Glutamine defended the kidneys versus lead intoxication via elevating endogenous antioxidants, reducing inflammation and carbonyl stress as well as improving insulin resistance and dyslipidemia

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Abstract

Objective(s):

Kidneys are primarily sensitive to lead (Pb) poisoning due to their cardinal role in lead excretion. Then, we studied the effect of glutamine (Gln) on lead nephrotoxicity in rats by assessing the histopathological and biochemical parameters (the renal NF-kβ expression, metabolic profile, oxidative stress, inflammatory markers, methylglyoxal (MGO), and glyoxalase-I activity).

Materials and Methods

Forty rats were allotted into four groups (ten rats in each): normal (N), Gln treated N, Pb intoxication (Pbi), and Gln treated Pbi. The treated groups took 0.1% Gln in drinking water for one month. To motivate lead poisoning, rats gained 50 mg/l lead acetate in drinking water for one month. Oxidative stress indices (total glutathione, its reduced and oxidized forms, and their ratios as well as advanced protein oxidation products, malondialdehyde, and ferric ion reducing power) and inflammatory markers (renal nuclear factor-kβ expression, interleukin 1β level, and myeloperoxidase activity) were measured. Further, metabolic profile (fasting blood sugar, insulin, insulin sensitivity, lipid profile, and atherogenic index) and renal dysfunction parameters were also determined. Pb-induced renal damage and dysfunction were assessed with Histopathological examination.

Results

In the kidney of Pbi rats, the glomerulus was damaged. Gln prevented kidney damage and reduced kidney dysfunction parameters. In addition, Gln decreased oxidative stress and inflammation in sera and kidney homogenates as well as improved insulin resistance, dyslipidemia, and carbonyl stress *($p < 0.001$)*.

1. Introduction

Lately, industrial developments are increased lead (Pb) levels in the environment [1]. Pb causes damage to the nervous, reproductive, hematopoietic respiratory, and excretory systems (liver and kidneys) [2]. Pb related nephrotoxicity is one of the principal mute characteristics of lead toxicity [3]. Kidneys are primarily sensitive to the toxic effect of lead due to their cardinal role in lead excretion [4]. In addition, there are proteins in the kidneys that have a high affinity for pb, which elevates their susceptibility to lead toxicity [5]. Deterioration in membrane function and its constitutional fullness following membrane oxidative degradation of polyunsaturated fatty acids probably have a cardinal role in Pb related nephrotoxicity [6]. Nuclear factor- kβ (NF-kβ) up-regulation after pb motivation oxidative stress has a primary role in toxic effects on different organs [9, 10]. Thus, lowering the NF-kβ signaling pathway is a potential target for preventing and ameliorating Pb toxicity [11].

Recently, chelating agents are used for the treatment of Pb toxicity but their failure to away the intracellular Pb [7] and reverse lead-induced oxidative stress, loss of essential metals, and liver or renal dysfunction [8] leads to the investigation of alternative treatments. Glutamine (Gln) is the most plentiful plasma free amino acid in plasma and has a pivotal role in multiple tissue maintenance. Gln not only has antioxidant, anti-inflammatory, and chelating activities but also plays a cardinal role in glutathione synthesis [9]. We recently reported Gln protective effect against lead-induced hepatotoxicity in rats via a down-regulating effect on the hepatic NF-kβ signaling pathway. There are evidences that documented Pb poisoning induces insulin resistance, dyslipidemia, and carbonyl stress or methylglyoxal (MGO) accumulation, oxidative stress, and inflammation [10–13]. Then, we recently reported Gln protective effect against lead-induced hepatotoxicity in rats. Then, we studied the effect of Gln on lead nephrotoxicity by assessing the histopathological and biochemical parameters (the renal NF-kβ expression, metabolic profile, oxidative stress, inflammatory markers, methylglyoxal (MGO), and glyoxalase-I activity).

