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Hepatorenal protective effect of flaxseed protein isolate incorporated in lemon juice against lead toxicity in rats

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ABSTRACT

Finding renal and hepatoprotective agents preferably with antioxidant activities against environmental pollutants especially lead which can adversely affect liver and kidney is a great demand. In the current study, flaxseed protein isolate (FPI) was extracted from defatted flaxseed meal. Amino acids profile, antioxidant capacity and solubility of the extracted FPI were determined. The solubility of FPI in the acidic media was exploited in preparation of lemon juice with FPI. Twenty four male rats were assigned to four groups; normal control, lead intoxicated (oral daily dose of 60 mg/kg b.w. in distilled water for four weeks), lead intoxicated and orally administrated with daily dose equal 1 ml of lemon juice as well as lead intoxicated and orally administered with FPI (daily dose equal 100 mg/kg) in 1 ml of lemon juice. The oral administration of FPI incorporated in lemon juice suppressed the elevation in kidney functions, lipid peroxidation of kidney tissues, urinary protein and creatinine as well as liver functions caused by lead intoxication. Additionally, lemon juice with FPI combated the reduction of GSH of kidney tissues. It was revealed also that lemon juice without FPI suppressed the elevation in kidney and liver functions caused by lead. It can be concluded that flaxseed protein isolate is a good source of protein with potent antioxidant activity. Additionally, lemon juice and FPI are considered protective sources of kidney and liver against lead toxicity.

1. Introduction

Environmental pollutants are major burdens on human health. Lead is one of these pollutants entering the body through digestion of contaminated food and water, as well as through inhalation a polluted air and dust [1]. Cellular damage is induced by the accumulated lead in tissues via the oxidative stress caused by the excessive reactive oxygen species in addition to reduction in the activities of cellular antioxidants [2,3]. Lead attaches with various biomolecules among them enzymes, regulatory and signaling proteins which changes their composition and makes them unable to perform their functions [4]. In addition to that, lead causes inhibition of cellular energy production, DNA damage as well as apoptosis subsequent to mitochondrial impairment [5]. Also, the production of several pro- and anti-inflammatory cytokines is inhibited by lead [6]. Thus, lead can adversely affect various organs among them brain, liver and kidney [7]. The traditional chelating agents used as curatives for lead intoxication may apply negative effects in addition to its incapability of alleviating some of lead effects commonly the oxidative stress [8]. So, there is a need for protective agents and remedies not only capable of avoiding the negative effects of lead but also possess antioxidant activities.

Flaxseed is considered one of the most common oilseed, however, it contains other nutritional constituents make it a potent functional food. One of the most important nutritional constituents of flaxseed is protein which is considered an important source of nutrients in addition to its biological benefits. Flaxseed protein is high in arginine, aspartic acid and glutamic acid. Also it was reported that flaxseed protein contain high contents of cysteine and methionine that can improve the antioxidant system in the human body and potentially elevate the stability of DNA during cell division [9]. The biological effects of flaxseed protein were rarely studied [10], but in general the biological activity of different dietary proteins has been established to be related to the formation of bioactive peptides through their release from their parent proteins after the food digestion in the gastrointestinal tract. These peptides are able to cross the digestive epithelial barrier and reach the blood vessels, which allow them to reach peripheral organs and apply their beneficial effects in the organism [11]. It was hypothesized that the flaxseed protein isolated from defatted flaxseed meal may be beneficial as protective agent against the negative effects of lead on the liver and kidney. So, the current study was designed to evaluate the

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protective effect of flaxseed protein isolate incorporated in the lemon juice against the hepato and nephro-toxicity induced by lead acetate in the rats.

2. Materials and methods

2.1. Materials

Flaxseed meal was obtained from a local factory "Peacock" Tanta, Gharbeya, Egypt. Lemon fruits (*Citrus limon*) were purchased from local market to prepare lemon juice.

2.2. Animals

Adult male Wiaster rats weighing 176.25 ± 19.18 g as Mean ± SD (10 weeks old) were used. Animals were provided by the animal house of National Research Centre, Cairo, Egypt. The animals were kept individually in stainless steel metabolic cages at room temperature (24 ± 2 °C and 40–60 % relative humidity) under 12 h light and dark cycles and allowed free access to food and water.

