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Original Article

Antagonistic effects of thiamine versus lead acetate exposure-correlated with hepato-renal toxicity in diabetic and non-diabetic rats: A stereological and biochemical survey

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Abstract

Background and aims: The present study aimed to investigate the protective effect of thiamine on the toxicity of lead acetate (PbAc) in the liver and kidneys of diabetic rats. To evaluate the effect of thiamine, stereological criteria, and biochemical processes were used.

Methods: Forty-eight female rats were used and divided into eight groups of six animals. G I: served as the control group; G II: diabetic group; G III: PbAc group; G IV: thiamine group; G V: diabetes + thiamine group; G VI: PbAc + thiamine group; G VII: diabetes + PbAc + thiamine group; G VIII: diabetes + PbAc group.

Results: The total volume of hepatocytes in the liver and the volume of cortex, medulla, and glomerulus in the kidney were significantly increased in the both diabetic and PbAc groups compared to control animals. Moreover, the sinusoids and central vein volumes showed a significant decrease in both the diabetic and PbAc groups compared with the controls. The PbAc and diabetic groups showed higher total cholesterol (TC), very low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), malondialdehyde (MDA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (Cr), and lower high-density lipoprotein cholesterol (HDL-C) concentrations than the control group. It was discovered that thiamine significantly alters the levels of the desired parameters, bringing them closer to the control group.

Conclusion: Thiamine is a potent antidiabetic agent, and this compound supplementation possesses hypoglycemic properties and has an effect on the hepatorenal structure in diabetes rats.

Keywords: Stereology, Biochemical parameters, Rats, Thiamine, Diabetes

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Introduction

The environment in which we live contains varying amounts of heavy metals, some of which can play an important role in nutrition, while others are dangerous chemical substances for human health. The mechanism of lead acetate (PbAc) toxicity has been studied by many researchers for years and they accept PbAc as a pervasive environmental pollutant that remains for a long time and spreads out strongly in the environment (1). PbAc is a multi-organ toxic substance that affects most of the body's various organs, including hematopoietic tissues, the liver, kidneys, the brain, and testes, and ultimately reflects its destructive effects on their functional efficiency (2). PbAc is absorbed through the duodenum with the involvement of a divalent metal transporter and distributed to soft tissues along with red blood cell proteins (3). The liver plays a vital role in the detoxification process and can protect many other organs in the body when exposed to various sources of toxins. The examinations have revealed that the liver is the principal reservoir of PbAc, reserving about one-third of the lead absorbed, which is traced by the kidneys (4). As a result of PbAc toxicity, the balance between the prooxidant and antioxidant systems will be disrupted. Oxidative stress results from oxidative imbalance and overproduction of reactive oxygen species (ROS), depletion of cellular antioxidants, and consequent DNA damage (5).

Vitamins such as thiamine are transported by high-affinity carriers across the plasma membrane, although the rate of this transfer has been shown to be slow overall (6) Thiamine can also be absorbed by liver cells, red blood cells, and other cells with active transmission (7). Once inside the cells, they are used as precursors to various compounds, as metabolic cofactors, and as regulators of oxidative stress (8) In agreement with the present observations, studies have shown that measured poisoning changes some parameters of stereology in the kidney (9) and hepatotoxicity changes in the liver (10, 11).

Vitamins are vital nutrients that play an important role in human nutrition and health and possess complex structures. The first known B vitamin was vitamin B1, which was eventually presented as thiamine. Since thiamine can only be stored in the body for a short time, regular consumption of thiamine in the diet is necessary to maintain proper blood levels before it can be easily excreted (12,13). Following the decarboxylation of pyruvic and ketoglutaric acids, thiamine directs the production of energy, fat, and nucleotide metabolism processes, and its function is determined as a coenzyme. All organisms need different amounts of thiamine to function properly; nevertheless, the dietary requirement for thiamine is about 1.5 mg/dL for adults, which is commensurate with the calories received from the diet (14). The therapeutic potential of thiamine has been widely used to protect tissues such as the liver and kidneys against PbAc-induced fat peroxidation. The therapeutic use of thiamine in some rare genetic disorders (Wilson's disease) is clearly stated. In 2011, Shelin demonstrated that thiamine administration could activate thiamine-dependent enzymes that are inhibited by copper^{++}(15). Such laboratory studies prove that thiamine has been able to effectively play the role of an antioxidant. Advances in research have shown that thiamine can induce systemic acquired resistance through signaling pathways related to salicylic acid and Ca^{2+}(16).

