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Original Article

The Effect of *Moringa oleifera* on High Fat Diet and Streptozotocin Induced Diabetic Rats

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Abstract

Moringa oleifera has been used in many regions of the world at the level of folk medicine as a remedy for numerous diseases including the treatment of type 2 diabetes. This study aims to investigate the antidiabetic activity of the methanolic extract of *M. oleifera* leaves in type 2 diabetes mellitus in animal model induced by fat rich diet and the use of the diabetogenic agent, streptozotocin (STZ) in male Wistar rats. A daily dose of 300 mg/kg of *M. oleifera* extract (*MOE*) was administered to diabetic male Wistar rats that had been induced by high fat diet feeding for one month followed by intraperitoneal injection of 40mg/kg of streptozotocin. After three weeks of *MOE* treatment, blood glucose concentration, lipid profile markers, urea, and creatinine were assayed. Food intake was measured daily and body weight was measured weekly. After three weeks of treatment, *MOE* significantly decreased the blood glucose of the tested rats, and a marked decrease of triglyceride also was noticed. Regarding renal function indicators, *MOE* significantly decreased the serum urea level of diabetic rats. However, no change has been noticed in the serum creatinine level in all experimental groups. The findings in the present study suggest that *M. oleifera* can significantly alleviate hyperglycemia, and hyperlipidemia in diabetic rats. *M. oleifera* could be a potential treatment of type 2 diabetic patients.

Keywords: Type 2 diabetes mellitus, herbal extract, hyperglycemia, glucose, diabetes complications, Wistar rats.

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1. Introduction

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia that results from insulin deficiency, insulin resistance, or a combination of both. The persistent hyperglycemia and associated metabolic abnormalities of diabetes are often associated with secondary damage in multiple organ systems. Diabetes mellitus is classified into two essential types, type 1 (T1DM) and type 2 (T2DM), T1DM results from autoimmune-induced destruction of the insulin producing β-cells of the islets of Langerhans in the pancreas that leads to insulin deficiency

while T2DM diabetes is a heterogeneous and multifactorial complex disease that involves interactions of genetic susceptibility, and environmental risk factors such as sedentary lifestyle, dietary habits, and obesity. These factors act simultaneously to induce peripheral insulin resistance and insufficient insulin secretion by the pancreatic β-cells which give rise to the development of T2DM [1]. In T2DM, the persistent hyperglycemia is injurious to the β-cells and peripheral tissues and leads to serious events such as cardiovascular disease, nephropathy, neuropathy, retinal damage, and peripheral gangrene [2]. This pathological effect has been termed glucotoxicity as hyperglycemia is responsible for the production of glycation end products that generate cytokine from macrophage, generation of the free radicle, and endothelial cell dysfunction. All of these factors are implicated in the occurrence of diabetic complications [3]. Glucotoxicity, free radicles, lipotoxicity, and inflammatory mediators have been reported to decrease β-cell function and survival [4–6]. Different oral drugs classes are available that act by several mechanisms for the treatment of diabetes hyperglycemia. A diabetic patient may need more than one drug to control blood glucose within an acceptable level and finally may end up with the use of multiple drugs including insulin [7]. The search for new drugs that offer therapeutic advantages over the present drugs as protection and regeneration of the pancreatic β-cells and prevention of diabetes-induced tissues and organ damage is substantial to reduce the disease burden and severity. This is true especially from the plant kingdom as the plants represent a tremendous source of different phytochemicals. In recent years, numerous studies have shown promising findings regarding the antidiabetic activity of different plants and herbs. These findings have been demonstrated in diabetes animal models treated by herbal extracts [8]. The antihyperglycemic activity of numerous herbs in experimental diabetes is thought to be mediated by their antioxidants constituents as the improvement of the hyperglycemia is associated with a significant decrease of oxidative stress markers such as Malondialdehyde and hydrogen peroxide, and elevations in the activity of catalase. These findings have shown that free radicles such as superoxides, hydroxyl, and lipid peroxides may be involved both in the pathogenesis and the complication of the disease [9–11]. The other investigated mechanisms for the activity of herbal antidiabetics include suppression of α-amylase and α-glucosidase, insulin release stimulation, β-cells regeneration, and glucose transporter 4 (GLUT4) regulation [12]. Numerous phytoconstituents have been isolated with antidiabetic effects, these include saponins, polyphenols, ellagitannins, and triterpenes [13]. *M. oleifera* is a plant originally belongs to India, and now it is found in Asia, Europe, Africa, and other regions [14]. In Iraq, the interest is increasing at the level of traditional medicine for cultivation and use of different parts of the tree for a variety of conditions including T2DM. *M. oleifera* leaves contain many compounds like protein, minerals, antioxidants, and used by

