ANTI- DIABETIC EFFECT OF MORINGA OLEIFERA LEAF EXTRACT ON RATS

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Abstract
Moringa oleifera is commonly known as drum stick tree (due to slender, long, triangular seed pods), horse radish tree (the roots taste similar to that of horse radish), benoil tree (as the benzoil is extracted from the tree). Moringa is a fast growing drought resistant tree. In the present review we find out the character of Moringa oleifera cultivation, collection, medicinal and common uses. Helpful utilization of leaves of M. oleifera has been assessed in diabetes due to its conceivable ability to diminish blood glucose and lipids oxidation after ingestion, as consequence of the polyphenols content and others compounds. By the by most outcomes have been acquire from leaf extricate, hence this study would utilize leaf powder as the normal method of utilization of populace to know impacts over harmfulness glucose, fatty substances, cholesterol, corporal weight, and prevalent gatherings of microbiota.

Keywords: Moringa oleifera powder, Glucose, Genotoxicity, diabetes, methanolic extract, hyperglycemia; nephrotoxicity; phytochemical; antioxidant; flavonoids; inflammatory properties.

INTRODUCTION
Diabetes is a chronic condition. It is essential to maintain healthy blood sugar levels when suffering from diabetes. A healthy diet and lifestyle combined with your medication can result in controlled blood sugar levels. If you are a diabetic, keeping a constant check on your blood sugar levels can help you prevent the complications linked with this chronic condition. You might have come across several foods and drinks that can naturally help you lower blood sugar levels. The GI score of foods and drinks also plays a significant role. One of the foods that are known to control blood sugar levels is moringa and its leaves. Moringa is commonly known as drumsticks to find out the benefits of moringa and its leaves for diabetics.
Figur : 1 . M.Oleifera Leaf Extract on Diabetes of rats

The leaf of this plant has been reported to possess antioxidant and medicinal properties that may be helpful in the treatment and management of diabetes and its associated complications. The leaves are consumed as food as they consist of nutrients, cancer prevention agents and macronutrients to further prevent dietary inadequacies [5]. Anyway the investigation of impact of organic mixtures of various piece of Moringa oleifera plant have brought different activity, as M. oleifera, should be tried first for wellbeing in suitable in vitro and in vivo models, prior to being utilized in human wellbeing [6, 7]. Disregarding nutraceutical valuable properties, the various mixtures of the plant present particular pharmacological impacts, including poisonousness profiles, which have not yet been totally explained. Additionally, global guidelines connecting with human wellbeing request that all sort of drug and nutraceutical items are tried for their security, and the best approach to guaranteeing this is to direct poisonousness tests in fitting in vitro and in vivo models.

Therefore, for toxicological evaluation it has been used animal models to reveal histopathological damage. There have been used in biological assay aqueous and ethanol extract of leaf in different doses, meanwhile leaf powder studies have been most done in clinical research. Thus it can be use in vivo models to bring more information about powder leaf consumption effect on different diseases. For instance, enlistment of exploratory diabetes in rodents is a helpful model to concentrate on movement of hypoglycemic specialists over hyperglycemia and it is ramifications where it has been noticed the conceivable cell reinforcement and antidiabetic impacts through plasma glucose, triacylglycerol and cholesterol checking, tiny sore perception, marker chemical, serum and lipid peroxidation estimating and for figuring out the way physiology. Likewise the exploratory creature model of diabetes mellitus should be possible by compound enlistment utilizing streptozotocin or alloxan which diabetogenic activity has been utilized and demonstrated in various creature species, with various defeat of organization or dietary stratus. People and mouse genotype is practically the
same between them, this impacts digestion, as it turns out to be exceptionally equivalent with people, and various kinds of mice contrast in their reaction to high fat eating regimen and aversion to metabolic illnesses. BALB/c mice are normally impervious to the improvement of high fat eating regimen instigated weight and advancement of diabetes thus.

