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Some Physiological Effects of Momordica charantia and Trigonella foenum-graecum Extracts in Diabetic Rats as Compared with Cidophage®

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***Abstract*—**This study was conducted to evaluate the anti-diabetic properties of ethanolic extract of two plants commonly used in folk medicine, Mormodica charantia (bitter melon) and Trigonella foenum-graecum (fenugreek). The study was performed on STZ-induced diabetic rats (DM type-I). Plant extracts of these two plants were given to STZ diabetic rats at the concentration of 500 mg/kg body weight ,50 mg/kg body weight respectively. Cidophage® (metformin HCl) were administered to another group to support the results at a dose of 500 mg/kg body weight, the ethanolic extracts and Cidophage administered orally once a day for four weeks using a stomach tube and; serum samples were obtained for biochemical analysis. The extracts caused significant decreases in glucose levels compared with diabetic control rats. Insulin secretions were increased after 4 weeks of treatment with Cidophage® compared with the control non-diabetic rats. Levels of AST and ALT liver enzymes were normalized by all treatments. Decreases in liver cholesterol, triglycerides, and LDL in diabetic rats were observed with all treatments. HDL levels were increased by the treatments in the following order: bitter melon, Cidophage®, and fenugreek. Creatinine levels were reduced by all treatments. Serum nitric oxide and malonaldehyde levels were reduced by all extracts. GSH levels were increased by all extracts. Extravasation as measured by the Evans Blue test increased significantly in STZ-induced diabetic animals. This effect was reversed by ethanolic extracts of bitter melon or fenugreek.

***Keywords*—**Cidophage®, Diabetic rats, Mormodica charantia, Trigonella foenum-graecum

I. Introduction

DIABETES mellitus (DM) is a syndrome of disturbed energy homeostasis caused by the abnormal metabolism of carbohydrates, proteins and fats. It is the most common endocrine-metabolic disorder worldwide [1] among these natural products.

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Fenugreek seed (*Trigonella foenum-graecum L.*) has been shown to reduce glucose levels in type 2 diabetes and may help do so in type 1 (insulin-dependent) diabetes [3] *Momordica charantia*, also referred to as *bitter melon* or bitter gourd, is commonly known as vegetable insulin and has been used as a traditional anti-diabetic remedy for many years [4].

Most prior studies with these plants were focused on their actions on hyperglycemia and/or insulin metabolism. However, their effects on peripheral circulation and vascular pathology are still unclear. Thus, the present study was undertaken to investigate the effects of extracts of these plants on vascular permeability in peripheral circulation and their effects on nitric oxide and oxidative stress in rats with streptozotocin-induced diabetes.

II. Materials and Methods

A. Experimental Animals

Male Sprague-Dawley rats weighing 200 to 220 gm were housed in the Physiology Department, Faculty of Veterinary, Mansoura University. Animals were left for one week to acclimatize. Rats were kept six rats per cage and were provided with a standard diet and water *ad libitum*.

B. Streptozotocin-Induced Diabetic Animal Model

The induction of diabetes was performed using the diabetogenic compound streptozotocin (STZ) [5]. In our study, a single dose of 50 mg/kg of streptozotocin STZ (Sigma Chemical Company, St. Louis, Missouri) in 0.1 M citrate buffer (0.1 M citric acid, 0.1 M trisodium citrate, pH 4.5) was administrated intraperitoneally in a total volume of 1.0 ml. After 3 days of STZ injection, blood samples were taken from tip of the tail and hyperglycemia was confirmed by measuring blood glucose levels directly using a glucometer (One-Touch technology, Roche Group, UK). Animals showing fasting blood glucose levels higher than 250 mg/dl were considered diabetic and were included in the study. Treatment was started after 3 days of induction of diabetes and continued for 4 weeks.