2. Materials and Methods

2.1. Materials

Lead acetate, perchlorate sodium, acetonitrile, ethanol, thiobarbituric acid, CaCl2, NaCl, and EDTA, H_2O_2, sodium mono, and dihydrogen phosphate citric acid, were bought from Merck Company (Germany). Reduced and oxidized glutathione (GSH and GSSG, respectively), Gln, and paraoxon, and Trizol reagent were purchased from Sigma Aldrich Chemical Co (USA). Biochemical kits were taken from Pars Azmoon Company (Tehran, Iran). SYBER Green qPCR Master Mix 2x and cDNA synthesis kit were received from Yekta Tajhiz Azma Company (Iran).

2. 2. Rat Model of lead intoxication

Male Wistar rats with weights of 175 ± 10 was prepared from the Faculty of Veterinary Medicine, Tehran University, Iran. Animals are maintenance in temperature 22 ± 1°C under a 12/12 h light/dark cycle with free access to food and water. After two weeks, 40 male Wistar rats were randomly divided into four groups (10rats in each): normal rats (N), lead intoxication (Pbi), and two similar groups under glutamine (Gln) treatment, respectively N (Gln) and Pbi (Gln). Lead toxicity was motivated in rats with a solution of 50 mg/l lead acetate in drinking water orally for 30 days. Treated groups received 0.1% of Gln in drinking water daily for 30 days. Moreover, the intake of Pb and Gln through drinking water inhibits the stress in rats [14]. The dose of the treatment was chosen according to our recent study [11]. This study was permitted by the Ethics Committee of Ardabil University of Medical Sciences (IR.ARUMS.REC.1398.170). After 16 hours of fasting and anesthetizing with an intraperitoneal (i.p) injection of ketamine & xylosine (respectively, 90 &10 mg/kg body mass), blood samples were accumulated from their heart and transferred into the test tubes with and without EDTA. Serum samples were ready via 10 min

centrifugation of blood at 1500g, 4 C˚ and stored at − 70°C until measurements. Kidneys were removed and stored at -70˚C for biochemical and histopathological analysis.

2.2.1. Determination of Biochemical Parameters

Lead content in rat's kidney was measured with the flame atomic absorption spectrophotometer (PerkinElmer, 3100) at 283.3 nm. Fasting blood glucose (FBG), triglyceride (TG), total cholesterol (TC), LDL, HDL, creatinine (Cr), urea, urine protein excretion (PU) was assessed by commercial kits (Pars Azmoon, Tehran, Iran). The cardiovascular indices were calculated with calculating LDL/HDL and MPO/PON-1 ratios. Glomerular filtration rate (GFR) was computed via equation.1 (Eq. 1) [15].

$$\mathrm{Eq.1GFR} = \frac{\mathrm{Urinecreatinine}}{\mathrm{serumcreatinine}} \times \frac{\mathrm{Urinevolume}}{\mathrm{Bodyweight}}$$

A rat insulin enzyme-linked immunosorbent assay (ELISA) kit (ZellBio GmBH, Germany) was applied for measuring insulin levels. Further, the homeostasis model assessment of insulin resistance (HOMA) was estimated. Besides, the percentage of beta-cell action (%B), and the percentage of insulin sensitivity (%S) were calculated with the HOMA2 calculator software [16]. Finally, Kidney index of rat was computed (liver weight/rat weight × 100%).

2.2.2. Determination of glycation, oxidative stress, inflammatory, antioxidant, and anti-glycation markers in serum and kidney homogenate

Methylglyoxal (MG) was analyzed by HPLC through detecting the DNPH derivatives at a wavelength of 330 nm. [21]. The Glo-I activity was measured by determining the primary rate generation of S-D-lactoylglutathione at 240 nm [22]. Oxidative stress markers as malondialdehyde (MDA) were measured base on the detection of the absorbance of thiobarbituric acid at 532 nm. Advanced oxidation protein products (AOPP) were determined with spectrophotometric detection at a wavelength of 340 nm [17]. First LDL oxidation products or conjugated dienes (CD) were assessed at 234 nm [18] and last LDL oxidation products or fluorescent LDL oxidation products (FLOP) were quantified with a fluorimeter at 360 nm. [19]. The ferric ion reducing antioxidant power (FRAP) in samples was determined spectrophotometrically at a wavelength of 700 nm [20]. Reduced glutathione (GSH) and oxidized of it (GSSG) were measured with UV-HPLC at 210 nm [21]. Moreover, total glutathione was computed by adding GSH to GSSG. Then, the ratio of GSH to GSSG was calculated. The activity of paraoxonase-I (PON1) was done by detecting the p-nitrophenol absorbance within one minute at a wavelength of 412 nm [22]. The sera activity of CAT was quantified with a changed Abi method [23]. Shortly, 5 µl serum was added to a mixture (containing 10 mm of H2O2 in equal volume phosphate buffer 50 mm, pH = 7 and saline) and read absorbance at 240 nm to 20 seconds.

