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Biochemical effects of *Cleome droserifolia* on alloxan -induced diabetes in rats: Role of insulin, oxidative stress and inflammation

M.A.Nagy
Chemistry Department, Faculty of Science, Beni-Suef University, (EGYPT)
E-mail : nagy_bio@yahoo.com

ABSTRACT

Background : Oxidative stress associated with insulin –dependent diabetes mellitus is a risk for inflammatory disorders that induce renal dysfunction. *Cleome droserifolia* is a widely used for its antioxidant and antiinflammatory effects. This study aims to evaluate antihyperglycemic, antihyperlipidemic, antioxidant and anti inflammatory effects of methanolic *Cleome droserifolia* extract (MCD) against alloxan induced diabetes in male albino rats and explore possible effects of MCD on insulin and oxidative stress profile. Biochemical observations were further substantiated with histological examination of pancreas and kidney. **Method :** This study was carried on 30 male albino rats weighing 190±10, 90 days old, classified into 3 groups, control (NC), diabetic (DC) and treated diabetic group (MCD). Alloxan was given in a dose of 150 mg/kg intraperitoneally (IP). **Result :** Alloxan produced significant increase in serum glucose, Triglyceride (TG), Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C), urea and creatinine (Cr), renal reduced glutathione (GSH), renal nitrotyrosine, Renal TNF-α. On other hand, alloxan produced significant decrease in insulin levels and activity of renal catalase (CAT), renal glutathione peroxidase (GSH-Px) and renal superoxide oxide dismutase (SOD).Oral MCD (0.31 g /kg body weight of rats daily for 30 days)significantly ameliorated these effects. **Conclusion :** Treatment with MCD ameliorated DM and its related late consequences. Furthermore, it has antioxidant and antiinflammatory effects. Commonly, MCD is a way to surmount the diabetic state and it has antioxidant and antiinflammatory effects. It may be a promising adjuvant to anti diabetic therapy.

KEYWORDS

Cleome droserifolia;
Diabetes mellitus;
Lipid profile;
Oxidative stress;
Inflammation.

INTRODUCTION

Diabetes Mellitus (DM) is a heterogeneous disorder characterized by a progressive decline in insulin action (insulin resistance), followed by the inability of β-cells to compensate for insulin resistance (pancreatic beta cell dysfunction[27].

Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defense system and thus promotes de novo free radicals generation[22].

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The source of oxidative stress is a cascade of ROS leaking from the mitochondria. This process has been associated with the onset of type 1 diabetes (T1DM) *via* the apoptosis of pancreatic β-cells, and the onset of (T2D) *via* insulin resistance[23].

Chronic and sustained high toxic levels of ROS are associated with several pathological conditions including inûammatory diseases and the complications of diabetes[13]. Tumor necrosis factor-α (TNF-α) is an important proinflammatory cytokine involved in the pathogenesis of autoimmune T1D. Low-grade inflammation is a common feature in subjects with T2D. Heart disease, the metabolic syndrome and T2D all have in common the increased concentration of circulatory cytokines as a result of inflammation[16].

Nitrotyrosine (N-tyr) was initially proposed to be a speciûc marker of increased oxidative stress related to the generation of peroxynitrite from nitric oxide in vascular endothelial cells and other tissues. Although several non-nitric oxide biosynthetic pathways for N-tyr have also been described, in all cases tyrosine nitration is believed to involve free radical chemistry reactions[12].

DM is associated with a large number of lipid abnormalities. which in turn depend on the extent of insulin deficiency, insulin resistance, obesity, diet and the presence of concomitant primary and other secondary causes of hyperlipemia. In diabetic hyperlipemia, a series of bizarre lipoproteins and other lipids appear and interaction of this with oxidative stress and free radicals leads to enhanced lipid peroxidation in plasma, tissues and membranes, causing extensive tissue damage[31].