many underdeveloped or developing populations as food integrators in nutrition, and in traditional medicine. In traditional medicine, the leaves have been administered as a remedy for numerous diseases ranging from infectious as typhoid fever and malaria, to chronic as diabetes mellitus and hypertension [15]. The recognized phytochemicals in its leaves are alkaloids, flavonoids, phenolic acids, glucosinolates, isothiocyanates, tannins, vitamins, and others. Of those, quercetin, kaempferol flavonoids, and chlorogenic acids have been reported to be the likely active compounds responsible for its hypoglycemic effect [16]. The animal antidiabetic effect of *M. oleifera* leaves has been well documented. Ndong et al. found that the glucose level in the blood of diabetic and nondiabetic rats had been decreased significantly by the leaves in comparison with the controls [17]. The administration of *M. oleifera* ethanolic leaves extract for one week to diabetic rats that had been induced by alloxan resulted in an obvious reduction of the serum glucose according to a study carried out by Kar et al. [18]. To the best of my knowledge, the antihyperglycemic activity of *M. oleifera* leaves extract in the T2DM animal model was investigated in one study that was carried on Sprague-Dawley rats model induced by using fat rich diet feeding for two weeks and STZ injection, and according to this study, the ethanolic *M. oleifera* leaves extract significantly decreased the blood glucose concentration, and improved the glucose tolerance and insulin resistance [19]. The present study aims to explore the antihyperglycemic, antihyperlipidemic, and to evaluate the renal function and the renal protective effects of the methanolic leaves extract of the plant *M. oleifera* in high fat diet-STZ T2DM Wistar rats model. This model simulates the sequence of pathophysiological events of the disease in humans as the high fat feeding for a relatively long duration results in obesity and subsequent insulin resistance, and later on, insulin deficiency is caused by STZ.

2. Materials and Methods

2.1. Chemicals and Materials

STZ was supplied from Medchem Express (Monmouth Junction, NJ, USA). Diethyl ether 98% was supplied by Thomas Baker (Chemicals) (Mumbai, India). Analytical reagent grade was used for all solvents (methanol 97%, distilled water), and chemicals (trisodium citrate dehydrate, and citric acid) used in the present work. One-call plus glucometer (ACON Laboratories Inc. San Diago, USA) was used for blood glucose monitoring.

2.2. Moringa oleifera Extract (MOE) Preparation

The leaves were collected from a local farm in Basrah city south of Iraq, Abu AL-kasiab Township in November, identified and authenticated by the Department of Biology, College of Science-Basrah University, and a voucher specimen (No. 17332) was deposited in the Herbarium of Basrah University. The leaves were washed, dried in the shade, and milled by electrical blinder into a fine powder. Later on, 2 L. of methanol (97%) was used for

maceration of 500 g. of the powdered leaves for 48 hs. The mixture was then stirred by the reflex hot plate magnetic stirrer (Heidolph MR 3001, Germany) at 50 ºC for 6 hs. The mixture then was filtered by a Whitman No. 1 filter paper, and a reduced pressure was used to concentrate the mixture by vacuum rotary evaporator (Heidolph, Germany). The concentrate was dried by a water bath (Memmert) at 50 ºC. The 35 g. of black-greenish and sticky yield was kept in a tight glass container and stored at 4 ºC until use.

2.3. Preparation of Normal and High Fat Diets

In the present study two types of diets were used, normal control diet (CON), and high fat diet (HFD). The CON and HFD were prepared according to the American Institute of Nutrition (AIN)-93 recommendations with some modification [20]. The components percent of both types are explained in Table 1. The difference is the animal fat (beef fat) percent with a difference in the caloric density, whereas a similar amount of cornstarch in the CON was replaced by the beef fat in the HFD. The Kcal. Energy percents of CON are carbohydrate 57 %, 32 % protein, 11 % fat, while the high-fat diet (HFD) kcal. energy percent are carbohydrates 28 %, protein 27 %, fat 45%. The diet formulation was prepared in Department of Animal Production, College of Agriculture by Dr. Alfred Sulaka karomy. All the components are crushed, mixed thoroughly, and compressed into pellet diet by a pellet press machine. Animal fat contains a high percentage of saturated fatty acid which gives rise to the development of obesity and diabetes.