Animals were divided into the following groups (6 rats each):

- Group (1): healthy rats that served as normal controls
- Group (2): rats that received STZ only and served as diabetic controls
- Group (3): diabetic rats that received daily doses of 500 mg/kg BW Cidophage®
- Group (4): diabetic rats that received daily doses of fenugreek ethanolic extract (50 mg/kg BW),
- Group (5): diabetic rats that received daily doses of bitter melon ethanolic extract (500 mg/kg BW)

N.B.: (All groups received their doses by stomach tube)

C. Preparation of the Ethanolic Extracts

Bitter melon fruits were cultivated at the Faculty of Agriculture-Mansoura University. Fenugreek was purchased from local commercial sources in Mansoura city. A total of 250 g of either ground dry fenugreek seeds or ground bitter melon was extracted with 1.0 L of 95% ethanol for 5 days. The extract was evaporated to dryness in a rotavapor (Air Blow Equipment, Chennai, India) at 40–50°C under reduced pressure. A semisolid material was obtained (15–20 g) and stored at 0–4°C until use. When needed, the residual extract was resuspended in distilled water and used in the study at the previously stated concentrations.[6]

D. Blood Sampling

Four weeks after STZ injection, food was withdrawn for 12 hours. The fasting animals were sacrificed and blood samples were collected into clean centrifuge tubes. The blood samples were allowed to coagulate and were centrifuged at 3000 rpm for 20 minutes to separate the blood serum. Separated serum was stored at -20°C for subsequent biochemical analyses.

E. Biochemical Analyses

Serum glucose levels were determined according to a method described by [7] Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to the method of [8] The determination of serum creatinine was performed according to [9]. Serum cholesterol and HDL-cholesterol levels were determined according to the method of [10]. Determination of serum triglycerides was performed according to [11]. Serum LDL-cholesterol was determined according to [12]. Determination of serum reduced glutathione (GSH) was performed according to [13]. Determination of nitric oxide (NO) was performed according to the method of [14]. Serum lipid peroxide (Malondialdehyde) was determined according to [15]. Serum insulin was determined by automated insulin immunoassay using an Elecsys autoanalyzer (Roche Diagnostics Mannheim, Germany) according to the manufacturer's instructions [16].

F. Measurements of Microvascular Permeability (Evans Blue Assay)

The Evans blue (EB; a tetrasodium diazo salt) extravasation test was used to measure vascular permeability [17]. Evans blue (20 mg/kg) was injected into the caudal vein, where it rapidly bound to plasma albumin. After 10 minutes, animals were killed and samples of dorsal skin were taken for determination of the extravasated Evans blue. Half of the skin sample was dried at 60°C for 24 hours, and a dry/wet weight ratio was calculated to avoid underestimation of EB dye concentration due to local edema. The other half was placed in a formamide solution (4 mL/g wet tissue) for 24 h for dye extraction. The extracted amount of EB dye was determined by spectrophotometry at 620 nm using a 96-well microplate photometer. The concentration of EB was then calculated from a standard curve and expressed as µg of EB per g of dry tissue [18].

G. Wound Creation

Wounds were created three days after the induction of diabetes. Under anesthesia, the backs of all the rats were shaved and skin wounds were prepared (2.5 cm in diameter and ~ 0.1 mm deep) [19]. Animals were sacrificed at days 3 and 28 after wound creation. Skin samples were excised and fixed in 10% formalin. Slides were stained with hematoxylin and eosin (H&E) for examination by light microscopy.

H. Histopathological Analysis

Pancreas and skin wound tissues from each rat were fixed overnight in 10% buffered formalin solution and embedded in paraffin. Sections (4 µm) were prepared and stained with H&E.

I. Statistical Analysis

Data were analyzed by analysis of variance using the general linear model procedure in SAS (SAS Institute, 2004) [20].

III. Results

A. Serum Glucose Levels

Serum glucose levels were significantly increased in the diabetic group when compared with the control group. Four weeks post treatment, glucose levels were significantly decreased in all treated groups in the following order of effectiveness: Cidophage®, bitter melon, and fenugreek (Table I).

