

The Ameliorative Effect of *Cleome droserifolia* (Samwa) on Myocardial Injury Associated with Diabetes in Male Rats

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ABSTRACT

Background: Diabetes is an imperative risk factor for cardiac complications development. *Cleome droserifolia* (Cd) is used in Egyptian traditional medicine for treatment of diabetes mellitus. **Aim:** The aim of the present study was to measure the possible protective effects of ethanolic extract of Cd against cardiomyopathy in Streptozotocin (STZ) induced diabetic rats. **Materials and Methods:** Experimental diabetes was induced by intraperitoneal injection with a single dose of STZ (45 mg/kgbw). Oral dose Cd extract was administered daily for four weeks (310 mg/kg bw). **Results:** Diabetic rats exhibited an increase in glucose level and lipid profile as well as cardiac pro-inflammatory, pro-apoptotic markers and oxidative stress, while a decrease was recorded in antioxidant enzymatic activities. On the other hand the administration of diabetic rats with Cd extract for four weeks resulted in a significant amelioration in the most of the parameters mentioned above when compared with diabetic rats. **Conclusion:** The data clearly indicated that the treatment with *Cleome droserifolia* exerts a therapeutic protective nature in cardiac complications due to diabetes by decreasing oxidative stress and cardiac damage which may be attributed to its antioxidative potential.

Keywords: Diabetic rats, Streptozotocin, Heart injury, *Cleome droserifolia*.

INTRODUCTION

Diabetes mellitus is a rapidly growing disease world-wide that is estimated to be present in 6.6% of the international population and projected to be increased by 7.8% in 2030,⁽¹⁾. Treating diabetic patients is multifaceted in all aspects and they require objectives and optimum information in order to obtain the maximum benefits of their treatment and avoid complications. It is considered of clinical and public health significance, as it adversely affects personal health, health-related quality of life, life expectancy and has significant implications on the health care system⁽²⁾. Epidemiological evidence shows that diabetes frequently results in severe complications, such as cardiovascular disease, cerebro-vascular disease, micro-vascular disorders (e.g. nephropathy, retinopathy, neuropathy, sexual dysfunction) and diabetic foot disorders⁽³⁾.

Heart muscle injury has been reported in both human and animal models of diabetes. Results from clinical and experimental diabetes mellitus indicate that free radical induced oxidative stress play a significant role in organelle transformation and cardiac dysfunction⁽⁴⁾. Both type 1 and 2 diabetes cause damage to myocyte which occurs through the pathways utilizing reactive oxygen species (ROS)⁽⁵⁾.

In human and experimental diabetes, heart sickness is associated with a marked apoptosis. This loss of cells can lead to fibrosis, contractile deficiency, and subsequent heart dysfunction⁽⁶⁾.

Globally, there is renewed interest in natural products (NPs) as a starting point for drug discovery and development for different diseases⁽⁷⁾. NPs are structurally diverse and serve as a valuable source for novel molecular scaffolds in drug development⁽⁸⁾. Traditional anti-diabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities, or as simple dietary adjuncts to the existing therapies⁽⁹⁾. *Cleome droserifolia* (Cd) is used by herbalists in Egypt as a hypoglycemic agent, and its decoction is widely used by the Bedouins of the southern Sinai for the treatment of diabetes. It is also used in treatment of various disorders such as diarrhea, fever, inflammation, liver diseases, bronchitis, skin diseases, malaria fever and in the treatment of scabies and rheumatic fever. It is rich in bioactive compounds as flavanoids, flavonol glycosides, alkaloids, tannins and steroids⁽¹⁰⁾. So far as plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. Flavonoids as a one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics as they possess radical scavenging properties⁽⁸⁾.

AIM OF THE WORK

The present study is objected to investigate the probable hypoglycemic, hypolipidemic and anti-inflammatory effects of *Cleome droserifolia* on

cardiac myopathy in relation to its antioxidant and anti-apoptotic effect in STZ-induced diabetic rats.

MATERIALS AND METHODS

Chemicals

Streptozotocin was purchased from Sigma Chemical Company (St. Louis, MO 6, USA). All other chemicals used for this study were of analytical grade.