Diabetic renal dysfunction whose incidence is up to 47.66% is the most common and difficult diabetic microvascular complication to treat and has become the first cause of end-stage renal disease. It is reported that about 43% of the chronic renal failure (CRF) patients on dialysis are DN, 60% case fatality of diabetes mellitus (DM) patients are DN, DM patients who died of renal failure are 17 times more than non-DM patients[33].

Herbal remedies that stem from egyptien traditional medecine hold a great promise against DM. The dried herb of *Cleome droserifolia* (Forssk.) Del., is a plant of the Cleomaceae family. It is present in the deserts, especially the Eastern desert, Red Sea region, Sinai, Gebel. Its decoction of leaves and stems is widely used by the Bedouins of the southern Sinai for the treatment of diabetes.

Methanolic extract of leaves and stems for *Cleome droserifolia* (MCD) is rich in Bioactive compounds as flavanoids, flavonol glycosides, alkaloids, tannins and Steroids as shown in Figure (2.8). So far as plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics as they possess radical scavenging properties[24]. The present work was conducted to evaluate the possible hypoglycemic, hypolipidemic, antioxidant and anti inflammatory properties of MCD in alloxan - induced type II iabetes mellitus (DM).

MATERIAL AND METHODS

Materials

Experimental animals

300White male albino rats weighting about 190±10 g were used as experimental animals in the present investigation. The animals were housed in standard polypropylene cages and maintained under controlled room temperature (22±2 °C) and humidity (55±5%) with 12 h light and 12 h dark cycle and were fed a standard diet of known composition, and water ad libitum. The chow was purchased from El-Gomhoria Company, Cairo, Egypt. They were housed for two weeks for accommodation. Our work was carried out in accordance with the guidelines of El Minia University for animal use. These animals were used for induction of *Diabetes mellitus*

Plant material preparation of MCD

The freshly collected leaves and stem part of *Cleome droserifolia* were washed with distilled water and air-dried under the control conditions and powdered. The powdered plant material was percolated with petroleum ether to remove fatty substances; the marc was further exhaustively extracted with of 80% methanol for 3 days. The extract was filtered, concentrated on rotavapour and then freeze-dried under high vacuum (1.3 Pa) and at temperature of - 40± 2C°. The

extract will be dissolved in 0.5 g Carboxy methyl cellulose (0.5w/v) for oral administration. The extract was prepared at the Department of agriculture chemistry, Faculty of Agriculture, Minia University.

Experimental design and animal grouping

Experimental *Diabetes* was induced in 20 rats by a a dose of 150 mg/kg intraperitoneally (IP)[40]. Above 200 mg/dl blood glucose level were considered diabetic rats. These DM animals were divided into 2 groups, one served as untreated DM group, the other as treated DM group, which was given MCD as 0.31 g /kg body weight of rats daily for 30 days Control rats were injected with same volume of vehicle (saline buffer). At the end of the experiment, all groups were bled by vein puncture, fasting blood samples were centrifuged, sera were kept at -80°C right the time of analysis, meanwhile, kidney and brain were collected, blotted between 2 filter papers, frozen directly into liquid Nitrogen, then kept at -80°C till tissue biochemical investigations.

Methods

Biochemical investigations in blood and tissue

Biochemical estimations in blood and serum insulin: Fasting blood glucose (FBG) concentration of all the three experimental groups was determined by glucometer during different phases of the experiment by withdrawing blood from the tail vein. Serum insulin was assayed in the Radioactive Isotopes Unit, Central Department of Scinentificial Analysis and Test, National Research Center (Dokki, Giza) by radioimmunoassay kits of DPC (Diagnostic Products Corporation, Los Angeles, USA) [Maier et al.,1974]. For estimating serum lipid profile, serum was isolated from the blood collected by cardiac puncture under mild ether anesthesia from overnight fasted rats on day 30th of MCD treatment and serum total cholesterol (TC)[6] and triglyceride (TG)[11] using diagnostic kits (Erba Mannheim Cholesterol kit, Transasia Bio-Medicals Ltd., Daman). Results were expressed in mg/dl. On other hand, serum urea and creatinine[38] were also measured.

