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Effect of cadmium on glucose, lipid profile and oxidative stress in streptozotocin-induced diabetic and non-diabetic rats

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Abstract

Cadmium (Cd), highly toxic heavy metal, has been considered as a possible risk factor for diabetes and its complications. However, the underlying mechanisms of Cd-induced diabetes are not clear. The present study was designed to evaluate the effects of Cd on the oxidative system in the diabetic and non-diabetic rats. Rats were divided into the following groups of 8 animals each: control (C), diabetic (D), Cd-exposed (1 mg/kg/bw) and diabetic-exposed to Cd (1 mg/kg/bw) (D + Cd- exposed) groups. Diabetes was induced by streptozotocin (intraperitoneally (i.p.)) at a single dose of 60 mg/kg. Cd (i.p.) was administered 3 days after streptozotocin (STZ) administration to the end of the study. After 4-weeks, blood was drawn to determine the changes of glucose, serum superoxide dismutase (SOD), catalase (CAT), glutathione-S-transfrase (GST) and glutathione peroxidase (GPx) activities as well as the levels of reduced glutathione (GSH), malondialdehyde (MDA) and lipid profile. The results indicated that Cd exposure aggravated increased blood glucose, MDA, triglycerides, total cholesterol, LDL-C as well as decreased GSH levels and the activities of antioxidant enzymes in diabetic rats (p<0.001). These results suggest that Cd exposure deteriorates diabetic effect and its complications in STZ-diabetic model by induction of oxidative stress.

Keywords: cadmium; diabetes; lipid profile; oxidative stress; rats

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1. Introduction

Diabetes is chronic progressive diseases that are connected with a combination of environmental factors, lifestyle and genetics [1]. Although many risk factors have been identified, such as diet, obesity and other lifestyle factors, however, there may be other as yet unidentified environmental factors that influence whether or not an individual will become diabetic, or whether mild or immature diabetes progresses to a more developed disease state [2].

Cadmium (Cd), is one of the major toxic pollutants in an environment that has many applications in the production of polyvinyl chloride (PVC), batteries and pigments [3]. Cd-induced cellular toxicity has been described in various targets, including metallo enzyme interference, thiol protein alterations, energy metabolism inhibition, DNA and membrane structure/function alterations, and excessive oxidative damage [4]. As a result of the extensive use of Cd in industry and its extensive dissemination in the environment, numerous studies have focused on the identification of Cd-induced diseases in exposed human populations [3, 4]. In some cases, the results of these epidemiological studies have suggested a

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possible link between Cd exposure and diabetes. It has shown that cd-induced hyperglycemia was associated with increased lipid peroxidation, decreased insulin release, increased activation of gluconeogenic enzymes and impaired insulin receptor [5,6]. Till now, there are only a few reports investigating the relationship between chronic cadmium exposure and diabetes occurrence. On the other hands, the detailed effects and mechanisms of Cd on blood glucose regulation and also the association between Cd exposure and diabetes and the corresponding mechanism of this association are still unclear. Previous investigations have indicated that Cd insert diabetogenic effects through damage to pancreatic β-cell, decrease insulin secretion and enhance glucose intolerance [7, 8].

Recently, a number of studies reported that the oxidative stress contributes to the progression of diabetes and its complications, which is correlated with oxygen free radical production, non-enzymatic protein glycosylation, auto-oxidation of glucose, destruction glutathione metabolism, alteration in antioxidant enzymes and lipid peroxides generation [9, 10]. Growing study shows that toxic metals result in overproduction of reactive oxygen species (ROS) eliciting oxidative stress damage that lead to many pathophysiological processes and development of disease [6]. Moreover, it has been reported that Cd-induced toxic effects are related to the condition of oxidative stress in tissues [6]. However, the exact mechanisms through which Cd induced diabetes and dyslipidemia or worsened the diabetic conditions have not been elucidated. Thus, the aim of this study was to investigate the effects of Cd on the diabetic and non-diabetic rats and oxidative stress parameters as possible mechanisms.

