

Original Research Article

Antidiabetic potential of *Moringa oleifera* Lam. leaf extract in type 2 diabetic rats, and its mechanism of action

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Abstract

Purpose: To explore the antidiabetic potential of *Moringa oleifera* leaf extract in type 2 diabetic rats, and the underlying mechanisms.

Methods: Streptozotocin (STZ) at a dose of 40 mg/kg was given to high fat diet (HFD)- fed rats to induce type 2 diabetes. *M. oleifera* leaf extract at doses 100, 200 and 400 mg/kg were given to 3 groups of type 2 diabetic rats. The area under curve (AUC) of glucose and homeostasis model assessment of insulin resistance (HOMA-R) were calculated using appropriate formulas, whereas levels of glucose, insulin, peroxisome proliferator activated receptor- γ (PPAR γ), dipeptidyl peptidase-IV (DPP-IV) and inflammatory cytokines (IL-6, IL-1 β and TNF α) were assayed using ELISA kits.

Results: The leaf extract of *M. oleifera* significantly reduced the levels of glucose, insulin and cytokines in treated type 2 diabetic groups ($p < 0.05$). DC group had significantly increased AUC for glucose, whereas the extract-treated groups showed significant decrease in glucose AUC. There was significant decrease in insulin sensitivity parameters, as indicated by increase in HOMA-R and decrease in PPAR γ levels in the DC group ($p < 0.05$). However, treatment with the *M. oleifera* extract reversed this trend via marked decrease in HOMA-R level and significant rise in PPAR γ level. In contrast, the extract had no effect on DPP-IV concentration in diabetic treated groups ($p < 0.05$).

Conclusion: These results indicate that *M. oleifera* leaf extract mitigates hyperglycemia in type 2 DM by modulating hyperinsulinemia, PPAR γ and inflammatory cytokines. Thus, the extract is a potential source of drug for the management of type 2 DM.

Keywords: *Moringa oleifera*, Diabetes mellitus, Streptozotocin, Peroxisome proliferator activated receptor- γ , Dipeptidyl peptidase IV

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INTRODUCTION

Type 2 diabetes mellitus (DM) is believed to be the most prevailing chronic metabolic disorder

with direct negative impact on the health of affected individuals. The 9th edition of International Diabetes Federation of Middle East and North Africa (IDF MENA) region reported

that in 2019, 426 million people were diagnosed with diabetes all over the world, out of which more than 55 million people are in the MENA region, and it has been projected that this number will increase to 108 million by 2045 [1]. It has been established that adipose tissue secretes biologically active mediators (adipocytokines or adipokines) which play an imperative role in the emergence of insulin resistance and type 2 DM [2].

PPAR γ belongs to nuclear receptor superfamily, and it regulates the expressions of adipokines. The activation of PPAR γ in visceral adipose tissue exerts inhibitory effect on inflammatory cytokine genes expression, thereby reducing the expressions of inflammatory cytokines such as IL-1 β , IL-6, and TNF α . Previous studies reported that the activation of PPAR γ improves insulin sensitivity [3].

The management of diabetes with minimum adverse effect is a huge challenge to clinicians. Numerous attempts have been made to manage hyperglycemia in diabetes with synthetic drugs, but the drugs used in clinical practice are either too expensive or have undesirable side effects or contraindications [4]. Plant-derived medicines have received great attention due to their minimum side effects and relative affordability, when compared to synthetic drugs [5]. Thus, there is need to discover medicines from natural sources which can be used as alternatives to synthetic drugs for type 2 DM. *Moringa oleifera* (*M. oleifera*) commonly known as drumstick tree belongs to the family *Moringaceae*. It is a fast-growing tree widely distributed in Red sea area (Jazan), Bangladesh, India, Pakistan, Afghanistan and parts of Africa where it is used in folk medicine [6]. The plant is cultivated on large scale for medicinal purposes in southern parts of Jazan. It is an edible plant with high nutritional and medicinal values, and it is used for the treatment of many diseases, hence the name "miracle tree" [7]. Phytochemical analyses of the leaves of *M. oleifera* have revealed that they are rich in minerals such as potassium, calcium, iron and phosphorous; vitamins A, C, D and E; amino acids and antioxidants such as flavonoids, β -carotene, and vitamin C [8]. Different parts of *M. oleifera* plant have been comprehensively studied in the treatment of various diseases such as typhoid fever, arthritis, malaria, swellings, skin diseases, parasitic diseases, hypertension, diabetes and liver disorder, and also for boosting the immune system in immuno-compromised patients, especially HIV/AIDS-related symptoms [9]. The traditional use of *M. oleifera* in diabetes is supported by previous published scientific studies on experimental animals [10]. Recently, it