TABLE I
EFFECTS OF DIFFERENT TREATMENTS ON THE INDICATED BIOCHEMICAL PARAMETERS

Group	Glucose mg/dl	Insulin uU/mL	AST IU/L	ALT IU/L	Creatinine Mg/dL
Normal control	98.33 ± 4.84^{d}	5.29±0.005^{a}	30.66 ± 1.20^{d}	8.33 ± 1.86^{e}	0.54 ± 0.08 ^{c}
Diabetic control	611.33 ± 7.26 ^{a}	2.11±0.06^{e}	81 ± 2.08^{a}	38.33 ± 2.60^{a}	1.56 ± 0.09 ^{a}
Cidophage®	106.33 ± 4.80 ^{d}	4.61±0.16^{b}	31 ± 2.08^{d}	12 ± 0.58^{de}	0.61 ± 0.01 ^{c}
Fenugreek	130.33 ± 6.48 ^{c}	4.18±0.04^{c}	35.33 ± 0.33^{cd}	13.66 ± 0.67^{cd}	0.6 ± 0.06 ^{c}
Bitter melon	115 ± 7.09^{cd}	4.50±0.17^{b}	32 ± 3.61^{d}	13 ± 1.15^{cd}	0.57 ± 0.03 ^{c}
LSD	19.82	0.36	7.015	4.289	0.1666

Values are mean ±S.E. Values denoted by different letters in each column were significantly different at (P< 0.05).

B. Serum Insulin levels

Serum insulin levels were significantly decreased in the diabetic group compared to the control group. Treatment with Cidophage® and bitter melon significantly increased insulin levels, and there was no significance difference between the diabetic group and the fenugreek group (Table I).

C. Liver Enzymes

After four weeks of treatment, both AST and ALT levels were significantly decreased by the extracts, with the order of effectiveness being Cidophage®, bitter melon, and fenugreek (Tables I).

D. Serum Creatinine Level

There was a significant increase in creatinine levels in the diabetic group. Four weeks post-treatment, creatinine levels were reduced by bitter melon, Cidophage®, and fenugreek, in that order (Table I).

E. Serum Lipid Profile

There were significant increases in the levels of serum cholesterol, triglycerides and LDL and significantly decreased HDL levels in the diabetic group, indicating disrupted lipid metabolism. Four weeks post treatment, cholesterol, triglycerides, and LDL levels were reduced significantly in the following order: bitter melon, Cidophage®, and fenugreek (Table II). HDL levels were significantly increased by all treatments in the following order of effectiveness: bitter melon Cidophage®, and fenugreek (Table II).

F. Free Radicals and Antioxidants

Significantly increased serum nitric oxide and malonaldehyde levels and significantly decreased reduced glutathione levels were observed in the diabetic group. Four weeks post treatment, serum nitric oxide and malonaldehyde levels were significantly reduced by all compounds in the following order: bitter melon, Cidophage®, and fenugreek (Table III). Reduced glutathione levels were increased by all treatments in the order fenugreek, bitter melon, and Cidophage® (Table III).

TABLE II
EFFECTS OF DIFFERENT TREATMENTS ON LIPID PROFILE PARAMETERS

Group	Cholesterol mg/dl	Triglycerides mg/dL	LDL Mg/dL	HDL Mg/dL
Normal control	114.33 ± 4.37 [b]	119 ± 2.08[b]	48.53 ± 4.65 [b]	42 ± 0.90 [a]
Diabetic control	204.67 ± 5.49 [a]	245.67 ± 6.39[a]	141.51 ± 6.23 [a]	14.03 ± 2.04 [d]
Cidophage®	97.33 ± 4.91[cd]	111 ± 5.29[bc]	34.61 ± 4.46 [c]	40.53 0.63[ab]
Fenugreek	106 ± 4.04[bcd]	118 ± 4.16[b]	45.10 ± 4.68[cbd]	37.3 ± 1.48[bc]
Bitter melon	93.67 ± 2.85 [d]	105 ± 2.65[c]	31.18 ± 3.28 [d]	41.48 ± 1.02 [a]
LSD	13.183	12.535	14.35	3.835

Values are mean ±S.E. Values denoted by different letters in each column were significantly different at (P< 0.05).