2.3. Diet

A balanced diet (10 % protein as casein, 10 % corn oil, 10 % sucrose, 60.5 % corn starch, 5 % cellulose, 3.5 % salt mixture and 1 % vitamin mixture) was prepared in accordance with the AIN-93 diet [12]. Salt and vitamin mixtures were prepared in accordance with AIN-93 formulation [12].

2.4. Methods

2.4.1. Preparation of flaxseed protein isolate (FPI)

Flaxseed meal which was already hydraulic pressed was subjected to defatting using a soxhlet apparatus and n-hexane as defatting solvent. The defatted meal was spread to dry then ground in coffee mill to obtain a finely divided material suitable for further extraction. Generally, the alkaline solution (0.02 N NaOH) was first added to the defatted flaxseed meal and stirred for two hours to solubilize the proteins. The mixture was then centrifuged, and the pH of supernatant was adjusted by dilute acid (6 N HCl) to precipitate the proteins at its isoelectric point (4.2–4.8). Precipitated protein was then separated by centrifugation and the precipitate was washed several time with water followed by ether and acetone then dried and saved in refrigerator until used.

2.4.2. Water-Holding Capacity (WHC) and Oil-Holding Capacity (OHC) of FPI

Water-Holding Capacity (WHC) and Oil-Holding Capacity (OHC) of FPI were determined as described by Alfredo et al. [13] and expressed as g of water or oil held/g sample.

2.4.3. Determination of FPI protein content

The total protein content of FPI was determined according to the method of AOAC. [14].

2.4.4. Determination of amino acids profile of FPI using HPLC

The amino acids profile of FPI was determined using HPLC-Pico-Tag method according to Millipore Cooperative (1987). The Pico-Tag method was described by Heinrikson and Meredith [15] and Cohen et al. [16]. The Pico-Tag method, was developed commercially by Waters Associates, was an integrated technique for amino-acid analysis. Phenylisothiocyanate (PITC, or Edman's reagent) was used for pre-column derivatization, while reversed-phase gradient elution high-performance liquid chromatography (HPLC) separates the phenylthiocarbamy (PTC) derivatives which were detected by their UV absorbance. The chromatographic analysis using HPLC was carried out using the following gradient of Pico-Tag solvent A and B (P/N 88108 and 88112).Sample was injected and loaded on Pico-Tag amino acids column (150 × 3.9 mm) stainless steel. Detection of the PTC derivatives is by ultraviolet absorption measurements using a fixed wavelength (254 nm) Waters detector. Before injecting of the sample, the illustrated was calibrated by two injections of the standards.

2.4.5. Determination of FPI solubility at different pH from 1 to 12

One gram of the prepared FPI was dissolved in 50 ml distilled water. The pH of the solutions was adjusted to the desired value using 1 N NaOH or 6 N HCl. the solution was stirred by homogenizer for 3 min and 2500 rpm then left over night for complete hydration. The pH was adjusted again then the solution was centrifuged, the supernatant was used for determination of solubility of protein and antioxidant activity.

2.4.6. Evaluation of antioxidant activity of soluble FPI at different pH (1:12)

The antioxidant activity of the soluble FPI at each pH was measured by 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and hydrogen peroxide (H_2O_2) scavenging activity.

2.4.6.1. Determination of the free radical-scavenging assay (DPPH). The DPPH radical has a strong absorbance at 517 nm due to its unpaired electron and giving the radical a purple color. But upon reduction with an antioxidant, its absorption decreases due to the formation of its non-radical form, DPPH-H [17] that was based on the method of [18] with some modification. Results were expressed as percentage inhibition of the DPPH using the following equation:

Inhibition of DPPH (%) = Absorbance control-Absorbance sample/ Absorbance control X 100.