According to the official census of the World Health Organization, about 3% of the world's population suffers from some type of diabetes (17). Diabetes-induced hyperglycemia, in addition to accelerating the onset of disease complications, leads to damage to nucleic acids, membrane lipids (18), and cellular proteins (19). Subsequently, the oxidative stress induced by diabetes critically and severely affects insulin receptors and glucose transport proteins (GLUT) (20). Due to the fact that medications for diabetics may have unpleasant side effects, so attention has been drawn to vitamin supplements (such as thiamine), which may be beneficial in minimizing side effects (14). Lipid peroxidation, which is the result of the destructive effects of diabetes, can be inhibited by thiamine through the adsorption of superoxide anions and hydroxyl radicals, and therefore cell membranes are protected against lipid peroxidation (21).

Based on this, the effects of thiamine on liver and kidney damage during lead poisoning were investigated in diabetic and non-diabetic rats, and in the meantime, the volume values of stereological profiles, lipid peroxidation, and liver and kidney markers were investigated.

Materials and Methods

Animals and Grouping

A total of *48 male Wistar rats* with an *age* of about 8-9 *weeks* old and an average weight of 170-200 *g* were procured from the animal house, department of veterinary medicine, Shahrekord University, and maintained in the animal house of anatomy department of faculty of basic veterinary sciences. The animals *were* housed *in a* room with *a controlled temperature (*20 ± 2 °C*) and* a *relative humidity* of 37%. Rats received water *ad libitum* and were fed standard diet pellets, containing 15% proteins, 4.5% fiber, 3% fat, 5.5% ash, and 1.5% salts and vitamin mixture. The rats were randomly divided into eight groups (n = 6, each).

- Group I: (Control): non-diabetic rats, received a standard diet and distilled water
- Group II: (diabetes): alloxan monohydrate, 180 mg/kg body weight (i.p.)
- Group III: (PbAc): 200 ppm PbAc dissolved in distilled water (i.p.)
- Group IV: (Thiamine): thiamine (30 mg/kg body weight, IP)
- Group V: (diabetes + Thiamine): diabetic rats received thiamine (1%) at a dose of (30 mg/kg, b.w., IP).
- Group VI: (PbAc + Thiamine): rats received PbAc (200 ppm, i.p.) along with thiamine (1%) at a dose of (30 mg/kg, b.w., IP)
- Group VII: (diabetes + PbAc + Thiamine): diabetic rats received PbAc (200 ppm, IP) along with thiamine (1%) at a dose of (30 mg/kg, b.w., IP)
- Group VIII: (diabetes + PbAc): diabetic rats received PbAc (200 ppm, IP) (14).

At the end of the 20th day, blood samples were collected from the heart under general anesthesia. Blood sampling was performed to determine serum levels of malondialdehyde (MDA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine (Cr), and lipid peroxidation including total serum cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) and triglyceride (TG) levels. The samples were centrifugated for 10 minutes at 3000 rpm at 4°C using a universal centrifuge (Hettich, Tuttlingen, Germany) set. Clear serums were obtained and stored at -70°C until use. The animal was killed by exsanguination under ether (Et_2O) anesthesia (Merck Darmstadt, Germany).

In addition, liver and kidney tissues were removed by laparotomy after the dissection of surrounding tissues. Specimens are trimmed and fixed in a 10% neutral buffered formalin solution for histological examinations. Samples were processed by routine and standard paraffin embedding and serially sectioned into 5 µm thickness and then slides were stained with H&E.

Diabetes Induction

Diabetes was induced in the experimental rats with an IP injection of alloxan monohydrate (180 mg/kg body weight; Sigma-Aldrich, USA) in PBS solution (pH = 7.4). Fasting blood glucose was measured in the blood of the induced rats after 72 h from the injection. Rats with fasting plasma glucose levels greater than 200 mg/dL (hyperglycemia) were considered diabetic rats (14).

Stereological Study

Hepatocytes, sinusoids, and central veins volumes (mm^3) in the liver and cortex, medulla and glomeruli volumes (mm^3) in the kidney were measured by stereological methods (Figure 1A, B and Figure 2C, D). The Cavalieri principle was applied to estimate the total volume of the liver and kidney (22). In order to reduce the sampling variance, our selection was based on the order of the fragments according to the "smooth pattern". In the term "smooth pattern", the pieces obtained by the macroscopic incision of a tissue (preferably with a fixed piece thickness) are arranged in a rhombus-like pattern so that the size decreases from the middle to both ends (23). The fragmentation process takes place in a systematic random sampling pattern, in which case samples are randomly selected in a uniform way (14). The hepatic fragments (at least three pieces per animal) are embedded together, after processing and embedding the tissues in paraffin, 5 µm thick sections were obtained by an automatic rotating microtome device. The sections were stained using hematoxylin and eosin. The liver and kidney were analyzed considering the hepatocytes, sinusoids, central vein, cortex, medulla, and glomeruli. The following formula is used to estimate the volumetric values [V(total)] of the interest (Vv) on sections (24).

$$V\,(total) = t.\ a(p).\ \Sigma P$$

Where t is the thickness of the section, a(p) is the interval point area and ΣP is the total number of points that touch appropriate areas in the sections.