2.4. Animals

Young male Wistar rats with a body weight of 180-200 g. were supplied from College of Veterinary Medicine-Basrah University. The experiments of animals were done during daytime in accordance with the animal use regulations approved by the committee of animal care and use in College of the Veterinary Medicine. The Best effort was done to decrease the animals suffering and pain throughout all study procedures. Every 3 rats were housed in a polypropylene cage with ad libitium supply of food and water and 12 hs. light/12 hs. dark cycle and a temperature of 21 ± 4 ◦C. Rats acclimatization was done for one week before the initiation of the two-month study duration.

2.5. Induction of T2DM

The induction of T2DM was performed by two sequential phases. In the first phase, the high fat diet was used for feeding the rats for one month, while the second phase involved the injection of STZ. The rats had fasted at 7 a.m., and at 2 p.m. were injected intraperitoneally with 40 mg/kg of STZ that dissolved in fresh 0.1M sodium citrate buffer (pH 4.5) [21]. Both STZ and citrate buffer solutions were kept cold by the use of ice bags and STZ kept in the dark condition during the work to avoid degradation of heat and light sensitive STZ. All the rats in other groups were injected with 0.5 ml of vehicle sodium

citrate buffer. 6 hs. Post-STZ injection, the drinking water was replaced with a 10% glucose solution for 48 hs. to prevent fatal hypoglycemia that occurs as a result of massive β-cell necrosis and sudden release of insulin. After three days of STZ injection, the diabetes mellitus was confirmed by measurement of glucose concentration in blood samples that were withdrawn from the tail vein by the use of blood glucose meter, which was higher than 300 mg/dl for all rats that had been injected with STZ. The rats weight was measured weekly and food intake was measured by calculation of the food leftover in the cage.

2.6. Experimental Design

A total of 24 rats was included in the present study and divided into 4 groups, n = 6 rats for each group. The first is the (CON) group that was fed with the normal diet and continued on this diet until the end of the study. The second is (HFD) group that was fed with HFD during the whole study duration. The third group was the diabetic control group (DM) where the rats had been fed for one month with HFD and then injected with STZ. The fourth group is diabetic-*M. oleifera* extract-treated group (DM-*MOE*) that was treated daily with a dose of 300 mg/kg body weight of *M. oleifera* leaves methanolic extract for 3 weeks. The leaves extract had been suspended in distilled water and then given by oral gavage at 10 p.m. So finally, there were four Wistar rats groups:

1. CON group: Fed with normal control diet (CON) for the two months-study duration.

2. HFD group: Fed with high fat diet (HFD) for the two months-study duration.

3. DM diabetic rats control group: T2DM was induced by feeding Wistar rats by HFD for one month followed by STZ injection and continued on HFD to the end of the study.

4. DM-*MOE* group: Diabetic group as in the previous group treated with *MOE* for three weeks.

2.7. Biochemical Analysis

At the end of the study, diethyl ether was used for animals anesthetization through the inhalational route by ether impregnated-cotton ball inside a small chamber, and blood samples were withdrawn from the heart. The blood samples were then centrifuged at 3000 rpm for 15 min. The serum samples were analyzed by an automated analyzer (Mindray B-200) for biochemical parameters which were glucose concentration, serum lipid profile that included triglycerides, cholesterol, and HDL. Renal function indicators including serum urea and creatinine level were measured to evaluate renal function. Measurement of aspartate transaminase (AST) and alanine transaminase (ALT) as hepatic function indicators was to evaluate liver function.

2.8. Statistical Analysis

Statistical Package for the Social Sciences software (SPSS version 22) was used to perform the statistical analysis. The mean ± SD was used to express the results and One-Way Analysis of Variance (ANOVA) followed by the Tukey post hoc test was applied to compare between the means of the

groups and a *P* value less than 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Body Weight and Food Intake

The results for the body weight and food intake for different groups are presented in Figure 1A, and 1B respectively. The rats in the HFD group showed a significant increase in the body weight in comparison with the rats in the CON group ($p < 0.001$), while the HFD rats showed a significant increase in the body weight in comparison with the diabetic rats ($p < 0.001$). On the other hand, the body weight of the diabetic-*M. oleifera* treated group was not different from that of the diabetic group. The results of food intake showed that the CON rats food consumption was significantly high in comparison with that of the HFD rats ($p < 0.001$). Despite the higher food intake of the CON group, the body weight of the HFD group was significantly higher than that of the CON group. The food intake did not show a significant difference among the HFD group, the diabetic DM group, and the DM-*MOE* group.