2. Material and Methods

2.1 Chemicals

All purified enzymes, coenzymes, substrates, standards, buffers, kits and also cadmium chloride ($CdCl_{2)}$ and other chemicals were purchased from Sigma-Aldrich Chemical (St. Louis, USA). The Pars Azmoon kit was purchased from the Pars Azmoon Company (Pars Azmoon Co., Tehran, Iran).

2.2 Animals

Wistar albino rats (2 months; 200 ± 13 g) were bred at the university experimental animal care center. Animals were maintained under standard environmental conditions and had free access to standard rodent feed and water. The study was conducted in Mashhad Medical University Experimental Animal Research Laboratory. Protocols were approved by the Ethical Committee (The Ethical Research Committee of Mashhad University of Medical Sciences).

2.3 Experimental design

32 male Wistar albino rats were randomly allotted to four experimental groups (n=8 per group) as follows: group 1, control (C); group 2, diabetic (D), group 3, exposed to cadmium (1 mg/kg/bw) (Cd-exposed) and group 4, diabetic-exposed to cadmium (1 mg/kg/bw) (D + Cd-exposed). Rats were kept in their own cages at constant room temperature (21 ± 2 °C) under a normal 12-h light/dark cycle with free access to food and water. The animals were housed according to regulations for the Welfare of experimented animals. On the first day of the study, the diabetic groups were given streptozotocin (STZ) in a single intraperitoneal (i.p.) injection at a dose of 60 mg/kg for induction of diabetes. Blood was extracted from the tail vein for glucose analysis 72 hours after STZ injection. Rats with blood glucose levels higher than 250 mg /dl were accepted as being diabetic (Ames One Touch glucometer). In the control group (C), physiological saline (i.p.) was injected as a vehicle. Cadmium chloride ($CdCl_2$), (i.p. injection) was administered to the non-diabetic rat from 1 day and to the diabetic group from 3 days after STZ administration; these injections were continued to the end of the study (for 4 weeks). The blood glucose level was recorded at weekly intervals (Pars Azmoon kit). At the end of the period of this study, the animals were anesthetized with ether and blood was subsequently collected from the retro orbital sinus. Blood and sera were separated by centrifugation at 3000 rpm for 10 min for glucose, lipid profile, reduced glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transfrase (GST) assays.

2.4 Measurement of Blood Glucose

Glucose concentrations were measured with the Ames One Touch glucometer (One-Touch Basic; Lifescan, Johnson and Johnson, New Brunswick, NJ) in rat tail vein

blood. Blood glucose was estimated using the diagnostic kits (Pars Azmoon kit, IRI) on an automatic analyzer (Abbott, model Alcyon 300, USA).

2.5 Measurement of Serum Lipid Profile

The concentrations of triglycerides, total cholesterol, LDL-C and high-density lipoprotein cholesterol (HDL-C) in serum were estimated by using diagnostic kits (Pars Azmoon kit, IRI) on an automatic analyzer (Abbott, model Alcyon 300, USA)

2.6 Measurement of Serum MDA

The accumulation of lipid peroxides was measured in the serum. The formation of MDA, an end product of fatty acid peroxidation was measured spectrophotometrically at 532 nm by using a thiobarbituric acid reactive substance (TBARS), essentially by the method of Genet et al. (2002). Results are expressed as μmole of MDA formed/mg protein [11].

2.7 Measurement of Serum GSH

Reduced glutathione (GSH) content was estimated according to the method of Ellman (1959). The procedure is based on the reduction of Ellman's reagent by –SH groups of GSH to form 2-Nitro-5-mercaptobenzoic acid, the nitromercaptobenzoic acid anion has an intense yellow color which can be determined spectrophotometrically. Serum GSH contents were measured spectrophotometrically at 412 nm using DTNB as the reagent [12].

2.8 Measurement activities of antioxidant enzymes

SOD activity was determined by the method of Kakkar et al. 1984 [13]. The activity of CAT was determined by the method of Sinha 1972 [14]. GPx activity was estimated by the method of Rotruck et al. 1973 (15). GST activity was determined by the method of Habig et al. 1974 [16].