Preparation of Plant Extract:

The freshly collected leaves of *Cleome droserifolia* were washed with distilled water and air-dried under the control conditions and powdered. Ethanolic extract was prepared by soaking 100 g of the dry powdered plant materials in 1 L of 95% ethanol⁽¹¹⁾. The extracts were filtered after 48 h, first through a Whatmann filter paper and then through cotton wool. The extracts were concentrated using a rotary evaporator with the water bath set at 40°C. The remained residual part of the herb was further extracted with ethanol and the filtrate was concentrated under vacuum using rotary evaporator. The resultant extract dissolved in 20% dimethyl sulfoxide (DMSO) at a dose of 1 ml/kg b.w⁽¹²⁾ then stored in a glass container at -20°C pending bioassay.

Experimental Animals and Induction of Diabetic Rats:

Adult male Wister albino rats, initially weighing 180-200g, were purchase from Biological Products & Vaccines (VACSERA) Cairo, Egypt, and housed in the animal house of Zoology Department, Faculty of Sciences, Mansoura University. They were housed under standard laboratory conditions of light (12:12 h L: D cycle), temperature (23 ± 2°C) and relative humidity (55 ± 5%). The animals were provided standard rat pellet feed and tap water *ad libitum*. Maintenance and treatment of all the animals was done in accordance with the principles of Institutional Animal Ethics Committee constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Egypt.

After two weeks of acclimatization, rats were divided into 5 groups, each of 10 rats. Induction of diabetes was done by a single intraperitoneal STZ dose (45 mg/kgbw) injection, that was dissolved in freshly prepared 0.1 M citrate buffer. Blood samples were collected from the tail tip and the confirmation of hyperglycemia was achieved two days after STZ injection⁽¹³⁾.

Experimental Protocol Design:

After two weeks of acclimatization, rats were randomly divided into five main groups each comprising of 10 rats. **1- Control group:** Rats fed a normal diet. **2- DMSO group:** Rats received orally 20% dimethylsulfoxide (1ml/kg b.w) diluted in H₂O daily using gastric tube. **3- *Cleome droserifolia* treated group.** Rats orally received extract of *Cleome droserifolia* 310 mg/kg b.w. dissolved in 20% DMSO. **4- Diabetic group:** Rats injected intraperitoneally with a single freshly prepared STZ at a dose of 45 mg/kg b.w. **5- Diabetic group treated by *Cd*:** Diabetic rats as described in group 4 received *Cleome droserifolia* dissolved in DMSO (310 mg/kg b w) daily by gastric tube for 4 weeks⁽¹⁴⁾.

Four weeks post diabetes induction, all animals groups were sacrificed. Blood samples were collected in clean centrifuge tubes. In another tube blood samples were placed with EDTA then centrifuged the collected plasma kept for measuring glycosylated hemoglobin. Heart muscles were obtained for further investigations in heart homogenates.

Preparation of heart homogenate:

A portion of left ventricle of the heart was weighed and homogenized in ice-cold tris buffer using tephlon homogenizer, the homogenate was centrifuged at 5,000 rpm for 15 min, and the supernatant obtained was stored frozen at -20 °C until use. Estimation of tissue oxidative stress by measuring Malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) as well as antioxidant activities, superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH), apoptotic markers P53, BAX and antiapoptotic marker BCL-2 were estimated in the homogenate.

Biochemical Assays

The glucose and insulin levels in serum and the HbA-1c levels in blood were determined using kits supplied by Spinreact (St. Esteved'en Bas Girona, Spain), Abcam (Cambridge, MA, USA) and BioSystems (Barcelona, Spain), respectively. The lipid profiles in serum, including total lipids (TL), triglyceride (TG), total cholesterol (TC), high density-lipoprotein (HDL), low density -lipoprotein (LDL) and very low -density lipoproteins (VLDL), were estimated according to Biodiagnostic kits (Dokki, Giza, Egypt). Interleukin 6 (IL-6), interleukin 1beta (IL-1β) and tumor necrosis factor alpha (TNF-α) in the serum were assessed using enzyme-linked immunosorbent assay (ELISA) kits provided by R&D Systems (Minneapolis, MN, USA) and Bioscience (San Diego, CA, USA) according to the manufacturer's instructions.

Creatine kinase-muscle bone (CK-MB) and lactic dehydrogenase (LDH) activities in the serum were determined using kits purchased from Elitech (Puteaux, France). Lipid peroxidation was assessed by determining the amount of malondialdehyde (MDA). The hydrogen peroxide (H₂O₂) concentrations were estimated according to Biodiagnostic kits (Dokki, Giza, Egypt). The superoxide dismutase (SOD), catalase (CAT) activities and glutathione (GSH) content were assayed using kits provided by Biodiagnostic (Dokki, Giza, Egypt).

