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THERAPEUTIC EFFECT OF MULBERRY LEAF EXTRACT ON CARDIAC OXIDATIVE STRESS IN TYPE 2 DIABETIC MALE RATS

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ABSTRACT
Diabetic complications result from long-term hyperglycemia that causes macrovascular and microvascular damage, endangering the heart. Therefore this study was conducted to evaluate the cardioprotective effect, antioxidant activity and anti-diabetic effect of mulberry (Morus alba L.) leaf extracts in diabetic rats. Streptozotocin was injected in male Wistar rats to induce type 2 diabetes. After confirmation of diabetes, animals were treated orally with mulberry dried leaf extracts (600 mg/kg) everyday for 4 weeks. Mulberry leaf extract was effective in lowering blood glucose, Glycosylated hemoglobin, Nitric oxide, malondialdehyde (MDA) and increasing insulin level and markers of cardiac oxidative stress including superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPX). Lipid profiles including total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-c), and very low-density lipoprotein cholesterol (VLDL) were significantly decreased whereas high-density lipoprotein cholesterol (HDL-c) and adipsin were significantly increased in the treated diabetic rats which were treated with Mulberry leaf extract in comparisonto the diabetic control ($p<0.05$).The results confirm the effect of mulberry on modulation of glucose metabolism through correcting hyperglycaemia, increasing insulin secretion, and improving cardiac oxidative stress parameters in STZ- induced diabetic rat model and also emphasize the effectiveness of Adipsin as a novel prognostic biomarker for cardiovascular diseases.

KEYWORDS: *Diabetes, Cardiac Oxidative stress, Mulberry leaf extract, Adipsin.*

1. INTRODUCTION
One of the most widespread health problems is diabetes type 2, particularly in developed countries like Japan, Europe, and North America. According to the World Health Organization (WHO), 300 million individuals will have diabetes worldwide by 2025 **(Anonym, 2011).**

Diabetes has a considerable impact on Egypt's morbidity, mortality, and medical care funding. 15.6% of Egyptians between the ages of 20 and 79 have type 2 diabetes (T2D). Egypt has the tenth-highest number of T2D patients in the world, according to the International Diabetes Federation (IDF) **(Hegazi et al., 2015)**.

Diabetic complications result from long-term hyperglycemia that causes macrovascular and microvascular damage, endangering the heart, brain, kidneys, peripheral nerves, eyes, and feet **(Borghetti et al., 2018)**. An association between heart failure and diabetes has been reported **(Irshad and Muddasarul, 2020).** Epidemiological studies have shown a 2.5-fold increased risk of heart failure among diabetes patients compared to their healthy age-matched counterparts, indicating the need for studies on diabetic cardiomyopathy (DCM) **(Devereux et al., 2000; Lorenzo- Almoros et al., 2017)**.

Chronic hyperglycemia results in myocardial structural and functional alterations that induce heart failure that is not related to other conventional cardiovascular risk factors and, ultimately, DCM **(Karamitsos et al., 2008).** Additionally, DCM may be one of the main causes of death in people with diabetes, so it is imperative to create a medicine that can successfully treat DCM **(Borghetti et al., 2018).** Due to its effectiveness, affordability, and safety, herbal therapy has lately received recognition as a potential diabetes treatment technique **(Gulsin et al., 2019).** Many nations have a large mulberry (Morus alba) population, and their dried leaves are utilized extensively in herbal medicine **(Han et al., 2020)**.

In traditional Chinese medicine, mulberry leaves are used to cure diabetes. Recent studies have revealed that mulberry leaves contain polysaccharides, flavonoids, alkaloids, volatile oils, and other active components that

