetic rats. Rats were fasted for 20 h before the oral administration of a sucrose solution (2.5 g/kg body weight) with (treated group) or without (control group) ethanol extract of *M. oleifera* (500mg/kg body weight). Values are means and standard deviations represented by vertical bars (n=8). Result derived from repeated-measures ANOVA and adjusted using Bonferroni correction.

Effect of *M. oleifera* on gut motility

M. oleifera extract increased the gastrointestinal motility significantly ($p<0.05$) at both 500 mg/Kg and 1000 mg/Kg doses in figure 7.

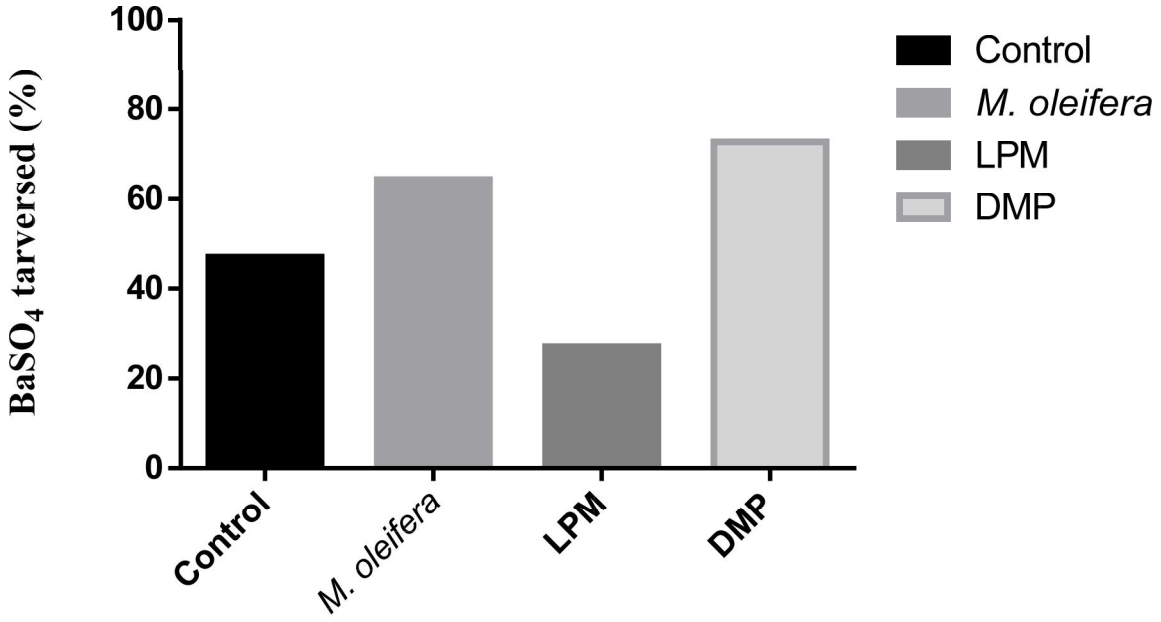

Figure 7: Effects of ethanol extract of *M. oleifera* on gastrointestinal motility (by $BaSO_4$ traversed).

Rats were fasted for 20 h before the oral administration of ethanol extract of *M. oleifera* or water (control). Enzyme activity was determined and $BaSO_4$ was administered at 60min. Motility was measured over the following 15 min. Acarbose (ACB) (200 mg/Kg) and Loperamide (LPM) 5 mg/Kg) & Domperidone (DMP) (10 mg/Kg) were used as reference controls for disaccharidase activity test and gastrointestinal motility test respectively (n=12). Mean values and significance ($p<0.05$) were derived from repeated-measures ANOVA and adjusted using Bonferroni correction.

Effect of *M. oleifera* powder on *in vitro* glucose dialysis retardation index (GDRI)

M. oleifera powder reduced the amount of glucose present in the dialysate. GDRI 36.97% and 46.20% at 30 min and 60 min respectively ($p<0.05$; Table 1)

Table 1: Retarding effect of insoluble fiber of *M. oleifera* on the glucose movement (glucose dialysis retardation index).

Treatment	Dialysis for 30 min		Dialysis for 60 min	
	Glucose in Dialysate(mmol/L)	Glucose dialysis retardation index (%)	Glucose in Dialysate(mmol/L)	Glucose dialysis retardation index (%)
M. oleifera	0.75±0.18*	36.97	0.92±0.12*	46.20

CMC 1000 mg	0.62±0.11*	42.86	0.83±0.07*	51.46
Control	1.19±0.21	0	1.71±0.14	0

Data are presented as Mean ± SD (n=4). Glucose dialysis retardation index = control (100%) – fiber (% of control value). Mean values marked with an asterisk (*) were significantly different from those of respective control groups ($p<0.05$) (derived from repeated-measures ANOVA and adjusted using Bonferroni correction).

Effect of *M. oleifera* powder on α-amylase activity

The effect of *M. oleifera* powder on starch digestibility was determined by the alteration in the glucose concentration in the dialysate with time. There was no significant change, compared to control, in the glucose content at every time points ($p<0.05$; Figure-8).