The inflammatory markers as IL-1β and TGF-β were measured using the ELISA kits (ZellBio GmBH, Germany). The activity of myeloperoxidase (MPO) was determined by reading the absorbance of oxidized Guaiacol at wavelength 470 nm. Concisely, 10 µl serum was added to a mixture (50 mM Potassium Phosphate Buffer with 100 mM Guaiacol and 0.0017% (w/w) Hydrogen Peroxide, pH 7.0 at 25°C) and read absorbance at 470 nm until 4 min.

2.2.3. Gene expression of the renal NF-κB

TRIzol reagent (Invitrogen, USA) was used for the removal total RNA from the hepatic tissue. The extracted RNA quantity and quality and were determined at 260 nm and by 260/280 nm ratio with a Nanodrop spectrophotometer. cDNA was made by reverse transcription following the manufacturer's protocols (MBI Ferments, Lithuania). qRT-PCR was done with a standard SYBR-Green PCR kit (Toyobo, Japan), and gene specific PCR amplification was performed using the ABI 7300 (Applied Biosystems, Germany). B-actin (ACTB) was applied as a housekeeping gene for normalization of gene expression data. RT- PCR primer sequences were as follows: NF-kβ: 5´-CCTGTCTGCACCTGTTCCAA-3´ (forward) 3´ACTCCTGGGTCTGTGTTGTT-5´(reverse)

ACTB: 5´-GGAGAA GATTTGGCACCACACT-3´ (forward)

3´-CGGTTGGCCTTAGGGTTCAGA-5´ (reverse).

For computing relative gene expression levels was used the $2^{-\Delta\Delta CT}$ method after normalization to the mRNA level of β-actin. The first ΔCT is the difference in threshold cycle between the target and reference genes: ΔCT = CT (NF-kβ)-CT (ACTB) [24].

2.2.5. Pathological study

Sections of kidney

Renal samples were fixed in a buffer solution containing 10% formalin and processed for paraffin embedding. Then, sections were stained with hematoxylin-eosin (H&E) and observed under light microscopy for histopathological parameters [25].

2.3. Statistical analysis

All data were expressed as mean ± S.D (standard deviations). The normal distribution of the results was confirmed by the Kolmogorov-Smirnov test. Comparing different variables in all four groups was done with a multiple analysis of variance (MANOVA-TUKEY) test and the correlation between Pb sera levels and FBS, HOMA, LDL/HDL, MGO, GLO-I, GSH/GSSG, CD, FLOP, MDA, AOPP, FRAP, CAT, PON-I, IL-1β, and MPO was determined by multiple regression analysis using SPSS version 16. significance was defined as $p < 0.05$.

3. Results

Table 1 represents the metabolic profile (FBS, insulin, HOMA, %B, %S, lipid profile, and atherogenic index) and renal function markers (Urea, Cre, PU, and, GFR, and KWI) in the rat groups with or without receiving Gln. FBS, insulin, HOMA, TG, TC, atherogenic index, urea, Cre, PU, and KWI were highest, and %B, %S and GFR were lowest in the untreated Pbi group. Gln compensated for the cited changes in metabolic profile and renal function parameters in Pbi (Gln) group *(P < 0.001).*

Table 2 shows effect of Gln on lead acetate, total glutathione, GSH/GSSG, FRAP, CAT, MDA, AOPP, MPO in both serum and kidney homogenate as well as the renal NF-kβ/BACT of all rat groups. Lead level, MPO activity, and the renal NF-kβ/BACT were less and total glutathione and FRAP were more in Gln treated N and Pbi groups than untreated ones. However, GSH/GSSG and CAT only were higher in Pb (Gln) than Pb group as well as MDA and AOPP were lower *(p < 0.001)*.