have hypoglycemic, hypolipidemic, antioxidant, anti-aging, and other effects. Mulberry leaf extract (MLE) is frequently used in the treatment of diabetes as a result **(Gurukar and Chilkunda, 2018; Wang et al., 2018; Wu, 2019; Zhang et al., 2019).** This investigation examined the potential cardioprotective, antioxidant, and anti-diabetic properties of mulberry (Morus alba L.) leaf extracts in diabetic rats.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Streptozotocin was purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA). Leaves of Morus alba were collected from a local farm. All other chemicals used in this study were of analytical grade. Rat protein Carbonyl Eliza kit was purchased from Bioassay Technology Laboratory Co., Shanghai, China. Cardiac oxidative stress parameters including SOD, CAT, NO, GSH, GPX and MDA were measured in cardiac tissue using Eliza kit purchased from CUSABIO BIOTECH CO.,Ltd . Troponin I (cTn-I) and Adipsin were measured in sera samples using Eliza kit purchased from CUSABIO BIOTECH CO., Ltd. Rat HbA1c was measured in sera samples using Eliza kit purchased from LifeSpan BioSciences, Inc.

2.2. Determination of serum cholesterol level:

The cholesterol is determined after enzymatic hydrolysis and oxidation according to the method of **Richmond, (1973)**.

2.3. Determination of serum HDL-cholesterol level:

Serum HDL - cholesterol was determined according to the method of **Lopez-Virella, (1977)**. The serum low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL) were calculated by the Friedewald formula **(Friedewald et al., 1972)**
As follows:
LDL-c = Total cholesterol – [HDL-c + (TG/5)] VLDL-c = TG/5.

Caclulation of antiatherogenic index (AAI):
The antiatherogenic index (AAI) was calculated according to the formula of **Guidoand Joseph (1992)**.

$$\text{AAI (\%)} = \frac{\text{HDL-cholesterol}}{\text{Total cholesterol - HDL-cholesterol}} \times 100$$

2.4. Measuring blood glucose levels

A glucometer (GlucoPlus Inc., Quebec, Canada) with a maximum measuring capacity of 600 mg/dL) was used for measuring blood glucose levels.

2.5. Determination of serum insulin level

Serum insulin level was assayed by solid-phase radioimmunoassay technique (RIA) according to the method of **Burtis and Ashwood, (1994).**

2.6. Determination of Cardiac oxidative stress parameters

Superoxide dismutase (SOD) and glutathione peroxidase (GPX), reduced glutathione (GSH) activities, catalase (CAT) activities and the malondialdehyde (MDA) content in heart tissues were determined using commercially available kits. The samples were analyzed using a miltonroy-1201 spectronic spectrophotometer.

2.7. Preparation of Mulberry Leaf Extracts (MLE) and Isolation

Mulberry Leaf Extracts were prepared according to the method ofTond et al., (2016) Leaves of Morus alba were collected from a local farm. It was washed thoroughly under running tap water, shade dried, and ground to a fine powder using an electric blender. 2200g of dried leaves powder were extracted three times with 96% ethanol by maceration at room temperature. The mixture wasfiltered, evaporated in vacuum evaporator to give 112g of extract. The obtained dry extract was suspended in water followed by extraction with hexane, $CHCl_3$ and ethyl acetate for three times consecutively**.**

2.8. Experimental animals

Twenty-four albino rats weighed 180 g ± 10 g on average were used in the present study. They were obtained from the animal house of Atomic Energy Authority. They were caged under controlled environmental and nutritional conditions (25°C and 55–60% relative humidity). Rats were provided with standard diet and tap water ad libitum. Rats were adapted to the laboratory environment for one week before commencement of the study.

2.9. Experimental design

In this experimental study, 24 male albino rats randomly divided into 4 groups (6 rats in each group). One of them was selected randomly as control (**Group 1**) it was treated only with placebo (citrate buffer) intraperitoneally. **Group 2 (Mullberry group)** received mulberry leaf extracts only (600 mg/kg) every day for 28 day. **Group 3 (Diabetic group)** diabetic control received normal diet and water ad libitum (type 2 diabetes was induced by administration of streptozotocin (55mg/kg of body weight). **Group 4 diabetic + mulberry leaf extracts** (600 mg/kg) every day for 28 day.