A 36-point grid was superimposed on images of tissue sections, and the volume density (Vv) of liver components (including hepatocytes, sinusoids, central veins, and fibrous tissue) was obtained using the point-counting method and calculated by the following formula (23).

$$V\,(constituent) = Vv\,(constituent) \times V\,(liver/kidney)$$
$$Vv = P\,(constituent)/P\,(reference)$$

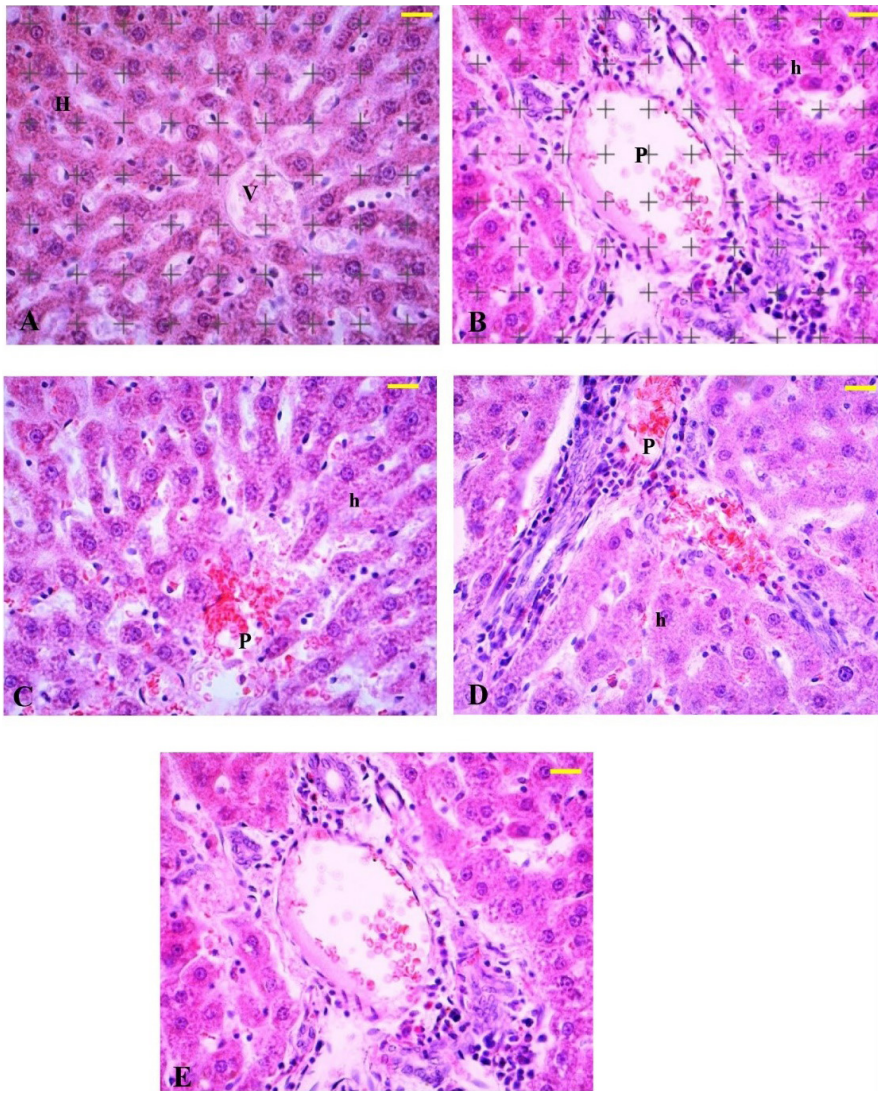

Figure 1. Photomicrographs showing the Estimation of the Volume of the Hepatic Structural Components. The number the points hitting the structure is divided by the total points. Volume density (Vv) of hepatocyte, sinusoids, and central vein were obtained. (A) Control group; (B) PbAc administered group; (C) Diabetes + thiamine group; (D) Diabetes + PbAc + thiamine group; E: Diabetes + PbAc. V: central vein; P: portal vein; h: sheets of hepatocytes; (H&E staining, resolution × 400; scale bar 40 µm). HV (10^3 mm^3); Sinusoids volume (mm^3); Central vein volume (mm^3)