Table 3 compares sera early and end LDL oxidation products (CD and FOLP), MGO, IL-1β, TGF-β, and enzymes activities (PON-1 and Glo-I) in all rat groups. LDL oxidation products, MGO, IL-1β, TGF-β in normal and lead toxicity rat model were lower than untreated ones and Glo-I and PON-1 activities were higher *(p < 0.001)*.

The histopathological kidney alternations via lead toxicity of Pb and Pbi (Gln) groups were represented in figure 2a-2d. In the kidney of Pb rats, the glomerulus was shrunk and damaged (1a) and a cast or remnants of fallen cells can be seen in the middle of the tubule lumen (1b). Moreover, epithelium cells have fallen into the lumen in Pbi (1c). The cited histopathological changed did not observed in Pbi (Gln) and infiltration and inflammation in the kidney of treated rats were less than untreated (1d).

Table 4 exhibits the correlation between sera lead level with FBS, HOMA, LDL/HDL, MGO, GLO-I, GSH/GSSG, CD, FLOP, MDA, AOPP, FRAP, CAT, PON-I, IL-1β, and MPO. Here, sera lead level had high positive correlation with FBS, HOMA, LDL/HDL, MGO, CD, FLOP, MDA, AOPP, IL-1β, and MPO but it had a high negative with other parameters (*P < 0.005*).

4. Discussion

We researched the effect of glutamine on lead nephrotoxicity in rats. Pb motivated renal damage and dysfunction through insulin resistance, dyslipidemia, oxidative stress, inflammation induction, and methylglyoxal accumulation. Glutamine prevented Pb related nephrotoxicity by improving the metabolic profile and a decrease in Pb concentration, oxidative stress, inflammation induction, and methylglyoxal accumulation.

Pb participated in renal damage and dysfunction in rats by harming and shrinking the glomerulus (Fig. 1a), shedding casts, cellular debris(1b), and epithelium cells (1c) in the middle of the tubule lumen. A decrease in GFR and elevating Cre, urea, urinary protein excretion, KWI (Table.1), and TGF-β (Table 3) supports renal dysfunction in rats following lead toxicity induction. Gln interrupted the cited histopathological changes and ameliorated renal function. Further, it reduced infiltration and inflammation in the kidneys of rats (1d). Gln reno-protective effect is presumably via diminishing Pb

levels in sera and kidney homogenates (Table 1) and sera MGO and IL-1β levels (Table 3). Glo-I is the most important factor against MGO accumulation or carbonyl stress. A decrease in Glo-I activity and an increase in IL-1β level cause nephropathy and atherosclerosis [26, 27]. Moreover, its antioxidant and anti-inflammatory activities (Tables 2 and 3) as well as its improving effect on metabolism (Table 1) decreased renal dysfunction. Gln antioxidant property confirms via raising total glutathione, GSH/GSSG, CAT in the sera and kidney homogenates, and sera PON-1. Receiving lead acetate in drinking water elevated Pb concentration in the rat sera and kidneys. Gln through chelating property and a beneficial effect on glutathione metabolism decreased Pb level in sera and kidney homogenates (Table 2). Formerly, a decrease in Glo-I activity in lead exposure wheat and an increase in its activity with glutathione supplementation has been stated [10].

The renal NF-kβ activation has a cardinal role in kidney dysfunction. Then, inhibition of the pathway decreases kidney injury intensity [28]. IL-1β [29], MGO, Oxidized LDL, and MPO through NF- kβ signaling initiation and oxidative stress motivation lead to renal injury [30, 31]. The renal NF-kβ pathway (Table 2) and its inducers (Table 3) were higher in Pbi groups. Gln reno-protective confirms via its reductive effect on NF-kβ signaling and its activators. In addition, the treatment increases Glo-I activity. Glo-I activity mediates the kidney prone to nephropathy [32]. The effect of Gln on the renal NF-kβ pathway and its inducers has not been given.