was reported that *M. oleifera* leaf extract demonstrated antidiabetic and antioxidant effects in experimental animal models of diabetes [11]. However, previous studies on the antidiabetic potential of *M. oleifera* were focused on assessment of its efficacy, while its possible underlying mechanisms in type 2 DM are yet to be established. The traditional and medicinal values of *M. oleifera* which are supported by previous scientific findings formed the basis of the present research. Therefore, the current research was conducted to explore antidiabetic potential of the plant extract in a rat model of type 2 DM, and its underlying mechanisms.

EXPERIMENTAL

Animals

Male Wistar rats weighing 100 - 120g were procured from the Medical Research Centre (MRC), a research unit of Jazan University. The rats were separated into six groups, having 6 rats in each. Standard laboratory temperature of 25 °C was maintained throughout the experimental period. This research was approved by the ethical approval committee of Jazan University (approval no. 0107/307/1439) and accomplished according to international guidelines [12].

Collection of *M. oleifera* leaves

Moringa oleifera plant is grown for commercial purposes in Jazan region of Saudi Arabia. A local farmer was contacted to provide the leaves of *M. oleifera* for research purpose. The *M. oleifera* plant was identified based on the literature, ethical knowledge and relevant resources, and the leaves were collected from the plant.

Extraction of *M. oleifera* leaves

The leaves were washed with water and kept under shed to dry. The dried leaves were crushed to powder with a commercial blender, and the powdered material (1 kg) was extracted by percolation using ethanol (95 %). The filtrate was filtered and left to dry at room temperature for 7 days. The extract was further subjected to freeze-drying to form a concentrate. The dried extract (concentrate) was kept in airtight container at 4 °C. Prior to administration, the extract was suspended in 1% CMC in distilled water.

Chemicals and biochemicals (including kits)

Streptozotocin was purchased from Sigma Aldrich Pvt Ltd. ELISA kits for insulin, PPAR γ , DDP-IV, IL-1 β , IL-6 and TNF α were purchased

from MyBioSource, USA. All other chemicals and biochemicals from different manufacturers were purchased through authorized local suppliers in the Kingdom of Saudi Arabia.

Preparation of high fat diet (HFD)

HFD was prepared in the research laboratory using normal pellet diet (NPD), animal fat (lard), and a mixture of coconut oil and butter ghee. In this process, NPD (1000 g) was crushed into powder and blended with animal fat (20 %), coconut oil (10 %) and butter ghee (10 %). The mixture was then made into pellets and kept in a tray under shade to solidify. The dried pellet of HFD was stored in a refrigerator and given to groups II, III, IV and V rats in place of NPD for 4 weeks.

Standardization of STZ dose for induction of type 2 DM

Different doses of STZ were standardized to find a suitable dose for the induction of type 2 DM in HFD-fed rats. Streptozotocin (STZ) was dissolved in 0.1 M citrate buffer, pH 4.5. For this purpose, a pilot study was performed using 3 different doses of STZ (35, 40 and 45 mg/kg). The STZ was intraperitoneally (i.p) injected into 3 different groups of rats, and their fasting glucose levels were checked after 72 h. At a dose of 40 mg/kg, STZ increased blood glucose level above 200 mg/dL in all rats, clearly indicating development of type 2 DM. Therefore, 40 mg/kg was selected as the dose of STZ for induction of type 2 DM in HFD-fed rats.

Experimental model of type 2 DM

Streptozotocin (STZ) at a dose of 40 mg/kg was administered to groups II, III, IV and V rats to induce type 2 DM [13]. Fasting blood glucose level was determined after 72 h of STZ injection to confirm induction of type 2 DM. Rats with fasting glucose level ≥ 200 mg/dL were assumed to be diabetic.