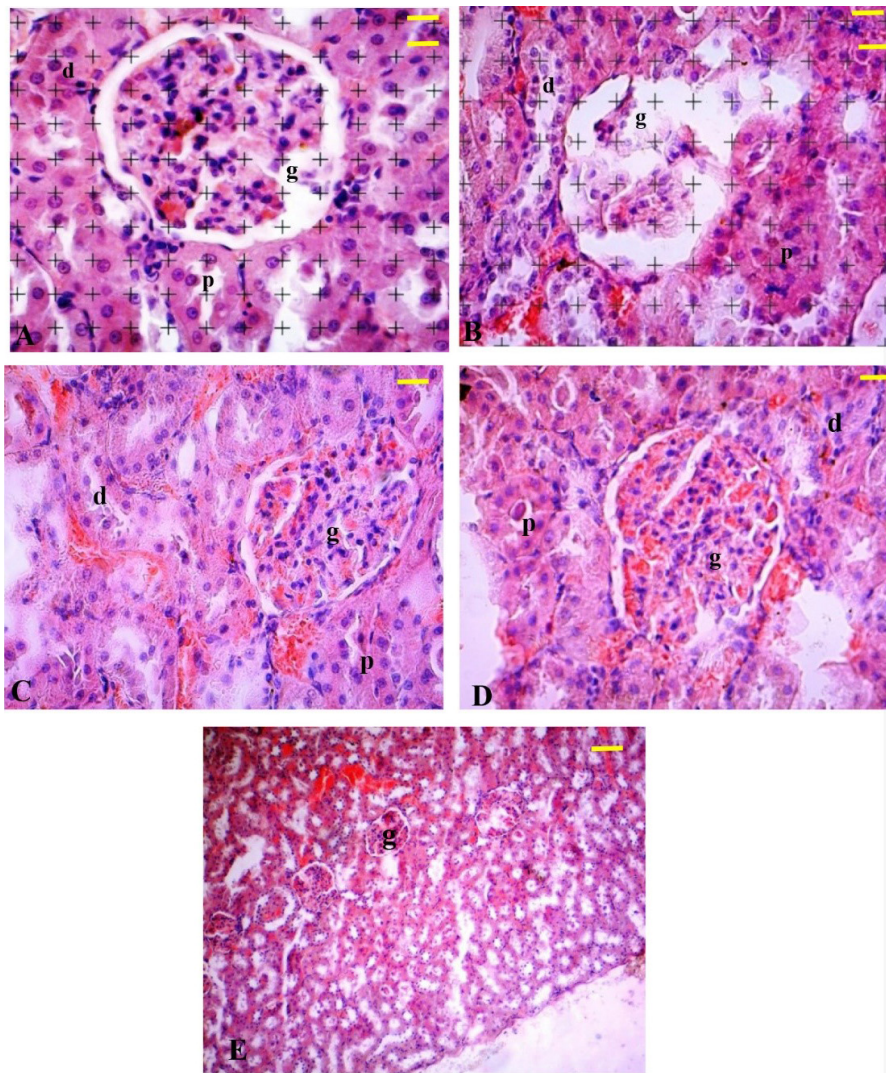

Figure 2. Photomicrographs Showing the Estimation of the Volume of the Renal Structural Components. Estimation of volume density using point counting method. A gird of points was placed on the images of the renal sections. Volume density (Vv) of renal cortex, medulla, and glomeruli were obtained. (A) Control group, (B) PbAc administered group; (C) Diabetes + thiamine group; (D) Diabetes + PbAc + thiamine group; E Diabetes + PbAc group. p: proximal convoluted tubules; d: distal convoluted tubules; g: glomeruli; (H&E staining, resolution × 400; scale bar 30 μm)

Where *P (constituent)* and *P (reference)* were the number of points hit on the constituent's profile and on the reference space, respectively.

Measurement of Serum Glucose

Serum glucose levels were measured using a glucose analysis kit (Pars Azmoon Co, Tehran, Iran). The principle was based on the bi-enzymatic assay comprising enzymatic oxidation of glucose to gluconic acid, which yielded hydrogen peroxide, and then the reaction of hydrogen peroxide with 4-amino antipyrine and phenol to yield the colorimetric product which is measured at 500 nm. The results were reported as mg/dL.

Estimation of Malondialdehyde Level

MDA as the end product of lipid peroxidation reacts with Thiobarbituric acid (TBA) and yields a colored complex that can be measured spectrophotometrically (25). In this study, 2 mL of TBA reagent containing 0.375% TBA, 15% TCA, and 0.25 mol/L HCl was added to 1 mL of serum from all groups. The mixture was placed in boiling water for 50 min, cooled to room temperature, and centrifuged at 1000 rpm for 10 minutes. Thereafter, the absorbance of the supernatant was read at the wavelength of 535 nm against the blank reference. The results were calculated by using a molar extinction coefficient for MDA of 1.56×10^5/M/cm. The concentration of MDA was expressed as nmol/mL (26).