For determination of tissue biochemical parameters, rats were sacrificed by decapitation and incisions were immediately done for separation of the kidney. The isolated kidney was quickly weighted and dissected into pieces, homogenized in volumes of ice cold de-ionized water to yield 20% W/V homogenate using ice cold Teflon homogenizer (Potter Elvehjem type). Also, renal reduced glutathione (GSH)[9], nitrotyrosine[20], TNF-α[14] and activity of renal (CAT),[37] GSH-Px[34] (SOD)[15].

Histopathological examination of pancreas

Some pancreas were cleaned and fixed in 10% neutral buffered formalin solution. After dehydration in graded ethanol solutions and in toluene, they were embedded in paraffin Sections of 3–5µm thickness were stained with hematoxylin and eosin (H.E.) for histopathological examination.

Histopathological examination of kidney

Some Kidney were cleaned and fixed in 10% neutral buffered formalin solution. After dehydration in graded ethanol solutions and in toluene, they were embedded in paraffin. Sections of 3–5µm thickness were stained with hematoxylin and eosin (H.E.) for histopathological examination.

Statistical analysis

Statistical analysis was carried out using Graph Pad Instat software (version 3, ISS-Rome, Italy). Groups of data were compared with ANOVA, followed by Tukey-Kramer (TK) multiple comparisons post-test. Values of $P < 0.05$ were regarded as significant. Data were expressed as mean ± standard error (SEM).

TABLE 1 : Effect of MCD on studied blood parameters in diabetic rats, compared to normal control (Values are expressed in M±SE, N= 10 for each group)

Parameter	NC	DC	MCD
Glucose (mg/dl)	112.3±9.8	465±41.8 ***[a]	139±7.4 [b]
Insulin (ng/ml)	1.18±0.07	0.48±0.07***[a]	0.90±0.04 [b]
Triglycerides (mg/dl)	74.2±6.8	178.2±10.4***[a]	110.3±7.1 [b]
T Cholesterol (mg/dl)	87.2±8.4	163.2±7.9***[a]	99.26±5.6 [b]
H.D.L-C (mg/dl)	63.2±5.7	39.3±4.5***[a]	46.2±3.2 [b]
L.D.L-C (mg/dl)	13.5±7.3	53.2±4.8***[a]	30.2±2.9[b]

a*Significantly different from control at P < 0.001.**
b*Significantly different from DM at P < 0.001**

RESULTS

Serum glucose levels were significantly increased, but insulin levels were decreased in alloxan diabetic rats

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TABLE 2 : Effect of MCD on studied renal parameters in diabetic rats, compared to normal control (Values are expressed in M±SE, N= 10 for each group)

Parameter	NC	DC	MCD
Renal MDA nM MDA/mg protein	110.23±14.36	974.23±28.12***[a]	638.24±39.58[c]
Renal GSH mg/gm tissue	15.23±1.06	9.36±0.89***[a]	34.26±2.96[b]
Renal Nitrotyrosine (nM)	124.36±8.13	253.68±4.43***[a]	183.34±6.24[b]
Renal NO (nmol/mg protein)	1.87±0.17	4.24±0.3***a	2.05 ±0.31***b
Renal CAT µ moles H2O2 decomposed/min/mg protein	274.39±12.36	163.26±10.36***[a]	316.36±24.26[c]
Renal GSH-Px µg GSH consumed/min/mg protein	752.36±73.13	375.62±34.11***[a]	700.21±70.36[b]
Renal SOD Units/min/mg protein	350.28±24.18	274.29±21.48***[a]	331.06±24.39[b]
renal TNF-α (pg/gm)	151±8.5	614±52.9***[a]	483.2±39.72[b]
renal caspase-3 (U/mg protein)	0.87±0.06	1.9±0.19***[a]	1.14 ± 0.12[b]
Urea (mg/dl)	25.41±2.14	66.14±2.14***a	31.2±2.11 b
Creatinine (mg/dl)	0.65±0.07	1.31±0.01***a	0.87±0.03 b

a*Significantly different from control at P < 0.001. b***Significantly different from DM at P < 0.001**

compared to control, and treatment with MCD significantly reduced glucose but non significantly elevated insulin in relation to diabetic rats. Both triglycerides, total cholesterol and LDL-C levels were significantly increased, while HDL-C was significantly decreased by induction of diabetes, these figures were significantly ameliorated after MCD treatment (TABLE 1). Activity of renal (CAT), (GSH-Px) and (SOD) were significantly decreased in the alloxan group compared to control, treatment with MCD significantly ameliorated these changes (TABLE 2):.