Treatment of groups

Wistar rats (n=36) were divided into six different groups. Group I (normal control, NC) was administered normal saline (1 ml/kg, p.o) for 21 days. Group II (diabetic control, DC) received STZ injection (40 mg/kg, i.p) after 4 weeks of HFD. Group III (diabetic treated) was given *M. oleifera* extract (100 mg/kg, orally) for 21 days. Group IV (diabetic treated) received *M. oleifera* (200 mg/kg, orally) for 21 days. Group V (diabetic treated) received *M. oleifera* (400 mg/kg, orally) for 21 days. Group VI (*M. oleifera* treated only)

was given only *M. oleifera* (400 mg/kg, orally) for 21 days.

Blood collection and serum preparation

Blood was taken from the retro-orbital plexus of each rat after 72 h (to check induction of type 2 DM) and at the end of the study (after 21 days of *M. oleifera* dosing). The blood samples were collected in serum separation tubes and serum samples recovered after centrifugation were used for assay of biochemical parameters.

Biochemical assays

Glucose

Glucose level was estimated using assay kit which is based on the principle of glucose oxidase peroxidase [14].

Oral glucose tolerance test (OGTT)

The area under curve (AUC) of glucose was estimated using trapezoidal method [15]. The AUC was used for estimation of OGTT. Glucose solution (2 g/kg) was given to 8 h-fasted rats, and blood was withdrawn at the start (zero time) and subsequently at time intervals 30, 60 and 120 min. Glucose levels were determined in each blood sample. The AUC of glucose was calculated as shown in Eq 1.

$$AUC = [(fasting\ value + value\ at\ 30\ min) \times 0.25] + [(value\ at\ 30\ min + value\ at\ 60\ min) \times 0.25] + [(value\ at\ 60\ min + value\ at\ 120\ min) \times 0.5] \dots\dots\dots (1)$$

Insulin assay

Insulin levels were assayed with ELISA kit which works on simple sandwich ELISA principle. In this technique, standards and test samples (serum) were added to a 96-well pre-coated with mouse specific anti-insulin antibody. Then, monoclonal antibody labelled with horseradish peroxidase (HRP) was added to the micro-plate well and subjected to incubation, after which the micro-plate was washed to remove free insulin. The TMB substrate reagent was added, and the absorbance of the yellow colour formed was measured at 450 nm in an ELISA reader. The concentrations of insulin in test samples were estimated by extrapolating the OD values of the samples from a standard calibration curve.

Calculation of HOMA-R

Insulin resistance was calculated based on HOMA-R as shown in Eq 2 [16].

$$\text{HOMA-R} = \{(F_i \times F_g)/22.5\} \dots\dots (2)$$

where F_i and F_g are fasting insulin ($\mu\text{U/ml}$) and fasting glucose (mmol/l), respectively. The value of glucose in mg/dL was converted to mmol ($18 \text{ mg/dL} = 1 \text{ mmol}$).

Peroxisome proliferator activated receptor gamma (PPAR γ) assay

ELISA kit was used to measure PPAR γ in an assay based on simple sandwich ELISA principle. In this technique, standards and test samples (supernatants of abdominal adipose tissue homogenates) were added to a 96-well micro-plate pre-coated with mouse-specific PPAR γ antibody. Then, biotinylated detection antibody and horseradish peroxidase (HRP) conjugates were added to each well of micro-plate and left to incubate, after which the micro-plate was washed to remove unbound components. Then, the TMB substrate reagent was added, and the absorbance of the yellow colour formed was measured at 450 nm in an ELISA reader. The concentrations of PPAR γ in test samples were determined by extrapolating the OD values of the samples from a standard curve.

Dipeptidyl peptidase IV (DPP-IV) assay

The concentration of DPP-IV was estimated in serum using sandwich ELISA principle. In this technique, standards or samples were added to a micro-plate pre-coated with anti-DPPIV antibody. Then, biotin-conjugated detection antibody and horseradish peroxidase (HRP) were added to micro-plate and washed with wash buffer. Thereafter, TMB substrate was added, and the absorbance of yellow colour formed was measured at 450 nm in an ELISA reader. The concentrations of DPPIV in test samples were determined by extrapolating the OD values of the samples from a standard curve.