G.Level of Vascular Permeability

There was a significant increase in vascular permeability in the diabetic group compared to the normal group. Four weeks post treatment, EBET levels were significantly reduced by all compounds in the following order: Cidophage®, bitter melon and fenugreek (Fig. 1).

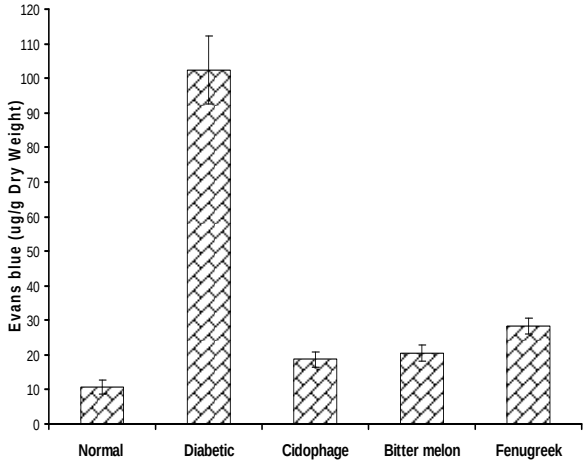

Fig. 1 Vascular permeability as indicated by Evans Blue skin assay

TABLE IV
FOLD CHANGE OF THE STUDIED BIOCHEMICAL PARAMETERS FROM THE CONTROL VALUES AFTER THE DIFFERENT TREATMENT

	STZ	Cidophage®	Bitter melon	Fenu greek
Glucose	6.22	1.08	1.17	1.33
Insulin	0.40	0.87	0.86	0.79
AST	2.64	1.01	1.04	1.15
ALT	4.60	1.44	1.56	1.64
Creatinine	2.91	1.13	1.06	1.11
Cholesterol	1.79	0.85	0.82	0.93
Triglycerides	2.06	0.93	0.88	0.99
HDL	0.33	0.97	0.99	0.89
LDL	2.92	0.71	0.64	0.93
GSH	0.49	0.99	0.97	0.96
NO	20.18	2.10	2.33	2.43
Permeability	9.60	1.75	1.94	2.66

H. Histopathology of the Pancreas

The histological structures of the pancreases of the normal controls (Fig. 2A) showed predominant exocrine pancreatic tissue composed of acini with draining ductules. Moreover, each islet was separated from the acini by a reticular membrane and was arranged in an anastomosing cellular plate or in cords of α cells, β cells, D cells and F cells. The pancreatic islets of diabetic rats (Fig. 2B) revealed significant architectural disarray that sometimes extended into the surrounding exocrine tissue. Islets were damaged and shrunken in size and the infiltration of very few lymphocytes was observed. In treated diabetic rats (Fig. 2C and 2D), the endocrine component of the pancreas (the islets of Langerhans) retained normal histology, with scattered nodules within the substances of the exocrine pancreas, and exhibited no pathological changes (i.e., no signs of pancreatitis).

I. Histopathological Results of Skin Wounding

1. Three Days Post-wounding

In the normal control group, the wound gaps were filled with blood clots (consisting of fibrin, neutrophils and blood platelets), and the content of inflammatory cells (mainly neutrophils) increased to a peak at three days. Later, the neutrophils were replaced by macrophages (Fig. 3A). The inflammatory response covered the wounds with thick crusts (necrotic inflammatory cells, tissue and bacterial colony). Re-epithelialization was seen starting from the wound edges. In the diabetic control group, the wounds showed fewer inflammatory cells compared with the non diabetic group (Fig. 3B). The wounds of diabetic rats treated with fenugreek showed an increased number of inflammatory cells compared with the diabetic group (Fig. 3C). The wound healing of diabetic rats treated with bitter melon showed an inflammatory phase with more inflammatory cells than observed in Groups 2 and 3 but still fewer than in the control group (Fig. 3D).

