

**Effect of *Moringa oleifera* Extract on Serum Glucose and Interleukin-1, Interleukin-2 and Tumor Necrosis Factor  $\alpha$  in Streptozotocin-Induced Diabetic Rats.**

**Siham A.Wadee<sup>(1)</sup>, Entedar R. Sarhat<sup>(2)</sup>, Rajaa S. Najim<sup>(3)</sup>**

<sup>(1)</sup> Dept. of Pharmacology, Collage of Veterinary Medicine, Tikrit University.

<sup>(2),(3)</sup> Dept. of Basic Science, Dentistry Collage, Tikrit University

**Abstract:**

**Background:** Diabetes Mellitus presents a major challenge to healthcare systems around the world. One of the outcomes of diabetes is oxidative stress that is caused by the effect of hyperglycemia. Recent studies indicate that, oxidative agents in diabetes result in many complications such as cardiovascular disease, nephropathy, retinopathy and neuropathy.

**Aim of the study:** This study was designed to evaluate the effect of *Moringa oleifera* (MOE), in Streptozotocin (STZ)-induced diabetes in wistar rats.

**Methods:** The rats were randomly divided into three groups of 10 animals each: Group I, healthy control; Group II, diabetics; Group III, diabetics treated with MOE (100 mg/ kg) for 12 weeks. Diabetes was induced by a single intra-peritoneal injection of Streptozotocin (STZ, 65 mg/kg). Finally, blood samples were collected and analyzed for glucose and interleukin-1 (IL-1), interleukin-2 (IL-2), and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ).

**Results;** result revealed that, oral administration of MO extract caused a significant decrease in serum glucose IL-1, and TNF- $\alpha$  levels ( $183 \pm 41.10$  mg/dl), ( $25.7 \pm 1.5$  pg/ml), ( $10.7 \pm 2.0$  pg/mL) respectively, whereas increased in IL-2 ( $5.0 \pm 1.92$  U/mL) in diabetic rats treated with MO extract when compared to levels of diabetic and control groups ( $298.2 \pm 8.09$  mg/dl), ( $97.24 \pm 3.21$  pg/ml), ( $13.4 \pm 2.21$  pg/mL), and ( $4.08 \pm 1.17$  U/mL) respectively.

**Conclusions:** It appears that, *Moringa oleifera* extract had both antihyperglycemic and decreased the activity of IL-1, and TNF- $\alpha$ , and increased IL-2 on STZ-induced diabetic rats. Further studies are needed to determine its protective effects on the other diabetes complications.

**Keywords:** Diabetes Mellitus, *Moringa oleifera* Extract, Interleukin-1,2, Tumor Necrosis Factor.

## 1. Introduction

Today, Diabetes mellitus (DM) is a major worldwide health problem leading to markedly increase mortality and serious morbidity. Every 30 seconds, one person in the world loses a leg due to lack of awareness of diabetes and diabetes controls<sup>(1)</sup>. Its categorized by persistent hyperglycemia, glucosuria and polyuria<sup>(1)</sup>.

Cytokines are small polypeptides with a wide range of inflammatory, metabolic and immunomodulatory properties. They are manufactured by macrophages, lymphocytes, monocyte, dendritic cells, neutrophils, endothelial cells and fibroblasts. Cytokines are the mean of communication between immune and non-immune cells<sup>(3)</sup>.

*Moringa oleifera* Lam. (*M. oleifera*) is belonging to the family Moringaceae. It is commonly called drumstick or horseradish tree, which is a widespread growing plant in tropical and subtropical areas<sup>(4)</sup>.

The *Moringa* plant provides a rich and rare combination of zeatin, quercetin,  $\beta$ -sitosterol, caffeoylquinic acid and kaempferol. Various preparations of *M. oleifera* exhibited antioxidant, antibiotic, hypotensive, anti-ulcer, anti-inflammatory and anti-cancer properties<sup>(5)</sup>.

Considering the antihyperglycemic properties of MOE, this study was designed to evaluate the effects of MOE extract in streptozotocin-induced diabetes of rats. In this context, green tea can rightly be mentioned as a plant of considerable interest.

Adipose tissue macrophages are responsible for almost all adipose tissue tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) expression and significant amounts of interleukin-6 (IL-6). Concentrations of acute-phase response markers and mediators of inflammation such as cytokines like TNF- $\alpha$  and interleukin-6 are also raised in people with T2DM. This finding has led to the suggestion that raised concentrations of pro-

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inflammatory cytokines and the resultant acute-phase response may underlie much of the metabolic clustering due to obesity and diabetes mellitus<sup>(6)</sup>.

This study examined the hypothesis that MOE supplementation decreases levels of TNF- $\alpha$ , IL-6, MCP-1, and

hyperglycemia in diabetes. To examine this hypothesis, we studied the effect of MOE supplementation on blood levels of TNF- $\alpha$ , IL-6, MCP-1, glucose, glycosylated hemoglobin, and oxidative stress in streptozotocin-treated diabetic rats

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**Materials and Methods:-**

**Preparation of Moringa oleifera extract.**

The air-dried powdered aerial parts of Moringa oleifera (2 kg) were extracted by cold percolation with 95 % ethanol (3 x 4 L) till exhaustion. The ethanol extract was concentrated under reduced pressure to give 250 g of a brown residue.