Where, Absorbance control is the absorbance of DPPH solution without extract, Butylated Hydroxyl Toluene (BHT) was used as positive control. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

2.4.6.2. Estimation of H_2O_2 scavenging activity. Hydrogen peroxide exhibits weak activity in initiating lipid peroxidation, however, its potential to produce ROS (reactive oxygen species), such as hydroxyl radical through fenton reaction is very high. The H_2O_2 scavenging ability of each extract was determined according to Sfahlan, et al. [19].

2.4.7. Animal treatments and grouping

Twenty four rats were adapted for one week then divided into four groups (n = 6) as follows:

Group 1: normal control.

Group 2: lead acetate, rats of this group were given lead acetate dissolved in distilled water by oral gavage at daily dose equal 60 mg/kg of body weight for four weeks [20,21].

Group 3: lead acetate + lemon juice, rats of this group were orally administrated with daily dose equal one ml of freshly prepared lemon juice then orally given lead acetate in distilled water at daily dose equal 60 mg/kg of body weight for four weeks.

Group 4: lead acetate + lemon juice FPI, rats of this group were orally administrated with FPI at daily dose equal 100 mg/kg of body weight dissolved in one ml of freshly prepared lemon juice then orally given lead acetate in distilled water at daily dose equal 60 mg/kg of body weight for four weeks.

All rats' groups were fed on balanced diet all over the study period. During the experiment, body weight and food intake were recorded weekly. At the end of the study total food intake, body weight gain and feed efficiency ratio were calculated. The animal experiment has been carried out according to the Ethics Committee, National Research Centre, Cairo, Egypt and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals

(Publication No. 85-23, revised 1985).

2.4.8. Biochemical parameters of urine, blood and tissues

At the end of the study period, urine was collected for 24 h from each rat to be subjected to determination of protein according to Koerbin et al. [22] and creatinine according to Larsen [23] with calculation of creatinine clearance. Blood was withdrawn from each rat to be subjected to determination of plasma levels of creatinine [23] and urea [24] in addition to the activities of lactate dehydrogenase (LDH) according to Zimmerman and Weinstein [25], aspartate transaminase (AST) and alanine transaminase (ALT) according to Reitman and Frankel [26], alkaline phosphatase (ALP) according to Bessey et al. [27] and Gama-GT (γ- GT) according to [28] as well as the levels of total protein and albumin according to Rheinhold [29] and Doumas et al. [30] in succession with calculation of globulin and albumin/globulin (A/G) ratio. Via the dissection, liver and kidney were immediately separated from each rat and weighed. Kidney tissues were analyzed for malondialdehyde (MDA) as indicator of lipid peroxidation according to Ohkawa et al. [31] and reduced glutathione (GSH) according to Sedlak and Lindsay [32].

2.4.9. Statistical analysis

Statistical analyses were done using SPSS version 22. The results of antioxidant activity of flaxseed protein isolate at different PH (1:12) as well as the results of the animal experiment were expressed as mean ± standard error (SE) and analyzed statistically using one-way analysis of variance (ANOVA) followed by Duncan test. The statistical significance of difference was taken as $P \leq 0.05$.

3. Results

Total protein content of FPI was 93.5 %. Water holding capacity of FPI was 11.4 g/g while oil holding capacity was 1.47 g/g. The amino acids profile of flaxseed protein isolate (FPI) was illustrated in Table 1. As shown, flaxseed protein isolate was rich in arginine amino acid.

Fig. 1 revealed that the solubility of FPI increased at pH 1 and 2. The least solubility of flaxseed protein isolate was at its isoelectric point around pH 5. With increasing pH towards alkaline the solubility increased.

Table 2 declared that FPI in acidic medium showed higher scavenging radicals activity than in alkaline medium and there was no scavenging radicals activity at basic medium (11–12 pH). In contrary, the hydrogen peroxide scavenging activity % was high at both elevated and low pH, while the least scavenging activity of FPI was at its isoelectric point.

Table 1
The amino acids profile of flaxseed protein isolate (FPI).

Amino acids	g/100 g sample
Aspartic acid	6.57
Glutamic acid	8.47
Serine	6.76
Glycine	2.19
Histidine	6.96
Arginine	13.97
Threonine	6.94
Alanine	6.55
Proline	3.43
Tyrosine	5.03
Valine	3.96
Methionine	2.88
Cysteine	0.17
Isoleucine	6.26
Leucine	4.92
Phenylalanine	4.29
Lysine	2.70

Hydroxyproline and tryptophan not determined.