2. Twenty-Eight Days after Wound Creation

The wounds created in the control group showed mature epidermis with epidermal papillae beside mature fibrous tissue, with few remaining inflammatory cells (Fig. 4A). In the diabetic group, the created wounds showed complete re-epithelialization of the dermis with an absence of epidermal papillae; crust remnants were still observed. The dermis

showed less mature granulation tissue infiltrated with numerous inflammatory cells (Fig. 4B). The wounds of diabetic rats treated with fenugreek showed complete re-epithelialization with apparently normal epidermal thickness. Granulation tissue infiltrated with macrophages was seen in the dermis (Fig 4C). The wound healing of diabetic rats treated with bitter melon showed results similar to the previous group except for the presence of epidermal papillae, more collagen fiber and fewer inflammatory cells (Fig. 4D).

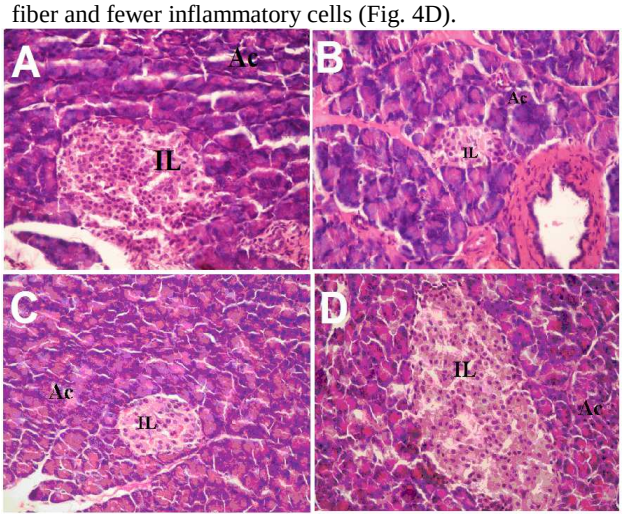

Fig.2 Photomicrographs of pancreas sections showing: A. normal rat pancreas with normal acini (Ac) and islets (IL) containing β-cells. B: pancreas of diabetic control rat with shrunken islets. C: pancreas of diabetic rat treated with 50 mg/kg b.wt of fenugreek. D: pancreas of diabetic rat treated with 500 mg/kg b.wt of bitter melon (H&E, 10X)

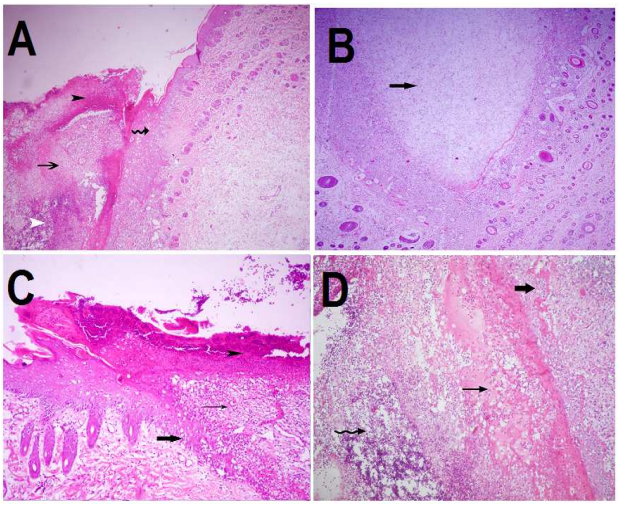

Fig.3 Photomicrograph of skin sections 3 days post wounding. **A**: Skin of a normal control rat showing crust (arrowhead) covering a blood clot represented by fibrin (arrow) and neutrophils (yellow arrowhead), and blood platelets alongside re-epithelialization (corrugated arrow). **B**. Skin of a diabetic rat showing fewer inflammatory cells in the inflammatory phase**. C.** Skin of a diabetic rat treated with bitter melon, with a thick crust (arrowhead), granulation tissue (thin arrow) and re-epithelialization (thick arrow). **D.** Skin of a diabetic rat treated with fenugreek showing an increased number of neutrophils (corrugated arrow) and fibrin (thin arrow) alongside re-epithelialization (thick arrow) H&E, 10X