2.10. Induction and selection of type 2 diabetic rats

Type2 diabetes was induced in male wistar rats by a single-doseintraperitoneal (IP) injection of 55mg/kg b.w. STZ. STZ was dissolved in citrate buffer, pH 4.5 while the respective control rats were given vehicle citrate buffer **(Tond et al., 2016)**. Fasting blood glucose was measured in blood samples obtained from the tail viens of rats 48 h after STZ injection. Diabetes induction was confirmed through measurement of blood glucose level with glucometer and rats with blood glucose levels more than 126 mg/dl were considered diabetic and selected for further studies **(Shirwaikar et al., 2006)**

2.11. Sample Collection and Preparation.

Twenty-four hours after the last treatment, overnight fasted rats were sacrificed by cervical dislocation. Blood was collected and sera samples were obtained by centrifugation at 5000 rpm for 15 min then they were kept in the freezer at -80 ^{o} c to determine lipid profile parameters, troponin, creatine phosphokinase (CPK) and adipsin. Hearts were excised and washed. Samples from the heart were homogenized in cold phosphate-buffered saline (10% w/v), and
clear homogenate was collected to assay MDA, nitric oxide (NO), GSH, superoxide dismutase (SOD), catalase, protein carbonyl(PCO) and glutathione peroxidase (GPx) . Other samples were fixed in 10% formal saline solution, processed, embedded to obtain paraffin blocks and cut at 5-6 micron thickness sections. Sections were stained with Haematoxylin and Eosin.

2.12. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test using statistical software package (SPSS for Windows, V.16.0). All results were expressed as mean ± standard deviation (SD).

3. RESULTS

3.1 Effects of mulberry leaf extracts on lipid profile (Total cholesterol, Triglycerides, HDL, LDL, and VLDL).

The effect of mulberry leaf extracts administration on lipid profile was shown in table (1). Significant increase in the total cholesterol, triglyceride, VLDL and LDL concentrations were detected in the serum of type-2 diabetic rats compared to the control group (P<0.05). The administration of mulberry leaf extracts suppressed the increase in the total cholesterol, triglyceride, VLDL and LDL levels in the serum of diabetic rats. Serum HDL-c was significantly lowered by diabetes induction (P<0.05); however, it was higher in mulberry leaf extracts supplemented groups compared to the untreated diabetic groups (P<0.05).

Table 1: Effects of mulberry leaf extracts on lipid profile (Total cholesterol, Triglycerides, HDL, LDL, and VLDL).

	Parameters				
Group	**Total cholesterol (mg/dl)**	**Triglycerides (mg/dl)**	**HDL(mg/dl)**	**LDL (mg/dl)**	**VLDL (mg/dl)**
Control	90.4 ± 2.5 ^{a}	57.22 ± 4.3 ^{a}	39.98 ± 2.8 ^{c}	41.18 ± 1.18 ^{a}	18.42 ± 0.49 ^{a}
Mullberry	89.16 ± 6.03 ^{a}	50.26 ± 3.08 ^{a}	41.36 ± 4.08c	39.1 ± 1.11 ^{a}	19± 0.86 ^{a}
Diabetic	180.68 ± 3.1 ^{c}	101.17 ± 7.5 ^{c}	23.14 ± 2.8 ^{a}	55.6± 3.69 ^{c}	32.30 ± 2 ^{c}
Diabetic+	125.22 ± 5.9 ^{b}	68.72 ± 6.8 ^{b}	32.22 ± 2.3 ^{b}	47.12 ± 2.25 ^{b}	24 ± 1.5 ^{b}
Mullberry					

Data are represented as Means ± SD; values in the same column with different superscripts are significantly different at P < 0.05. (n =6 in each group)

3.2 Effect of mulberry leaf extracts on fasting blood glucose (FBG), insulin and Glycosylated hemoglobin (HbA1C %)

There was a statistically significant increase in levels of FBG and HbA1C% with a significant decrease in insulin levels in the diabetic group as compared to the normal control group (P < 0.05). Treatment with MLE (group 4) showed statistically significant decrease in levels of FBG and HbA1C%, with a significant increase in insulin levels as compared to the normal control group (P< 0.05) as shown in figure (1), (2)&(3).