Assay of inflammatory cytokines (IL-1 β , IL-6 and TNF α)

ELISA kits were used to measure levels of IL-6, IL-1 β and TNF α . The assay is based on simple sandwich ELISA principle. In this technique, standards and test samples (serum) were added to a 96-well micro-plate pre-coated with antibodies for IL-6, IL-1 β and TNF α . Then, biotinylated detection antibody and horseradish peroxidase (HRP) conjugates were added to each well of micro-plate and subjected to incubation. After the incubation period, the micro-plate was washed to remove free/unbound components. Then, TMB substrate reagent was

added, and the absorbance of the yellow colour formed was measured at 450 nm using ELISA reader. IL-6, IL-1 β and TNF α concentrations in test samples were calculated by extrapolating the OD values of the samples from a standard curve.

Statistical analysis

The results were analyzed by one-way ANOVA using GraphPad Prism 8.3. Groups were compared with each other and differences observed were deemed to be statistically significant at $p < 0.05$.

RESULTS

Blood glucose level

Diabetic control (DC) group had significantly increased glucose level than that of NC group ($p < 0.001$), which is depicted in Table 1. However, treatment with extract of *M. oleifera* (100, 200 and 400 mg/kg) led to significant decreases in blood glucose levels in comparison to DC group ($p < 0.001$). However, no significant difference in blood glucose level between *M. oleifera* per se group and NC group was documented ($p > 0.05$).

Oral glucose tolerance test

A significant upsurge in AUC of glucose was observed in DC group, relative to NC group ($p < 0.001$) which is illustrated in Table 1. In contrast, there were significant decreases in glucose AUC in groups treated with *M. oleifera* extract at doses 100, 200 and 400 mg/kg ($p < 0.001$), relative to DC group. However, no significant variation in AUC of glucose was documented between *M. oleifera* alone and NC group ($p > 0.05$).

Table 1: Effect of *M. oleifera* leaf extract on fasting glucose and AUC of glucose in type 2 diabetic rats

Group	Treatment	Glucose level (mg/dL)	AUC of glucose
I	Normal control group	92.83 \pm 5.94	12.92 \pm 0.51
II	Type 2 diabetic control group	292.00 \pm 11.82 ^a	37.80 \pm 0.74 ^a
III	Type 2 diabetic treated group	231.67 \pm 9.34 ^y	28.10 \pm 0.82 ^y
IV	Type 2 diabetic treated group	194.00 \pm 5.44 ^y	23.25 \pm 0.65 ^y
V	Type 2 diabetic treated group	153.67 \pm 7.26 ^y	19.32 \pm 0.73 ^y
VI	<i>M. oleifera</i> per se group	87.83 \pm 4.06 ^{ns}	11.85 \pm 0.48 ^{ns}

^a $P < 0.001$ compared to NC; ^y $p < 0.001$ compared to DC; ^{ns} $p > 0.05$ compared to NC

Insulin levels

A significant increase in insulin level was observed in DC group, relative to NC group (Table 2). However, daily dosing with extract of *M. oleifera* (100, 200 and 400 mg/kg) reduced insulin levels significantly ($p < 0.01$, $p < 0.001$), in comparison to DC group. Insulin levels in the group given *M. oleifera* only and NC group were comparable ($p > 0.05$).

HOMA-R level

Table 2, HOMA-R level was markedly increased in DC group, relative to NC group as shown in Table 2. ($p < 0.001$). However, the elevated level of HOMA-R was reduced significantly ($p < 0.001$) after administration of extract (*M. oleifera*) at doses 100, 200 and 400 mg/kg, relative to the DC group. There was no significant variation in HOMA-R level between only *M. oleifera per se* group and the NC group ($p > 0.05$).