3.2. Effects of MOE on the Blood Glucose and Lipid Profile of the Diabetic Rats

Table 2 presents the results of the blood glucose concentration and serum lipid profile. The diabetic rats showed a significantly higher blood glucose concentration (646 mg/dl) in comparison with both CON rats (162 mg/dl), and HFD rats (192.5 mg/dl), ($p < 0.001$). This obvious hyperglycemia in addition to other sings as polydipsia and polyuria indicated the occurrence of the diabetic state. The administration of *MOE* to the diabetic rats at a daily dose of 300 mg/kg by oral gavage for three weeks significantly decreased ($p < 0.05$) the blood glucose level from (646 mg/dl) in the diabetic rats to (421.3 mg/dl) in the DM-*MOE* group. Similar findings have been reported by other studies. The hypoglycaemic effect of *M. oleifera* ethanolic leaves extract was reported in diabetic Sprague-Dawley rats induced by two weeks of fat rich diet feeding plus STZ in the study conducted by Anyanwu et al where the administration of the ethanolic extract for 14 days at daily doses of 250 mg/Kg, and 500 mg/Kg. body weight decreased the level of blood glucose by 72%, and 70% respectively [19]. The difference between the present study and that conducted by Anyanwu et al is the HFD feeding period and the solvent used in the extract preparation. In the present study, the antidiabetic activity of the methanolic extract of *M. oleifera* leaves was investigated in Wistar rats that had been induced by a one month HFD feeding which had induced frank obesity followed by STZ, and the ability of *MOE* to decrease the blood glucose level in a significant manner in this model gives increased importance of the *M. oleifera* antidiabetic activity in T2DM. The administration of *M. oleifera* aqueous leaves extract at a daily dose of 200 mg/kg to the diabetic rats that had been induced by STZ decreased the fasting blood glucose level by 69%, and 67% according to two studies carried out by Dolly et al., and Hanan et al. respectively [22,23]. The increase in the

concentration of blood glucose of the rats in the HFD group (192.5 mg/dl) was not significant compared with the rats in the CON group (162.5 mg/dl). Table 2 presents the total cholesterol (TC), triglyceride (TG), and high density lipoprotein-cholesterol (HDL-C) serum levels. There was a significant increase ($P < 0.05$) in serum TC level of the diabetic rats (65.83 mg/dl) compared to the CON rats (43.33 mg/dl). The TC level of the HFD rats was not significantly different from the CON group and was intermediate between the CON group and the diabetic group. The treatment with *MOE* resulted in a non-significant lowering in TC level from 65.8 mg/dl in the diabetic rats to 54 mg/dl in the DM-*MOE* group. The serum levels of triglyceride and HDL did not show significant changes among all experimental groups. In this study, the T2DM animal model was induced by feeding male Wistar rats with HFD with fat from an animal source (sheep fat) for one month followed by the use of a single moderate dose (40 mg) of STZ and continued on the HFD diet till the end of the study. This model is used successfully by many studies with the use of low to moderate STZ dose (30-40 mg/kg body weight) to explore the antihyperglycemic activity of herbal extracts in T2DM. This model results in both peripheral insulin resistance, and insulin deficiency, the major pathological characteristics of T2DM as manifested by hyperglycemia, hyperlipidemia, glucose intolerance, and impaired insulin tolerance [24]. T2DM persistent hyperglycemia leads to serious complications as retinopathy that give rise to blindness, nephropathy which ends in renal failure, and neuropathy in addition to the macrovascular morbidity as coronary artery diseases and peripheral vascular diseases [2]. The lowering of the blood glucose level is the principal pharmacological goal in diabetes treatment as it limits the major disease complications that result from hyperglycemia and subsequent glucotoxicity which results in increased oxidative stress and multiorgan complications [25]. Several mechanisms have been proposed regarding the antihyperglycemic effect of herbal plants in diabetes as the plants are rich in many phytochemicals and antioxidants and *M. oleifera* is not an exception. In the present study, the treatment of the tested rats with *M. oleifera* leaves crude extract results in a significant lowering of the glucose concentration from 646 mg/dl to 421 mg/dl (34% reduction) (Table 1). *M. oleifera* leaves are extracted by different solvents and techniques. In this study, methanol was used as it shows a high content of phenols and flavonoids with high antioxidant activity and a good hypoglycemic effect [26,27]. Phenolic compounds have shown good activity in animals models of numerous diseases including psychological disease as the methanolic extract of *Thymus Kotschyanus* has hypnotic, anticonvulsant, and anxiolytic activity, which are likely attributed to the activation of $GABA_A$ receptors by phenolic compounds, while the ethanolic extracts of *Artemisia dracunculus* and *Stachys lavandulifolia* have an antidepressant-like activity that could be linked to the main components of the extracts which are the

