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ANTIDIABETIC EFFECT OF *Moringa oleifera* IN ALLOXAN INDUCED DIABETIC RAT

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ABSTRACT

Oral anti-diabetic agents have a number of serious adverse effects, thus, managing diabetes without any side effects is still a major challenge. Therefore, the search for many effective and safer hypoglycemic agents from herbal plants are being considered as an important area of investigation. The aim of this research was to investigate the effect of *Moringa oleifer*a (MO) on blood glucose levels, serum total cholesterol, hematological parameters, body weight and histopathological assessment of different organs in alloxan-induced diabetic rats. The rats were divided into 3 equal groups, each containing 15 individuals (n□15) as follows: normal control (C) group, diabetic control (DC) group and diabetic treatment (DT) group. Diabetes was induced in diabetic control (DC) and diabetic treatment (DT) group with alloxan @ 200mg/kg body weight intra-peritoneal. Diabetes was induced in diabetic control (DC) and diabetic treatment (DT) group with alloxan @ 200mg/kg body weight intraperitonially. Then DT group was treated with aqueous extract of MO @150 mg/kg body weight. After 42 days of treatment, MO extract reduced the amount of blood glucose significantly ($p<0.001$) in the group DT compared to DC from 31.41 ± 0.44 to 16.61 ± 0.33 mmol/L. Total cholesterol (TC) was lowered significantly ($p<0.01$) in DT compared to DC from 154.122 ± 3.41 to 108.489 ± 0.31mg/dL. In hematological study, DT group showed significant ($p<0.001$) elevation of erythrocyte count and demotion ($p<0.01$) of leukocyte count after 42 days of treatment in contrast with DC group. The body weight was also increased significantly ($p<0.001$) in DT group. Moreover, in histopathological examination, the MO therapy in diabetic rats showed mild regeneration of the pancreatic acini and islets of Langerhans compared to DC which indicate the organo-protective effect of MO. Based on present research it can be concluded that MO can be used as anti-diabetogenic agent in food.

Key Words: Moringa, antidiabetic, rat, kidney, pancreas

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder that is specified via hyperglycemia as a result of impaired insulin production, mode of action, or both (ADA, 2014). It is considered as one of the five leading causes of death in the world. Globally, 463 million people have diabetes and this number is projected to reach 578 million by 2030, and 700 million by 2045 (IDF, 2019). In Bangladesh, the number of detected diabetic patients reached 8.4 million along with the undetected diabetic patients almost 4.7 million (IDF, 2019). Oxidative stress due to chronic hyperglycemia during DM causes tissue and organ damage by increasing oxidation of carbohydrate, protein, lipid and DNA (Prasath and Subramanian, 2013; Yachamaneni and Dhanraj, 2017). Oxidative stress is more likely to be happened in the liver due to DM (Tolman *et al*., 2007). Different disorders related with glycogen and lipid metabolism are formed in liver by DM (Sánchez *et al*., 2000). Similarly, the oxidative stress also causes the diabetic kidney complications (Brownlee, 2001). Moreover, excessive hyperglycemic effects damage the β cells of pancreas, although the β cells can tolerate the oxidative stress most (Robertson *et al*., 2004). Treatment of diabetes with insulin or other modern drugs has series of adverse effects (Saxena & Vikram, 2004) and the cost is also increasing alarmingly in recent years (IDF, 2019); so, it is one of the crucial areas of investigation to find out new safer, easily available and more effective anti-hyperglycemic agents. *Moringa oleifera*, known in Bangladesh as 'Sajne', has been shown to possess hypoglycemic activity. The plant extract proved to have beneficial hypoglycemic effect possibly by insulin secreting action or by influencing the enzymes engaged in

glucose metabolism (Singh, 2011). Various phytoconstituents are found in MO like anthraquinone, 2-phenylchromenylium, a phenolic steroid, sitogluside (glycoside) and hemlock tannin (Putra *et. al*., 2022 and Zainab *et. al*., 2020). The objective of this study is to elucidate the possible antidiabetic activity of MO and its medicinal potency responsible for the protection of different organs.

MATERIAL AND METHODS

This study was conducted in the Department of Anatomy, Histology and Physiology, Sher-e-Bangla Agricultural University, Dhaka to evaluate the efficacy of MO extract in diabetic rats.