Also, Renal MDA, ntrotyrosine, NO, TNF-α, urea and Creatinine contents were significantly increased, in the alloxan group compared to control, treatment with MCD significantly ameliorated these changes (TABLE 2).

On other hand, Levels of renal GSH were significantly decreased in the alloxan group compared to control, treatment with MCD significantly changed these parameters (TABLE 2)In contrast, Activity of renal casaspase was significantly increased in the alloxan group compared to control, treatment with MCD significantly decreased the cctivity of renal casaspase (TABLE 2).

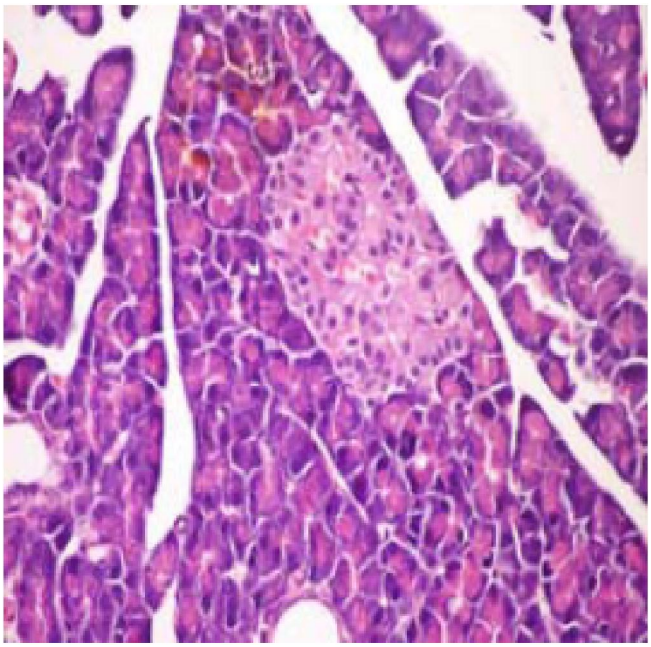

Figure 1 : Pancreatic tissue of normal male albino rats. The pancreas is subdivided by septa inti pancreatic lobules. The exocrine portion of the pancreas consists of pancreatic acini while endocrine portion consists of islets of Langerhan (H& E x 400).

DISCUSSION

Methanolic extract of leaves and stems for *Cleome droserifolia* (MCD) is rich in Bioactive compounds as flavanoids, flavonol glycosides, alkaloids, tannins and Steroids. So far as plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators[24].

Alloxan is toxic glucose analogues that preferentially accumulate in pancreatic beta cells *via* the Glucose transporter -2 (GLUT2). In the presence of intracellular thiols, especially glutathione; alloxan generates reactive oxygen species (ROS) in a cyclic redox reaction with its reduction product, dialuric acid. Autoxida-