Oxidative stress has a central role in the pathophysiology of renal dysfunction. Lead toxicity causes renal damage via lipid peroxidation and protein oxidation. Lead motivates oxidative stress via the MDA and AOPP elevation along with total glutathione, GSH/GSSG, CAT (Table 2), and PON-1 (Table 3) reduction. Here, Gln renal protective effect also validates its advantageous effect on glutathione metabolism and antioxidant enzymes (Table 2). Gln is a rate-limiting procreator for GSH synthesis [33] and with its antioxidant property increases GSH/GSSG [11]. Here for the first time, the effect of Gln on levels of total glutathione, GSH, GSSG, GSH/GSSG, AOPP, MDA, and the activities of CAT and PON-I in serum and kidney homogenate of lead toxicity rat model has been reported. Ox-LDL as another indicator of oxidative stress contributes to atherosclerosis and nephropathy [34, 35]. In this study, Pb causes the formation of early (CD) to end LDL (FOLP) oxidation products. Pb presumably elevates Ox-LDL due to a diminish in PON-1 (Table 3) activity and an increase in MPO activity (Table 2). Gln had guarding effect against nephropathy and atherosclerosis through a decline in LDL oxidation products resulting in an increase in PON-1 activity and a decrease in MPO activity. Recently, the effect of Gln on the cited oxidative stress and antioxidant markers in the liver of the lead toxicity rat model has been documented with us [11]. However, its effect on LDL oxidation parameters and renal oxidative stress markers has not been represented.

Up-raising IL-1β, NF-kβ signaling, insulin, and MGO contribute to renal dysfunction by insulin resistance and dyslipidemia induction [36, 37]. FBS, insulin, HOMA, TG, TC, and LDL/HDL in the Pbi group were more than in other groups (Table 1). Gln improved metabolism and insulin sensitivity in N and Pbi groups. The treatment's beneficial effect on insulin function confirms by raising β cell activity and insulin sensitivity percentages. Gln bettered metabolism and insulin sensitivity following its antioxidant property and a decrease in the NF-kβ pathway and its activators (Table 3). Among diverse NF-kβ inducers, IL-1β and

MGO have axial roles in insulin resistance and dyslipidemia. Previously interfering effect of Pb on rat brain glucose metabolism, insulin signaling [13] and dyslipidemia motivation in rats [12] has been represented. We reported for the first-time effect of Gln on renal NF-kβ signaling, metabolism (glucose and lipid), and insulin resistance in lead toxication.

Here the correlation between sera Pb levels with FBS, HOMA, LDL/HDL, MGO, Glo-I, GSH/GSSG, CD, FLOP, MDA, AOPP, FRAP, CAT, PON-I, IL-1β, and MPO was computed (Table 4). A high positive correlation between sera lead level and FBS, HOMA, LDL/HDL, LDL oxidation products, MGO, MDA, AOPP, IL-1β, and MPO along with a negative correlation with Glo-I, PON-1, CAT, GSH/GSSG, and FRAP satisfies elevating prone to insulin resistance and vascular complications such as nephropathy and atherosclerosis in lead intoxication following oxidative stress and inflammation progression.

Conclusion

Glutamine guarded the kidneys versus lead intoxication via elevating antioxidant markers, reducing inflammation and carbonyl stress as well as improving insulin resistance and dyslipidemia. Moreover, Gln reduced susceptibility to Pb related vascular complications such as atherosclerosis and nephropathy.

Declarations

Conflict of interest

There is no conflict of interest

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Authors' contribution

1) Authors make substantial contributions to conception and design, and/or acquisition of data, and/or analysis and interpretation of data: Mahdavifard S & Sekhavatmand N

2) Authors participate in drafting the article or revising it critically for important intellectual content: Mahdavifard S

3) Author gives final approval of the version to be submitted and any revised version: Mahdavifard S

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Tables

Table 1. The effect of glutamine on FBS, insulin, HOMA-IR and lipid profile in normal (N) and lead toxicity (Pbi) rats.