flavonoids [28,29]. Although the antihyperglycemic activity of *M. oleifera* was reported in some studies carried out on laboratory rats, the addition in the present study is the use of a fat rich diet followed by streptozotocin in the induction of the model, i.e induction of the T2DM model which represents an increased challenge against this effect of *M. oleifera* reported in previous researches and the significant lowering of glucose level in the present study highlights further aspects of the plant and its activity in diabetes. The dyslipidemia that occurs in T2DM is usually manifested as a disorder in one or more of the lipid profile parameters and the patients with uncontrolled T2DM show a reduction in HDL-C and increase in TG, TC, and LDL-C [30,31]. These lipid abnormalities considered as a risk factor for diabetic patients to develop atherosclerosis and coronary heart diseases and pathological deterioration of pancreases that lead to decreased insulin-secreting activity [32]. In this study, the diabetic group showed a significantly higher TC level and a marked increase in TG level with no change in HDL compared with the CON group. The use of *MOE* resulted in although non-significant but a marked decrease in TC, TG, and HDL of the DM-*MOE* group compared with the DM group. The ability of *M. oleifera* leaves to significantly decrease the elevated lipid profile markers was observed in several studies. Sai et al. reported that the use of 200 mg/kg daily dose of the aqueous *M. oleifera* leaves extract decreased the serum concentrations of TG, TC, VLDL-C, and LDL-C in diabetic rats that had been induced by STZ toward normal levels [33]. Other studies reported that the administration of *M. oleifera* leaves methanolic and aqueous extracts to the diabetic rats induced by alloxan significantly decreased the serum concentrations of TC, TG, and LDL-C while increased the HDL level along with hypoglycemic effect [27, 34].

3.3. Effects of MOE on Renal and Hepatic Biochemical Indicators

Table 3 shows the renal function indicators, urea, and creatinine that were measured to evaluate renal function. The results revealed a significantly higher serum urea level of the diabetic rats (81 % increase) compared with the CON control rats ($p < 0.05$), and the urea level of the diabetic rats was significantly decreased (32% decrease) ($p < 0.05$) when treated with *MOE*. No significant change in the serum creatinine level of the diabetic rats was noticed in comparison with the control CON rats. Measurement of hepatic function indicators AST and ALT showed no significant changes among all experimental groups. One of the life-threatening diabetes consequences in patients with prolonged hyperglycemia is diabetic nephropathy, which is an essential cause of chronic kidney disorders and end-stage renal disease. [35,36]. In many studies, the rats T2DM models induction by high fat diet and STZ exhibit a significant elevation in the renal biochemical indicators such as urea and creatinine in addition to renal pathological changes. These models are used to study diabetic nephropathy as the alteration in the renal function

indicators, and histopathological changes indicate the development of diabetes-induced renal damage [37]. In the present study, the elevation of the serum urea level in the diabetic group is significantly decreased by the administration of *MOE* indicating its ameliorative effect in diabetic nephropathy. The renal protective effect of *MOE* is in agreement with a review by Brilhante et al. who reported several studies that had observed the renal and hepatic protective properties of *M. oleifera* against the injurious effects of several drugs like rifampicin, acetaminophen, gentamicin, isoniazid, pyrazinamide which are mainly mediated by its leaves [14].

4. Conclusion

The present study demonstrates that *M. oleifera* has good antihyperglycemic activity, and antihyperlipidemic effects in the high fat-STZ induced T2DM rat model. *M. oleifera* could be a potential source of a drug for the treatment of T2DM after further phytochemical analysis to determine the active marker compound.

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Tables:

Table 1. The components of the diet types.

Components	**CON (% weight in g)**	**HFD (% weight in g)**
Cornstarch	34	15
Grounded wheat	17	5
Wheat bran	3	18
Beef tail fat	1	14
Soybean	33	35
animal protein	5	5
Calcium source	1.5	1.5
Vitamins and minerals mixture	1	1
Sucrose	4.5	5.5

CON, normal control diet; HFD, high fat diet.

Table 2. Blood glucose, and lipid profile of the rats after treatment.