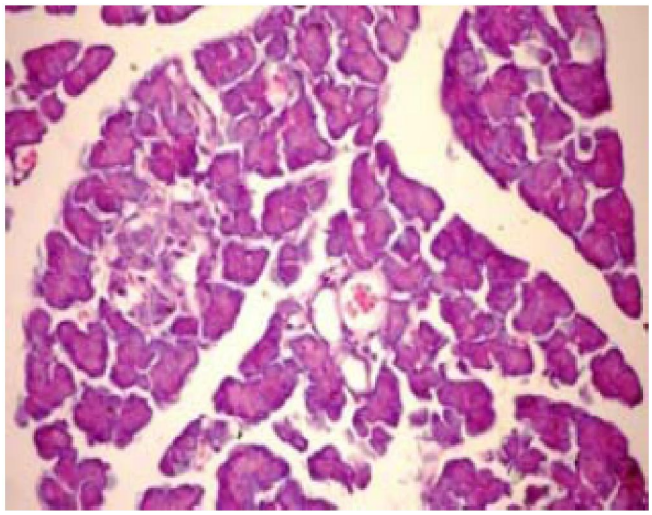

Figure 2 : Pancreatic tissue of diabetic rats. Normal architecture of the islets is disrupted islets of Langerhans exhibited hydrophobic cells, necrotic cells, vacuolizations and irregular hyperchromic nuclei (H& E x 400).

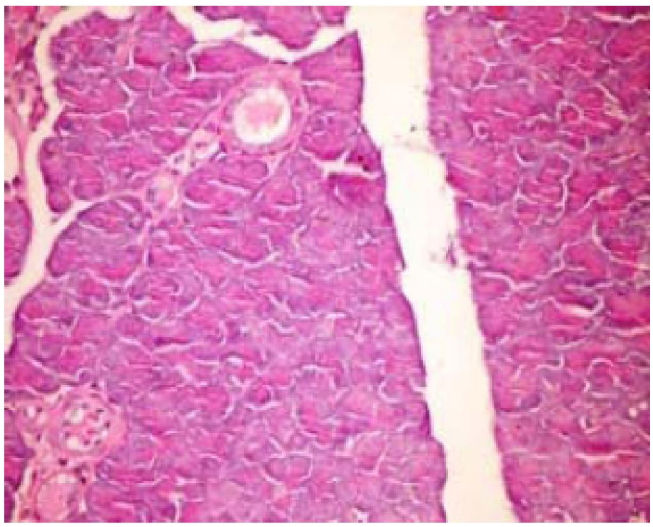

Figure 3 : Pancreatic tissue of diabetic rats treated with CDE (orally 0.31 g /kg body weight of rats for 30consecutive days). These still few vacuolizations and appear highly divided β-cells in the islets of Langerhans (H& E x 400).

tion of dialuric acid generates superoxide radicals, hydrogen peroxide and, in a final iron-catalysed reaction step, hydroxyl radicals. These hydroxyl radicals are ultimately responsible for the death of the beta cells, which have a particularly low antioxidative defence capacity.

The present investigation indicated that a single dose of alloxan (150 mg/kg) intraperitoneally to adult male albino rats (190±10 g) was suitable to induce histological changes of the islets of Langerhans characterized appearance, hypoinsulinemia and hyperglycemic state. The present dose as well as the observed histopatho-

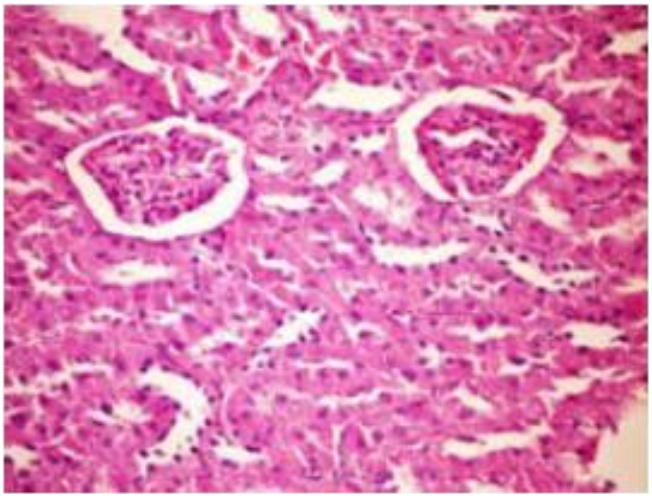

Figure 4 : Kidney of normal rat showing normal histological structure of renal parenchyma (H&E×400).

