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Anti-diabetic activity of the leaves of *Moringa oleifera* Lam. growing in Sudan on streptozotocin-induced diabetic rats

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ABSTRACT

Diabetes disease is a serious and costly health problem, particularly in developing countries where the medication is unavailable for all. Leaves of *Moringa oleifera* are used in Sudanese traditional medicine as anti- diabetic. This study aimed to evaluate this antidiabetic property on rats. aqueous leaf extract of *Moringa oleifera was* administered to STZ-induced rats representing type-2 and type-1 diabetes. Then, body weighting, fasting blood glucose (FBG), intravenous Glucose tolerance test (GTT) was evaluated in treated and untreated rats. Besides, the phytochemical screening of the plant. *Moringa oleifera* leaves showed anti-diabetic properties, being rich in phytochemicals of medical properties, supporting the traditional use. This edible plant is recommended as a food supplement for diabetic patients.

Keywords: Anti-diabetic, Diabetes Millets, *Moringa oleifera*, STZ-induced rats,

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INTRODUCTION

Diabetes disease is known since antiquity, it is mentioned in the writings of the ancient civilizations, particularly Egypt, Arabia, India, China and Asia Minor [1]. Recently, Diabetes Millets is highly prevalent worldwide when compared with many other diseases, it is fast becoming the epidemic of the 21st century. It is estimated that in the year 2000, there were 171 million diabetic peoples in the world. This estimation is expected to increase to reach 366 million diabetic peoples by 2030[2]. Diabetes mellitus is defined as a disease in which the body is unable to use and store glucose, properly. It is caused by the abnormality of carbohydrate metabolism which is linked to either low blood insulin level or insensitivity of target organs to insulin [3]. There are many types of diabetes, however, the two main types of diabetes are Type 1 (insulin-dependent), where the body is completely stop producing insulin, accordingly patients must take insulin injections daily to survive. Type 2 (non-insulin dependent) where the body is unable to produce enough insulin or the produced insulin does not work properly. Patients of type 2 mostly have a family history of diabetes (inherited), overweight or over 40 years of age. Interestingly, type 2 (non-insulin dependent) diabetes mellitus is much more prevalent than type 1 (insulin dependent) diabetes, affecting the people of both developed and developing countries [4,5]. Up to 250,000 children in developing countries under the age of 14 years have type 1 diabetes; around 38,000 of these children are in Africa [6].

The Moringa tree (*Morenga oleifera* lam.) belonging to family Moringaceae. It is a fast-growing tropical tree. It is called a miracle tree due to its numerous therapeutic benefits, this plant is also prescribed in ancient civilizations, it is well known, cultivated as a crop and consumed as vegetables in many African, Asian, Latin America and Caribbean countries besides its applications in traditional medicine; It is now cultivated as a crop in so many countries in Africa and Asia [7]. Recently, this plant which is naturally grown in Sudan, had attracted attention in the last years as a medicinal plant, it is now cultivated in many parts in Sudan and prescribed to diabetic persons by traditional healers. In Sudan, diabetes is now one of the major health problems, resulting in 10% of all hospital admissions and mortality[8]. Besides, diabetes remedies are extremely costly when compared to the low income of Sudanese people and there is a lack of governmental support or control on the pharmaceutical markets[9]. Accordingly, the diabetes drugs are now much more expensive than in many developed countries. Accordingly, such study could be of great value, in searching for an alternative, non-expensive and available anti-diabetic medicinal plants. This study is aimed to investigate the anti-diabetic activity of *Morenga oleifera* cultivated in Sudan on streptozotocin-induced diabetic rats.

MATERIALS AND METHOD

Plant collection, identification and extraction

Fresh leaves of *Morenga olifera* (5 kg) were collected manually from Khartoum state, from Moringa Project, Khrtoum North, Samrab area. Plant was identified and authenticated by the herbalists at Medicinal and Aromatic Plants Research Institute, The National Center for research, Kartoum, Sudan. Specimen was deposited at the herbarium. Moringa leaves were extracted twice with distilled water at temperature 60-70 ^{o}C repeatedly, for 48 h, using hot-plate with stirrer. The resulting extract was filtered using Whatman no.1 filter paper. The filtrate was freeze-dried using the freeze drier machine yielded a dry powder of Moringa leaves which kept directly into a dark clean bottle. On experiment the powder of leaves extract was reconstituted to be at a concentration 200mg/ml.