Flow cytometry study

Samples from the heart tissues were prepared as previously described for flow cytometry analysis. The cells were suspended in PBS with BSA, divided into aliquots and stored at 4°C for analysis. The flow cytometry analyses were performed on a FACS Calibur TM cytometer (BD Biosciences, San Jose, CA) using CellQuest Pro software (Becton Dickinson) for data acquisition and analysis.

P53, Bcl2 and Bax

Cell suspensions were prepared in a PBS/BSA buffer and were then incubated for 30 min with an anti-Bcl2 (100/D5) antibody and anti-Bax [6A7] antibody (ab5714) for flow cytometry analysis of Bcl-2 and Bax. A mouse anti-P53 “aa20-25” FITC, Clone: DO-1 was used for P53 analysis by flow cytometry. After 30-min incubation at room temperature, the cells were washed with PBS/BSA, centrifuged at 400×g for 5 min, resuspended in 0.5% paraformaldehyde in PBS/BSA and analyzed using flow cytometry.

The study was approved by the Ethics Board of Mansoura University.

Statistical Analysis

Data was subjected to statistical analysis using statistical software program Prism (GraphPad.Prism.v6.01). Means for different parameters, standard deviation of the means as well as the standard error were estimated. Differences between the means of different groups were determined using one way Prism with Duncan multiple comparison tests. The results were expressed as values as expressed as the mean and standard error ± SE (n=6).

RESULTS

Table 1 describes the diabetic and lipid profile in control and different experimental groups of rats. Diabetic rats presented a significant increase in

fasting blood glucose as well as HbA-1c percent while serum insulin was significantly decreased in the diabetic rats when compared to the control group. While the oral administration of *Cleome droserifolia* (Cd) to the diabetic rats for four weeks was significantly reduced the glycemia and HbA-1c and increased serum insulin levels, when compared to those of diabetic rats.as well as total lipids (TL), total cholesterol (TC), triglycerides (TG), LDL and VLDL levels after four weeks of treatment, in comparison to control rats while HDL show a significant decrease in STZ treated group. The administration of *Cleome droserifolia* ethanolic extract countered the levels of the serum parameters when compared to STZ treated group.

Table 2 recorded the effects of *Cleome droserifolia* ethanolic extract on cardiac and pro-inflammatory markers. The data showed a significant increase in cardiac markers, the creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) activity in STZ administrated group when compared to control rats group. While the treatment with *Cleome droserifolia* ethanolic extract countered these parameters. As well as, the results indicated a significant increase in the pro-inflammatory markers, IL-1B, IL-6 and TNF-α in STZ administrated group when compared to control rats group. But the treatment with *Cleome droserifolia* ethanolic extract ameliorates all of these parameters.

Table 3 represents the oxidative stress and antioxidant parameters as well as the pro-apoptotic markers in cardiac tissue of the control and different rats groups. The diabetic rats showed a significant increase in MDA and H₂O₂ levels when compared to those of control group, where a significant decline in these two parameters when diabetic rats treated with Cd extract was recorded. On the other hand, SOD, CAT and GSH activities were significantly decreased in the diabetic rats when compared to those of control rats group while the oral administration of diabetic rats Cd showed a significant elevation in the activities of SOD, CAT as well as the GSH level when compared to the STZ treated rats groups. The pro-apoptotic markers, P53, BAX and Bcl-2 in the control and different experimental groups of rats were represented in Table (3). The percent of P53 and BAX were significantly increased in the diabetic rats when compared to those of control group while Bcl-2 showed a decrease. While the oral administration of Cd to the diabetic rats showed a significant improvement in the percent of these parameters.

Table 1: Diabetic and lipid profiles in control and different rats groups.