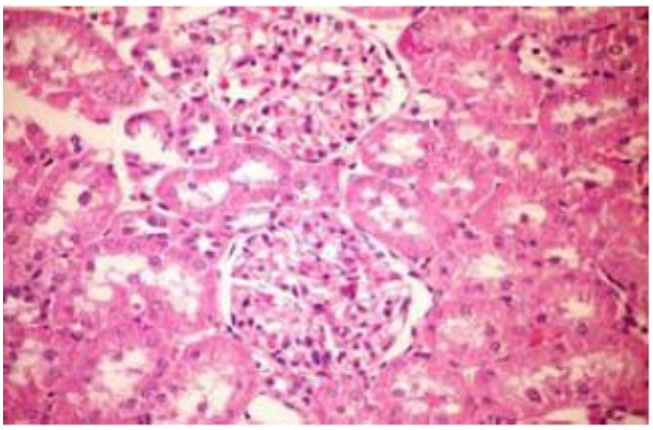

Figure 5 : Kidney of control diabetic rat showing vacuolization of endothelial lining glomerular tufts and epithelial lining renal tubules (H&E×400).

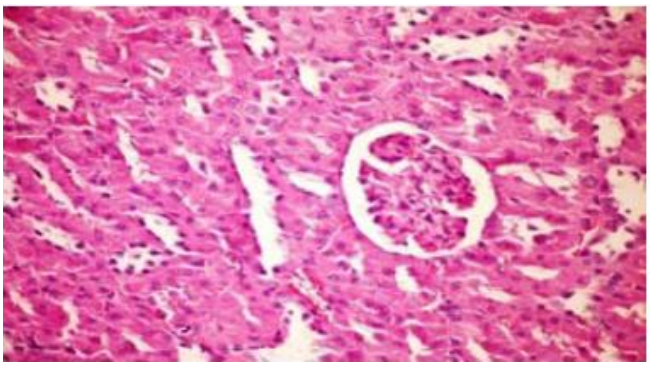

Figure 6 : Kidney of diabetic rat treated by CDE (orally 0.31 g /kg body weight of rats for 30consecutive days) showing no histological changes (H&E ×400).

logical and biochemical manifestations agree with the literature of[17].

A gradual loss of β-cells due to apoptosis significantly hinders insulin production and inhibits cell viabil-

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ity. During apoptosis, cells shrink; chromatin condenses; DNA is cleaved into pieces at inter nucleosomal regions. A proactive way to increase β-cell viability is to decrease apoptosis level in order to retain the cell population and increase insulin production[32].

Exposure of islets to alloxan shows significantly increased formation of peroxynitrite, NO and ROS with markedly elevated lipid peroxidation and reduced cell viability. Islets exposed to alloxan also show significantly increased mitochondrial membrane potential. Apparently, alloxan causes severe oxidative and cytotoxic stress to islets that is likely to compromise their insulin releasing capacity[18].

Oral administration of MCD causes significant decrease in levels of blood glucose in accordance with[21,28]. revealed that MCD has a hypoglycemic effect through potentiation of peripheral, hepatic insulin sensitivity and diminishing intestinal glucose absorption.

On other hand,[2] explained that flavonoids, the major active constituent in MCD are potential antidiabetic agents because they exert multiple actions that are both hypoglycemic (insulinomimetic action) and antihyperglycemic (insulin secretagogue). Also, ûavonoid-enriched extract from efficiently inhibited a-glucosidase activity and may inhibit the non-Na+ dependent facilated diffusion of monosaccharides in intestinal epithelial cells. Consequently, the parallel concentrative Na + dependent transport ATPase for monosaccharides gains efficiency[5].

Antidiabetic effect of MCD was further evidenced by histological observations made on the pancreatic tissue of MCD treated rats that show few vacuolization and highly divided β-cells in the islets of Langerhans

In our study, elevated levels of serum TC, TG, LDL and VLDL-cholesterol and decreased HDLcholesterol concentration in alloxan-induced diabetic rats are in accordance with[41] that represents the characteristic features of diabetic dyslipidemiaare that attributed to increased flux of free fatty acids into the liver secondary to insulin deficiency/ resistance which in turn resulting in excess fatty acid accumulation in the liver, which is converted to triglycerides.