Collection and acclimatization of rats*:*
Total forty-five (45) healthy mixed albino rats were collected from Jahangirnagar University, Savar, Dhaka. For three experimental trials, all the rats were grouped into 3 groups each containing 15 rats. Each group was kept in separate cages and arranged in rows according to group. The animals were fed with pellet at a recommended dose of 100 g/kg body weight. Drinking water was supplied ad libitum. Before starting the experiment, all the rats were acclimatized to this environmental condition for a period of one week.

Induction of diabetes:
To induce diabetes mellitus, alloxan injection was given @200 mg/kg body weight through intra-peritoneal route and this increased the blood glucose level ($p<0.05$) and at the same time body weight was decreased also (Federiuk *et. al*., 2004). In this experiment, polyuria, polydipsia and polyphagia after 24 hours of alloxan injection were observed. Rats with serum glucose level ranging between 150 mg/dl or above considered as hyperglycemic.

Experimental design:
In this study, a total of 45 rats (15 normal rats and 30 alloxan induced diabetic rats) were used for each trial. The rats were divided into 3 groups each containing 15 individuals (n=15) as follows:
Group A: Normal control group
Group B: Diabetic control group
Group C: Diabetic with MO treated group

Preparation and administration of MO leaf extract
Fresh, clean, and delicate MO leaves were collected from the tangled vegetation in the non-cultivated area of the Sher-e-Bangla Agricultural University campus. 100 mL of distilled water and 10 g of dried and powdered MO leaves were mixed for 24 hours to create the moringa aqueous extract, which was then kept at 4 °C. The combination was then passed twice through filter paper with a 2-µm pore size. The 100 mg/mL aqueous extract stock solution was either newly produced for each set of experiments or kept at 4 °C for up to 5 days (Tuorkey, 2016). During treatment, group C was administered with MO leaf extract at the rate of 150mg/kg/day for for 42 days treatment period.

Determination of blood glucose (mmol/L)
Blood samples were collected from tail vein on 0, 21st and 42nd day of experiment and blood glucose was determined by using glucose oxidase-peroxidase reactive strips and a glucometer (UNI-CHECK®, Visgeneer, Taiwan).

Determination of total cholesterol (mg/dL)
Blood samples were collected from tail vein on 42nd day of experiment and blood cholesterol was determined by using a blood testing meter (EasyMate® GCU, Bioptik Technology Inc., Taiwan).

Collection of blood for hematological tests
On 42nd day of experiment, blood was collected directly from the heart with the sterile syringe and needle after anaesthetizing the animals with Xylazine HCl® @ 12mg/kg and Ketamine HCl® @ 75mg/kg body weight intramuscularly (Burke, 1999). About 1mL of blood from the syringe was taken in the vacuum tube containing anticoagulant (K3EDTA) for hematological studies.

Determination of hematological parameters
Total erythrocyte count (million/cumm)
Blood sample was diluted 1:100 with RBC diluting fluid with the help of RBC pipette. It was mixed properly and a drop was placed under the cover slip of the hemocytometer and red blood cells were allowed to settle for 3 minutes. The number of RBC present in 16×5 squares was counted under (40X) microscope (Dacie *et al.,* 1958 and D'Armour *et al*., 1965).

Total leukocyte count (thousand/cumm)
Blood sample was diluted 1:20 with WBC diluting fluid with the help of WBC pipette. It was mixed properly and a drop was placed under the cover slip of the hemocytometer and white blood cells were allowed to settle for 3 minutes. The number of WBC present in 16×4 squares was counted under (10X) microscope (Dacie *et al*., 1958 and D'Armour *et al.,* 1965).