Parameter	Groups (Ten rats in each group)			
	N	N (Glutamine)	Pbi	Pbi (Glutamine)
Fasting blood sugar (mmol/L)	4.67± 0.21	4.13 ± 0.19 *	7.12 ± 0.38 *	5.85 ± 0.26 *, #
Insulin (µU/L)	15.19 ± 0.83	14.96± 0.77	18.39 ± 1.20 *	16.01 ± 1.04 #
HOMA-IR	3.15± 0.19	2.74 ± 0.13*	5.81 ± 0.32*	4.16 ± 0.27 *, #
β cell activity (%)	172.40 ± 8.41	217.50 ± 12.76*	87.70± 5.24 *	115.30 ±7.33 *, #
Insulin sensitivity (%)	52.30 ± 6.28	55.00 ± 7.31*	39.30 ± 2.10 *	47.00 ±3.09*, #
Triglyceride (mmol/L)	1.37 ± 0.09	1.35 ± 0.08	2.93 ± 0.17 *	1.87 ± 0.14 *, #
Total cholesterol (mmol/L)	1.83 ± 0.15	1.76 ± 0.13	3.22 ± 0.23 *	2.16 ± 0.19*, #
LDL (mmol/L)	0.27± 0.03	0.25 ± 0.02	1.16 ± 0.13 *	0.31 ± 0.04#
HDL (mmol/L)	0.94± 0.08	0.92 ± 0.07	0.72 ± 0.06 *	1.00 ± 1.02#
LDL/HDL	0.29± 00.03	0.27 ± 0.02*	1.61 ± 0.13 *	0.31 ± 0.04#
Urea (mmol/L)	11.28± 0.60	10.89 ± 0.56	35.07 ± 2.12 *	24.29 ± 1.30*, #
Creatinine (mmol/L)	63.30± 4.28	57.92 ± 0.02*	98.60 ± 6.23 *	75.96 ± 5.01#
Urinary protein excretion (mg/dL)	13.07± 0.86	11.68 ± 0.75	51.40 ± 3.02*	26.01 ± 1.84*, #
GFR (ml/min)	2.98± 0.22	3.04 ± 0.24	2.41± 0.14 *	2.68 ± 0.17*, #
Kidney weight index (%)	0.74 ± 0.05	0.69 ± 0.04	0.81 ± 0.07 *	0.76 ± 0.06#

* Indicates Significant difference with group N ($p < 0.001$)

# Indicates Significant difference with group Pbi ($p < 0.001$)

Table 2. Effect of glutamine on levels of total glutathione, reduced glutathione (GSH), oxidized glutathione (GSSG), and ratio of them in serum (µmol/L) and kidney homogenate ((nmol/mg protein)) of normal (N) and lead toxicity (Pbi) rats.

Parameter		N	N (Glutamine)	Pbi	Pbi (Glutamine)
Lead content	Serum (µg/L)	3.63± 0.29	2.29 ± 0.12*	20.72± 1.51*	7.84 ± 0.57*, #
	Kidney (µg/g dry tissue)	3.78 ±0.36	1.94± 0.23*	18.26± 1.36*	6.40 ± 0.49*, #
Total glutathione	Serum (µmol/L)	137.89± 8.34	160.87 ± 10.42*	73.05± 4.17*	103.69 ± 6.30*, #
	Kidney (nmol/mg protein)	162.26 ±9.76	181.06± 11.24*	121.60± 7.52 *	144.03 ± 8.94*, #
GSH/GSSG	Serum	5.98 ±0.28	6.14± 0.35	1.50± 0.09 *	2.57 ± 0.18 *, #
	Kidney	5.41± 0.24	5.79± 026	2.99± 0.13*	410 ± 0.19 *, #
CAT	Serum (U/mg protein)	126.00 ±7.09	130.84± 7.62	44.32± 3.52 *	84.91 ± 5.74 *, #
	Kidney (U/mg protein)	150.19± 9.45	152.64± 10.38	62.76± 3.32*	108.43 ± 6.92 *, #
MDA	Serum (µmol/L)	12.68 ±0.65	10.73± 0.51	136.35± 8.49 *	58.19 ± 4.31 *, #
	Kidney (nmol/g tissue)	4.14± 0.28	3.89± 0.24	36.34± 2.41*	16.81 ± 0.97 *, #
AOPP	Serum	8.71 ±0.56	8.08± 0.49	32.84± 2.18 *	17.46 ± 1.14 *, #
	Kidney	3.19± 0.21	2.83± 0.16	37.08± 2.30*	20.13 ± 1.18 *, #
FRAP	Serum	625.69 ±37.04	665.83± 40.35*	381.74± 22.38 *	498.57 ± 27.62 *, #
	Kidney	1.34± 0.07	1.60± 0.09*	0.66± 0.04*	1.12 ± 0.06 *, #
MPO	Serum (U/mg protein)	0.75 ±0.08	0.49± 0.06*	4.63± 0.57 *	1.42 ± 0.18 *, #
	Kidney (U/mg protein)	1.22± 0.15	0.84± 0.14*	2.53± 0.35*	1.26 ± 0.13 *, #
NF-kβ/BACT	Kidney	0.26± 0.04	0.11± 0.02*	4.29± 026*	1.48 ± 0.08 *, #