Experimental animals

About 60 male albino Wistar rats of same age and body weight 150-200 g was used in this study. Rats were housed in well-prepared cages at an ambient temperature of 25-30 ^{o}C and 45-55% relative humidity with a 12 h each of dark and light cycle. Animals were fed special standard pellet diet (Laboratory animal feed pellets, Saudi Arabia) and water *ad libitum*. Experiments on these animals were performed followed the criteria of the international ethical committee of animal care.

Phytochemical investigation

The aqueous leafe extract of *Moringa oleifera* was investigated for presence of some secondary metabolites of medical properties which known as phytochemical compounds (Vinoth et al., 2012) [10]

Induction of Diabetes Mellitus

Rats were fasted for 24 h before the induction of diabetes by streptozotocin (STZ) intra-peritoneal injection. Rats were initially divided into 3 groups. For the first group, 20 normal rats were separated to be induced milled diabetes (MD) or type- 2 diabetes. For the second group, 20 normal rats were separated to be induced severe (SD) diabetes of type 1 diabetes. For the third group, 20 normal rats were separated to serve as negative control which was injected by a single dose of 0.5 ml normal saline. Rats from the second and third groups were injected by single intramuscular injection of STZ at the dose of 4mg or 7 mg/0.5 ml of physiological saline/100 g body weight/rat, respectively. All rats were fasted to the next day (given water only but no food). Fasted rats were subjected to Fasting blood glucose testing (FBG) using a single touch glucometer (FreeStyle, UK). Only rats having fasting blood glucose level more than 250 mg/dl were separated and considered as SD or type-1 diabetes and rats having fasting blood glucose level more than 90 mg/dl but less than 250 mg/dl were

separated and considered as MD or type-2 diabetes. Separated groups were used throughout the experimental schedule [11].

Experimental design

Thirty rats were selected and divided into five equal groups of six as follows:

i) Control group (C):

Rats of this group received single intra-peritoneal injection of physiological saline (0.5 ml/100 g body weight/rat) and FBG ranged from served as negative control.

ii) Mild diabetic group (MD):

The rats were made diabetic by a single intramuscular injection of STZ (4 mg/0.5 ml physiological saline/100 g body weight/rat), with FBG ranging between 90-250 mg/dl, served as type -2 diabetic.

iii) Severe diabetic group (MD):

The rats were made diabetic by a single intramuscular injection of STZ (7 mg/0.5 ml physiological saline/100 g body weight/rat), with FBG more than 250 mg/dl, served as Type-1 diabetic.

iv) Mild diabetic and M. oliefera supplemented group (MD+M):

The MD rats were forcefully fed aqueous leaves extract of M. oliefera at the dose of 80 mg/0.5 ml distilled water/100 g body weight/d/rat after 24 h of for next 14 days.

v) Severe diabetic and M. oliefera supplemented group (SD+M):

The SD rats were forcefully fed aqueous leaves extract of M. oliefera at the dose of 120 mg/0.5 ml distilled water/100 g body weight/d/rat after 24 h of for 14 days.

Animals of C, MD and SD groups were subjected to forceful feeding of 0.5 ml distilled water/100 g body weight/d for 14 d to keep all the animals at same type of treatment condition in respect to aqueous leaves extract of M. oliefera supplemented groups which were administered 0.5 ml distilled water/100 g body weight/day (at a concentration of 200mg/ml). Adequate food pellets were supplemented to all groups. Oral administration in rats was done using special long-mouth syringe.

On the starting day of extract supplementation to MD and SD rats, fasting blood glucose (FBG) was monitored of all the animals in each group. On the 15th day of experiment, 4 h before animal sacrifice, intravenous Glucose tolerance test (GTT) was performed [11.]