Determination of Body weight (g)
Body weights of the rats of all groups were recorded before treatment (on day 0) and during treatment period i.e. 7th, 14th, 21st, 28th, 35th and 42nd day by using weighing machine

Histopathological Evaluation of Different Organs

Tissue Preparation for Light Microscopy

The rats were sacrificed by being given ketamine (80 mg/kg) and xylazine (10 mg/kg) anesthesia intramuscularly (Giroux *et. al.*, 2016) at the end of treatment and tissues (less than 5 cm thickness) of liver, kidney & pancreas were collected for performing the histopathology. Then the tissues from each rat were fixed with 10% neutral buffered formalin for 24 hours and then dehydrated in the graded alcohol, cleared in xylene, embedded in paraffin. The sections were cut at 5 µm thickness by sliding microscope (Leica SM2010R) (Plate 13) and placed on the surface of warm water in a water bath and the flattened sections were floated onto a coated glass slide. Then the glass slides containing sections were dried in a slide warmer (XH-2002) overnight. The prepared slides were stained using Hematoxylin and Eosin (H&E) and then examined under the light microscope (G-206LED, Taiwan) and photographs were captured for further morphological analysis.

Statistical analysis:

All data were expressed as mean ± SEM (n=15). Statistical differences among the groups were calculated using analysis of variance (ANOVA) followed by a Post-Hoc Test (Bonferroni Correction) in Microsoft Excel Spreadsheet Software 2023. Differences with $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

The research was performed to investigate the antidiabetic effects of MO on blood glucose serum total cholesterol (TC), hematological parameters i.e. TEC and TLC, body weight, and histopathological assessment of different organs.

Effects on blood glucose level

The effects of MO on blood glucose level to control diabetes in rats are presented in Table 1 In DT group, MO extract was found to significantly ($P<0.001$) reduce blood glucose levels to 25.11±0.39 and 16.61 □ 0.33mmol/L in 21st and 42nd day, respectively comparing with the DC group that is agreement with the previous studies (Oyedepo *et. al.*, 2013 and Attanayake *et. al.*, 2015). This may have occurred due to the direct cytotoxic activity of alloxan on the pancreatic β-cells, which disrupted insulin secretion and may be the cause of the hyperglycemic effect (Rohilla and Ali, 2012; Gupta *et. al.*, 2012; Etuk, 2010). Abd El Latif *et. al.* (2014) suggested that the aqueous extract of MO leaves revealed hypoglycemic properties by reducing the manifestation of the pyruvate carboxylase enzyme in the liver and by using its antioxidant properties to repair destroyed hepatic cells and β-cells. Additionally, supplying MO leaf extract to diabetic rats generated enhanced glucose absorption in the liver and muscle, as well as increased glycogen synthase activity and contents, which might be the reason for the reduced glucose level in the MO treated group observed in our experiment (Olayaki *et. al.*, 2015).

Table 1. Effects of MO on Blood Glucose (mmol/L)

Groups	Day 0 (mmol/L)	Day 28 (mmol/L)	Day 56 (mmol/L)
Control (C)	4.71 ± 0.46	6.81 ± 0.30	6.28 ± 0.24
Diabetic control (DC)	6.07 ± 0.26	29.9 ± 0.45	31.41 ± 0.44
Diabetic Treatment (DT)	6.03 ± 0.16	25.11 ± 0.39	16.61 ± 0.33*

Data are shown as mean□SEM (n□15 samples per group). p<0.05 significant when compared to the group DC.

Effects on serum total cholesterol

The effects of MO on serum total cholesterol (TC) are shown Table 2 At the end of the experimental period, it was found that, TC level was raised significantly ($P<0.001$) on diabetes induced rats (group DC) in comparison to control (C) group. Total cholesterol value decreased significantly ($P<0.001$) in Diabetic Treatment (DT) group by treating them with MO in alloxan induced diabetic rats. Alloxan causes the production of excess fatty acid in plasma that enhances the liver conversion of home fatty acids into phospholipids and cholesterol (Rhoads *et. al*., 1976). Following the treatment with MO extract, the serum total cholesterol level was significantly reduced ($P<0.001$). This result is similar with Omodanisi *et. al.*, (2017) where they proved that administration of MO extracts tends to reduce the cholesterol level.

Table 2. Effects of MO on Total Cholesterol (mg/dL)

Groups	Total Cholesterol (mg/dL)
Control (C)	106.011 ± 0.31
Diabetic control (DC)	154.122 ± 3.41
Diabetic Treatment (DT)	122.336 ± 1.14*

Data are shown as mean±SEM (n=15 samples per group). p<0.05 significant when compared to the group C

Effects on hematological parameters

Effects on total erythrocyte count (TEC)

Effects of MO on total erythrocyte count (TEC) of diabetic rats are shown in **Table 3** After induction of diabetes by alloxan TEC values were significantly (P<0.001) reduced in DC group to 5.56 ± 0.08 million/cumm compared to control (C) group. On the other hand, TEC values were significantly (p<0.001) increased up to 9.02 ± 0.17 in DT group compared to DC group. Similar effects of MO extract were found found by Osman *et. al.*, (2012).