Parameters	Animal Groups				
	Control	DMSO	<i>Cd</i>	Diabetes	Diabetes+ <i>Cd</i>
Glucose (mg/dl)	100.00± 5.60	101.00 ± 3.03	99.00± 1.94	477.00 ± 20.90 ^a	159.00± 11.78 ^{a,b}
Insulin (µIU/ml)	0.33 ± 0.06	0.34 ± 0.04	0.35 ± 0.06	0.14± 0.07 ^a	0.21 ± 0.02 ^{a,b}
HbA-1c (%)	2.69 ± 0.06	2.79 ± 0.08	2.90 ± 0.10	5.93±0.14 ^a	3.73 ± 0.31 ^{a,b}
Total lipids (mg/dl)	403.00 ± 1.20	402.00 ± 0.57	404.00 ± 1.50	505.00 ± 0.88 ^a	455.00 ± 1.15 ^{a,b}
Total Cholesterol (mg/dl)	98.88 ± 2.52	99.25± 1.10	99.62 ± 1.73	152.3 ± 1.25^a	129.8±1.70^{a,b}
Triglyceride (mg/dl)	104.0 ± 3.81	103.0 ± 3.20	104.2 ± 1.83	152.3 ± 1.25 ^a	125.2 ± 3.31 ^{a,b}
LDL-C (mg/dl)	39.34 ± 1.15	38.52 ± 1.11	38.48 ± 1.23	88.77 ± 1.53 ^a	68.51 ± 1.31 ^{a,b}
VLDL-C (mg/dl)	20.8± 0.76	20.30 ± 0.95	20.70 ± 0.421	35.20 ± 1.30 ^a	25.03 ± 0.66 ^{a,b}
HDL-C (mg/dl)	42.00±1.79	40.82± 2.01	42.35± 0.88	15.35± 3.05 ^a	35.73±1.58 ^{a,b}

The values are expressed as the means ± SE (n=10 for each group). DMSO: Dimethyl sulfoxide. *Cd*: *Cleome droserifolia*. a and b Significant change comparing to control and diabetic group respectively. LDL-C: Low density lipoprotein Cholesterol, VLDL-C: Very Low density lipoprotein Cholesterol, HDL-C: High density lipoprotein Cholesterol.

Table 2: Cardiac and pro-inflammatory markers in the serum of control and different experimental groups.

Parameters	Animal Groups				
	Control	DMSO	<i>Cd</i>	Diabetes	Diabetes+ <i>Cd</i>
CK-MB(U/L)	94.00± 3.83	94.00± 2.06	93.00± 3.82	480.00± 22.56 ^a	116.00±6.10 ^{a,b}
LDH (U/L)	990.00± 26.0	1061.00± 21.0	1093.00± 12.48 ^a	3430.00± 41.2 ^a	1527.00 ± 13.8 ^{a,b}
IL-1B (pg/ ml)	0.10 ± 0.03	0.10 ± 0.03	0.09± 0.04	0.40 ± 0.04 ^a	0.13 ± 0.05 ^{a,b}
IL-6 (pg/ ml)	0.13 ± 0.01	0.14 ± 0.002	0.12 ± 0.004	0.43 ± 0.006 ^a	0.18 ± 0.01 ^{a,b}
TNF -α (ng/ ml)	0.152± 0.04	0.147 ± 0.02 ^a	0.132± 0.01 ^a	0.382 ± 0.04 ^a	0.174± 0.02 ^{a,b}

The values are expressed as the means ± SE (n=10 for each group). DMSO: Dimethyl sulfoxide. *Cd*: *Cleome droserifolia*. a and b Significant change comparing to control and diabetic group respectively. CK-MB: creatine kinase- myocardial band, LDH: lactate dehydrogenate, IL-1B: Interleukin -1B, IL-6: Interleukin- 6, TNF: Tumor Necrotic factor.

Table 3: Oxidative stress and antioxidant parameters as well as the pro-apoptotic markers in cardiac tissue of the control and different rats groups.

Parameters	Animal Groups				
	Control	DMSO	<i>Cd</i>	Diabetes	Diabetes+ <i>Cd</i>
MDA(nmol/g wet tissue)	69 ± 0.74	66 ± 1.44	63 ± 2.62	166± 0.91 ^a	74± 1.90 ^{a,b}
H ₂ O ₂ (nmol/g wet tissue)	12.71± 0.80	11.77± 0.58	11.71± 0.72	30.14 ± 0.68 ^a	14.68 ± 0.28 ^{a,b}
SOD (nmol/g wet tissue)	46.1± 2.1	47.2± 3.01	46.6± 3.29	21.9 ± 2.87 ^a	39.8 ± 3.14 ^{a,b}
GSH (nmol/g wet tissue)	21.76 ± 0.09	22.07± 0.87	21.48± 0.31	11.07± 0.88 ^a	16.48± 1.11 ^{a,b}
CAT (nmol/g wet tissue)	42.21 ± 0.81	42.87± 0.96	43.15± 0.79	17.89± 0.63 ^a	39.43± 0.87 ^{a,b}
P53 %	18.56 ± 1.20	18.86 ± 1.09	20.00 ± 0.90	42.92 ± 0.64 ^a	25.86 ± 1.03 ^{a,b}
BAX %	12.71 ± 0.98	13.09± 0.76	12.63 ± 1.04	36.04 ± 1.42 ^a	20.04 ± 0.76 ^{a,b}
BCL-2 %	46.69± 1.06	45.78 ± 0.84	44.74 ± 1.13	21.49 ± 0.54 ^a	38.18 ± 1.49 ^{a,b}