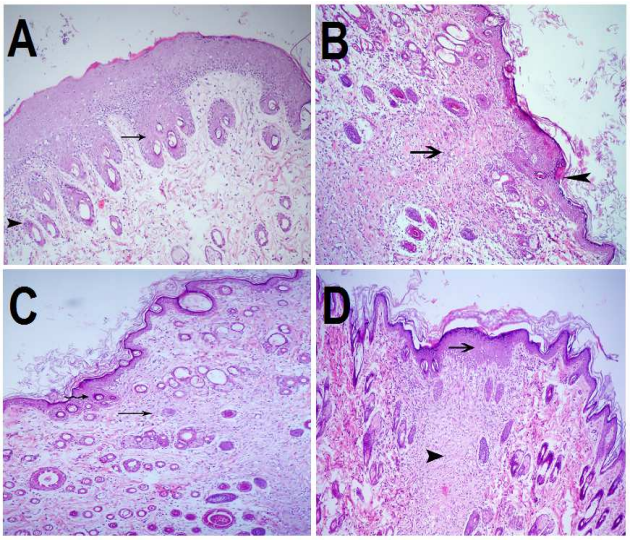

Fig. 4 Photomicrographs of skin sections 25 days post wound creation. A. Skin of a control rat with a mature epidermis with epidermal papillae (arrow) alongside mature fibrous tissue and few inflammatory cells (arrowhead). B. Skin of a diabetic rat showing re-epithelialization of the dermis with the absence of epidermal papillae alongside crust remnant (arrowhead) and less mature granulation tissue infiltrated with numerous inflammatory cells (arrow). C. Skin of a diabetic rat treated with fenugreek showing complete re-epithelialization with apparently normal epidermal thickness (corrugated arrow). Granulation tissue infiltrated with macrophages was seen in the dermis (arrow). D. Skin of a diabetic rat treated with bitter melon showing complete re-epithelialization (arrow) with apparently normal epidermal thickness alongside granulation tissue infiltrated with macrophages (arrowhead). H&E, 10X

IV. Discussion

Diabetes mellitus complications include cardiovascular disease, chronic renal failure, retinal damage, and poor wound healing. Poor healing of wounds, particularly of the feet, can lead to gangrene and require amputation [21]. In diabetes, hyperglycemia often leads to various peripheral vascular complications [22]. The present study shows that the administration of bitter melon (BM) ethanolic extract (500 mg/kg BW) normalized fasting blood glucose levels to 1.17 times that of nondiabetic healthy control rats in comparison to a 6.22-fold increase in untreated STZ diabetic rats. Fenugreek reduced the blood glucose levels to 1.33 times that of non-diabetic healthy control rats.

Normally, blood glucose levels are tightly controlled by insulin, a hormone produced by the pancreas. Insulin lowers the blood glucose level in response to an influx of carbohydrate. In the present study, serum insulin levels were significantly reduced with the induction of diabetes. Treatment with Cidophage® and bitter melon significantly enhanced insulin secretion after 4 weeks of treatment. Fenugreek normalized the effect of STZ injection on insulin secretion to a lesser degree. These data confirm the theoretical mechanism of bitter melon in normalizing blood glucose levels by enhancing

insulin secretion [23],[24]. The liver is an important insulin-dependent tissue that plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes [25],[26]. In the present study, the induction of diabetes by STZ in rats induced elevated ALT and AST liver enzymes. These results are in accordance with previously published studies reporting that the increase in ALT activity in diabetes is usually due to hepatocellular damage and is usually accompanied by an increase in AST activity [27]. Moreover, AST and ALT activity have been used as indicators of liver function [28]. In the present study, at four weeks post-treatment, both AST and ALT levels were normalized by Cidophage®, bitter melon, and fenugreek, in that order. The reversal of AST and ALT activity in our treatment of diabetic groups to near normalcy is evidence of the prevention of cellular and tissue damage by these herbal extracts. These results are in agreement with a previous study reporting that bitter melon significantly improved liver functions [29].