2.9 Measurement of Serum Protein Content

Protein content was determined by the method of Bradford et al. (1976), using bovine serum albumin (BSA) as a standard [17].

3. Statistical analysis

All biochemical tests for accordance were carried out at least twice. Each group consisted of eight rats. One way analysis of variance (ANOVA) was performed and the Tukey *post hoc* test was used for multiple comparisons. Statistical analyses were performed using the InStat 3.0 program. The results are expressed as mean ± SEM.

4. Results

During the experimental period (4-week), STZ-diabetic rats exhibited significant ($p< 0.001$) hyperglycemia compared with the control rats. The experimental groups are compared to the control at each time point (Figure 1). Cd significantly increased blood glucose levels in the non-diabetic rat compared with the control rats ($p< 0.001$) (Figure 1). Cd significantly increased blood glucose levels in the diabetic compared with the non-exposed diabetic rats ($p< 0.01$) (Figure 1). STZ-injected rats showed a significant increase in the serum levels of triglycerides, total cholesterol and LDL-C, with a significant decrease in the serum HDL-C level versus to the control group ($p< 0.001$) (Table 1). Cd increased the serum levels of triglycerides, total cholesterol and LDL-C ($p< 0.01$; $p< 0.01$; $p< 0.05$, respectively), and reduced serum HDL-C level ($p< 0.05$) at the end of the experiment in the control rats. Also, Cd increased the serum levels of triglycerides, total cholesterol and LDL-C ($p< 0.05$; $p< 0.001$; $p< 0.001$ respectively) at the end of experiment in the diabetic rats compared with the non-exposed diabetic rats (Table 1). STZ injection produced significant changes in the oxidative stress parameters in the serum of diabetic rats 4 weeks after diabetes induction, as shown by the increased MDA ($p<0.001$) (Figure 2a) and decreased the GSH content ($p<0.01$) (Figure 2b) and th activities of SOD, CAT, GST and GPx compared with the control group ($p< 0.001$; $p< 0.05$; $p<0.05$; $p< 0.05$ respectively) (Figure 3). Cd significantly increased the serum MDA ($p<0.05$) (Figure 2a) and decreased the GSH content ($p<0.05$) (Figure 2b) and the activity of SOD compared with the control rats ($p< 0.01$) (Figure 3). In addition, Cd significantly increased the serum MDA ($p<0.001$) and decreased the GSH content ($p<0.05$) compared with the non-exposed diabetic rats (Figure 2).

Table 1. Plasma lipid profile (mg/dl): total cholesterol, triglycerides, LDL-C, HDL-C, in control (C), unexposed diabetic (D) and diabetic rats exposed to Cd (D + Cd-exposed) (n=8, for each group)

parameters	C	D	Cd-exposed	D + Cd-exposed
Total cholestrol (mg/dl)	53.37 ± 2.65	76.87 ± 3.06 ***	67.12±5.63**	101.88 ± 3.95***,+++
Triglycerides (mg/dl)	38.21 ±3.22	64.10 ± 4.51***	49.48±2.89**	98.11 ± 5.50***,+
LDL-C (mg/dl)	21.34 ± 5.33	48.55 ± 4.33***	39.14±3.11*	76.93 ± 3.60***,+++
HDL-C (mg/dl)	38.97 ± 3.59	19.11 ± 2.88***	27.11±3.23*	11.13 ± 2.34***

Values are the mean ± SEM.
Statistical significance for the difference between the data of the control group vs other groups: *; p <0.05, **; p <0.01,***; p <0.001.
Statistical significance for the difference between the data of unexposed diabetes group vs diabetic rats exposed to Cd: +; p <0.05, +++; p <0.001.