The restorative utilization of M. oleifera leaves has been assessed in diabetes in light of their conceivable ability to diminish blood glucose fixations after ingestion since they contains polyphones, for example, quercetin-3-glycoside, rutin, kaempferol and glycosides. Decline in glucose as a result of M. oleifera treatment can be seen in various tests as: fasting blood glucose, oral glucose resistance test and post prandial glucose on diabetic rodents, in a normal lessening of 25% or more.

Moringa oleifera (MO) has rich antioxidant content and diverse therapeutic abilities. Previous investigation identified MO with the ability to prevent the occurrence and complications of diabetic-induced kidney injury through its protective effect on the oxidative status and inflammatory cytokines in the kidneys of diabetic rats. This plant has been reported to have some analgesic, anti-diabetic, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antibacterial properties and plays beneficial roles in modern medicine. Remarkably, studies with animal models on markers of oxidative stress and correlation with antioxidant properties in vivo and in vitro systems have been reported, ely. In vivo studies revealed antioxidant capacity of the aqueous extract of MO leaf to possess the potency of increasing the antioxidant status and reduce lipid peroxidation in a dose-dependent manner, while in vitro demonstrated high antioxidant capacity, thereby showing protective effects against ROS. Edoga and others reported anti-diabetic and hypotensive activities of MO in albino rats, as the plant played a role as a hypoglycemic agent in lowering blood glucose levels and preventing further cellular damage.

Previous studies on the extracts of MO using chromatographic and spectroscopic techniques revealed the presence of notable phenolic compounds such as kaempferol, quercetin, catechin, Gallic acid, caffeic acid, p-coumaric acid, vanillin, ferulic acid, protocatechuic acid, cinnamic acid and epicatechin. These secondary metabolites identified from MO extract have been linked to various biological profiles including antioxidant, anti-tuberculosis, analgesic, anticancer, anti-diabetic, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antibacterial and antimicrobial and antimalarial activities exhibited by this plant. Some histological assessment of the pancreatic segment of diabetic rodents uncovered degenerative changes in cells, which were fundamentally switched after treatment with concentrate of MO. Against hepatotoxic impact of Moringa oleifera and Vernonia amygdalina (VA) separates in streptozotocin (STZ) actuated diabetic rodents uncovered that solitary and joined concentrates of MO and VA have hepato-defensive impacts and might be successful in decreasing liver harm.

Antioxidant characterestic of MO has not been extensively estimated, and specific phytochemicals in MO methanolic leaf extract have not been comprehensively evaluated. These were explored in our study with the overarching aim of clarifying the potential of Moringa oleifera as a food supplement and bringing to the fore its
stunning capabilities that could feasibly revolutionalsepharmacological products in the treatment and management of diabetes. Furthermore, due to its numerous scientific and health claims, it is appropriate to further investigate the potential treatment regime of Moringa oleifera on diabetes in STZ-induced diabetic animal models that can be used to advance clinical techniques on its ameliorative activities. It is anticipated that the outcome will support the ethnomedicinal information of this plant, especially

**METHODS**

**Plant material**

*Moringa oleifera* leaves were collected from a private herbarium. The leaves were washed and oven dried at 40 °C for 24 h, until the moisture content reached 10%. The dry leaves were pounded, and the powder was kept at room temperature until use.

**Extract Preparation**

Green leaves of MO were washed, air dried and mixed to fine frame. Separate was ready from 1 kg of MO powder through consistent blending in n-hexane for 24 h. The buildup was re-separated in 80% (v/v) methanol. At room temperature for 24 h. The methanolic separate was dissipated to dryness in vacuum utilizing turning evaporator and put away at −4 °C for use and physiochemical screening.

**Ethical Statement**

All tests were acted in consistence with the rule for the government assistance of trial creatures by the Public Foundations of Wellbeing and as per the rules of Institutional Creature Care. This study was approved by the Institutional Animal Ethics Committee at the Faculty of Health Science.

**Study Design**

**Experimental Animals**

Experimental rats used for this study were adult male rats weighing about 200 g and 250 g, and aged 10 weeks. Forty-eight rats were randomly divided into 4 groups. Rats were housed in a well-ventilated animal facility in stainless steel cages (beddings composed of ground sterilized maize cobs) with 5 rats per cage to allow free mobility. Conducive temperature of 22 ± 2 °C, humidity 55% ± 5% and a normal period (12 h light/12 h dark) was maintained.