Table 2: Effect of *M. oleifera* leaf extract on insulin and HOMA-R levels in type 2 diabetic rats

Group	Treatment	Insulin level ($\mu\text{U/mL}$)	HOMA-R
I	Normal control group	5.44 \pm 0.32	1.25 \pm 0.10
II	Type 2 diabetic control group	10.03 \pm 0.27 ^a	6.71 \pm 0.41 ^a
III	Type 2 diabetic treated group	8.68 \pm 0.19 ^x	4.97 \pm 0.28 ^x
IV	Type 2 diabetic treated group	7.97 \pm 0.25 ^y	2.83 \pm 0.24 ^y
V	Type 2 diabetic treated group	7.25 \pm 0.31 ^y	1.14 \pm 0.09 ^y
VI	<i>M. oleifera per se</i> group	5.25 \pm 0.27 ^{ns}	54.66 \pm 1.56 ^{ns}

^a $P < 0.001$ compared to NC; ^x $p < 0.01$, ^y $p < 0.001$ compared to DC; ^{ns} $p > 0.05$ compared to NC

PPAR γ levels

PPAR γ level in adipose tissue was significantly reduced in DC group, in comparison to NC group ($p < 0.001$; Figure 1). However, daily administration of extract of *M. oleifera* led to significant increase in PPAR γ levels, in comparison to DC group ($p < 0.001$). In contrast, no significant variation in PPAR γ level was documented between *M. oleifera* alone and NC group ($p > 0.05$).

DPP-IV concentration

There was markedly higher concentration of DPP-IV in the DC group in comparison to NC group ($p < 0.01$; Figure 2). Daily administration of

M. oleifera leaf extract demonstrated no significant decrease in DPP-IV concentration at doses 100, 200 and 400 mg/kg, when compared to DC group ($p > 0.05$). In contrast, no significant variation in DPP-IV level was documented between *M. oleifera* alone and NC group ($p > 0.05$).

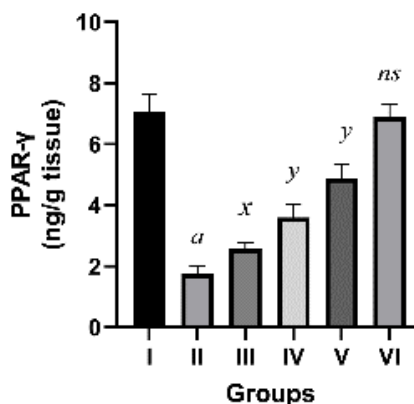


Figure 1: PPAR γ levels in the supernatant of adipose tissue homogenate in NC, DC and *M. oleifera* treated groups. ^a $p < 0.001$ versus NC; ^x $p < 0.01$, ^y $p < 0.001$ versus DC; ^{ns} $p > 0.05$ versus NC

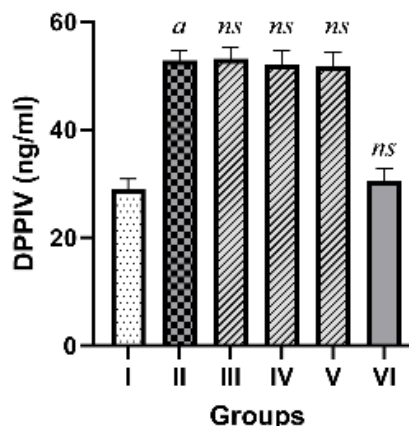


Figure 2: DPP-IV concentration in serum of NC, DC and *M. oleifera* treated groups. ^a $P < 0.001$ versus NC; ^{ns} $p > 0.05$ versus DC and NC

Cytokines (IL-6, IL-1 β and TNF α) levels

The levels of IL-6, IL-1 β and TNF α were augmented significantly in DC group, when compared to NC group (Figure 3, Figure 4 and Figure 5). However, following daily administration of *M. oleifera* extract (100, 200 and 400 mg/kg), there were marked decrease in the levels of these cytokines (IL-6, IL-1 β and TNF α), relative to the DC group ($p < 0.001$). However, no significant variation in levels of IL-6, IL-1 β and

TNF α were documented between *M. oleifera* alone and the NC group ($p > 0.05$).