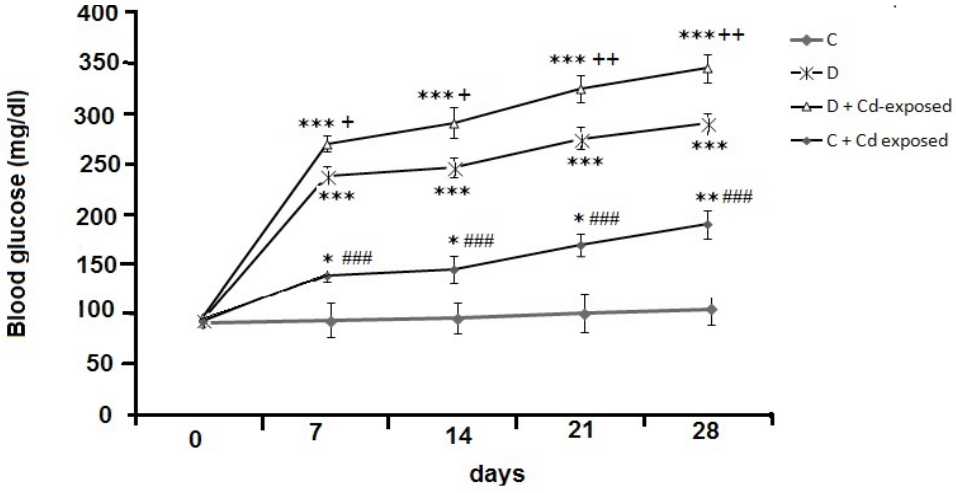


Figure 1. Blood glucose level (mg/dl) in control (C), unexposed diabetic (D) and diabetic rats exposed to Cd (D + Cd-exposed) (n=8, for each group).Values are the mean ± SEM. the experimental groups are compared to the control at each time point.
Statistical significance for the difference between the data of the control group vs other groups:***; p <0.001.
Statistical significance for the difference between the data of unexposed diabetes group vs diabetic rats exposed to Cd: ++; p <0.01, +++; p <0.001. Statistical significance for the difference between the data of Cd exposed group vs diabetic rats exposed to Cd: ###; p <0.001.

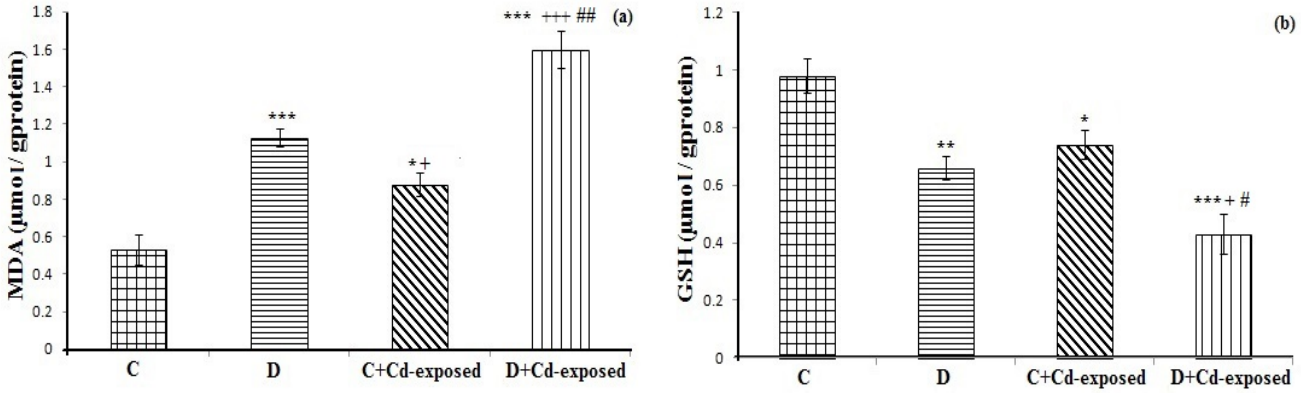


Figure 2. Serum MDA (a) and GSH (b) levels (µmol/gprotein) in control (C), unexposed diabetic (D) and diabetic rats exposed to Cd (D + Cd-exposed) (n=8, for each group).Values are the mean ± SEM.
Statistical significance for the difference between the data of the control group vs other groups: *; p <0.05,**; p <0.01,***; p <0.001.
Statistical significance for the difference between the data of unexposed diabetes group vs diabetic rats exposed to Cd: +; p <0.05, +++; p <0.001. Statistical significance for the difference between the data of Cd exposed group vs diabetic rats exposed to Cd: #; p <0.05, ##; p <0. 01.