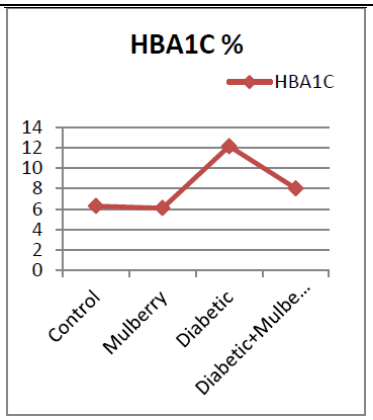

Figure (1): Effects of mulberry leaf extracts on Glycosylated hemoglobin (HbA1C %)

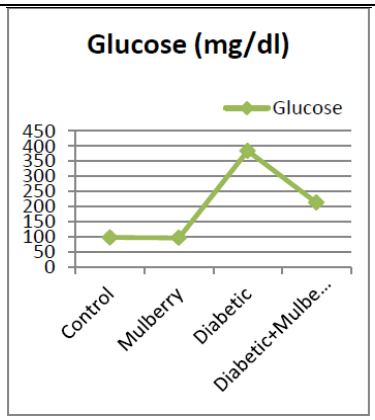

Figure (2): Effects of mulberry leaf extracts on blood glucose levels

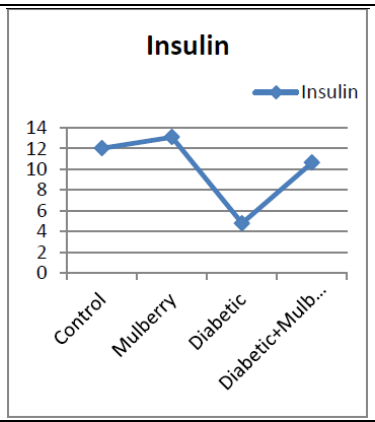

Figure (3): Effects of mulberryleaf extracts on insulin levels

Data are presented as the mean ± standard deviation of the mean (n =6 in each group). P <0.05

3.3. Mulberry leaf extracts alleviate cardiac oxidative stress in the diabeticrats

Oxidative stress has been implicated in the pathogenesis of type 2 diabetes and its complications. As shown in figure (4)&(5) SOD, GPX and CAT activities decreased significantly ($p<0.05$) in diabetes group as compared to control group, whereas figure (6) indicates a significant decrease in the GSH content ($p<0.05$) in the same group as compared to control group. On the other hand, the diabetes group treated with mulberry leaf extracts showed significant ($p<0.05$) increases in the GSH content, SOD, GPX and CAT activities as represented in figure (4),(5) &(6) .

The test results of the mean values of MDA level in normal and experimental groups were given in figure (6). Diabetic group manifested significant increments ($p<0.05$) in levels of MDA compared to that in the normalcontrol group. Whereas, significant reduction in MDA levels were detected in thediabetes group treated with mulberry leaf extracts.