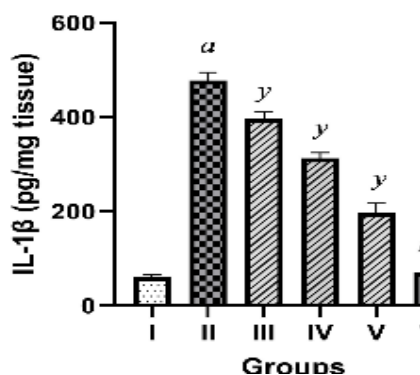


Figure 3: Levels of IL-1 β in NC, DC and *M. oleifera* treated groups. ^a $P < 0.001$ versus NC; ^y $p < 0.001$ versus DC; ^{ns} $p > 0.05$ versus NC

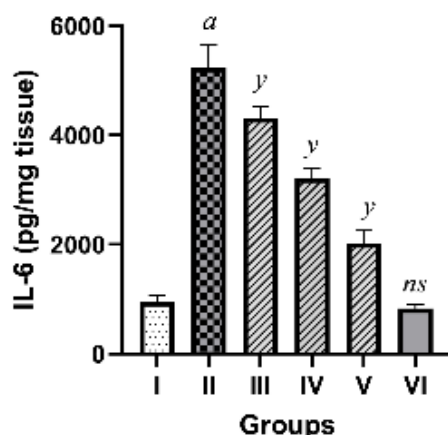


Figure 4: Levels of IL-6 in NC, DC and *M. oleifera* treated groups. ^a $P < 0.001$ versus NC; ^y $p < 0.001$ versus DC; ^{ns} $p > 0.05$ versus NC

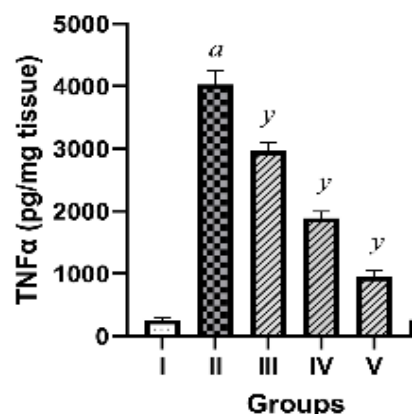


Figure 5: Levels of TNF α in NC, DC and *M. oleifera* treated groups. ^a $P < 0.001$ versus NC; ^y $p < 0.001$ versus DC; ^{ns} $p > 0.05$ versus NC

DISCUSSION

Type 2 DM is the most prevalent form of diabetes: it accounts for more than 90 % of all diabetes cases throughout the world. It is characterized by persistent hyperglycemia, which is associated with reduced secretion of insulin and/or decreased insulin sensitivity in target tissues that leads to development of insulin resistance. Continuous and prolonged hyperglycemia in diabetes is generally associated with microvascular and macrovascular complications [17]. Previous research findings have indicated that low-dose STZ injection to high fat diet-fed animals produced marked hyperglycemia and poor glycemic control, as evidenced in increased levels of HbA1c and impairment of glucose tolerance [13].

The present study has demonstrated that low-dose STZ administration produced sustained hyperglycemia in HFD-fed Wistar rats. However, administration of *M. oleifera* extract for 21 days significantly reduced glucose level, which clearly indicates antihyperglycemic effect. These findings are supported by previous published research asserting that *M. oleifera* leaf extract significantly reduced glucose levels in type 2 DM [18]. However, the study did not establish the mechanism underlying antihyperglycemic effect of the extract. Hence, the present study was performed to investigate and establish the potential mechanism underlying antihyperglycemic effect of *M. oleifera* in a rat model of type 2 DM.

If left untreated, sustained hyperglycemia may lead to impairment of glucose tolerance (IGT) which eventually results in type 2 DM. It has been suggested that impairment of glucose tolerance is due to decreased utilisation of glucose by peripheral tissues [19]. An investigation of OGTT is usually carried out to determine whether the patient has IGT, especially when the fasting blood glucose level is within the normal range. The present study revealed that AUC of glucose was significantly higher in type 2 DC group subjected to OGTT by administration of glucose solution (2 g/kg). However, administration of *M. oleifera* leaf extract led to significant reduction in AUC of glucose after an OGTT.