* Indicates significant difference with group N ($p < 0.001$)

[#] Indicates significant difference with group Pbi ($p < 0.001$)

Table .3. Comparison the levels of glycation, oxidative stress and inflammatory markers in the all groups

Parameter	Groups			
	N	N (Gln)	Pb	Pb (Gln)
CD (µmol/L)	12.58 ± 0.71	7.05 ± 0.46[*]	93.24 ± 5.98 [*]	40.31 ± 2.32 [*, #]
FOPL (µmol/L)	205.14 ± 11.66	174.02 ± 9.74[*]	456.39 ± 22.80 [*]	287.16 ± 16.58 [*, #]
MGO (µmol/L)	11.72 ± 0.69	6.84 ± 0.52[*]	39.60 ± 2.14 [*]	18.54 ± 1.10 [*, #]
IL-1β (pg/ml)	305.94 ± 15.74	276.31 ± 13.16[*]	506.22 ± 27.50 [*]	380.81± 19.97 [*, #]
TGF-β (pg/ml)	27.68 ± 0.32	21.08 ± 0.26[*]	72.41 ± 3.95 [*]	44.37 ± 3.11 [*, #]
Glo-I (U/ml)	40.59± 2.74	48.27 ± 3.18[*]	22.74 ± 1.37 [*, #]	31.42 ± 2.06 [*, #]
PON-I (U/ml)	126.27± 8.02	135.31± 9.46[*]	77.69 ± 4.12[*]	91.83± 7.19[*, #]

* Compared N group with other groups ($p < 0.001$)

Compared Pbi group with other groups ($p < 0.001$)

g-Alb: glycated albumin; g-LDL: glycated LDL; MG: methylglyoxal; AGEs: advanced glycation end products; CD: conjugated dines; FOPL: fluorescent oxidation products of LDL; AOPP: advanced oxidation end products; MDA: malondialdehyde; GSH: reduced glutathione; IL-1β: interleukine-1β; Glo-I: glyoxalase-I; PON-I: paraoxonase-I; CAT: catalase; MPO: myeloperoxidase

Table .4. Correlation between sera lead level with FBS, HOMA, LDL/HDL, MGO, GLO-I, GSH/GSSG, CD, FLOP, MDA, AOPP, FRAP,CAT, PON-I, IL-1β, and MPO

Parameter	r	P value <
FBS (µmol/L)	0.957	0.005
HOMA (µmol/L)	0.992	
LDL/HDL	0.962	
MGO (µmol/L)	0.988	
Glo-I (U/ml)	-0.956	
GSH/GSSG	-0.971	
CD	0.971	
FLOP	0.988	
MDA	0.984	
AOPP	0.995	
FRAP	-0.960	
CAT	0.960	
PON-I (U/ml)	-0.868	
IL-1β	0.958	
MPO	0.976	

g-Alb: glycated albumin; g-LDL: glycated LDL; MG: methylglyoxal; AGEs: advanced glycation end products; CD: conjugated dines; FOPL: fluorescent oxidation products of LDL; AOPP: advanced oxidation end products; MDA: malondialdehyde; GSH: reduced glutathione; IL-1β: interleukine-1β; Glo-I: glyoxalase-I; PON-I: paraoxonase-I; CAT: catalase; MPO: myeloperoxidase