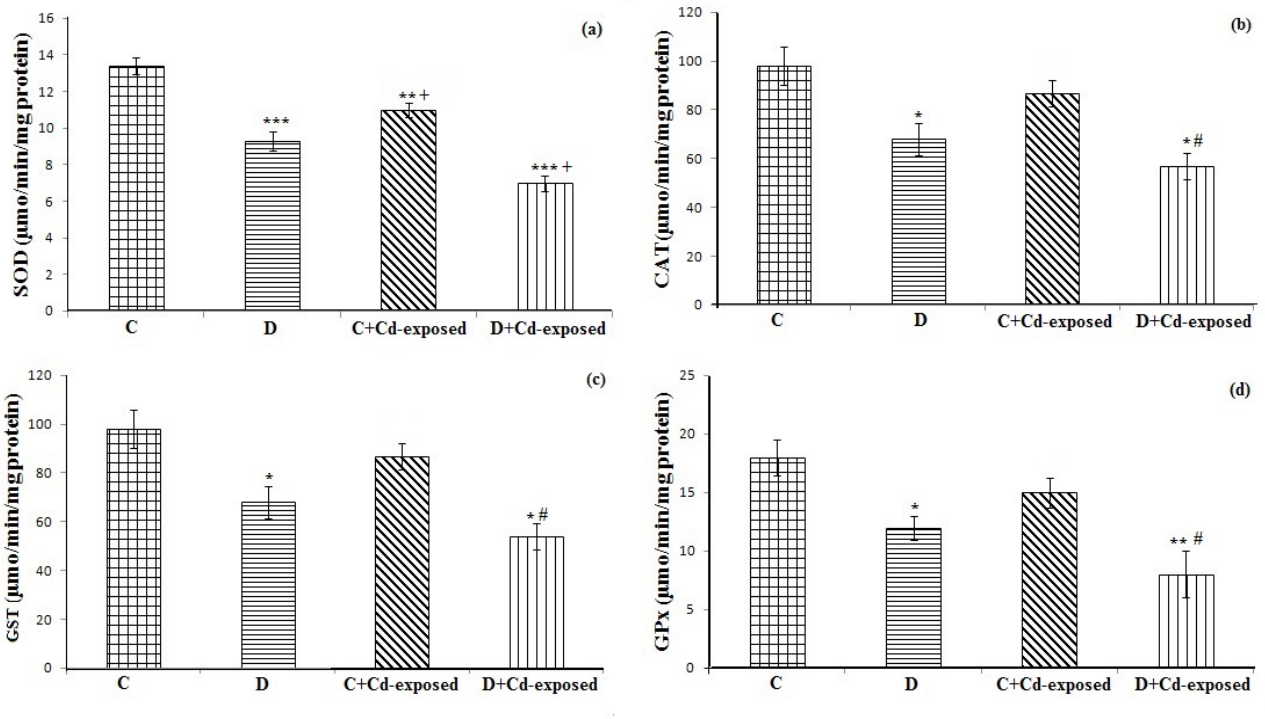


Figure 3. Activities of SOD (a), CAT (b), GST (c), GPx (d) in serum (µmol/min/mg protein) of control (C), unexposed diabetic (D) and diabetic rats exposed to Cd (D + Cd-exposed) (n=8, for each group).Values are the mean ± SEM.
Statistical significance for the difference between the data of the control group vs other groups: *; p <0.05, **; p <0.01,***; p <0.001. Statistical significance for the difference between the data of unexposed diabetes group vs diabetic rats exposed to Cd: +; p <0.05. Statistical significance for the difference between the data of Cd exposed group vs diabetic rats exposed to Cd: #; p <0.05.