Liver tissue participates in the uptake, oxidation and metabolic conversion of fatty acids, the synthesis of cholesterol and phospholipids and the secretion of specific classes of serum lipoproteins. In diabetes, fatty acids are increasingly taken up by the liver and, after esterification with glycerol phosphate, are deposited as triglycerides. As a result, diabetic liver steatosis develops [30]. In the present study, at four weeks post-treatment, the observed increase in serum cholesterol in diabetic rats could be due to increased cholesterogenesis [31]. The present study showed a decrease in liver cholesterol, triglycerides, and LDL in diabetic rats after treatment with bitter melon, Cidophage®, and fenugreek, in that order. This reduction may be attributed to an increased clearance and decreased production of the major transporters of endogenously synthesized cholesterol and triglycerides. HDL levels were increased by all treatments in the following order: bitter melon, Cidophage®, and fenugreek. These data are in agreement with other studies reporting the ability of some plants, such as bitter melon [33] and fenugreek [34], to modulate lipid profiles.

Diabetes mellitus affects the kidney and is the leading cause of diabetic nephropathy [34]. Several studies have shown the presence of lipid deposits in the kidney of diabetic humans may play an important role in the pathogenesis of diabetic kidney disease [35]. Levels of serum creatinine reflect the kidney functions [36]. It has been reported that the rate of glomerular cell (podocyte) apoptosis is increased in rats with streptozotocin-induced diabetes mellitus [37]. In agreement with this study, we found that there was a significant increase in levels of serum creatinine after STZ injection. Four weeks post-treatment, creatinine levels were reduced by all treatments in the following order: bitter melon, fenugreek, and Cidophage®. These results are in agreement with [38], who found that fenugreek could reduce creatinine in alloxan-induced diabetes. Moreover, our results agree with other researchers who found that bitter melon reduces serum creatinine and kidney weight and improves glomerular filtration [39].

In diabetes, there is an increase the production of reactive oxygen species (ROS) [40],[41]. ROS can be effectively eliminated by several intracellular and extracellular antioxidant systems [42]. When the generation of ROS exceeds antioxidant defense mechanisms, these unstable molecules interact with biologic macromolecules such as lipids, proteins and DNA and lead to structural changes and functional abnormalities. It has been reported that increased oxidative damage (measured as levels of malondialdehyde (MDA) or its product thiobarbituric reactive substances (TBARS)) and lowered antioxidant defenses (measured as the activities of antioxidant enzymes or vitamins E or C) are the underlying mechanisms of diabetes complications. An increase in TBARS levels promotes DNA and protein alterations, including changes in the activities of enzymes implicated in lipid metabolism and the free-radical-scavenging process [40],[43]. Similarly, increased levels of nitric oxide end products have been reported in DM patients of [41]. Marked production of NO leads to pathological changes in various physiological systems [44], [45], leading to peripheral vascular diseases [46],[47]. Glutathione, the primary endogenous antioxidant, has a multifaceted role in antioxidant defense and is a direct scavenger of free radicals and is a cosubstrate for peroxide detoxification by glutathione peroxidases [48]. In agreement with these studies, we found that MDA and NO were increased in diabetic rats compared to the control group. Moreover, in the diabetic group, reduced glutathione was decreased, indicating a disruption in the balance of the redox system. Four weeks post-treatment, serum nitric oxide and malonaldehyde levels were reduced by all treatments, in order of effect: bitter melon, Cidophage®, and fenugreek.