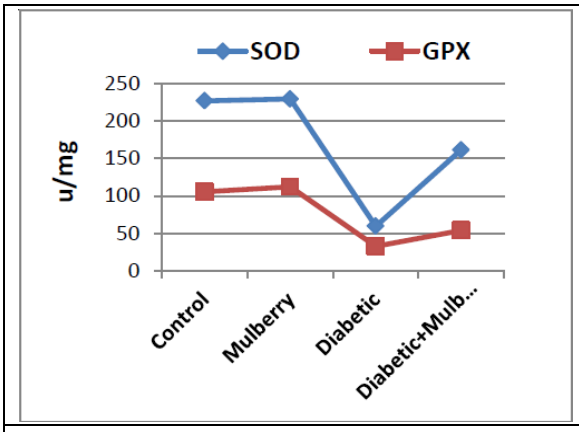

Figure (4): Effects of mulberry leaf extractson SOD and GPX activities

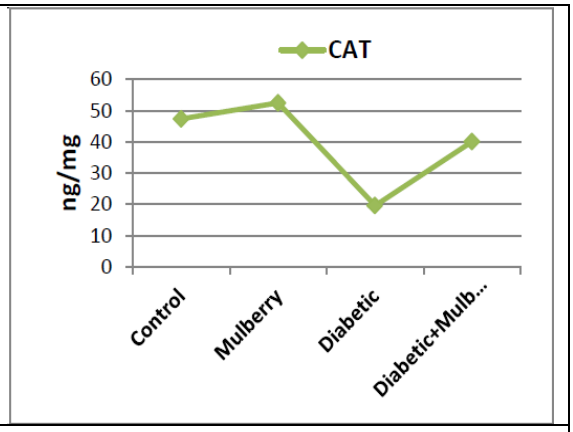

Figure (5): Effects of mulberry leaf extracts on CAT activities

Data are presented as the mean ± standard deviation of the mean (n =6 in each group). P <0.05

3.4. Effects of mulberry leaf extracts on levels of Nitric oxide and protein carbonyl content (PCO)

Tissue NO and PCO content in control and diabetic rats were shown in figure (6). We found a significant increase ($P<0.05$) in NO and PCO content in diabetic group compared with their respective controls. While, the treatment of diabetic rats with mulberry leaf extracts resulted in significant reduction ($P<0.05$) in NO and PCO content near the normal values.

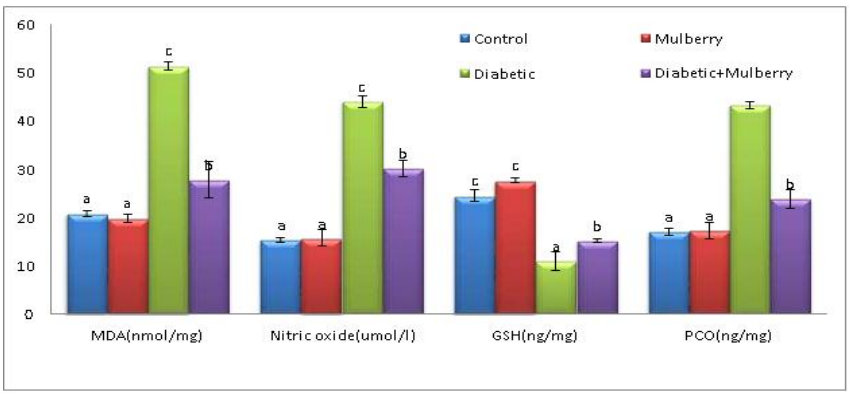

Figure (6): Effects of mulberry leaf extracts on levels of MDA, Nitric oxide, GSH and PCO.Data are presented as the mean ± standard deviation of the mean (P <0.05) (n =6 in each group).

3.5. Effects of mulberry leaf extracts on levels of Troponin, Adipsin and creatine phosphokinase (CPK).

According to the data tabulated in table (2), Troponin and CPK levels were significantly increased ($P<0.05$) in diabetes rats as compared to those found in the control group. On contrast, the Troponin and CPK levels were significantly ($P<0.05$) decreased in diabetic group treated with mulberry leaf extracts as compared to the untreated rats. On contrast, as shown in table (2), Adipsin was significantly ($P<0.05$) decreased in diabetic group as compared to those found in the control group. Treatment of diabetic group with mulberry leaf extracts resulted in significant increase ($P<0.05$) in Adipsin as compared to the untreated rats.

Table (2): Effects of mulberry leaf extracts on levels of Troponin , Adipsin and creatine phosphokinase (CPK).

	Parameters		
Group	**Troponin (pg/ml)**	**CPK (U/l)**	**Adipsin (ng/ml)**
Control	21.38± 1.46 [a]	647.4 ± 30.08 [b]	8.67 ± 0.28 [c]
Mullberry	18.66 ± 3.03 [a]	548.56 ± 39.68 [a]	8.41 ± 0.29 [c]
Diabetic	51.62 ± 2.29 [c]	1308.4 ± 44.03 [d]	5.68 ± 0.16 [a]
Diabetic+	35.78 ± 3.4 [b]	857.72 ± 24.97 [c]	6.66 ± 0.11 [b]
Mullberry			

Data are represented as Means ± SD; values in the same column with different superscripts are significantly different at P < 0.05. (n =6 in each group).