Effects on total leukocyte count (TLC)

Error! Reference source not found.3 represents the effects of MO on total leukocyte count (TLC) in alloxan induced diabetic rats. The results indicated that after alloxan administration, TLC was significantly (P<0.001) increased in DC group compared to control group. After getting treatment, there was a significant (P<0.001) reduction of white blood cells in DT group compared to the DC group. In contrast to our findings Ibrahim *et. al.,* (2014) reported that Moringa seeds enhanced (p<0.05) the WBC count growing rabbits.

Table 3. Effects of MO on Hematological Parameters

Groups	TEC (million/cumm)	TLC (thousand/cumm)
Control (C)	8.39 ± 0.19	8.62 ± 0.22
Diabetic control (DC)	5.56 ± 0.08	10.27 ± 0.32
Diabetic Treatment (DT)	9.02 ± 0.17*	8.41 ± 0.05

Data are shown as mean±SEM (n=15 samples per group). *p<0.01 highly significant when compared to the group DC.

Effects on body weight

Table 4 shows the effects of administration of MO on body weight of alloxan induced diabetic rats. After induction of diabetes in rats, there was a significant (P<0.001) reduction in body weight of diabetic control (DC) group rats to 152.62±0.75g. After treating with MO a significant (P<0.001) increase in body weight was observed to 164.00±1.27g in diabetic treatment (DT) group compared to diabetic control (DC) group rats.Results of the present study support the findings of these results were in agreement with the findings of Packirisamy et. al. (2018) and Basyony et. al. (2016) who also observed significant increase in body weight after treatment with *MO* extract in hyperglycemic animals. These results suggest that the weight gain could be due to the availability of phytochemicals such as important amino acids and vitamins, particularly those that are fat-soluble.

Table 4. Effects of MO on Body weight (g)

Groups	Day 0	Day 14	Day 28	Day 42
Control (C)	177.43±2.91	180.19±2.46	182.1±2.07	183.03±2.04
Diabetic control (DC)	171.14±1.03	164.85±0.91	159.62±1.15	152.62±0.75
Diabetic Treatment (DT)	157.48±1.93	160.25±1.11**	162.09±1.36**	164.00±1.27**

Data are shown as mean±SEM (n=15 samples per group).** p<0.01 highly significant when compared to the group T_0.

Histopathological Assessment of Different Organs

Histopathological Study of Pancreas

In Fig. 1, the pancreatic specimen of the control (C) group exhibited normal exocrine acini (AC) and well-organized islets of Langerhans (IL). In the case of the diabetic control group (DC), degenerated AC was observed, and no islets were seen in the specimen. In Fig. 2, The MO leaf solution given to diabetic rat (DT) pancreatic tissue displayed regenerated AC. Mild restoration of islets was also observed but not as like as in the group C. A number of researchers have reported on the histological alterations of the islets of Langerhans in diabetics. These changes might be quantitative, such as a decrease in quantity or size, or qualitative, such as necrosis and degeneration. In a study, thickened septa, congested RBC, and atrophied β-cells along with vacuolation in the islets were evident in the diabetic control group which was different from our findings (Al-Qudah *et. al*., 2016). In the present study, degenerated pancreatic acini with no islets were found in the diabetic group (DC). This might be related to the breakdown of pancreatic cells caused by alloxan toxicity, resulting in necrosis (Lenzen, 2008). The islet cells were prominent and showed typical histological features in the C group and slightly regenerated islets were seen in in MO treated (DT) group.