5. Discussion

Diabetes mellitus is a metabolic disease that characterized by hyperglycemia and disturbance metabolism of lipids, carbohydrates and proteins [5]. Recently, a number of studies reported that the oxidative stress contributes to the progression of diabetes and its complications, which is correlated with oxygen free radical production, non-enzymatic protein glycosylation, auto-oxidation of glucose, destruction glutathione metabolism, and alteration in antioxidant enzymes and lipid peroxides generation [5]. Recently, it has been indicated that metal-induced oxidative stress might be related to the initiation and progression of diabetes [9]. Lipid peroxidation is considered as an intermediate pathway that involved in the metal exposure and diabetes [9]. However, the exact mechanism of metal-induced diabetes is not clear. The suspetible of pancreatic β cell to oxidative stress are parobably factor [9].

In the present study, cd injection increased serum glucose, lipid profile as well as MDA levels and reduced serum GSH, SOD levels in the non-diabetic rats. The results of the present study indicate that intraperitoneal injection of Cd significantly worsened the adverse metabolic effects in rats received STZ. Cd exposure to the diabetic rats showed a significantly aggravate the increased levels of glucose, lipid profile and MDA as well as a distinct reduction in the activities of SOD, CAT, GST and GPx (antioxidant enzymes) in STZ diabetic rats, indicating an increased plasma oxidative stress. Additionally, decreased GSH, SOD, CAT, GST and GPx levels after Cd administration may play an additional role in increasing oxidative stress. The results confirmed previous findings reported by other investigators that toxic metals induce excessive amounts of ROS eliciting oxidative stress damage that results in many pathophysiological processes and development of disease [18]. Oxidative stress induced by oxygen derived free radicals, is a disturbance in the pro-oxidant and antioxidant balance, which results in functional cell damage, undesirable biological reactions, and involved in several pathological processes such as diabetes [18]. Pancreatic β-cells, which function in insulin biosynthesis/secretion in mammals, are the greatest target for ROS attack than other cell types. The mitochondria of β-cells can also produce excessive levels of ROS in these cells. This event combined with a failure of the ROS defense

system, lead to the relatively high susceptibility of β-cells to oxidative stress damage [19]. Previous studies have been indicated that similarly Cd could induce oxidative stress damage and suppress of insulin production in the pancreatic islet β-cells in vivo and in vitro [18, 19].

Our data indicate Cd administration can cause increases in blood glucose, although, the mechanism responsible for Cd-induced hyperglycemia has not yet clarified, however, our finding for the first time illustrated the overproduction of oxidative stress by Cd might be involved in the hyperglycemia in the rats. These findings may constitute the predominant mechanism in complications of hyperglycemia induced by Cd.

Diabetes mellitus often accompanied by lipid abnormalities, including increased LDL-C and cholesterol. These abnormalities may be further exacerbated by the increased oxidizing environment which enhances the production of oxidized LDLs (oxLDLs), glycated LDL and oxysterols (formed from the oxidation of cholesterol). It has been proposed that these oxidized lipid products can bind to specific receptor proteins or activate inflammatory proteins which produce ROS. The import of oxLDLs in the vascular wall is a major mechanism by which ROS and oxidative stress induce atherosclerosis. The results of the present study indicate that Cd accelerates hyperlipidemia in diabetic rats [20]. Both experimental and epidemiological studies show that exposure to Cd may disturb lipid metabolism and contribute to the development of cardiovascular diseases [21]. It has been shown that imbalance of oxidative stress causes hyperlipidemia [21], therefore, Cd may increase the serum lipid profile by disturbing oxidative systems [22]. In the present study, it was shown that Cd administration caused a significant increase in the levels of MDA in plasma, with an overall lipid profile in STZ diabetic rats. The main aldehydic lipid peroxidation product, MDA, amplifies cellular damage due to their ability to covalently modify critical biomolecules, including lipids, proteins and nucleic acids, hence disturbing glucose regulation [23]. The important forms of cellular damage caused by Cd are lipid per-oxidation, protein oxidation and thiol depletion [21]. Cd due to its per-oxidative properties induces oxidative damage to various macromolecules, such as lipids [21]. The important target site for Cd action is cellular membranes and this toxic metal cased lipid peroxidation [22]. Therefore, the aggravation of variable measurements in STZ-diabetic rats after Cd exposure may suggest the increased toxic effect of Cd in STZ diabetic rat that could be mediated through over production of oxygen free.