As observed from the results (Table 3), rats treated orally only with lead acetate recorded less body weight gain than normal rats. On the other side, rats treated with flaxseed protein isolate incorporated in lemon juice along with lead acetate recorded slight increase in body weight gain whereas, rats treated with lemon juice along with lead acetate showed notable reduction in the body weight from 176.17 g to 168.33 g.

It was revealed (Table 4) that the treatment with lead acetate caused significant ($p \leq 0.05$) increase in the absolute kidney weight. Additionally, rats treated with only lead acetate exhibited high significantly values of plasma urea, creatinine and LDH more than those of normal rats. Also, the treatment with lead acetate induced oxidative stress which was revealed via the results of lipid peroxidation and GSH. The results disclosed the modulator effect of lemon juice either without or with flaxseed protein isolate on the elevation either in the absolute kidney weight or in the kidney functions (urea, creatinine and LDH). In addition, administration of lemon juice either without or with flaxseed protein isolate especially lemon juice with flaxseed protein isolate suppressed either the elevation of MDA or the reduction of GSH.

In the same context, the urine volume as well as the values of urinary protein and creatinine (Table 5) significantly ($p \leq 0.05$) elevated in rats treated with only lead acetate. While, rats administrated with lemon juice either without or with flaxseed protein isolate especially lemon juice with flaxseed protein isolate showed significant reduction in urine volume as well as the values of urinary protein and creatinine.

Table 6 showed slight change in the liver weight and significant elevation in the activities of each AST, ALT, ALP and GGT of rats treated with only lead acetate while the values of total protein, albumin and globulin diminished in this group. On the other hand, the administration of lemon juice either without or with flaxseed protein isolate repressed either the increasing in the activities of each AST, ALT, ALP and GGT or the reduction in the values of total protein and albumin.

4. Discussion

The results of the present study revealed that flaxseed protein isolate was rich in arginine amino acid. The essential amino acid profile of FPI was similar to those observed by Kaushik et al. [33] who also reported that flaxseed protein isolates rich in arginine and low in lysine which makes it favorable in heart-healthy foods and infant formulas. High purity flaxseed protein isolate was prepared via removal of mucilage before extraction of protein from defatted flaxseed meal at 60 °C especially that Kaushik et al. [33] declared that the purity of FPI depends on temperature for removal of mucilage which hinders the extraction of protein.

The pH-dependent FPI solubility is due to the positive and negative charges which promote interaction with water and facilitate protein solubilization. While the absence of electrostatic repulsion because of neutralization of charges in protein causes diminish of solubility near isoelectric point. Tirgar et al. [34] also found that the solubility of flaxseed protein concentrate increased either in low acidic or in alkaline medium. The solubility and free radical scavenging activity of FPI in the acidic media were exploited in preparation of lemon juice with FPI. The hepatorenal protective effect of the prepared lemon juice with FPI against lead-induced toxicity and oxidative stress was evaluated in rats.

The harmful effects of lead on liver or kidney either in mice or rat have been declared in several studies [35–39]. Additionally, Gargouri et al. [40] touched upon the damaging effect of lead on the kidney of offspring of pregnant rats which intoxicated with lead acetate. Also it was shown that the developing of kidney diseases in humans may be occur as a result of high blood level of lead via affecting transportation in proximal tubules and decreasing of creatinine clearance [41]. The chronic toxicity of lead acetate stems from generation of various pro-inflammatory cytokines such as TNF-α and IL-4 in serum and tissues, accumulations of ROS in addition to inhibition of the expression of IL-10, a potent anti-inflammatory cytokine [42]. Andjelkovic et al. [43]