ALT Activity Assay

ALT activity was measured by using the Pars Azmoon kit (Tehran, Iran). The reaction is evaluated based on the transferring of an amino group from alanine to α-ketoglutarate resulting in the production of glutamate and pyruvate. Then, in a parallel reaction, the measurement of the absorbance change of NADH concentration at 340 nm is assayed based on the pyruvate reaction with lactate dehydrogenase. ALT activity in serum samples was measured as U/L.

AST Activity Assay

AST activity was measured by using the Pars Azmoon kit (Tehran, Iran). The reaction is based on the reversible transamination between aspartate and α-ketoglutarate to form glutamate and oxaloacetate. AST activity is assayed by monitoring the rate of NADH oxidation at 340 nm in the presence of oxaloacetate and malate dehydrogenase. AST activity was measured as U/L.

Creatinine Levels

The serum Cr was measured based on the Jaffe method (Pars. Azmoon Kit, Tehran, Iran) in which creatinine reacts with picric acid to form a reddish complex (27).

Lipid Profile Assay

TG and TC concentrations were measured using an enzymatic colorimetric assay (Pars Azmoon kits, Tehran, Iran, catalog numbers: GPO-PAP, CHOD-PAP) by an automatic serum auto-analyzer (BT3000, Italy). HDL-C was measured by using enzymatic (Bionic Diagnostic Kits, Tehran, Iran) according to the colorimetric with the utilization of an auto analyzer. LDL-C concentrations were calculated.

Statistical Analysis

The study information was entered into SPSS statistical software (IBM corporate, Chicago, IL, USA) and was analyzed by means of the LSD test as a post hoc test. $P<0.05$ was considered statistically significant.

Results

Effect of Thiamine on Body Weight and Glucose Concentration

Body weight was the same in rats from all study groups at the beginning of the experiment. Twenty days thereafter, body weight was higher in all treated rats with alloxan (G II and V), but the groups that received PbAc (G III, VI, and VII) had lower body weight than the control group. Meanwhile, the administration of thiamine reduced the changes in the diabetic and PbAc-poisoned groups. Serum glucose levels were significantly increased in alloxan-induced diabetic rats (375 ± 21 mg/dL, G II) compared with the non-diabetic control group (98 ± 13 mg/dL, G I). These values in the diabetic-treated group plus thiamine (235 ± 8 mg/dL, G V) showed a considerable decrease compared to the diabetic group alone (G II). On the other hand, blood glucose levels in PbAc-treated rats (423 ± 26 mg/dL, G III) alone and along with thiamine (415 ± 24 mg/dL, G VI), as well as diabetic rats that received PbAc along with thiamine (461 ± 27 mg/dL, G VII) showed an increase compared to the control group.

Effect of Thiamine on Stereological Findings

Information on the volume of the liver, the hepatocytes, the sinusoids, and the central veins is provided in Table 1 for the control and treatment rats. In the diabetic and PbAc groups (G II and III, respectively), the volumetric values of the liver and hepatocytes were significantly higher, and conversely, sinusoids and central vein volumes were lower compared to the control and thiamine groups ($P<0.05$). When all the mentioned parameters were analyzed, a significant difference was observed between PbAc-poisoned diabetic rats along with thiamine (G VII) compared to the control group ($P<0.05$). Volumetric parameters measured in the liver after injection of thiamine in the diabetic rats (G V) were not significantly different from those in the control group ($P>0.05$). Administration of thiamine (30 mg/kg) along with PbAc (G VI) significantly ($P<0.05$) decreased the hepatocytes volume and increased sinusoids and central veins volumes when compared to PbAc-treated rats (G III) (Figure 1A-1D).

Table 2 shows the stereological assessment in particular groups based on the parameters of kidney weight and volumes of kidney structural components including volumes of kidney, cortex, medulla, and glomeruli. The PbAc group, followed by the diabetic group (G II and III, respectively), had the highest kidney weight ($P<0.05$). A tendency to decrease kidney weight was observed in the experimental groups receiving a single treatment of thiamine (G V, VI, and VII) compared with the control group. Nevertheless, a significant increase was still evident in group VII compared to the control group ($P<0.05$). In general, the volumes of cortex, medulla, and glomeruli in group II (diabetic rats) were higher than in the control, while administration of thiamine to

Table 1. The Mean ± Standard Deviation of the Liver (10^3 mm^3), Hepatocytes (10^3 mm^3), Sinusoids (mm^3), and Central Vein (mm^3) Volumes in Control and Treatment Groups