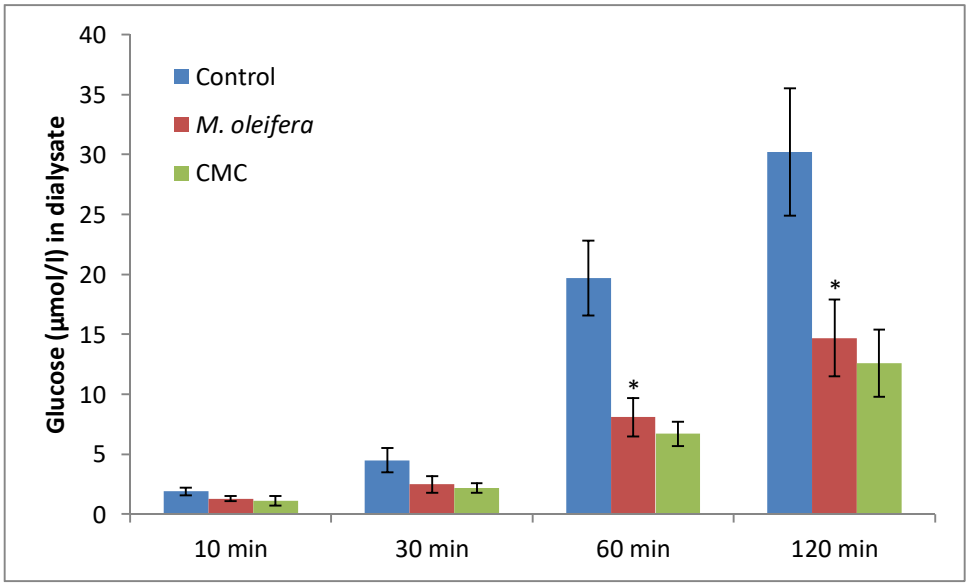

Figure 8: Effect of *M. oleifera* powder on α-amylase activity

Effect of *M. oleifera* powder on *in vitro* glucose adsorption capacity

M. oleifera powder showed some capacity of glucose adsorption in the presence of different levels of glucose in the solution. This activity of glucose adsorption was found to persist from higher level of glucose to even very low level of glucose present in the solution (Figure-9).

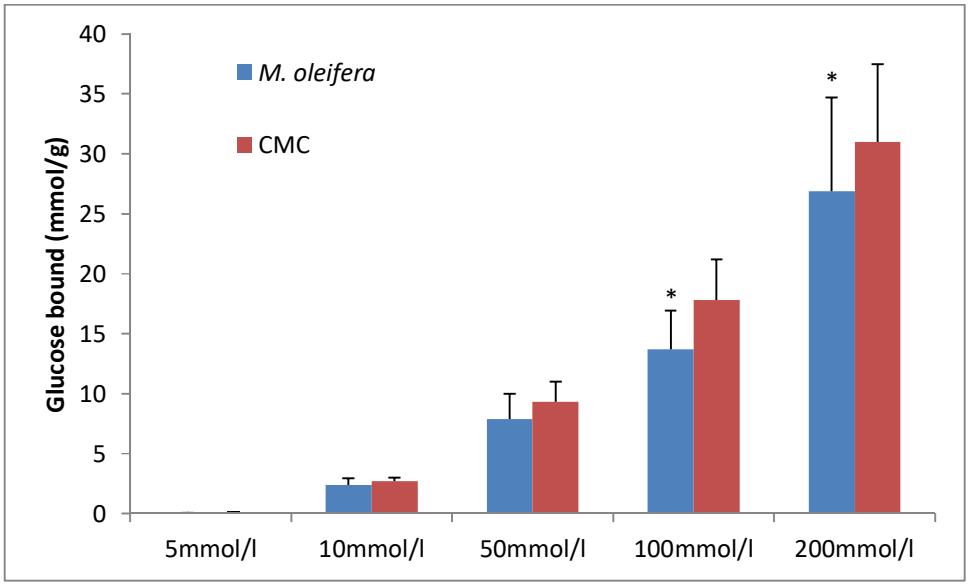

Figure 9: Effect of *M. oleifera* powder on *in vitro* glucose adsorption capacity