Histopathological Examination of Liver

In Fig. 3, the liver tissue of the C group represented proper sinusoids, hepatocytes with a distinct nucleus, and an endothelium-lined central vein. In alloxan-induced diabetic group (DC) revealed distinct hepatic cords along with regular sinusoidal space (Fig. 4). The MO extract administered group (DT) demonstrated improved hepatic cords with sinusoids (Fig. 5) which was similar to the Control group. The liver is an

essential organ for regulating appropriate blood glucose levels because it acts on insulin disposal and generates inflammatory cytokines. A study carried out by Abdollahi *et. al*., (2010) demonstrated that STZ-induced hyperglycemic rats showed dispersed and degenerated necrotic cells, and dilated cytoplasmic hydropic with microvesicular flattening of the hepatic tissue which was in contrast with the findings of our study. Another report evident necrotic hepatocytes with granular cytoplasm and dilated sinusoids in the hyperglycemic rats that were also different from ours (Nagilla and Reddy, 2017). However, in the present investigation, the liver of the MO treated group of rats did not exhibit any abnormalities. This might have occurred due to the duration of the treatment, dose of the alloxan, and amount of the MO extracts given to the treated groups.

FIGURE WITH LEGENDS

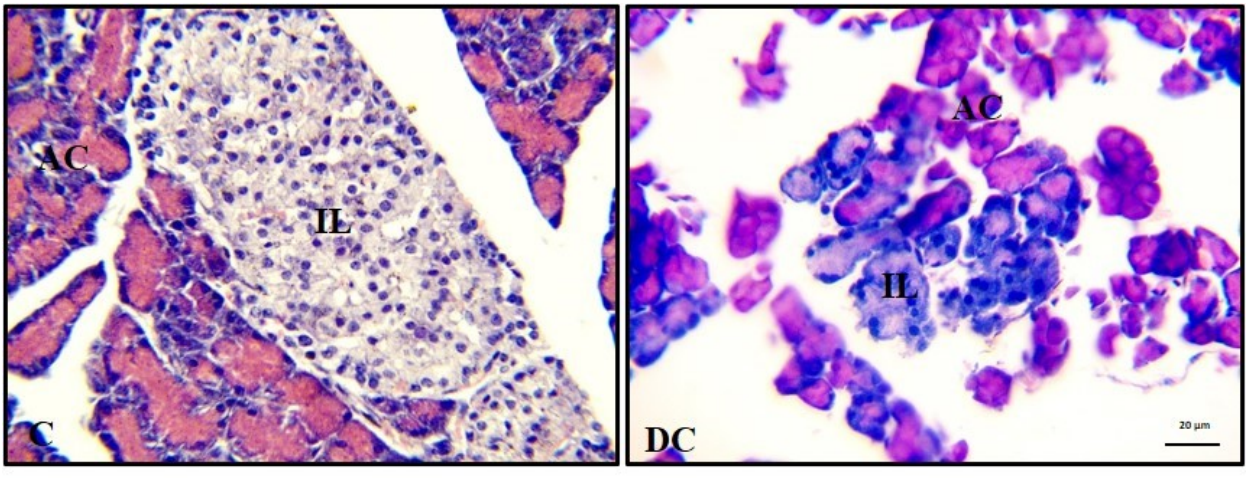

Figure 1. Histopathological examination of pancreas (C vs DC) stained with H&E (40X) (C) the pancreas of rats from the control group showing normal exocrine acini (AC) with normal islets of Langerhans (IL). (DC) pancreas of rat from alloxan-induced diabetic control group showing degenerated pancreatic acini (AC) and no islets was seen. Bars = 20 µm.