**Induction of Diabetes:** A diabetic state was induced in the rats by injecting intraperitoneally (i.p.) freshly prepared streptozotocin in citrate buffer (0.1 M pH 4.5) to overnight fasted rats at a dose of 55 mg/kg. Blood was obtained from the rat’s tail to confirm a diabetic state using a glucometer. A stable glucose level of (>18 mmol/L) confirmed hyperglycemia, and only diabetic rats were included in the study.
Diabetic Model and Treatment

Sample size assurance was performed to gauge the base number of rodents expected to fundamentally separate two gatherings (control and diabetic) at a p-value of 0.05 and certainty time frame in light of distinction in implies (glucose levels) utilizing LaMorte's Power Mini-computer. A Microsoft Excel bookkeeping sheet downloaded from the site was utilized. A sum of four rodents for every gathering was determined, yet we chose to build this worth up to five.

A dose of *Moringa oleifera* (250 mg/kg) is the most suitable based on preliminary investigations in our research centre. Forty-eight rats were randomly divided into four groups of twelve rats each; NC—Normal non-treated control, NC + MO—*Moringa oleifera* treated control rats, DM—diabetic rats and DM + MO—*Moringa oleifera* treated diabetic rats. NC and DM (control groups) received distilled water while MO and DM + MO (experimental groups) received *Moringa oleifera* extract at a dose of (250 mg/kg/b.wt.). Distilled water was used as the diluent for reconstructing the extract and administered via oral gavage for 6 weeks.

At the end of the treatment, rats were fasted overnight and anaesthetized intraperitoneally with sodium pentobarbital injection (60 mg/kg). Sodium pentobarbital was used to ensure unconsciousness of rats while death occurred as well as guaranteeing rapid and painless death. This procedure was carried out in the animal house. Blood samples were obtained via the rat’s abdominal aorta into a lithium heparin plasma separator tubes and serum clot activator tubes. The whole kidneys were quickly excised from each rat, washed in ice-cold phosphate-buffered saline, blotted, weighed and frozen in liquid nitrogen.

**Blood and Homogenate Preparation**

Blood samples were centrifuged at 4000 g for 10 min at 4 °C and afterward put away at −80 °C to get plasma and serum. Kidneys (200 mg) were homogenized on ice in 2000 µL super cold phosphate support saline (PBS, 50 mM pH 7.5). Homogenates were centrifuged at 15,000 rpm for 10 min at 4 °C. The supernatants were aliquoted and put away at −80 °C for assessment of biochemical boundaries.

**Statistical analysis**

Differences between treatments were identified with an analysis of variance (ANOVA; SPSS-PC, version 20.0). In cases where the standard deviation differed between groups, Mann-Whitney U and Kruskal Wallis multiple comparison nonparametric tests were applied with a 95% (p < 0.05) confidence level.
RESULTS & DISCUSSION

The fast revelation of different restorative plants and regular items with hostile to diabetic possibilities has given an exceptional mediation throughout the entire existence of numerous illnesses including diabetes [39]. The reason for the utilization of various plants as original solutions for diabetic complexities can’t be overemphasized [40,41]. Hyperglycemia-actuated oxidative pressure has been demonstrated to be effectively engaged with the beginning and movement of diabetes, prompting different inconveniences, for example, cardiovascular sicknesses, nephropathy, removal of appendages and visual impairment [42-44]. The component of STZ (C8H15N3O7) as a poison used to prompt hyperglycemia in exploratory creatures includes its harmful impact on the beta cells of the pancreatic islet [45]. Subsequently, ROS are framed during this cycle and an outpouring of responses happen prompting expanded degrees of superoxide extremists, hydrogen peroxide, and hydroxyl revolutionaries with likely harming impacts on cell macromolecules in the creatures.