RESULTS AND DISCUSSION

Phytochemical properties

The preliminary phytochemical screening of the aqueous extracts of *Moringa olifera* was carried out in order to investigate the bioactive principles which may be the main factor behind the anti-diabetic activity. As represented in Table (1), the phytochemical analysis of

the aqueous extract of *Moringa olifera* revealed presence of some bioactive ingredients such as saponins, tannins, flavonoids and terpenoids. This is in harmony with the findings of Abalaka et al.[12] who mentioned that the leaves of *Moringa oleifera* have many phytochemical secondary metabolites of great pharmacological properties, such as alkaloids, flavonoids. Arun and Rao [13] also published that leaves of *Moringa olifera* revealed presence of Alkaloids, Flavonoids, Carbohydrates, Tannin and Phenolic Compounds. As well, Vinoth et al.[14] observed that alkaloids, tannins, flavonoids, terpenoids, saponins Glycoside and compounds reducing were present in the different extracts which were water, ethanol, and chloroform. Meaning that, leaves of *Moringa oleifera* is a rich source of phytochemical principals of medical properties. Although, there is a need for further research on these phytochemical compounds to find which compound(s) have the expected anti-diabetic effect.

Table 1: Phytochemical analysis for the aqueous leaves extract of *Moringa olifera*

Solvent used in extraction	Saponins	Tannins	Allkaloids	Flavonoids	Volatile oils	Terpenoids
Water	+	+	–	+	–	+

+ = present, – = absent.

Effects on Body weight

The findings of this study revealed that there are significant differences between the mean body weight of the control group and the other treated groups (Type-2 and type-1 diabetic groups) as shown in Table (2). Type-1 diabetic rat group showed loss of weight more than type-2 diabetic group, however, there was no significant difference between them at $p<0.05$. In general, the streptozotocin is a toxic substance (Wang et al., 2011) (15). Loss of weight is a preliminary and sensitive index of toxicity (Raza et al., 2002) (16). Also, the loss of body weight associated with STZ-induced diabetes could be due to dehydration and catabolism of fats or breakdown of tissue proteins, with consequent wasting of muscle (Kimani et al., 2015) (17). Any decrease in the body weight could be due either the general discomfort which led to a low drinking and feeding rate in the treated animals or to the effect of the suspension on the internal organs (Oyewole et al., 2007, Brodie et al., 1970) (18,19). Also, the loss of weight could be related to the plant itself, where the administration of this plant may lead to loss of weight. Idohou-Dossou (2011) (20) reported that some some loss of weight observed when group of women administered *Moringa oliefera* for 3 months, but it was not statistically significant when compared to the control group.

Table 2: The effect of treatment on body weight of different rat groups

Rat group	Mean body weight in grams ± standard deviation	
	At the beginning of experiment	After 14 days of treatment
Control (no treatment)	175.6 ± 18.2	246.2 ± 10.6
Type 2 diabetes	172.0 ± 19.5	191.8 ± 14.7

Type 1 diabetes	172.6 ± 9,5	162.6 ± 15.8
Type 2 diabetes +Moringa treatment	175,6 ± 8.1	187.4 ± 10.8
Type 1 diabetes +Moringa treatment	169.2 ± 8.3	161.4 ± 19.2

Effects on Fasting Blood Glucose test (FBG)

The results showed significant decrease in FBG after 14 days treatment in respect to control group (Table 3), meaning that Supplementation of aqueous extract of leaves of *Moringa oleifera* to type-2 and type-2 diabetic rat groups resulted some recovery of Fasting Blood Glucose when compared to the control showing its anti-hyperglycemic potency. This interesting finding suggesting future studies at long term treatment. The streptozotocin which was injected to the treated rat groups affects on the pancreatic beta cells and develops diabetes in rats, to be either type-1 or type- 2 depending on dose taken (Wang et al., 2011)[13]. Accordingly, this result supporting the folk use of *Moringa oleifera* leaves extract for monitoring diabetes. This result agrees with (Jaiswal et al., 2009 and Yassa and Tohamy, 2014)[20,21] who mentioned that *Moringa oleifera* leaves have a significant effect on therapy on hyperglycemic rats at the dose of 200 mg/kg body weight per day for 21 days treatment (Jaiswal et al., 2009) [20] and after 8 weeks treatment (Yassa and Tohamy, 2014) [21].