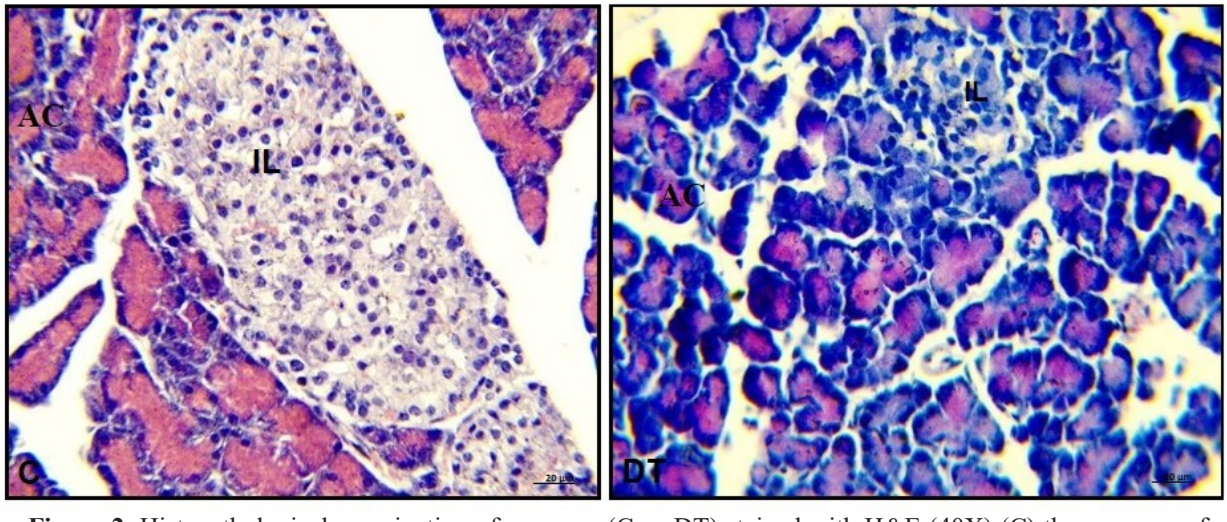

Figure 2. Histopathological examination of pancreas (C vs DT) stained with H&E (40X) (C) the pancreas of rats from the control group showing normal exocrine acini (AC) with normal islets of Langerhans (IL). (DT) pancreas of diabetic rat treated with *M. oleifera* leaf solution @150mg/kg bwt. showing regenerated acini (AC) with mild restored islets. Bars = 20 µm.

Histopathological Examination of Kidney

In Figure 15, the histopathological investigation of the kidney from the C group stated a regular arrangement of glomerulus (G) surrounded by Bowman's capsule (BC). In the case of the diabetic control (T0) group, increased capsular space was seen. The MO extract given group (DT) demonstrated normal histoarchitecture of BC and G that was similar to the C group (Figure 16). Diabetic nephropathy is the gradual

development of renal failure due to hyperglycemia. Increasing levels of blood glucose and glycosylated protein along with elevated oxidative stress cause hemodynamic alterations in the renal tissue of alloxan-induced rats, which in turn cause impaired kidney function (Pansare *et. al.*, 2021). Kumari *et.al.*, (2021) revealed that alloxan-induced oxidative stress causes renal tissue destruction, most notably vacuolation in the Bowman's capsule and glomerulus, which is in contrast with our findings. Research directed by Teoh *et. al.*, (2010) discovered that the STZ-induced diabetic group had elevated mucopolysaccharide deposits in the PCT (proximal convoluted tubule), deteriorated glomerulus with thickened BM (basement membrane) in the renal tissue which were not found in our study. The histopathology of the current experiment revealed no abnormalities in the MO treated group. So, we hypothesize that the length of the treatment, dosage of the extracts, and method of extract preparation, management of the rats might be responsible for this outcome.