Diabetes was induced intraperitoneally in rats by a single dose of streptozotocin (55 mg/kg) and treated with methanolic extract of *Moringa oleifera* (250 mg/kg b.wt) for six weeks. Forty-eight (48) adult male strain rats were randomly divided into four groups: normal control (NC), *Moringa oleifera* treated control rats (NC + MO), diabetic rats (DM) and *Moringa oleifera* treated diabetic rats (DM + MO). Estimation of antioxidant capacity, total polyphenols, flavonoids and flavonols content of *Moringa oleifera* extract was performed and serum biochemical markers were evaluated. Antioxidants such as catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD) activities, glutathione (GSH) and inflammatory biomarkers were determined in the kidney. Results showed high antioxidant capacities of MO extract and improved serum biochemical markers, whilst lipid peroxidation (MDA) levels were reduced in non-diabetic and diabetic rats after MO treatment when compared to normal control. Subsequent administration of MO led to an increased concentration of serum albumin, globulin and total protein with a decrease in the level of MDA, and improvements in CAT, SOD, GSH, GPx, (tumour necrosis factor-alpha)TNF-α and (interleukin-6)IL-6. MO contains potent phytochemical constituents that offer protective action against diabetic-induced renal damage, reactive oxygen species (ROS) and inflammation and could therefore play a role in reducing diabetic complications, particularly in developing countries such as in Africa where the majority cannot afford orthodox medicine.

**Effect of Moringa oleifera on Kidney Weight, Relative Kidney Weight and Plasma Glucose Levels of Rats**

The effect of MO on kidney weight, relative kidney weight and blood glucose levels of rodents is shown in Table 1. The distinction in kidney weight between non-diabetic treated rodents (NC + MO) and normal control isn’t critical. Nonetheless, *Moringa oleifera*-treated control rodents (NC + MO) showed a critical (p < 0.05) decline when contrasted with the diabetic control (DM). Kidney loads of diabetic control rodents expanded altogether (p < 0.05) when contrasted with ordinary control (NC). After treatment of diabetic rodents (DM + MO) with MO, a critical (p < 0.05) decline was seen when looked at to diabetic control (DM). Comparative
outcomes were seen in the general kidney weight. Raised blood glucose level was seen in the diabetic gathering when contrasted with ordinary control. Plasma glucose level diminished fundamentally ($p < 0.05$) in diabetic rodents after treatment when contrasted with diabetic controls (DM).

Table 1. Effect of Moringa oleifera on kidney weight, relative kidney weight and plasma glucose levels.

<table>
<thead>
<tr>
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<th>NC</th>
<th>NC+ MO</th>
<th>DM</th>
<th>DM+MO</th>
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<tr>
<td>Kidney weight (g)</td>
<td>1.90 ± 0.17</td>
<td>1.80 ± 0.17 b</td>
<td>2.38 ± 0.18 a</td>
<td>2.13 ± 0.20 a,b</td>
</tr>
<tr>
<td>Relative kidney weight (g/100 g)</td>
<td>0.60 ± 0.04</td>
<td>0.57 ± 0.03 b</td>
<td>1.10 ± 0.08 a</td>
<td>1.03 ± 0.08 a,b</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>8.42 ± 0.82</td>
<td>5.01 ± 0.53 a,b</td>
<td>28.08 ± 1.12 a</td>
<td>26.22 ± 0.61 a,b</td>
</tr>
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</table>

NC (Normal control), NC + MO (Moringa oleifera-treated control rats), DM (Diabetic rats), DM + MO (Moringa oleifera-treated diabetic rats). Values are presented as mean (SD). $a p < 0.05$ values are significant compared with non-diabetic control. $b p < 0.05$ values are significant compared with diabetic control.