3.6. Histological analysis of the Haematoxylin and Eosin stained heart specimens:

Examination of heart sections of the rat animals from the control group showed classical histological appearance as the cardiomyocytes were oriented longitudinally with characteristic branching and anastomosing pattern. The myocytes exhibited acidophilic sarcoplasm in which large central ovoid pale- stained vesicular nuclei were situated and transverse crossstriations were clearly seen. The fibroblasts were observed in the narrow interfiber spaces (Fig. 7). However, sections of diabetic group showed evident histological affection. The cardiac myocytes of diabetic group appeared fragmented, disrupted and separated with apparent increase in the interfiber spaces. Moreover, some interfiber spaces were occupied by fibrosis and aggregation of mononuclear cellular infiltration. The cardiac myocytes exhibited deeply stained eosinophilic sarcoplasm with pyknotic nuclei. Dilated congested blood vessels were detected. While in Diabetic+Mullberry (group 4), most cardiac myocytes achieved nearly normal appearance, showing long slender, branched, and anastomosing fibers with centrally placed vesicular nuclei. Meanwhile, some cardiac muscles fibers were still fragmented.

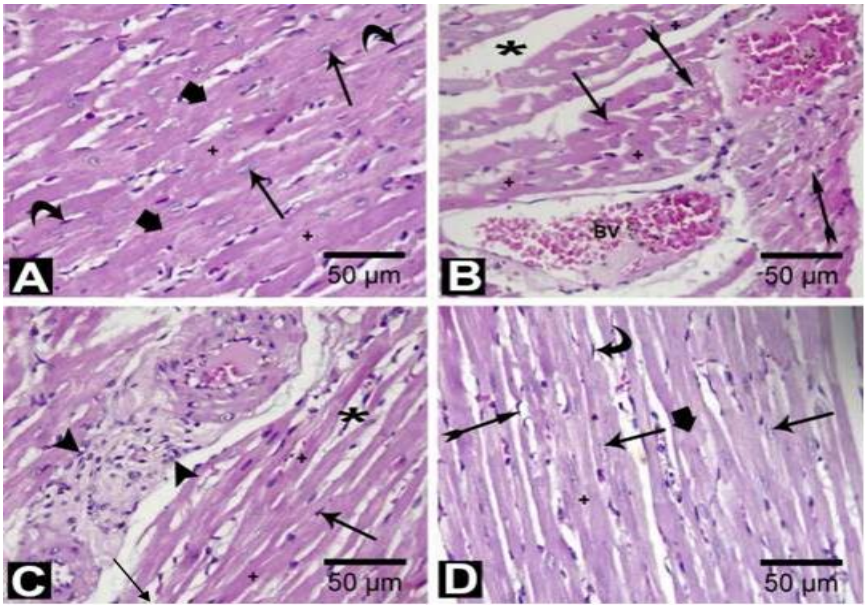

Figure 7: Photomicrograph of the myocardium a section of an adult rat of control Group (A), diabetic group (B, C) and Diabetic+Mullberry group (D) showing: A) cardiac myocytes with centrally located oval vesicular nuclei (arrow), acidophilic sarcoplasm (+) and with transverse cross-striations (short thick arrow). The fibroblasts (curved arrow) were observed in the narrow interfiber spaces. B,C): diabetic group showing fragmented, disrupted and separated the cardiac myocytes (bified tailed arrow), cardiac myocytes deeply stained eosinophilic sarcoplasm (+)with pyknotic nuclei (arrow), increase in the interfiber spaces(*), fibrosis and aggregation of mononuclear cellular infiltration (arrowhead) and dilated congested blood vessels (BV). D) The Diabetic+Mullberry group showing most of cardiac myocytes exhibit normal appearance, with centrally placed vesicular nuclei (arrow), acidophilic sarcoplasm (+) and with transverse cross- striations (short thick arrow), some cardiac myocytes are still fragmented ((bified tailed arrow).