Groups		LV (10^3 mm^3)	HV (10^3 mm^3)	Sinusoids Volume (mm^3)	Central Vein Volume (mm^3)
G I	Control	8.6 ± 2.15	0.75 ± 0.08	2.60 ± 0.24	1.35 ± 0.09
G II	Diabetes	11.8 ± 2.21*	0.98 ± 0.09*	2.10 ± 0.12*	1.12 ± 0.08*
G III	PbAc	21.1 ± 4.32*	1.20 ± 0.12*	1.60 ± 0.18*	0.94 ± 0.05*
G IV	Thiamine	8.5 ± 1.20	0.75 ± 0.07	2.57 ± 0.25	1.38 ± 0.08
G V	Diabetes + thiamine	9.1 ± 2.65	0.78 ± 0.08	2.42 ± 0.19	1.28 ± 0.07
G VI	PbAc + thiamine	18.9 ± 3.21	0.93 ± 0.09**	2.35 ± 0.14**	1.27 ± 0.04**
G VII	Diabetes + PbAc + thiamine	18.2 ± 3.34*	1.1 ± 0.10*	1.80 ± 0.16*	1.04 ± 0.06*
G VIII	Diabetes + PbAc	20.1 ± 4.25*	1.22 ± 0.14*	1.61 ± 0.15*	0.93 ± 0.06*

LV: liver volume; HV: hepatocyte volume.
*Significantly different from control and thiamine groups; **Significantly different when compared to the PbAc treated group ($P<0.05$).

diabetic rats reduced these values significantly compared to group II ($P<0.05$). Except for kidney volume, there was a significant difference ($P<0.05$) between the PbAc + thiamine group (G VI) and the PbAc group (G III) in the name of the cortex, medulla, and glomeruli volumes (Figure 2A-2D).

Effect of Thiamine on Lipid Parameters

The mean of lipid parameters including TC, HDL-C, LDL-C, VLDL-C, and TG is shown in Table 3. Throughout the study period, no significant differences were observed between the control group in comparison with those of the thiamine group (G IV) ($P>0.05$). The diabetic group (G II) in all the named lipid parameters, with the exception of the HDL-C which showed a decrease, displayed a significant increase compared to the control group ($P<0.05$). Thiamine treatment noticeably improved alterations in whole-body lipid parameters in diabetic rats (G V), as characterized by lower TC, LDL-C, VLDL-C, and TG, and higher HDL-C in comparison with the control group ($P<0.05$). Rats that received only PbAc showed a significant decrease in HDL-C and a significant increase in serum TC, LDL-C, VLDL-C, and TG in comparison to the normal control group ($P<0.05$). Thiamine at a dose of 30 mg/kg body weight significantly reduced total serum LDL-C, VLDL-C, and TG in the PbAc + thiamine group (G VI), while in the PbAc + thiamine group, the HDL content increased significantly when compared with animals treated with PbAc.

Effect of Thiamine on Serum MDA, ALT and AST Activities and Cr Level

The MDA, ALT, AST, and Cr levels of alloxan-induced diabetic rats (G II) were significantly increased compared with those of the control and thiamine groups ($P<0.05$). After treatment of the diabetic rats with thiamine, measured parameters showed the healing process but were not significantly different from the control group ($P>0.05$). The experimental group treated with PbAc (G III) had statistically higher contents of MDA, ALT, AST, and Cr compared to values in the control group ($P<0.05$). However, serum concentrations of MDA, ALT, AST, and Cr in the PbAc + thiamine group were significantly lower than in the PbAc group alone ($P<0.05$). Diabetic rats contaminated with PbAc and treated with thiamine (G VII) showed an increase in serum values of the mentioned parameters in comparison to the control group ($P<0.05$) (Table 4).

Discussion

Heavy metals such as PbAc are involved in environmental pollution that could originate from human industrial activity or occur naturally and lead to the destruction of most biological organisms (28). The results showed that PbAc induces oxidative stress, which in turn produces excess free radicals, and alters the enzyme system that inhibits free radicals or antioxidants; as a result, leading to disruption of membrane function (29). Based on reports and the use of a selected dose of PbAc, we have shown that

Table 2. The Mean ± Standard Deviation of Kidney Weight (mg), and the Volume of Renal Structures in Control and Treatment Groups

Groups		Kidney Weight (mg)	Kidney Volume (mm^3)	Cortex Volume (mm^3)	Medulla Volume (mm^3)	Glomeruli Volume (mm^3)
G I	Control	467 ± 36	342.2 ± 18.4	152.4 ± 11.3	132.1 ± 12.4	8.45 ± 0.28
G II	Diabetes	489 ± 47	364.5 ± 21.2	183.1 ± 12.6*	163.2 ± 14.3*	10.4 ± 0.23*
G III	PbAc	622 ± 52*	395.1 ± 26.3*	208.2 ± 14.1*	174.1 ± 13.4*	14.5 ± 0.32*
G IV	Thiamine	469 ± 21	344.3 ± 17.8	153.8 ± 10.7	133.5 ± 10.7	8.64 ± 0.25
G V	Diabetes + thiamine	477 ± 38	354.1 ± 18.7	171.4 ± 11.3	153.7 ± 11.9	9.8 ± 0.27
G VI	PbAc + thiamine	584 ± 43	389.6 ± 19.5	165.2 ± 13.6**	145.6 ± 11.8**	10.5 ± 0.31**
G VII	Diabetes + PbAc + thiamine	587 ± 40*	390.2 ± 20.4*	203.1 ± 14.1*	170.3 ± 15.2*	13.7 ± 0.21*
G VIII	Diabetes + PbAc	618 ± 49*	387.1 ± 28.1*	210.2 ± 15.2*	172.1 ± 14.8*	14.7 ± 0.37*