Diabetic rats injected with STZ showed elevated plasma glucose levels, which is indicative of hyperglycemia, an observation also reported by other authors [47,48]. However, treatment of rats with MO showed a significant decreased glucose level when compared to diabetic control (Table 1). This implies that Moringa oleifera is able to increase the ability of insulin to lower plasma glucose, suggesting its anti-diabetic activity. These results are consistent with other studies. [49,50]

Increased kidney size is a sign of acute inflammation and was observed in diabetic rats when compared to normal controls (Table 1). This study agrees with the findings of previous authors who reported that kidney enlargement may be due to hyperplasia (rapid production of the cell leading to enlarged tissues) and hypertrophy (enlargement of cell components) of the kidney. Treatment with MO reduced kidney size gained, showing a hypolipidemic effect of MO in the kidneys of diabetic rats. Promotion of excessive oxidative stress in the vascular and cellular milieu results in endothelial cell dysfunction, which is one of the earliest and most pivotal metabolic consequences of chronic hyperglycemia.

Estimation of Antioxidant Capacity, Total Polyphenols, Flavonoids and Flavonols Content of Moringa oleifera Extracts

Although other investigations have been led on MO, the cell reinforcement action of the methanolic leaf extract has been accounted for just somewhat. Our review developed this in a comprehensive antioxidant study (Table 2). The evaluation of all out cell reinforcement limits of methanolic concentrates of Moringa oleifera was directed utilizing three reciprocal tests: oxygen extremist absorbance limit (ORAC), Ferric reducing antioxidant power (FRAP), and Trolox equivalence antioxidant capacity (TEAC). The results were estimated as Trolox equivalent per gram as ORAC (3652.14 ± 113.32) _mol TE/L, and TEAC (96.09 ± 1.58) _mol TE/L ascorbic acid equivalent per gram as FRAP (1736 ± 3.08) AAE/L. Results showed high antioxidant capacity of MO to
In addition, high concentration of total polyphenols, flavonoids and flavonols content were estimated in the methanolic extracts of Moringa oleifera. Total polyphenols content of methanolic extract was (2454.00 _ 17.54) mg GAE/L, flavonoids (297.23 _ 30.00) mg CE/L and flavonols (148.70 _ 4.00) mg QE/L.

Table 2. Estimation of antioxidant capacity, total polyphenols, flavonoids and flavonols content of Moringa oleifera extracts.

<table>
<thead>
<tr>
<th>MO Methanolic Extract</th>
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<tbody>
<tr>
<td>ORAC (_mol TE/L)</td>
<td>3652.14 _ 113.32</td>
</tr>
<tr>
<td>FRAP (_mol AAE/L)</td>
<td>1736 _ 3.08</td>
</tr>
<tr>
<td>TEAC (_mol TE/L)</td>
<td>96.09 _ 1.58</td>
</tr>
<tr>
<td>Total polyphenol (mg GAE/L)</td>
<td>2454.00 _ 17.54</td>
</tr>
<tr>
<td>Flavonol (mg QE/L)</td>
<td>297.23 _ 30.00</td>
</tr>
<tr>
<td>Flavonoid (mg CE/L)</td>
<td>148.70 _ 4.00</td>
</tr>
</tbody>
</table>

Values are presented as (mean _ SD). TE (Trolox equivalent); AAE (ascorbic acid equivalent); CE (catechin equivalent); GAE (Gallic acid equivalent; QE (quercetin equivalent).

Clinical parameters

Body weight (g) and glucose were measured weekly using a triple-arm scale with a basket to hold the rats, respectively. At the end of the experimental period, a 12-h fast was imposed on the rats, after which they were sacrificed. Triglycerides (11503), cholesterol (11505), LDL (11585) and HDL (11557) were measured with commercial kits.

The underlying body weight for all gatherings was ±200 g. After alloxan enlistment, diabetic gatherings diminished in weight. During the two months, the body weight of the benchmark group was lower than that of the solid gathering treated with M. oleifera (246 g versus 263 g, separately), yet there was no massive distinction. The diabetic gathering treated with M. oleifera showed an expanded body weight in examination with both the diabetic gathering treated with glybenclamide and the untreated diabetic gathering (229 g, 190 g, and 173 g, separately) (Fig. 2). The diabetic gathering treated with M. oleifera was not the same as the untreated diabetic gathering (p < 0.05).
Weight (g/kg) of study groups during 8 weeks. Data are expressed as mean ± standard deviation per group

Glucose levels of 100 mg/dL were estimated in the exploratory rodents toward the start of the review (p > 0.05). After hyperglycemia enlistment, the diabetic gatherings showed glucose upsides of ±300 mg/dL (p > 0.05). In the subsequent week, glucose levels in the diabetic gathering treated with M. oleifera decreased in contrast with the untreated diabetic gathering. Then again, the benchmark group and the solid gathering treated with M. oleifera didn't show contrasts throughout this time span (p > 0.05). Triacylglycerol values were not different between diabetic gatherings (Fig. 3). The solid gathering treated with M. oleifera showed lower values (24 mg/dL) in contrast with the benchmark group (53 mg/dL).