Table 3: Mean values of fasting blood glucose test after 14 days treatment:

Rat group	After 14 days of treatment
Control (no treatment)	80 ± 9.1
Type 2 diabetes (MD group)	184 ± 15.3
Type 1 diabetes (SD group)	328 ± 41.1
Type 2 diabetes +Moringa treatment (MD+M group)	113 ± 32.6
Type 1 diabetes +Moringa treatment(SD+M group)	130 ± 30.0

*Mean in mg/dl ± standard deviation, Mean of 5 rats

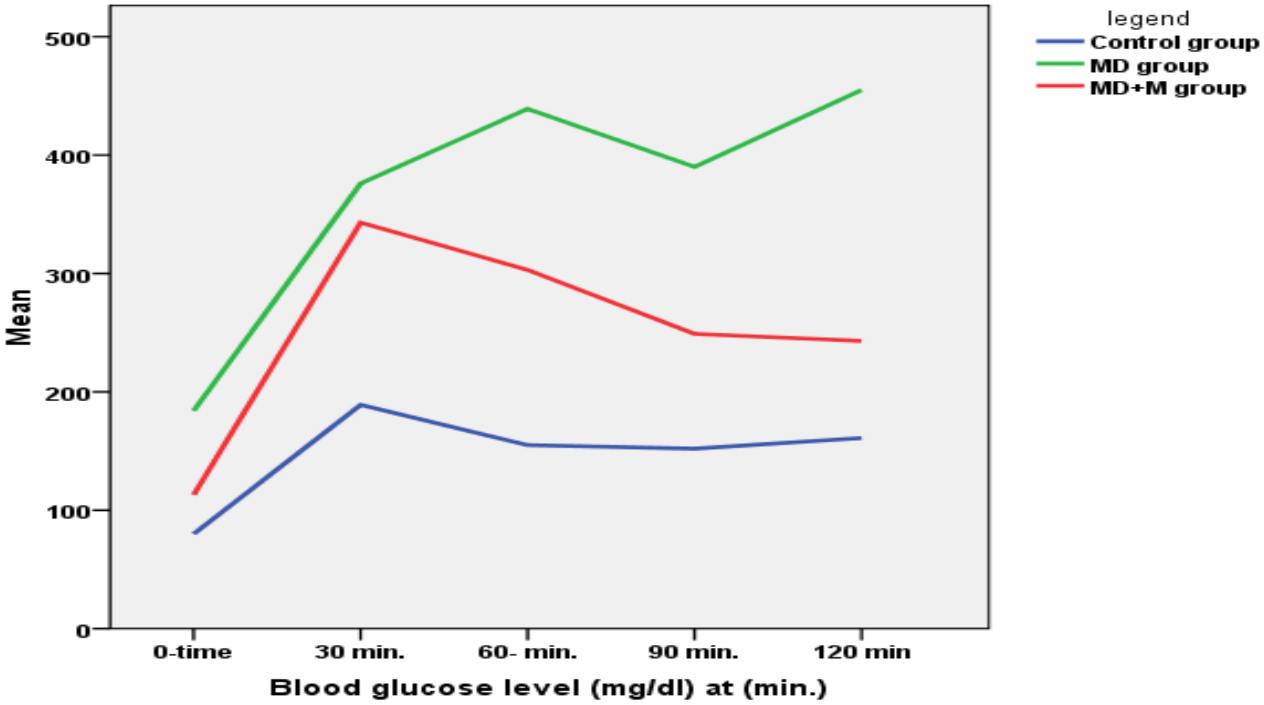
Effects on Glucose tolerance test (GTT)

It was found that *Moringa olifera* aqueous leaves extract revealed positive impact on the treated STZ-rat groups, particularly type-2 diabetic rat group (Table 4 and figure 1). This result means that *Moringa olifera* aqueous leaf extract improves glucose tolerance in diabetic rats. It is likely to be expected that the aqueous extract of the leaves has some direct effect by increasing the tissue utilization of glucose by inhibiting hepatic gluconeogenesis or absorption of glucose into the muscles and adipose tissues (Jaiswal et al., 2009)[20].