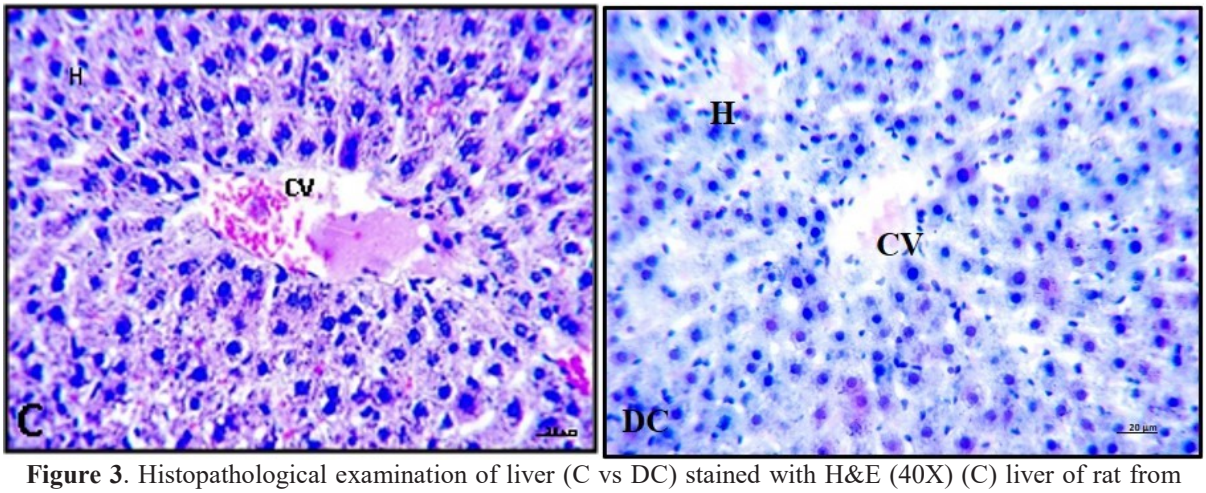

Figure 3. Histopathological examination of liver (C vs DC) stained with H&E (40X) (C) liver of rat from control group showing the regular arrangement of hepatocytes (H), central vein (CV) & sinusoids. (DT) liver of rat from alloxan-induced diabetic control group showing typical hepatocytes, hepatic cords with sinusoids. Bars = 20 µm.

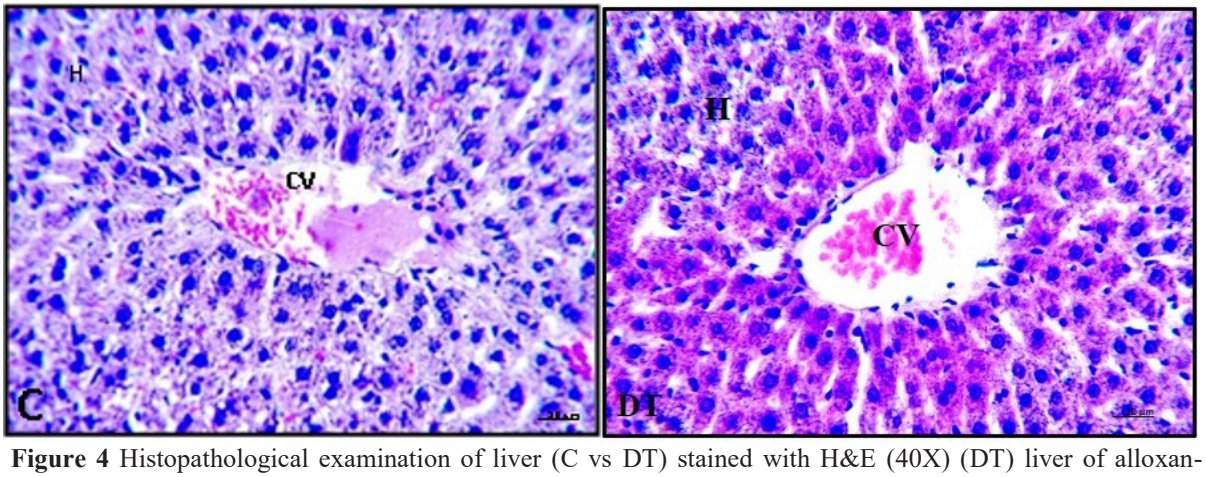

Figure 4 Histopathological examination of liver (C vs DT) stained with H&E (40X) (DT) liver of alloxan-induced diabetic rat treated with *M. oleifera* leaf extract @150mg/kg bwt. showing improved hepatic cords with distinct sinusoidal space. Bars = 20 µm.