A suppressing effect of the formation of GSH by Cd was shown in this study. The GSH reacts with free radicals and is a crucial substrate for glutathione peroxidase and glutathione-S-transferase, which involve in the cellular defense mechanisms against ROS [21]. It may be indicating that the ratio of GSH/GSSG inserts an important role in glucose homeostasis of diabetes because thiol groups are critical in intracellular and membrane redox state [22]. Cd induced a decrease in plasma GSH content, which might suppress the GSH/GSSG ratio and increase lipid peroxidation, thus aldehydic concentration, and hence aggravate serum glucose regulation [22].

In parallel, Cd markedly decreased the plasma antioxidant enzyme activities in diabetic rats.

SOD removes of superoxide radicals and catalase decomposes hydrogen peroxide to water and oxygen; hence, these enzymes may involve in the modulation of redox state of plasma [23]. These findings perfectly agree with those of Chang et al. 2013 who indicated the role of oxidative stress-mediated mitochondrial apoptosis in pancreatic β-cells [19].

In this study, prooxidant-antioxidant balance was evaluated by measuring MDA and GSH levels and activities of antioxidant enzymes in the exposed and non-exposed diabetic rats. The increased MDA and decreased antioxidant enzyme levels in the diabetic rats in our finding indicated that the balance changed towards pro-oxidation in STZ-induced diabetic rats. Cd exposure of diabetic rats in our study, which aggravated GSH content and antioxidant enzyme activities, may be due to an increase in free radical generation by Cd and decreased antioxidant defenses. It is a major mechanism that Cd contributes to the damage in some tissues including liver and pancreas. Cd accompanied by activation of ROS-related gene expression, leading to a higher mitochondrial oxygen radical generation, which may be responsible at least in part for the deteriorated hyperglycemia, hyperlipidemia and oxidative stress seen in the present study in the STZ- diabetic rats [24].

To the best of the authors' knowledge this is the first report that shows Cd deteriorates the adverse metabolic effects. Here, we found the evidence for oxidative stress as possible mechanisms of the aggravation of variable measurements in diabetes due to the Cd exposure. Therefore, Cd increases hyperglycemia, hyperlipidemia and MDA levels as well as decrease the activities of

antioxidant enzymes (SOD, CAT, GST and GPx), and GSH contents in the STZ diabetic rat model. Our data also for the first time show, the chronic Cd exposure increased serum glucose, lipid profiles, MDA levels and reduced serum GSH, SOD levels in the non-diabetic rats, however Cd cannot significantly reduce the other measured antagonist enzymes (CAT, GST and GPx).

The results of our study suggest an association between Cd exposure, elevated blood glucose levels and the development of diabetes. In addition, our experimental investigations suggest that Cd potentates or exacerbates diabetic complications. While the link between Cd exposure and elevated blood glucose and lipid profile levels has been established, however, the connection between Cd exposure and the antioxidant enzymes, lipid proxidation, and GSH levels in the diabetic and non-diabetic rats have been elucidated in our experiment. Therefore, Of the many diverse mechanisms by which Cd may increase blood glucose levels outlined above, there is considerable evidence that it may explain the mechanisms responsible for Cd exposure deteriorate diabetic effect in the STZ model of diabetes and its complications as well as non-diabetes by induction of oxidative stress. Further study is needed to determine the etiology of Cd-induced changes in blood glucose levels, and also detailed studies, including molecular research are required for the evaluation of the exact mechanism of Cd involved in diabetic complications.

6. Acknowledgments

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7. Conflict of interest statement

The authors declare that there are no conflicts of interest.

8. Refeerences

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