Previous research studies have demonstrated that obesity is correlated with insulin resistance (IR) in diabetic patients [20]. Insulin resistance is a condition in which normal concentration of insulin is unable to bind to insulin receptors in liver, muscle and fat cells, leading to inability to

accomplish adequate biological response in diabetic patients. When insulin resistance-like situation arises, the pancreatic β -cells compensate by secreting high levels of insulin to control hyperglycemia, resulting in hyperinsulinemia [21]. The results of this study clearly established significant hyperinsulinemia in type 2 DC group. However, daily administration of *M. oleifera* extract for 21 days significantly prevented hyperinsulinemia by reducing insulin levels. In the present study, it was assumed that the extract may ameliorate insulin resistance. Therefore, HOMA-R, an index for IR was calculated. The results indicated significant augmentation in HOMA-R level in type 2 DC group. However, *M. oleifera* leaf extract markedly reduced the level of HOMA-R.

In addition to IGT, there is an association between hyperinsulinemia and IR in type 2 DM. This is likely due to excessive secretion of insulin from pancreatic β -cells and downregulation of insulin receptor or dysfunction in post-receptor signalling. Previous research findings indicate that nuclear PPAR- γ modulation demonstrates a significant role in controlling hyperglycemia in type 2 DM [22]. Clinicians usually prescribe PPAR- γ agonists which act by improving the sensitivity of insulin towards its receptor and also by enhancing the recruitment of glucose transporters. In the present study, it was observed that PPAR- γ level was significantly reduced in type 2 DC group. However, *M. oleifera* leaf extract administration for 21 days significantly increased PPAR- γ levels in abdominal adipose tissue.

Dipeptidyl peptidase IV is a transmembrane glycoprotein which inactivates incretin hormones such as GLP-1 and GIP. These hormones maintain glucose homeostasis by stimulating insulin secretion and inhibiting glucagon secretion. Numerous inhibitors of DPP-IV are commercially available for treating type 2 DM [23]. In recent years, researchers have made efforts to identify new and effective anti-DPP-IV drugs from natural sources [24]. Therefore, the inhibitory potential of *M. oleifera* leaf extract on DPP-IV was investigated. The results revealed that the concentration of DPP-IV was significantly increased in type 2 DC group. However, *M. oleifera* leaf extract administration for 21 days did not produce any significant decrease in the concentration of DPP-IV. Thus, *M. oleifera* leaf extract had no inhibitory effect on DPP-IV.

It has been reported that inflammatory cytokines demonstrate significant role in IR pathogenesis via impairment in insulin receptor substrate (IRS) phosphorylation [25].

It has also been established by previous researchers that inflammatory cytokines stimulate I-kappa-beta kinase- β and I-kappa- α , thus facilitating insulin resistance by activating nuclear factor kappa- β (NFk β) [26]. In a previous work, it was reported that activation of inflammatory pathway increased the expressions of IL-6, IL-1 β and TNF- α and established an IR-like situation [20]. The present study revealed significant rise in levels of IL-6, IL-1 β and TNF- α in type 2 DC group. However, daily administration of *M. oleifera* extract significantly reduced elevated levels of these cytokines, thereby exerting anti-inflammatory properties.

CONCLUSION

This study has demonstrated that *M. oleifera* leaf extract reduces the levels of blood glucose, AUC of glucose, insulin and inflammatory cytokines (IL6, IL-1 β & TNF α) in type 2 diabetic rats. Moreover, the extract reduces HOMA-R values and improves PPAR γ levels, but has no significant inhibitory effect on DPP-IV. These findings suggest that *M. oleifera* leaf extract exerts antihyperglycemic effect through a mechanism involving modulation of hyperinsulinemia, PPAR γ and inflammatory cytokines, and could therefore be developed for the management of diabetes mellitus.

DECLARATIONS

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Disclosure statement

No conflict of interest is associated with this research.

Author's contribution

We declare that this work was carried out by Tarique Anwer, Mohammed M Safhi, Hafiz A Makeen, Saeed Alshahrani, Rahimullah Siddiqui, SM Sivakumar, Emad S Shaheen, and Mohammad Firoz Alam, and all responsibilities pertaining to claims relating to the content of this article will be borne by the authors.

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