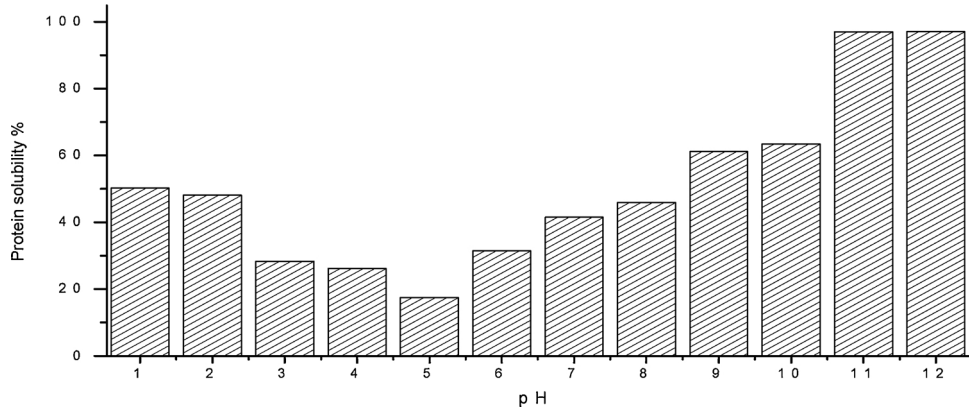

Fig. 1. Solubility of flaxseed protein isolate at different pH from 1 to 12.

Table 2
Antioxidant activity of flaxseed protein isolate at different PH (1:12).

pH	DPPH scavenging activity %	H_2O_2 scavenging activity %
1	$66.80^{a} \pm 1.10$	ND*
2	$27.40^{b} \pm 0.80$	$15.30^{c} \pm 0.50$
3	$21.90^{c} \pm 0.70$	$13.90^{d} \pm 0.60$
4	$9.30^{e} \pm 0.50$	$3.50^{f} \pm 0.20$
5	$11.90^{d} \pm 0.90$	$14.20^{d} \pm 0.70$
6	$7.80^{f} \pm 0.40$	$10.10^{e} \pm 0.90$
7	$12.50^{d} \pm 0.60$	$21.60^{a} \pm 1.20$
8	$7.80^{f} \pm 0.50$	$11.00^{e} \pm 0.80$
9	$9.80^{e} \pm 0.70$	$17.90^{b} \pm 0.60$
10	$7.80^{f} \pm 0.80$	$15.90^{c} \pm 0.90$
11	ND	$14.10^{d} \pm 1.00$
12	ND	$21.30^{a} \pm 1.20$
LSD at 5 %	1.195	1.413

* ND not detected. Different letters mean significant difference at 0.05 probability.

also concluded that the oxidative stress is the main mechanism of lead toxicity. As obvious via the results of the current study that lead acetate induced decrease in the body weight gain of lead intoxicated rats in compare to normal rats due to the negative effect of lead on the mass of muscles. On the other hand, flaxseed protein isolate incorporated in lemon juice attenuated this reduction in the body weight gain in compare with lemon juice alone which caused loss of weight. The harmful effects of lead acetate on kidney and liver in rats were confirmed via the results of the present work. The elevation in relative kidney weight of rats intoxicated with lead acetate was in consistent with Kaur and Sharma [44] who found the same results and attributed that to the expansion of glomerular and mesangial in addition to renal fibrosis. Also the oxidative stress caused by lead toxicity in the kidney tissues was confirmed in the results of the present work via the elevated lipid peroxidation and reduced GSH. The effect of lead on kidney tissues has been emphasized as it is known that lead is eliminated by the kidney. The absorbed lead binds to specific proteins in the proximal tubular cells (PCT) of the renal tubules, these complexes of lead-protein are implicated as typical intracellular inclusions in the acute lead nephrotoxicity [45].