Table 4: Effect of aqueous extract of *Moringa olifera* on glucose tolerance test (GTT) in STZ-induced diabetic rats

Group treatment	Blood glucose level (mg/dl) at (min.)				
	0	**30**	**60**	**90**	**120**
Control (no treatment)	80 ± 9.1	189	155	152	161
Type 2 diabetes (MD group)	184 ± 15.3	376	439	390	455
Type 1 diabetes (SD group)	328 ± 41.1	358	457	Hi Ketones!*	Hi Ketones!*
Type 2 diabetes +Moringa treatment (MD+M group)	113 ± 32.6	343	303	249	243
Type 1 diabetes +Moringa treatment (SD+M group)	130 ± 30.0	304	419	444	Hi Ketones!*

* Hi Ketones! = Higher than the device's ability to read



MD= Type-2 diabetic rats, MD+M= Type-2 diabetic treated with Moringa.

Figure 1: Effect of aqueous extract of *Moringa olifera* on glucose tolerance test (GTT) in type-2 diabetic rats

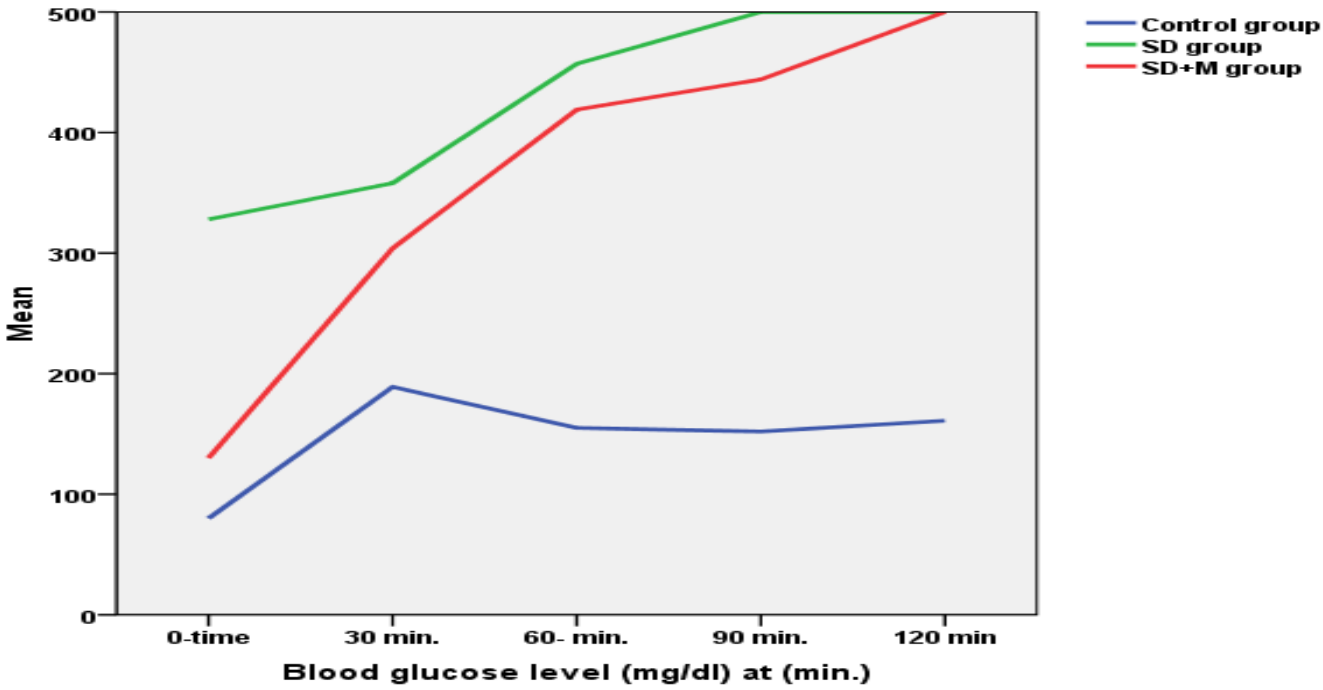


Figure 2: Effect of aqueous extract of *Moringa olifera* on glucose tolerance test (GTT) in type-1 diabetic rats

SD= Type-1 diabetic rats, SD+M= Type-1 diabetic treated with Moringa

CONCLUSION

Leaves of *Moringa olifera* which rich in bioactive phytochemical compounds, possesses significant anti-diabetic properties in treated rats. This study supports the traditional use in Sudan of Moringa in diabetes. Accordingly, it is recommended to introduce the edible leaves of *Moringa oliefera* in the diet of the diabetic people, which could monitor the diabetes beside to its nutritive value.

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