4. DISCUSSION

In this study we showed that 4 weeks treatment of diabetes group with mulberry leaf extracts significantly decreased triglyceride, cholesterol, LDL and increased HDL in blood in comparison to the control group and diabetes group, respectively. Also, our findings showed that treatment of diabetic rats with mulberry leaf extracts significantly decrease blood glucose levels, Glycosylated hemoglobin (HbA1C %) and significantly increased insulin leveles in comparision to control group. Diabetes

mellitus is associated with a cluster of interrelated plasma lipid and lipoprotein abnormalities, including reduced HDL cholesterol, a predominance of small dense LDL particles, and elevated triglycerides **(Erejuwa, 2012)**. There is an evidence that each of these dyslipidemic features is associated with increased risk of arteriosclerosis, cardiovascular disease and renal failure leading to death in patients with diabetes **(Qureshi et al., 2002)**.

In this work, we demenstrated that diabetes leads to dyslipidemia in rats and treatment with mulberry leaf extracts for 4 weeks reversed this abnormality in blood of diabetic rats. The extract of mulberry leaves enriched with DNJ, quercetin and kaempferol activated the expressions of AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor (PPAR)-a, leading to the increase in b-oxidation of free fatty acid and lipid breakdown **(Tsuduki et al., 2009; Kobayashi et al., 2015).**

Our results showed that, in all treated rats the increased levels of glucose were lowered significantly, but the level of FBG in treatment groups was still higher than the normal group. Additionally, in diabetic rats, degranulation or reduction of insulin secretion, arise from destruction of β-islet cells of pancreas. By the administration of MLE, the reduced glucose levels suggested that they likely increase the insulin secretion, which in turn, raise glucose uptake by tissues **(Lu et al., 2008)**. Elevation of the serum insulin in treated rats could be due to the insulinotropic substances, which induce the intact functional β-cells of the langerhans to produce insulin, or protect the functional β-cells from further deterioration, so that they remain active and produce insulin. It seems that the hypoglycemic effect of MLE is due to the increased level of serum insulin and the enhancement of peripheral metabolism of glucose **(Skim et al., 1999)**. On the otherhand, the hypoglycemic activity of mulberry leaves may be attributed to the high fiber content, the presence of trigonelline bases and moran A and/or moranoline in mulberry leaves.

Mulberry leaf extract also contain other compounds with significant hypoglycemic activity in diabetic rats **(Mohammadi et al., 2008)**. One of major constituent of Morus alba is deoxynojirimycin (DNJ) **(Butt et al., 2008)**. DNJ, as a competitive inhibitor of intestinal α-glucosidase, affecting carbohydrate digestion and absorption, resulting in suppressed postprandial hyperglycemia. Intake of DNJ inhibited D-glucose uptake at the intestinal brush border membrane because of its similar size and, to some extent, structure to D-glucose **(Skowron et al., 2014).**

In the present study, we illustrated that values of cardiac oxidative stress biomarkers (MDA, GSH, CAT, GPX and SOD) in the diabetes group were significantly changed. The possible sources of increased oxidative stress might include increased generation of free radicals or an impaired antioxidant defence system. Enhanced levels of free radicals were found in diabetes **(Mooradian, 2009)**. Oxygen-free radicals are toxic to tissue because of their high reactivity and ability to form covalent bonds nonenzymatically **(Ola et al., 2006)**. Extensive studies with biological materials have shown clearly that these active free radicals are able to produce chemical modifications in the cells and damage the proteins, lipids, carbohydrates and nucleotides.

Therefore, increased ROS causes cardiac dysfunction **(Quan et al., 2014)**. To overcome these consequences, cells have antioxidant defence systems, which scavenge the free oxygen radicals and suppress free radical chain and lipid peroxidation **(Uikey et al., 2003)**. Furthermore, this study found an increase in the MDA level in diabetes group compared with nondiabetes groups, supporting findings by other studies **(Moussa, 2008;Kumawat et al., 2009 ; Dawud et al., 2012)**. As an aldehydic product of lipid peroxidation, MDA is a biomarker of intensified lipid peroxidation and also indirect evidence of high free radical production in diabetes (**Dawud et al., 2012**).