Fig. 3 From: Effect of *Moringa oleifera* consumption on diabetic rats

Glucose values (mg/dL) in study groups during experiment. Data are expressed as mean ± error standard per group
DISCUSSION

Moringa oleifera has bioactive mixtures, the characteristics of which have been concentrated on lately to lay out a more logical reason for its utilization and to explain its organic movement. The leaves have been utilized as antidiabetic, antibacterial, and calming home grown drugs. There are concentrates on that show no gamble in utilizing M. oleifera leaves at different dosages; nonetheless, the greater part of these information have been gotten from concentrates on plant separate. Hence, the investigation of leaf powder utilization achieves new information the security of this plant while giving choices to establish protection without the deficiency of supplements, particularly since the leaf of this plant can be utilized as a vegetable in soup arrangements, cooked and blended in with ground nut cake or bundled in powder pills.

Furthermore, M. oleifera is a source of antioxidants, vitamins, and a protease-resistant glycoprotein that functions as dietary fiber. Moringa oleifera has antioxidant activity because it contains phenolic compounds and flavonoids, specifically three classes of phytochemicals: glucomoringin, flavonoids (quercetin and kaempferol) and phenolic acids (chlorogenic acid). These bioactive compounds can exert antioxidant and anti-inflammatory effects that could induce cellular protection, as can be observed in M. oleifera regulation of the formation of micronuclei in response to damage to the genetic material in cells. Micronuclei are chromosomal fragments or entire chromosomes that were not included into the daughter cell nuclei at mitosis. The erythrocyte micronucleus assay is a simple and minimally invasive method that detects in vivo structural or numerical chromosome damage. The results of this study show that M. oleifera regulates the formation of micronuclei, maintaining the basal values through an antioxidant effect that neutralizes free radicals that would otherwise affect DNA. Moreover, M. oleifera did not increase MNE frequency and maintains the ratio of PCEs and MNPCEs. In fact, these values were the same as the values in the control group. The doses tested in this study did not reach the LD50, and there was no observed histopathological damage in different organs. Previous studies have shown no toxicity or adverse effects in body organs for aqueous leaf extract in rats.

CONCLUSION

This study has demonstrated that M. oleifera leaf extract reduces the levels of blood glucose, AUCof glucose, insulin and inflammatory cytokines (IL6, IL-1β & TNFα) in type 2 diabetic rats. Moreover, The findings suggest that consumption of M. oleifera powder leaves could be beneficial in the diabetes mellitus rat model over glucose values and enterobacterias enumeration. However further research will be needed to evaluate the mechanisms of action over lipids and intestinal microbiota in diabetes mellitus to increase possible uses of M. oleifera in functional foods as a nutraceutical. Therefore, the study about leaf powder consumption can bring new knowledge about it safety and as an option of plant preservation without loss the nutrient, cooked and mixed with grounded groundnut cake or in powder pills. the experimental evidence from our study, results suggest that Moringa oleifera has an excellent ability to protect against oxidative damage due to its high polyphenols, flavonoids and flavonols content. Its use as a food supplement can be justified due to its
therapeutic benefits. Hyperglycemia was successfully induced in the animal model with STZ, which was confirmed in the blood and kidney biomarkers. Oxidative stress was observed in diabetic groups while treatment with a methanolic extract of MO ameliorated the effect. From our study, MO was also able to enhance antioxidant status and reduce lipid peroxidation, showing that MO has the potential to be used as an antidiabetic agent in the treatment and management of diabetes.

References