The values are expressed as the means ± SE (n=10 for each group). DMSO: Dimethyl sulfoxide. *Cd*: *Cleome droserifolia*. a and b Significant change comparing to control and diabetic group respectively. MDA: malondialdehyde, H₂O₂: hydrogen peroxide, SOD: superoxide dismutase, GSH: reduced glutathione, CAT: catalase.

DISCUSSION

A large number of studies reported that, diabetes mellitus (DM) is a great health problem⁽¹⁵⁾. In addition, by 2030 the expected number of diabetic patients is increasing very fast and may reach to 439 million⁽¹⁶⁾. Diabetes-associated with changes in the function and structure of the myocardium are called diabetic cardiomyopathy (DCM)⁽¹⁷⁾.

Streptozotocin (STZ) is one of the most substances commonly used to study diabetes in the rats. Death of pancreatic β -cells may exist by this toxin via the alkylation of DNA resulting in release of insulin and reduced DNA synthesis. Moreover, DNA fragmentation may also involve by means of production of reactive oxygen species⁽¹¹⁾. Insulin plays an important role in metabolism, causing an increase in carbohydrate metabolism, glycogen storage, fatty acids synthesis, amino acid uptake and protein synthesis⁽¹⁸⁾.

In the present study, a single injection of the rats with STZ (45mg/kg b.w.) resulted in significant elevation in serum glucose and blood glycosylated hemoglobin (HbA1c) levels but a significant decline in serum insulin was recorded when compared with control group. These changes in blood glucose and insulin concentrations reflect abnormalities such decreased insulin biosynthesis and secretion and impaired glucose utilization (oxidation, glycogenesis and gluconeogenesis). Forgoing experiments have proved that the main reason for STZ-induced cell death is alkylation of DNA⁽¹⁸⁾.

The elevation in HbA1c may be attributed to the higher levels of glucose because the excess glucose present in the blood reacts with hemoglobin to form HbA1c and the rate of glycation is proportional to the concentration of blood glucose, therefore, the measurement of HbA1c is supposed to be a very sensitive index for glycemic control⁽¹⁹⁾.

In the present work, STZ- diabetic rats showed a great disturbances in lipid profile as they exhibited a significant increase in serum total lipids (TL), total cholesterol (TC) and triglycerides (TG) in addition to serum low density lipoprotein (LDL-C) and very low density lipoprotein (VLDL-C) levels while a significant decrease in serum high density lipoprotein (HDL-C) level was reported when compared to the control group. The increase in the motivation of free fatty acids from the peripheral fat depots as due to insulin deficiency reflecting the abnormal

high concentration of serum lipids in diabetic rats. This is because the insulin inhibits the lipase enzyme. Thus, increase fatty acids level in serum promotes the liver conversion of them into phospholipids and cholesterol. Both phospholipids and cholesterol, side to side with high triglycerides formed in the liver, may be leaked into the blood in the form of lipoproteins⁽²⁰⁾.

In diabetes, the decreased level of HDL-C, together with increased LDL-C concentrations is the explanation of the increased cholesterol measure. About 60-80% of cholesterol is carried by LDL-C for that it represents the main cholesterol carrier in the blood. Cholesterol returned to liver is used by tissue and others⁽²¹⁾, but cholesterol may be deposited when there is a much LDL-C in blood. The main coronary risk factor enhancing atherosclerosis is the high levels of both TC and LDL-C. Based on this, the present study results of increased TC and LDL-C with decreased HDL-C concentrations in diabetic rats can be considered as indication for enhanced cardiac injury. The present results indicated a marked hyperlipidemia that characterized the diabetic state which is responsible for the risk factor for cardiovascular diseases.⁽²²⁾

Heart injury or disease can be assessed by both creatine kinase (CK) and lactate dehydrogenase (LDH). Both are energetic enzymes, which prominently present in cardiac tissue. Therefore, the present results of increased serum activities of both CK and LDH can be considered as indicator for myocardial damage, as evidenced here by the increased accumulation of both H_2O_2 and MDA in the cardiac tissue of diabetic rats⁽²³⁾.