Increased lipid peroxidation impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors (**Moussa, 2008**). Increased lipid peroxide may be due to the increased glycation of proteins in diabetes, and the glycated proteins might themselves act as a source of free radicals. There is a clear association between lipid peroxide and glucose concentration, which also may be thought to play a role in increased lipid peroxidation in diabetes **(Kumawat et al.,2012)**.

The study by Bhutia et al **(Bhutia et al., 2011)** noted significant increases of MDA level and fasting plasma glucose in poorly controlled type 2 diabetes. In the present study, the increased levels of MDA clearly show that diabetes patients were exposed to an increased oxidative stress through lipid peroxidation.

Our results demonstrated that the administration of MLE to diabetic rats restored oxidative stress biomarkers levels and decreased MDA levels. These effects of MLE are probably due to its hypoglycemic and free radical scavenging properties. MLE contains several compounds such as flavonoids glycoside that can act as a potent antioxidant. **(Enkhmaa et al., 2005)**

We observed in blood serum of type 2 diabetes group a significant decrease of reduced GSH level, GPx and CAT activities, with the exception of an increase in activity of SOD as compared with the control subjects. The depletion of GSH levels is in agreement with other studies **(Moussa, 2008 ;Kumawat et al., 2012)**. Moussa **(Moussa, 2008)** has found a link between hyperglycemia and GSH depletion. Hyperglycemia is, therefore, indirectly the cause of GSH depletion.

NO is afree radical which reacts with various molecules

to cause multiple biological effects. Under normal circumstances, the con- trolled production of NO (by eNOS) conveys antiapoptotic signal, inhibits platelet aggregation and adhesion to the vascular wall, besides playing an important role in cardiac contractile function. The vasodilatory function of NO is mediated via the activation of guanylate cyclase. NO also has cardio-protective effects **(Moncada and Palmer, 1991)**. Depending upon its source and level of output, NO can either play a cardio- protective or a detrimental role **(Balligand and Cannon, 1997)**.

iNOS is absent in the healthy heart but, as the name indicates, it is induced in cells under stress conditions, including hyperglycemia, oxidative stress and hyperinsulinemia. An increased myocardial NO production can cause nitration of actin and other cytoskeletal proteins in the myo- cardium. This alters their structure and may have damaging effects on the contractile function of myofilaments. NO also interacts with oxygen radicals and leads to the formation of peroxynitrite, apotent oxidant, which in high concentration causes tissue damage **(Balligand and Cannon, 1997)**.

Biomarkers have substantial utility in modern cardiovascular diagnosis, prognosis, and therapy. In the vast majority of cases, elevated circulating troponin is a marker for myocyte necrosis, whether from ischemia or other causes.

Histological examination of the diabetic heart revealed myocardium degeneration and increased collagen deposition. Oxidative stress, inflammation and cell injury are well-known causes of the cardiac fibrosis. Oxidative inflammatory histological changes in cardiac tissues indicated the myocardial injury such as deformation of nuclei of cardiomyocytes and disarrangement or disordered cardiac myofibrils. The structural cardiomyocytes change was probably due to the degeneration of the structural protein in mitochondria of the cytoplasm that occurred in protein degradation related to diabetes. These results are in line with past studies showing that oxidative stress and hyperglycemia frequently coexist **(Jemai and Sayadi, 2015)**. Mulberry leaf extract treatment dramatically decreased collagen production and fibrosis in diabetic rats' hearts by simultaneously boosting antioxidant defences and reducing chronic inflammation.

CONCLUSIONS

In conclusion, the study demonstrates that mulberry extracts significantly improve deranged lipid metabolism in STZ-induced diabetic rats. The suppression of oxidative stress may also contribute, at least in part, to the antidiabetic and the cardioprotective action of mulberry. The results suggest that mulberry leaves preparations can be used as a nutraceutical agent to ameliorate diabetes type 2 and might also be useful in preventing secondary diabetic complications including cardiovascular disease. Our results confirm that Adipsin is a novel prognostic biomarker for cardiovascular diseases.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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