The present study was done during the period from June 2017 to January 2018. on healthy adult albino male rats in the weight range of (150–200 gm), selected from an inbred group housed in specially designed cages and maintained under standard conditions

of temperature (23±10C) and humidity of (55– 60%) with a 12-hour light and 12-hour dark cycle for at least one week before use.

The animals were divided into three main groups (10 rats for each):

Group I: Non-diabetic control rats.

Group II: Diabetic control rats

Group III: Diabetic rats treated with 100 mg/Kg MO extract.

Serum glucose levels were determined enzymatically using standard methods by autoanalyzer SA1000. serum sample was analyzed for s IL-1, IL-2, TNF- $\alpha$

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levels using enzyme linked immune sorbent assay (ELISA).

Diabetes was induced by intravenous injection of streptozotocin (Sigma, St. Louis, Mo, USA) into the tail vein at a dose of 65 mg/kg body weight. STZ was extemporaneously dissolved in

0.1 M cold sodium citrate buffer, pH 4.5. Diabetes was confirmed through the measuring of fasting blood glucose levels 4 days after STZ injection from tail vein. Rats with fasting blood glucose  $\geq$  250 mg/dL with glycosuria were considered diabetic<sup>(7,8)</sup>.

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## Results

In the present study, the mean serum level of group II, TNF- $\alpha$ , IL-1 (298.2  $\pm$  8.09 mg/dl), (97.24  $\pm$  3.21 pg/ml), (13.4  $\pm$  2.21 pg/mL) respectively were significantly higher ( $P < 0.001$ ) than group I (control group) (152.16  $\pm$  12.63mg/dl), (5.29  $\pm$  1.50 pg/ml), (3.77  $\pm$  0.91pg/mL).

On the other hand, there is significant decrease in FBG, TNF- $\alpha$ , IL-1 in group III (183  $\pm$  41.10mg/dl), (25.7  $\pm$

1.5 pg/ml), (10.7  $\pm$  2.0pg/mL) versus group II (298.2  $\pm$  8.09mg/dl), (97.24  $\pm$  3.21pg/ml), (13.4  $\pm$  2.21pg/mL) respectively .

In this study, a negligible level of IL-2 (4.08  $\pm$  1.17 U/mL) was recorded in group II versus group I (7.22  $\pm$  2.028 U/mL), While, there is significant increase in IL-2 in groups III (5.0  $\pm$  1.92 U/mL), versus group II (4.08  $\pm$  1.17U/mL).

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**Table 3 - Effect of the orally administered Moringa oleifera extract on biochemical parameters .**

Groups	Fasting blood glucose (mg/dl)	TNF- $\alpha$ (pg/ml)	IL-1(pg/mL)	IL-2 (U/mL)
Group 1	152.16 $\pm$ 12.63	5.29 $\pm$ 1.50	3.77 $\pm$ 0.91	7.22 $\pm$ 2.028
Group 2	298.2 $\pm$ 8.09	97.24 $\pm$ 3.21	13.4 $\pm$ 2.21	4.08 $\pm$ 1.17
Group 3	183 $\pm$ 41.10	25.7 $\pm$ 1.5	10.7 $\pm$ 2.0	5.0 $\pm$ 1.92

P < 0. 001

### Discussion

The proinflammatory cytokine production is elevated in diabetes and in cases of elevated lipids. Diabetes induced abnormalities in fatty acid metabolism have the potential to influence macrophage cytokine release inducing upregulation of proinflammatory cytokines <sup>(9,10)</sup>. The proinflammatory cytokines IL-1, IL-2, and TNF- $\alpha$  may play important roles alone or in combination in the pathogenesis of DM <sup>(11,12)</sup>. TNF- $\alpha$  have been also determined as markers of the inflammatory response <sup>(13)</sup>. As well as higher glucose levels, increased TNF- $\alpha$  expression and enhanced superoxide production observed in the DM compared to the control. O<sub>2</sub><sup>-</sup> is known to quickly react with NO, and leads to the formation of ONOO- (peroxynitrite), which in turn is responsible for reducing NO biological

activity and decreasing vasodilation <sup>(14,15)</sup>.

The degree of effect of MO extract is different for different cytokines because different cytokines are regulated by a number of complex signal-transduction pathways. It is difficult to provide any specific explanation for varying effect of MO extract on different cytokines. The inhibitory effect of MO extract on proinflammatory cytokine inhibition may be mediated either by oxidative stress-dependent or -independent pathways <sup>(16,17)</sup>. It is possible that different cytokines are influenced to different degrees by oxidative stress or glycosylation of proteins caused by high glucose or diabetes, which may explain why the magnitude of the protective effect of MOE was quite different among the various cytokines in both cell-culture and rat studies.

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The current study concluded that, MO supplementation has the potential to, reduce the blood levels of proinflammatory cytokines, and thereby inhibit the pathogenesis of vascular inflammation in diabetes. No clinical trial has been done to determine whether MO supplementation can indeed delay or prevent diabetes-associated complications. The evidence that MO can inhibit markers of vascular inflammation must be explored at the clinical level to see whether MO can reduce levels of proinflammatory cytokines in the diabetic patient population. If so, then MO supplementation could be used as an adjuvant therapy for reduction of

vascular inflammation and CVD in diabetes. Further studies should be carried out to determine the protective effects of MO on diabetes complications.

**Conclusion:**

It appears that, Moringa olifera extract had both antihyperglycemic and decreased the activity of IL-1, and TNF- $\alpha$  , and increased IL-2 on STZ-induced diabetic rats. Further studies are needed to determine its protective effects on the other diabetes complications.

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