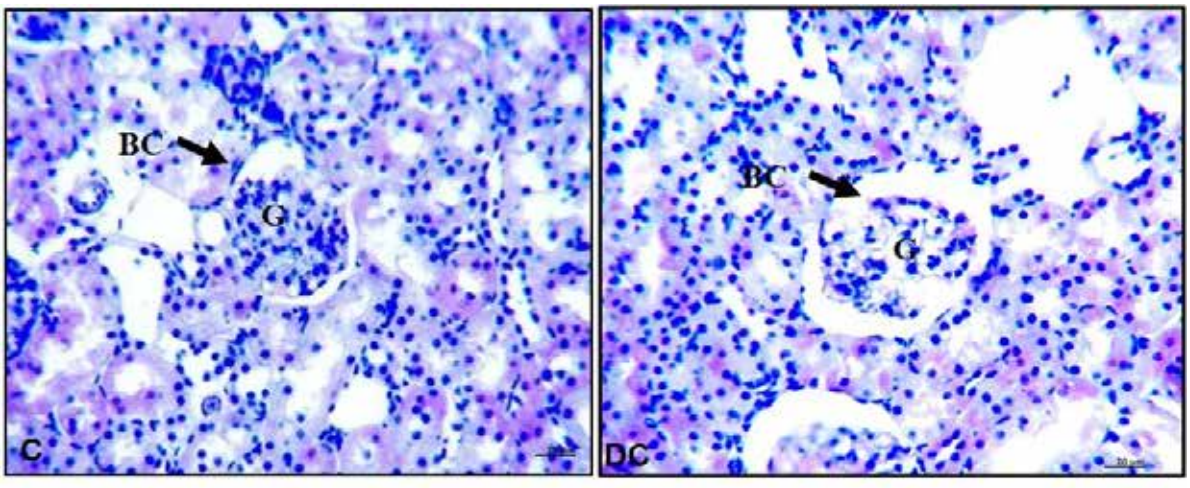

Figure 5. Histopathological examination of kidney (C vs DC) stained with H&E (40X) (C) kidney of rat from control group showing a normal structure of Bowman's capsule (BC) and glomerulus (G). (DC) kidney of rat from alloxan-induced diabetic control group exhibiting normal histological features of Bowman's capsule (BC) with glomerulus. Bars = 20 µm.

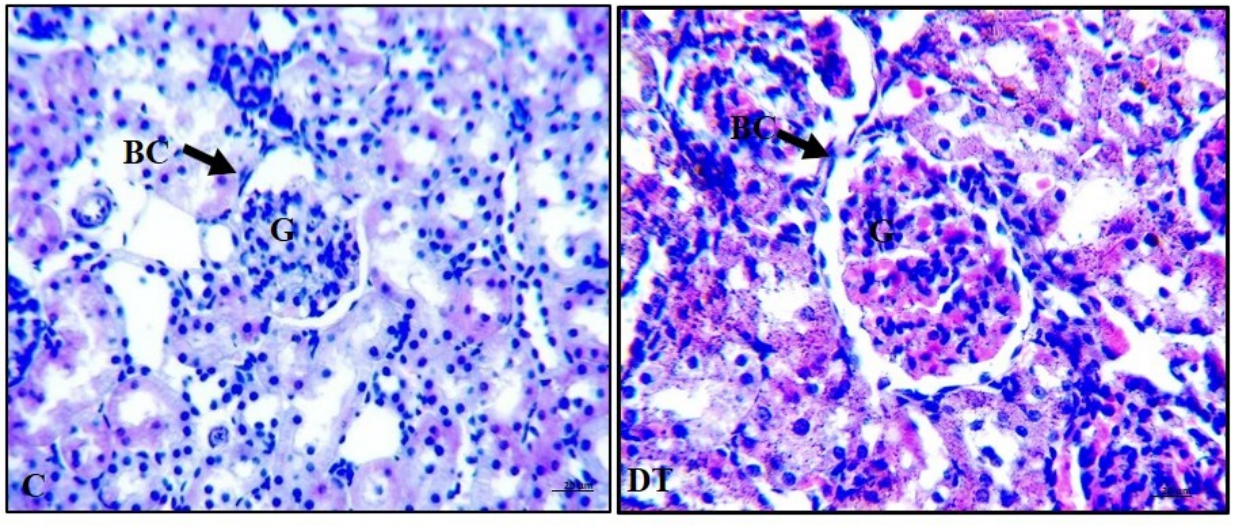

Figure 6. Histopathological examination of kidney (C vs DT) stained with H&E (40X) (DT) kidney of diabetic rat treated with *M. oleifera* leaf extract @150mg/kg bwt showing regular histoarchitecture of Bowman's capsule (BC) and glomerulus (G). Bars = 20 µm.`

CONCLUSION

In conclusion, the present study calls attention to the therapeutic use of MO in DM. The results of the current study demonstrated that MO was effective in lowering blood glucose, total blood cholesterol level with organ protective effects in alloxan induced rats. Further studies are necessary in terms of different doses and longer duration of MO to be considered as better therapeutic option for DM.

Conflict of interest:

The authors declared there is no conflict of interest.

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