Also, DM are related to increased levels of a different of adipokines (cytokines released from adipose tissue), including tumor necrosis factor- α (TNF- α), interleukin 1 β (IL-1 β), interleukin 6 (IL-6), and plasminogen activator inhibitor 1 (PAI-1), which associated to the inflammatory response. As fat mass increases, the levels of these pro-inflammatory cytokines typically increase⁽²⁴⁾.

In the present study, a significant increase in TNF- α , IL-1 β and IL-6 was recorded in STZ-group compared to control group. It is widely recognized that cardiomyocyte hypertrophy, cardiac inflammation, fibrosis, increased apoptosis, and metabolic abnormalities are present in diabetic cardiomyopathy. Hyperglycemia and metabolic disorders are interrelated to ROS overproduction. It's well-known that, chronic inflammation and fibrosis in

tissues of DM induce due to long-term exposure to oxidative stress. This event is leading to formation and progression of disease states. As well as the oxidative stress and inflammation play the main role in the pathogenesis and progression of diabetes-induced CVD. Different cytokines such as C-reactive protein (CRP) or oxidative stress-related proteins were consider as biomarker for the onset of CVD, where it shows an increased expression of inflammatory proteins⁽²⁵⁾.

The pathogenesis and progression of different forms of CVD are mainly dependent on the inflammation. Pro-inflammatory cytokines and chemokines are responsible for the chronic inflammatory process and may also contribute to the pathogenesis of diabetic cardiomyopathy. Moreover, inflammatory cytokines, such as TNF- α , IL-1 β and IL-6 concentrations are elevated in the serum of diabetic patients. For that, a link between this chronic inflammation and cardiac dysfunction can be suggested⁽²⁶⁾. It is well known that oxidative stress results from unbalance between the generation of oxygen derived radicals and the organism's antioxidant potential⁽¹⁷⁾. Diabetic state is often associated with oxidative stress, which is supposed to play an important role in the development of the diabetic complications. Reactive oxygen species (ROS) are reported to inducing cell dysfunction or death. In general, oxidative stress increases in diabetes because of multiple factors; dominant among these factors is glucose oxidation which leading to the production of free radicals⁽²⁷⁾. Other important factor includes the reduction of antioxidant defense including decreased cellular antioxidant levels and reduction in the activity of enzymes that dispose of the free radicals. The two mentioned factors lead to cellular oxidation reduction imbalance. This may be related to the association of STZ destruction of islet's cells and the generation of free radicals and rise in oxidative stress markers which would damage the inner endothelial tissue and would eventually be directly responsible for high blood glucose levels.⁽²⁸⁾

Exposure of islets of Langerhans to STZ causes severe oxidative and cytotoxic stress to islets that is likely to compromise their insulin releasing capacity. Oxidative stress which is the main cause of defective insulin secretion and increases of pancreas apoptosis are attributed to the overproduction of ROS or consumption of antioxidants may cause. Moreover, β -cell failure are resulted from the ROS produced by β -cell in