*Significantly different from control and thiamine groups; **Significantly different when compared to the PbAc treated group ($P<0.05$).

Table 3. Comparison of the mean (± standard deviation) lipid parameters in treatment rats

Groups		TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)	TG (mg/dL)
G I	Control	60.31 ± 5.62	31.18 ± 5.09	21.20 ± 3.92	5.24 ± 0.76	87.34 ± 4.05
G II	Diabetes	72.18 ± 4.21*	21.61 ± 4.07*	32.20 ± 4.74*	12.31 ± 0.47*	109.64 ± 6.7*
G III	PbAc	87.25 ± 5.30*	16.50 ± 2.16*	43.05 ± 5.39*	16.35 ± 1.26*	125.12 ± 7.26*
G IV	Thiamine	58.26 ± 5.84	31.32 ± 5.73	22.42 ± 3.24	4.71 ± 0.52	86.23 ± 3.15
G V	Diabetes + thiamine	63.21 ± 4.85	25.30 ± 3.75	23.12 ± 3.63	6.32 ± 0.65	92.25 ± 4.37
G VI	PbAc + thiamine	79.24 ± 5.82	24.50 ± 3.22**	28.12 ± 5.26**	9.35 ± 084**	95.65 ± 5.12**
G VII	Diabetes + PbAc + thiamine	85.21 ± 5.64*	20.13 ± 3.58*	40.42 ± 5.64*	14.52 ± 1.07*	121.3 ± 6.5*
G VIII	Diabetes + PbAc	79.25 ± 4.75*	15.81 ± 3.04*	44.08 ± 4.99*	17.06 ± 1.14*	127.13 ± 8.42*

TC, Total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very-long-density lipoprotein cholesterol; TG, triglycerides.
* Significantly different from control and thiamine groups; **Significantly different when compared to the PbAc treated group ($P<0.05$).

Table 4. Changes in MDA, ALT, AST and Cr of Normal and Experimental Animals

Groups		MDA (mM)	ALT (U/L)	AST (U/L)	Cr (mg/L)
G I	Control	4.2 ± 0.54	161.2 ± 15.5	134.7 ± 11.4	4.29 ± 0.31
G II	Diabetes	9.7 ± 0.85*	231 ± 18.4*	175.1 ± 14.3*	5.23 ± 0.25*
G III	PbAc	14.5 ± 1.24*	273.2 ± 21.3*	248.4 ± 19.6*	6.12 ± 0.34*
G IV	Thiamine	4.6 ± 0.38	158.1 ± 14.7	136.1 ± 10.6	4.42 ± 0.24
G V	Diabetes + thiamine	5.8 ± 0.47	187.2 ± 14.3	142.32 ± 10.9	5.35 ± 0.28*
G VI	PbAc + thiamine	8.2 ± 0.61**	185.3 ± 12.2**	144.5 ± 11.24**	5.79 ± 0.26
G VII	Diabetes + PbAc + thiamine	12.8 ± 0.64*	245.2 ± 11.7*	203.1 ± 12.4*	6.09 ± 0.33*
G VIII	Diabetes + PbAc	13.8 ± 1.03*	268.2 ± 22.6*	250.1 ± 18.7*	5.82 ± 0.46*

MDA, Malondialdehyde; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cr, creatinine.
* Significantly different from control and thiamine groups; **Significantly different when compared to the PbAc treated group ($P < 0.05$).

the administration of PbAc in control rats and/or diabetic rats induces toxic effects that are consistent with previous reports in the form of weight loss, changes in stereological parameters, and hematological parameters (30).

The results of the present study showed that exposures to a dosage of PbAc did produce significant changes in the mean body weight of rats. Likewise, the present study also showed an increase in blood glucose concentration in the PbAc-treated groups. However, thiamine largely diminished the blood glucose imbalance in the diabetic and PbAc-treated rats. In any form, the observed decrease in body weight and increase in blood glucose level was realistic, because many publications have reported that PbAc alters the desired parameters. An increase in blood glucose concentration has been reported due to the administration of PbAc (31).