Figures

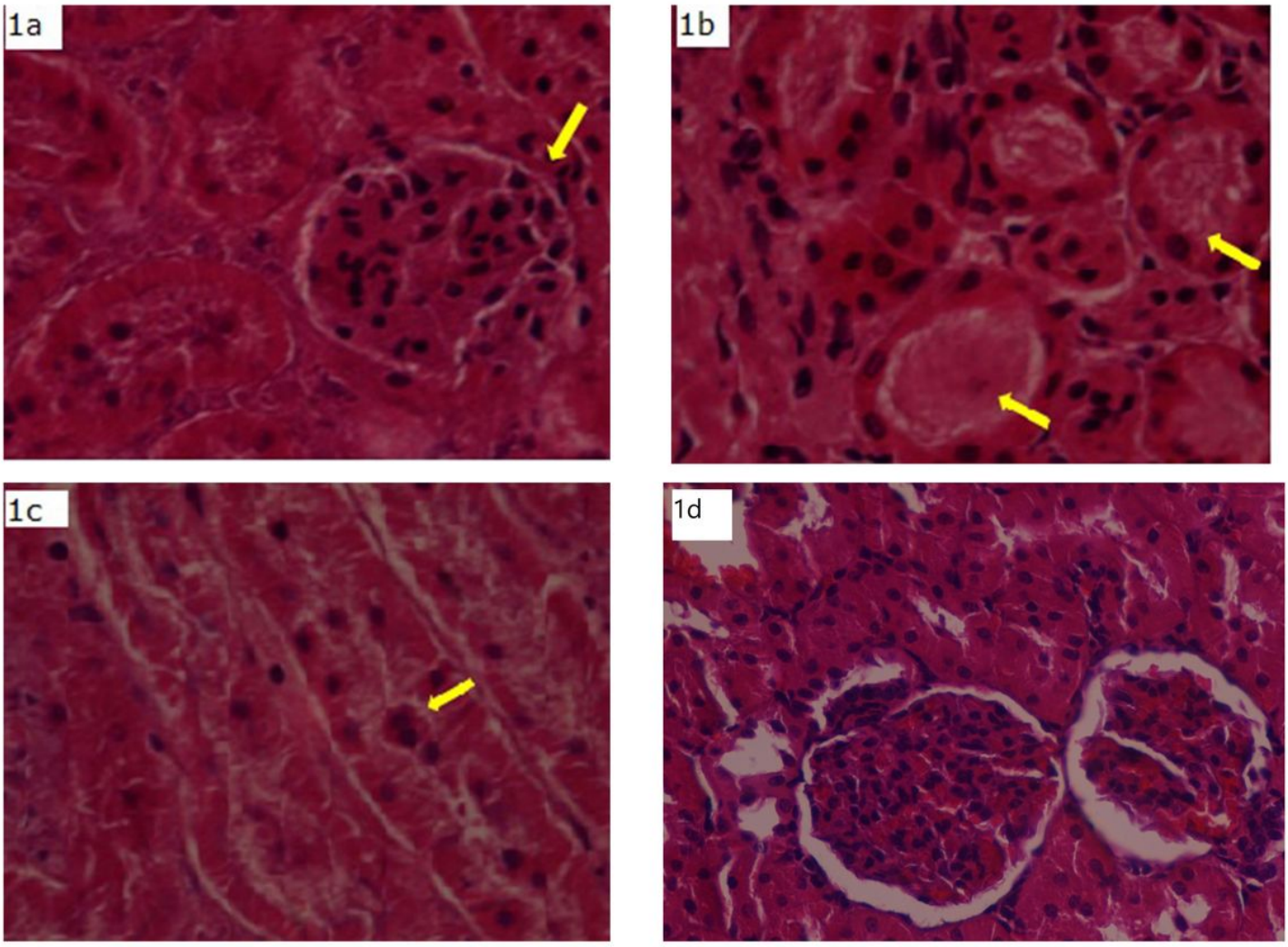

Figure 1

Histopathologic views (stained by H&E & original magnification ×400) of the liver in the lead toxicity (Pb) and glutamine treated one, Pb (Gln).

(a) The accumulation of lead residue in the Kupffer cells of liver of Pb rats, hyperplasia of Kupffer cells (arrow), a high number of binucleated hepatocytes (circle), congested sinusoids and autolytic cytoplasm (star) in the liver of Pbi group.

(b) Gln prevented the collection of lead in hepatocytes of the Pbi treated group and reduced other cited changes.