response to metabolic stress affect mitochondrial structure and function. Specifically, mitochondrial membrane phospholipids such as cardiolipin are oxidized by ROS; this leads to cytochrome c release and apoptosis which resulted from impairs membrane integrity⁽²⁹⁾. However, chronic diabetes is characterized by increased lipid peroxidation; the decrease in enzymatic and non-enzymatic antioxidant defense systems might be a reflection of lipid peroxidation process. In contrast, increased free radicals production which is react with polyunsaturated fatty acids in cell membranes resulting in lipid peroxidation, this is a reversible reaction leading to the elevated production of free radicals⁽³⁰⁾. Both type I and type II diabetes mellitus development were found to be mediated via lipid peroxide- damage. As low levels of lipooxygenase peroxides stimulate the secretion of insulin, the increase concentration of endogenous peroxides may initiate uncontrolled lipid peroxidation. This is leading the cellular infiltration and islet cell damage in type I diabetes. Increasing membrane fluidity and changing the activity of membrane- bound enzymes and receptors are the main characters of increase dimpairs membrane function sdue to the increased lipid peroxidation. Nearly all living organisms possessa common enzyme called catalase. It can catalyzeand decompose hydrogen peroxide to water and oxygen. One of the main cardiac oxidative stress preconditioning is H₂O₂. It is formed when the spontaneous dismutation of superoxide (O₂⁻) or catalyze reaction enzymatically using superoxide dismutase (SOD). Compared to other ROS, H₂O₂ is less reactive and easily crosses membranes, and diffuses from its original site of production⁽¹⁴⁾. Based on the mentioned above, the present findings of increased oxidative stress, as demonstrated by the elevated cardiac MDA and H₂O₂ concomitant with decreased cardiac antioxidants (SOD, CAT, and GSH) were recorded. For that events, proteins, lipids and nucleic acids the cell components are affected when the balance normally present in cells between radicals formation and protection against them is disturbed oxidative damage are exist. In this status, different blood enzymes have reported to be marker of cell damage with elevating cell membrane permeability. All these enzymes become measured in the serum, perhaps when they are leakage through diseased or damaged tissues, the membrane permeability are altered⁽³¹⁾. Previous studies documented that impaired

glucose tolerance impaired glucose tolerance is interrelated to the marked increase in the levels of proinflammatory cytokines and ROS. In addition, STZ-induced cardiac apoptosis was also evident from the DNA damage. A significant correlation between oxidative DNA damage and hyperglycemia was previously reported⁽³²⁾. Also, P53 and its regulated genes can be activated by hyperglycemia, leading to cardiomyocyte death⁽³³⁾. It is reported that P53 has the ability to induce apoptosis by an ROS dependent pathway. In contrast, Bcl-2 exhibits an antiapoptotic and anti-necrotic influence via its antioxidant effect on intracellular ROS. Bcl-2 can decrease lipid peroxidation by increasing cell resistance to ROS and blocking ROS production⁽³⁴⁾.

Drug development need to novel molecular scaffolds. Natural products (NP) are structurally diverse and serve as a valuable source for these novel molecular scaffolds. Traditional anti-diabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities, or as simple dietary adjuncts to the existing therapies. Globally, there is renewed concern in using NPs as a trigger beginning of drug discovery and remission of different diseases⁽⁷⁾. Pancreatic antioxidant activity of the drugs used in treatment of diabetes are dependent mainly on their protective strategy to protect β -cell from the out of proportional generation of free radicals⁽³⁵⁾. Valuable bioactive compounds such as flavonol glycosides, tannins, terpenes, flavonoids, Steroids, and alkaloids are the main constituents of *Cleome droserifolia* (*Cd*). The phenolics constitute of plant is one of the main groups of compounds which acting as primary antioxidants terminators for free radical. As well as, the flavonoids is one of the main universal and distributed groups of natural compounds. Radical scavenging characters of both phenolics and flavonoids are probably the most important natural properties they possess⁽¹⁴⁾.

In the present study, a significant decrease in glucose level and HbA1c % as well as increase in insulin level were observed in rats treated group comparing to STZ group. Multifaceted benefits may be existing from using *Cd* extract due to their valuable contents of several active constituents or compounds. These compounds can act by different modes of action to efficiency producing different biological pathways and to alleviate the diabetic symptoms. *Cd* possessing different mode of actions, these may be increasing

insulin secretion from the pancreas or inhibiting glucose absorption from intestine, as well as inhibiting glucose production from hepatocytes or enhancing glucose uptake into the peripheral tissue all these may be via the orchestrating of glucose transporters molecules⁽³⁶⁾. When compared to those of diabetic rats with no treatments, the treated ones with *Cd* for 30 days shows a significant reduction in glycemia as well as significant increase in serum insulin levels. This may explained with the major active constituent found in *Cd* the flavonoids, are the potential mediators for anti-diabetic action for their properties in possessing different hypoglycemic and antihyperglycemic characters. As well as, flavonoid-enriched extract contents which efficiently inhibited α -glucosidase activity and facilitating intestinal epithelial cells monosaccharides diffusion⁽³⁷⁾.