Oral administration of MCD causes significant decrease in the serum levels of glucose, triglycerides, total cholesterol and LDL-cholesterol in contrast to significant elevation in HDI.-cholesterol and body weight in accordance with[30].

Flavonoids in MCD inhibit the activity of cAMP-dependent protein phosphokinase, the consequence is that the cAMP concentration increases and that phosphorylation of the Hydroxy methyl glutaryl-CoA reductase, but endogenous cholesterol production is diminished. In addition, the flavonoids can interact with the enzyme protein phosphatase, which liberates the aliphatic phosphoesters from Hydroxy methyl glutaryl-CoA -CoA HMG-CoA reductase, thus restoring the activity of this. Thus, flavonoids inhibit HMG-CoA reductase by a dual mechanism[36].

Alloxan led to a significant increase in serum urea, serum creatinine, renal MDA, renal nitrotyrosine levels and activity of renal caspase-3. On other hand, Alloxan causes significant decrease in renal GSH content and significant decrease in activity of renal SOD, CAT and GPx. A similar effect was recorded by[3,38].

Santamaría et al[35] revealed that enhanced protein catabolism and accelerated amino acid deamination for gluconeogenesis is probably an acceptable postulate to interpret the elevated levels of urea and creatinine. Furthermore, Alloxan increased the productions of reactive oxygen species, enhanced lipid peroxidation and protein carbonylation in association with decreased intracellular antioxidant defense in the kidney tissue.

In addition, hyperglycemia enhanced the levels of proinflammatory cytokins (TNF-α)[4]. suggests that development of diabetic renal dysfunction may due to activation of endoplasmic reticulum stress that can mediate progressive endothelial damage through growth and migration of vascular smooth muscle and inflammatory cells, alteration of extracellular matrix, apoptosis of endothelial cells, over-expression of inflammatory cytokines as TNF-α.

Moreover,[7] revealed that DM increase the generation of NADPH oxidase-derived reactive oxygen species and induce apoptosis of glomerular epithelial cells (podocytes) and loss of podocytes contributes to progression of kidney disease.

On other hand[19],revealed that DM may impair the protein systems anchoring the podocyte foot processes in glomerular basement membrane, therefore blunting resistance of these cells to mechanical forces. Modulation by these factors of expression and activity of numerous structural and functional proteins results in the

(auto) inflammatory responses, dysfunction, apoptosis or necrosis of the podocytes

Also[26], revealed that TNFα-pathway has a broad range of inflammatory and apoptotic properties and dysregulation of these processes may contribute to injury of the diabetic kidney. In addition, the TNFα-pathway directly increases glomerular vasoconstriction and albumin permeability. Exposure of the kidney to TNFα increases mRNA expression of TNF receptors in renal tubulointerstitium and triggers cell death.

Oral administration of MCD causes significant decrease in level of seum urea and creatinine in accordance with[1].

Furthermore, oral administration of MCD causes significant decrease in renal MDA and significant enhancement in level of renal GSH and renal TNF-α and the activity of renal CAT, renal GSH-Px and renal SOD renal caspase-3 in accordance with[25].

The mechanism by which the MCD prevents renal oxidative stress may include an increasing rate of GSH or by induction of its synthesis or by a scavenger effect. Instead of the toxic reactive metabolites binding to glutathione and consume, they will be captured by the flavonoids[29].

Oral administration of MCD causes significant decrease in renal TNF-α in accordance with[10] due to its high content of flavonoids that inhibit eicosanoid synthesis. Reno- protective effect of MCD was further evidenced by histological observations made on the renal tissue of MCD treated rats that revealed showing no histological changes.

CONCLUSIONS

Treatment with MCD improved associated metabolic consequences to Type I DM, showing hypoglycemic, insulin sensitization, and antioxidant and hypolipidemic actions. The results suggest MCD as a beneficial adjuvant for the treatment of type I diabetes mellitus and possibly as a protector against long term nephropathy.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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