Groups	**Glucose (mg/dl)**	**TC (mg/dl)**	**TG (mg/dl)**	**HDL-C (mg/dl)**
CON	162.2 ± 42.3	43.3 ± 12.4	122.4 ± 41.2	28.8 ± 8.7
HFD	192.5 ± 49.5	51.8 ± 9.6	187.5 ± 55.7	32.0 ± 9.4
DM	646.7 ± 156.8***	65.8 ± 13.2*	202.7 ± 79.0	34.2 ± 8.4
DM-*MOE*	421.3 ± 147.3$^{\#}$	54.7 ± 15.8	165.0 ± 70.9	25.7 ± 15.1

Note: Values are mean ± SD., n=6. $^{***}p < 0.001$ compared with the normal diet control group, $^{*}P < 0.05$ compared with the normal diet control group, $^{\#}P < 0.05$ compared with the diabetic rat. Total cholesterol (TC), trigleceride (TG), high density lipoprotein-cholesterol (HDL-C). CON, normal diet control group; HFD, high fat diet group; DM, diabetic group; DM-*M0E*, diabetic-*Moringa oleifera* treated group.

Table 3. Renal and hepatic indicators of the rats after treatment.

Group	Urea	Creatinine	AST	ALT
CON	32.50 ± 9.48	0.631 ± 0.07	126.33 ± 37.02	40.00 ± 13.35
HFD	22.30 ± 2.06	0.665 ± 0.07	209.50 ± 55.30	65.20 ± 26.53
DM	58.70 ± 10.70 *	0.696 ± 0.03	241.66 ± 140.24	87.66 ± 45.84
DM-MOE	39.80 ± 14.99 #	0.701 ± 0.12	292.00 ± 228.97	86.16 ± 60.42

Note: Values are mean ± SD., n=6. $^{*}P < 0.05$ compared to the normal fat diet control group, $^{\#}P < 0.05$ compared to the diabetic rats group. aspartate transaminase (AST), alanine transaminase (ALT), CON, normal diet control group; HFD, high fat diet group; DM, diabetic group; DM-*M0E*, diabetic-*Moringa oleifera* treated group.

Figures:

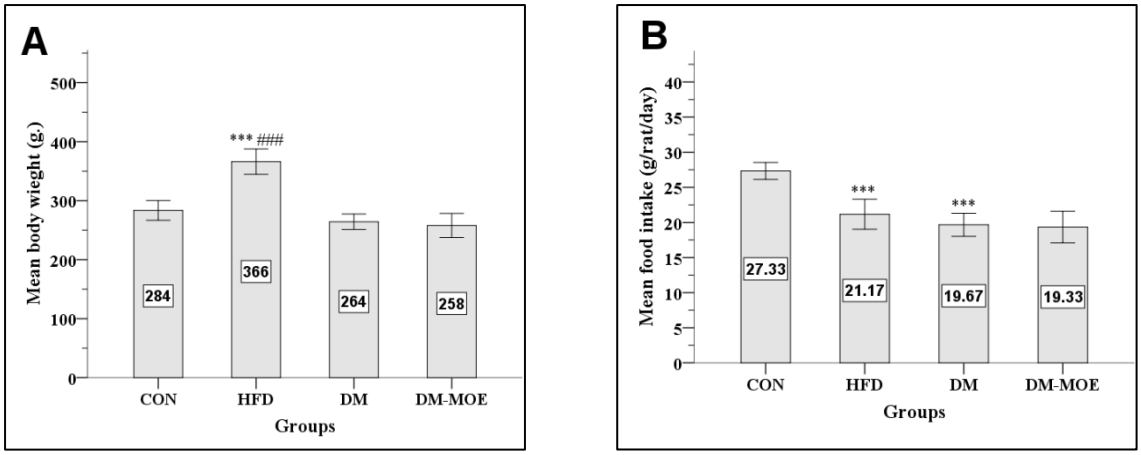

Figure 1. Body weight (A), and food intake (B) of rats in control, high fat diet, diabetic, and diabetic-*Moringa oleifera* treated groups. Body weight was recorded at the last day of the study and food intake was recorded in the last three days of the study. Values are presented as Mean ± SD (n=6). $^{***}P < 0.001$ compared with the CON control group. $^{###}P < 0.001$ compared to the diabetic rats group. CON, normal diet control group; HFD, high fat diet group; DM, diabetic group; DM-M0E, diabetic-*Moringa oleifera* treated group.