It was elucidated that administration with lemon juice either alone or with flaxseed protein isolate suppressed the deterioration of kidney and liver functions subsequent to lead intoxication. Also lemon juice with flaxseed protein isolate showed promising effect on suppression either the elevation of lipid peroxidation or the reduction of GSH caused by administration of lead acetate. The beneficial impact of flaxseed protein isolate in protection from the harmful effects of lead acetate may be stemmed from its antioxidant activity especially when incorporated in lemon juice (acidic medium, pH = 2). Additionally, the presence of vitamin c in lemon juice supported the protective effect of lemon juice either alone or with flaxseed protein isolate against the negative effects of lead acetate on liver and kidney which reflected through the results of the current study. It has been declared in several studies that vitamin c diminished the lead toxicity may be due to its antioxidant activity or its ability as a chelator of lead [46,47]. Moreover, there are other bioactive compounds in lemon juice to which the beneficial effect of lemon juice may be attributed such as naringenin (polyphenol, 4,5,7-trihydroxy-flavanone) that present in lemon juice by 0.38 mg/100 ml [48]. Naringenin demonstrated antioxidant, hepatoprotective and nephroprotective effects as reported by Zaidun et al. [49].

The potent of flaxseed protein isolate as protective source against kidney and liver toxicity may be attributed to the plenty of some amino acids especially arginine and glycine in it. Tkachenko et al. [50] disclosed that L-arginine as an efficient antioxidant reduced lipid peroxidation and increased the activity of glutathione in rats intoxicated with lead also suggested that L-arginine had protective effect against lead toxicity in rats. Alcaraz-Contreras et al. [51] concluded that glycine had antioxidant activity and ameliorative effect on oxidative stress and hepatic toxicity induced by lead in rats.

5. Conclusions

In addition to the nutritive value of flaxseed protein isolate as a source of protein, it exhibited potent antioxidant activity especially

Table 3
Growth performance parameters of studied experimental rat groups.

Parameters	Normal control	Lead acetate	Lead acetate + lemon juice	Lead acetate + lemon juice FPI
Initial body weight (g)	$176.17^{a} \pm 9.83$	$176.33^{a} \pm 8.87$	$176.17^{a} \pm 6.50$	$176.33^{a} \pm 8.02$
Final body weight (g)	$197.83^{a} \pm 12.47$	$183.83^{a} \pm 10.78$	$168.33^{a} \pm 7.93$	$185.33^{a} \pm 9.80$
Body weight gain (g)	$21.67^{b} \pm 3.12$	$7.50^{ab} \pm 4.56$	$-7.83^{a} \pm 8.74$	$9.00^{ab} \pm 11.01$
Total food intake (g)	$538.00^{b} \pm 13.75$	$506.83^{ab} \pm 13.33$	$465.33^{a} \pm 18.43$	$524.50^{b} \pm 26.28$
Food efficiency ratio	$0.04^{b} \pm 0.01$	$0.01^{ab} \pm 0.01$	$-0.02^{a} \pm 0.02$	$0.01^{ab} \pm 0.02$

In each row same letters mean non-significant difference while different letters mean significant difference at 0.05 probability. The data are expressed as mean values ± standard error.

Table 4
Kidney weight, functions and oxidative markers of studied experimental rat groups.

Parameters	Normal control	Lead acetate	Lead acetate + lemon juice	Lead acetate + lemon juice FPI
Kidney weight(g)	$1.25^{a} \pm 0.02$	$1.64^{b} \pm 0.05$	$1.38^{a} \pm 0.07$	$1.36^{a} \pm 0.06$
Kidney relative weight (%)	$0.64^{a} \pm 0.04$	$0.91^{c} \pm 0.07$	$0.82^{bc} \pm 0.03$	$0.74^{ab} \pm 0.02$
Urea (mg/dl)	$24.37^{a} \pm 1.63$	$46.73^{c} \pm 2.47$	$34.68^{b} \pm 2.97$	$33.27^{b} \pm 2.32$
Plasma creatinine (mg/dl)	$0.48^{a} \pm 0.04$	$1.23^{b} \pm 0.17$	$0.72^{a} \pm 0.05$	$0.68^{a} \pm 0.05$
LDH (U/l)	$215.19^{a} \pm 10.34$	$364.43^{c} \pm 15.28$	$304.51^{b} \pm 8.62$	$292.52^{b} \pm 9.06$
MDA (nmol/g)	$8.50^{a} \pm 0.62$	$21.74^{c} \pm 1.07$	$14.48^{b} \pm 1.55$	$11.59^{ab} \pm 1.11$
GSH (mg/g)	$124.55^{c} \pm 3.08$	$99.51^{a} \pm 3.80$	$105.33^{b} \pm 4.51$	$116.87^{c} \pm 4.03$

In each row same letters mean non-significant difference while different letters mean significant difference at 0.05 probability. The data are expressed as mean values ± standard error.