In diabetes, several mechanisms participate in the pathological changes observed in endothelial cells, including hyperinsulinemia, increased oxidative stress, and inactivation of NO [49],[50],[51]. Early in the course of diabetes, intracellular hyperglycemia causes abnormalities in blood flow and increased vascular permeability [52],[53]. In the present study, vascular permeability to albumin was assessed at the end of the experiment using Evans Blue dye [54]. We found a significant increase of Evans blue (EB) leakage in the skin of STZ-diabetic animals; this finding is in agreement with those of previous studies [55],[56] Four weeks post-treatment, dye extravasation levels were reduced by all treatments, in the following order: Cidophage®, bitter melon, and fenugreek. However, Cidophage® and bitter melon appear to exert higher but similar effects in reducing capillary permeability than fenugreek. Other studies have also reported the ability of other plant products to normalize capillary permeability [57].

The increase in capillary permeability is a sign of microvascular dysfunction at the arteriolar and capillary level. This dysfunction can lead to both structural and functional changes, especially in peripheral organs, accounting for a

group of disorders called peripheral vascular disease (PVD). PVD is a common and severe complication of diabetes and is characterized by a high prevalence, early development, and rapid progression. In diabetes, there is also an impaired collateralization of vascular ischemic beds [58]. In the diabetic foot, the thickened basement membrane is believed to impair migration of leukocytes and blood flow through the capillaries. These changes and an impaired neurogenic vasodilatory response result in an inability to achieve a normal hyperemic response, which is needed after foot injury, and an increased the risk of infection [59]. These findings account for the 15-fold increase in risk for lower extremity amputation seen in diabetic patients [60]. It has been reported previously that cutaneous wounds result in a decrease in antioxidant status as a result of the production of ROS. One research study reported that any diabetic ulcer that lasts for more than 4 weeks is usually an indication of a poor outcome and may lead to amputation [61]. Hence, the rate of wound healing plays an important role in the development of complications. Previous research studies have reported faster contraction of the wound area in normal rats when compared with diabetic rats [62]. In the present study, the diabetic group showed delayed wound healing compared with the treated diabetic group, as measured by histological observation. We found that the wounds contracted immediately after wound induction in normal rats, as shown by the rate of wound closure, which is similar to the findings of an earlier study [63]. In contrast, other research reports have described enlargement of wounds at the first day after wound creation [64]. The administration of bitter melon or fenugreek extract was found to stimulate cellular proliferation and migration through an unknown mechanism. This was evident from the histological studies, indicating the beneficial role of bitter melon and fenugreek extracts in accelerating wound healing. Moreover, we found that the groups treated with fenugreek and bitter melon showed increased amounts of granulation tissue when compared with the untreated group. These observations are in agreement with other studies reporting that the observation of rapid epithelial development in wound histopathology is considered a positive sign [65].

STZ-induced diabetic rats show damaged β-cells via the glucose transporter (GLUT2), and STZ also causes DNA alkylation [66]. These findings are in accordance with our histopathological results showing shrunken islets with distorted shapes and infiltration of lymphocytes compared to the control group. The treated animals showed more islets than the STZ control group and they were more comparable to normal rat islets, although there were individual differences. Enlargement of islets in diabetic animals post treatment was highest in bitter melon-treated rats followed by fenugreek-treated rats.

V. Conclusion

We concluded that the ethanolic extracts of the bitter melon and fenugreek herbs exhibit promising and safe antidiabetic activities in STZ induced type-1 DM in rats. The efficacy of these herbs was achieved by increasing insulin secretion and lowering blood glucose, lipid profiles, lipid peroxidation and nitric oxide levels. In addition, the plant extracts showed variable degrees of increases in HDL and GSH, resulting in capillary permeability normalization and accelerated wound healing. Hence, these herbs may be pursued for their clinical usefulness in the management of diabetes mellitus and its associated complications.

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