In the present study, a significant decrease in all the lipid profile measured (TL, TC, TG, LDL-C and vLDL-C) were observed in the STZ + *Cd* treated group comparing to STZ group, while a significant increase in HDL-C was noted. Meantime, the administration of *Cd* extract showed the significant elevation in different parameters that clarified by significant high concentration of HDL-C. These results may be attributed to the presence of flavonoids. Flavonoids in *Cd* are reported to inhibit the activity of cAMP - dependent protein phosphokinase⁽¹⁴⁾. Results of this may explained with the increases in cAMP concentration and that phosphorylation of the Hydroxy methyl glutaryl-CoA reductase, with the reduction of endogenous cholesterol production⁽³⁸⁾. Also, Helal⁽³⁹⁾ recorded that, the administration of *Cd* caused reduction in the levels of lipid profile with elevation in HDL-C. They attributed these results to the presence of flavonoids (Quercetin-3,7-O-dirhamnoside, Kaempferol -3,7-O-dirhamnoside, kaempferol -7-O-glucoside and 6-hydroxy kaempferol-3-O-glucoside). Flavonoids were found to reduce different stages in the initiation of atherosclerosis and endothelial damage through its antioxidant activity and free radical scavenging assay.

The present data reported that, *Cd* treatment significantly decreased the oxidative stress parameters as MDA and H₂O₂ in concomitant with increased the GSH levels and normalized the activities of SOD and CAT enzymes in the hearts of STZ+*Cd* treated rats. These results are agreement with a number of publications which demonstrating the anti-peroxidation effect of *Cd*

⁽¹⁴⁾. It is worth mentioning that *Cd* treatment significantly augmented the antioxidants level in the cardiac tissue, revealing its potential ability to improve the antioxidant mechanisms in the heart. These results indicate that *Cd* possesses potent antioxidant capacity that is responsible for cardio-protection against oxidative stress. Moreover; a significant decrease in both cardiac markers (CK-MB and LDH) was observed in the STZ + *Cd* treated group comparing to STZ group. *Cd* administration significantly ameliorated the elevated levels of LDH and CK-MB activities toward normal levels, indicating that *Cd* is an efficient cardioprotectant in diabetic conditions and can protect cardiomyocyte membrane integrity.⁽⁴⁰⁾

Myocardial inflammation may be the cause of high level of ROS resulting from sustained hyperglycemia, which is characterized by the production of large number of cytokines. Earlier studies documented that the pro-inflammatory cytokines and ROS levels were increased markedly and were related to with destruction of glucose tolerance. For that, as a hypothesis *Cd* can orchestrate due to its antioxidant properties different cellular activity during inflammation. The present study showed that *Cd* reduce IL-1b, IL-6 and TNF- α levels in STZ-induced diabetes in rats. Several recent experimental studies suggest anti-inflammatory effects of *Cd* polyphenols in other settings that are characterized by increased oxidative stress^(23,41). **El-Abhar⁽⁴²⁾**, concluded that cyclooxygenase and lipoxygenase play an important role as inflammatory mediators. Flavonoids inhibits both cyclooxygenase and lipoxygenase activities along with eicosanoid biosynthesis, thus diminishing the formation of these inflammatory metabolites. These data suggest that *Cd* could be an interesting nutritional source that contributes to preventing myocardial inflammation in diseases characterized by excessive oxidative stress, such as diabetes. To understand the mechanism underlying the cardioprotective action of *Cd* on cardiac apoptosis in diabetes, we investigated the involvement of the STZ-mediated extrinsic and intrinsic apoptotic cell death pathway in cardiac tissues. Using flow cytometric analysis the pro-apoptotic proteins were found to be regulated with *Cd* administration after progression of programmed cell death in cardiac tissues induced by STZ. This may be resulted by elevation of pro-apoptotic proteins, such as Bax and P53 and anti-apoptotic proteins, such as Bcl-2. These results explained a marked participation of both

mitochondrial and death receptor apoptotic pathways in the hearts of *Cd*-treated diabetic rats which provide guide that *Cd* has a major action in orchestrating apoptotic pathways in diabetes⁽⁴³⁾.

CONCLUSION

From this study we may conclude, *Cleome droserifolia* may possess a protective action versus heart muscle injury in STZ-induced diabetic rats. It could also reduce myocardial injury resulted from STZ administration in rats. Therapeutic involvements in these aspects may result in a targeted and technical-based treatment strategy versus myocardial injury following STZ-induced diabetic.

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