Through the stereological analysis in the present study, the protective role of thiamine on hepatic and renal tissue changes after PbAc administration in healthy and diabetic rats was confirmed. Based on our findings, no difference was found in all volumetric parameters of the structural components of the liver and kidneys together between thiamine-treated diabetic rats and the control group. On the other hand, the volumes of hepatocytes, sinusoids, and the central vein in the liver and the volumes of cortex, medulla, and glomeruli in the kidney showed a significant difference between the PbAc group treated with thiamine and the PbAc group. Thiamine administration can significantly reduce the stereological changes in the structural components of liver and kidney tissue caused by PbAc. As a result, the changes are returned to normal by thiamine, which may be evidence of the protective effect of thiamine against PbAc-induced tissue damage. This remark is reminiscent of those presented by Amiri et al, who illustrated that thiamine manages diabetes side effects through the improvement of measured stereological parameters in the pancreas of alloxan-induced diabetic mice (14). It is worth mentioning that the co-administration of thiamine with both diabetic and PbAc groups had considerable impacts on the desired stereological indices of the liver and kidney as compared with controls.

Organs such as the liver and kidneys are among the organs most affected by heavy metal toxicity, however, research has shown that serum biochemical factors are usually the first biomarkers of liver and kidney damage (29) To evaluate the therapeutic effects of thiamine on liver and kidney damages caused by PbAc, the serum levels of lipid parameters, ALT, AST, MDA, and Cr were measured in the blood of diabetic and PbAc-treated rats.

Research in recent years has shown that thiamine exhibits antioxidant activity (32). In fact, the results show that thiamine improved oxidative stress parameters in all treatment groups except PbAc-treated diabetic rats receiving thiamine.

Thiamine regulates basal metabolism in the form of coenzymes and (in some cases) non-coenzymes. The pyruvate dehydrogenase complex (PDC), which is involved in bioenergetic processes, is one of the most prominent thiamine coenzymes. The essential role of PDC in cellular metabolism and most of its clinical features are revealed following its deficiency, including mental retardation, ataxia, peripheral neuropathy, and structural abnormalities of the brain (33). Studies show that pathological accumulation of ROS in cells is associated with PDC deficiency. Mitochondrial manganese superoxide dismutase activity is severely reduced in PDC-deficient cells. In spite of the fact that the precise mechanism of thiamine against lead toxicity is unclear, it may be related to the formation of complexes between thiamine and lead, followed by its excretion. Thiamine has been suggested to facilitate the removal of lead from body fluids and other tissues by the formation of excretory complexes (34).

These results showed that treatment with PbAc significantly increased the serum level of TC, LDL-C, VLDL-C, and TG and decreased HDL-C levels. PbAc also increased the ALT, AST, Cr, and MDA values compared to the control group. PbAc, an environmental toxicant, can induce oxidative stress through the generation of ROS, thus the oxidative stress produced plays an active role in its toxic nature (35) Some research has shown that eventually, PbAc destroys the antioxidant defense system, which can be caused by PbAc-induced liver and kidney damage (29,36). It is well known that the increased serum levels of ALT, AST, and Cr correlate well with liver and

kidney injuries (29). Asiwe et al showed that the serum activities of AST and ALT in the blood serum of female rats were significantly increased in PbAc intoxication (37). In the present study, monitoring of evaluated lipid parameters showed significant changes in the PbAc-treated groups compared to the control group. As a result, PbAc can affect lipid composition by increasing arachidonic acid (essential unsaturated fatty acid in the membrane) and increasing lipid peroxidation (5). PbAc exposure has been linked to lipid peroxidation in recent studies (38). Wang et al showed that PbAc is able to produce specific lipid peroxidation and inhibit the activity of antioxidant enzymes (39). We found that thiamine markedly reversed the disruptive effects of lead. This suggested that thiamine could, at least partly, attenuate oxidative stress by modulating lipid peroxide levels in lead-treated rat liver and kidney.

Our study indicated that the administration of thiamine somewhat improves stereological criteria, serum biochemical levels of blood glucose, and lipid parameters in the liver and kidney of alloxan-induced diabetic rats.

Conclusion

Using stereological estimates, we showed that PbAc had significant effects on the volume of structural components in the liver and kidney in non-diabetic and diabetic rats. Thereafter, biochemical indices showed significant differences between the groups receiving PbAc compared to the control group. Thiamine was able to reduce the adverse effects of PbAc by reducing oxidative stress and improving liver and kidney function indicators and stereological parameters.

Authors' Contribution

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Competing Interests

All authors read and approved the final submitted article. None of the authors have conflicts of interest.

Data Availability Statement

Data are all contained within the paper and are also available from the corresponding author upon reasonable request.

Ethical Approval

The project was conducted in accordance with animal welfare standards for the species. Experimental procedures were approved by the animal care and ethics committee of Shahrekord University (IR.SKU.REC.1400.014).

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