Table 5
Urinary parameters of studied experimental rat groups.

Parameters	Normal control	Lead acetate	Lead acetate + lemon juice	Lead acetate + lemon juice FPI
Urine volume (ml)	$4.92^{a} \pm 0.47$	$12.25^{b} \pm 1.57$	$8.50^{ab} \pm 1.48$	$7.83^{a} \pm 1.35$
Urinary protein (mg/24 h)	$5.48^{a} \pm 0.35$	$11.10^{c} \pm 0.94$	$7.28^{b} \pm 0.54$	$6.85^{ab} \pm 0.54$
Urinary creatinine (mg/dl)	$38.93^{c} \pm 1.28$	$25.89^{a} \pm 1.13$	$29.03^{ab} \pm 0.94$	$31.11^{b} \pm 1.10$
Creatinine clerance (ml/min)	$0.28^{a} \pm 0.03$	$0.19^{a} \pm 0.04$	$0.26^{a} \pm 0.06$	$0.26^{a} \pm 0.05$

In each row same letters mean non-significant difference while different letters mean significant difference at 0.05 probability. The data are expressed as mean values ± standard error.

Table 6
Liver weight and functions of studied experimental rat groups.

Parameters	Normal control	Lead acetate	Lead acetate + lemon juice	Lead acetate + lemon juice FPI
Liver weight (g)	$5.15^{ab} \pm 0.15$	$5.28^{b} \pm 0.07$	$4.58^{a} \pm 0.18$	$4.96^{ab} \pm 0.28$
Liver relative weight (%)	$2.66^{a} \pm 0.18$	$2.93^{a} \pm 0.22$	$2.73^{a} \pm 0.09$	$2.68^{a} \pm 0.08$
AST (U/l)	$30.33^{a} \pm 2.24$	$45.50^{b} \pm 2.56$	$36.33^{a} \pm 2.18$	$32.33^{a} \pm 1.41$
ALT (U/l)	$22.50^{a} \pm 1.43$	$43.83^{b} \pm 2.15$	$27.50^{a} \pm 1.78$	$24.83^{a} \pm 1.49$
ALP(U/l)	$84.27^{a} \pm 6.89$	$142.71^{b} \pm 7.08$	$91.66^{a} \pm 7.44$	$87.26^{a} \pm 6.62$
GGT (U/l)	$12.41^{a} \pm 0.89$	$17.42^{b} \pm 1.05$	$14.94^{ab} \pm 0.87$	$13.24^{a} \pm 0.74$
Total protein (g/dl)	$8.05^{c} \pm 0.26$	$5.28^{a} \pm 0.16$	$6.78^{b} \pm 0.37$	$7.35^{bc} \pm 0.23$
Albumin (g/dl)	$4.13^{b} \pm 0.30$	$2.87^{a} \pm 0.23$	$3.69^{ab} \pm 0.42$	$3.94^{b} \pm 0.24$
Globulin (g/dl)	$3.92^{c} \pm 0.38$	$2.41^{a} \pm 0.27$	$3.10^{ab} \pm 0.08$	$3.04^{bc} \pm 0.06$
A/G ratio	$1.14^{a} \pm 0.19$	$1.33^{a} \pm 0.24$	$1.21^{a} \pm 0.15$	$1.16^{a} \pm 0.08$

In each row same letters mean non-significant difference while different letters mean significant difference at 0.05 probability. The data are expressed as mean values ± standard error.

when being soluble in acidic media (pH = 2–3) which was exploited in the preparation of lemon juice with FPI. This lemon juice with FPI exhibited hepatorenal protective effect against lead in rats in addition to its ameliorative effect against the oxidative stress induced by lead in rats. Also lemon juice exhibited protective effect against the negative effects of lead on liver and kidney. So, lemon juice and FPI are considered protective sources against kidney and liver toxicity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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