Discussion

Streptozotocin used in this study to induce type 2 diabetes in adult animals that causes DNA damage and generates superoxide radicals to destroy the beta-cells **[27]**. We have used glibenclamide in this experiment as a reference drug, which is a synthetic hypoglycemic agent and has been used as an antidiabetic drug in type 2 diabetic patients since 1973 and still being used **[28]**. This drug acts by stimulation of insulin release **[29]**. Only one oral dose of 500 mg/kg BW of *Moringa oleifera* ethanolic leaf extract was administered for evaluation of anti-hyperglycemic properties by increasing blood glucose tolerance in the normal rat, which was less potent than those of streptozotocin induced diabetic rat. *M. oleifera* leaves contain many powerful antioxidant phytochemicals, especially quercetin and kaempferol **[30]**. There are many reports about hypoglycemic activities of kaempferol derivatives from many medicinal plants such as *Sterculia rupestris* **[31]** and *Equisetum myriochaetum* **[32]**. Furthermore, they also improved chronic hyperglycemia impaired pancreatic beta cells viability and insulin secretion *in vitro* **[33]**. Quercetin, a strong antioxidant flavonoid revealed a protective effect against streptozotocin induced diabetes in rats by intraperitoneal injection of quercetin 15 mg/kg BW for 3 days prior to streptozotocin administration **[34]** and protected an Insulin Secreting cell line (INS-1) against oxidative damage **[35]**. It also exhibited hypoglycemic properties in diabetic rats **[37]**. Vessal *et al.* **[38]** reported that quercetin significantly increased hepatic glucokinase activities as an insulin-like effect.

Hyperglycemia causes cellular damage that hinders homeostasis of internal glucose concentration, which results in acutely altered cellular metabolism and long-term changes in cellular macromolecular content **[39-41]**. Postprandial glucose spike causes perturbation in endothelial cell function, **[42, 43]** and increases the risk of blood coagulation **[43]**. Hyperglycemic states also increases products of glycosylation, which has a significant influence in development of diabetes induced vascular disease **[44]**. Therefore, management of

hyperglycemic states is an important method of diabetes control. There are some basic pathways used anti-diabetic drugs includes, enhanced insulin secretion, enhanced sensitivity to insulin, improved peripheral glucose utilization, inhibition of glucose absorption, and inhibition of carbohydrate digestion **[45]**. *Moringa oleifera* leaves have shown promising glucose lowering effect in this study with chemically induced hyperglycemic rats. However, there are no reports have still been issued to reveal the tissue level mechanism of action of *M. oleifera*. In our current study, we have employed different techniques, through these which suggests one or more of the aforementioned modes of action.

Different studies have proved that blood glucose level in the upper normal range is a probable risk factor for cardiovascular disease, a common condition in case of chronic Type 2 diabetic patients **[46]**. In our study, fasting blood glucose remained unaffected in Type 2 diabetic rats in all groups apart from the "Glibenclamide" treated group. In glucose tolerance test, the peak glucose concentration after glucose induction in *Moringa oleifera* treated group at 500 mg/kg dose did not increased as happened in the control group.

To further ascertain, we measured the plasma insulin level of the test animals and found no significant increase in insulin secretion on *Moringa oleifera* administration. Therefore, increased insulin secretion from pancreatic beta-cell can be ruled out as possible mechanism of action.

An *in situ* intestinal perfusion of the GI tract shows marked reduction in glucose absorption. In $BaSO_4$ GI motility assay, intestinal motility was found to be significantly higher. Six Segment test showed significantly higher amount of unabsorbed sucrose in stomach, upper, middle and lower intestine in *Moringa oleifera* administered groups. The last three part of GI tract are most important for absorption of nutrients including sugars **[47]**. High unabsorbed sucrose content in the GI tract indicates that sucrose digestion is been reduced. Thus, a significantly higher concentration of sucrose have reached to the large intestine and caecum, which eventually remains unabsorbed and egested with faeces. An *in vitro* study was conducted to evaluate fiber binding capacity of the crude extract, no substantial effect was found that clearly demonstrates no dietary fibers are available in *Moringa oleifera*. Glucose are carried by specific transporter proteins **[48]**. Bound glucose is probably incapable of fitting the active site of these transporter proteins. This finding validates our initial findings in the gut perfusion experiments, which too showed no hindrance in glucose absorption. This is now fully understood that there is no glucose-fiber binding capacity of the crude extract.

Moringa oleifera in the α-amylase activity assay showed promising decrease in the catabolism of starch. As complex carbohydrates and disaccharides need to be broken down into simpler monosaccharides **[49]** prior to absorption, any inhibition of this catabolic process would retard sugar absorption, which in turn lowers glycemic peak. However, the precise mechanism of this inhibitory action remains to be studied.

Conclusions

This present study showed that ethanolic leaves extract of *Moringa oleifera* possessed hypoglycemic and anti-hyperglycemic properties in chemically induced type 2 diabetic rats, which suggest the presence of biologically active components that may be worth further

investigation and elucidation. Studies confirm the previous claims regarding anti-hyperglycemic action of *M. oleifera*. Additionally, we have elucidated that *M. oleifera* is capable of inhibiting absorption of glucose by inhibition of α-amylase. Therefore, its traditional use, as mentioned above is justified, and a primary mechanism of action is now known that was unrevealed until this study.

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Author contribution

PA and JMAH have designed the whole project. SBA, SMH and FFA was involved in the collection and identification of the plant material. SA and MH have collected and arranged all chemicals used in this project. PA, SA, SBA and FFA executed the designed project while SA assisted them in data collection, processing and interpretation. PA and SA wrote the manuscript and at the end all authors scrutinizes the paper and approved it.

Conflict of interests

All authors declares that there is no conflict of interest in this article.

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