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Original Research

Physiological and biochemical studies on the protective effect of *Ficus carica* leaf extract, vitamin C or their combination on liver toxicity induced by lead acetate in male rats

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ABSTRACT

Introduction: Lead is an environmental contaminant, which is toxic to organ systems in human and other animals. The present study investigated the possible protective role of *Ficus carica* leaf extract, vitamin C or the combined treatment in lead acetate-induced hepatotoxicity. **Methods:** One hundred and twenty-six adult male albino rats were divided into seven groups (n = 18). G_1 (control group) received distilled water. G_2 (lead acetate group) received lead acetate at a daily dose of 20 mg/kg body weight by gastric gavage. G_3 (*Ficus carica* group) received *Ficus carica* leaves extract at a daily dose of 200 mg/kg body weight by gastric gavage. G_4 (Ficus and lead group) received *Ficus carica* leaves extract followed by lead acetate after 20 minutes. G_5 (vitamin C group) received vitamin C at a daily dose of 200 mg/kg body weight by gastric gavage, G_6 (vitamin c and lead group) received vitamin C followed by lead acetate after 20 minutes. And, G_7 (Ficus, vitamin C, and lead group) received *Ficus carica* leaves extract and vitamin C followed by lead acetate after 20 minutes. The treatment extended for six weeks, blood and specimens were collected at a 2-week interval. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein (TP), direct bilirubin (DB), lipid peroxidation biomarker (Malondialdehyde (MDA)), antioxidants enzymes (Superoxide dismutase (SOD) and reduced glutathione (GSH)) in liver tissue and histopathological changes in liver were investigated. **Results:** Lead acetate caused significant increases in AST, ALT, ALP, DB and MDA levels. In addition, TP and level of SOD and GSH significantly decreased compared to the control group. The pre-treatment with the combination of *Ficus carica* and vitamin C improved liver parameters, the level of antioxidant enzymes as well as histopathological changes. **Conclusion:** The combination of *Ficus carica* leaf extract and vitamin C had a remarkable protective action against lead acetate induced- oxidative damage in rats.

Key words: Antioxidants, Ficus carica, Hepatotoxicity, Lead, Vitamin C

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INTRODUCTION

Lead (Pb) is a heavy metal that is harmful. Thus, lead is one of the toxicants and persistent environmental pollutants affecting all biological systems through exposure to polluted air, water, and food[1]. Lead has no beneficial role in body functions, cellular growth, proliferation, or signaling and there is no "safe" level of exposure has been reported[2,3]. Lead has detrimental effects in humans and animals[4]. The mechanisms of lead toxicity include oxidative damages in the cell membrane and other sub-cellular organelles, and disturbances in the process of gene expression[5].

Although the liver is not among the main targets for lead toxicity, it still vulnerable to lead toxicity[6]. A previous study covering 4.556 males conducted on individuals with occupational diseases from lead poisoning, reported an increase in total mortality with digestive system diseases including chronic hepatitis and cirrhosis[7]. In accordance, lead has been reported to induce Pb hepatotoxicity in terms of elevation in the levels of liver enzymes in the serum including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), and abnormal hepatic cholesterol metabolism[8].Although the mechanism of lead-induced hepatotoxicity is not very clear[9], oxidative stress is a plausible mechanism of lead hepatotoxicity by causing lipid and protein peroxidation resulting in damaging membrane integrity and fatty acid composition[10], through two different pathways; first the generation of reactive oxygen species and second, the depletion of antioxidants[11].

Medicinal plants play an everlasting and growing role in treating common ailments especially in developing countries. *Ficus carica* is one of the largest genera of medicinal plants[12]. *Ficus carica L.* has been

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reported to have numerous bioactive compounds including flavonoids, vitamins, enzymes, nicotinic acid, tyrosine, and other important constituents[13]. Previous studies investigating the hepatoprotective activity of *Ficus carica* leaf extract in rats with liver damage showed that the extractremarkably decreased the levels of AST, ALT, bilirubin, MDA equivalents and TP concentration in the serum[14,15]. It is likely that the antioxidant potential of *Ficuscarica* leaf extract is due to its high content of flavonoids[13]. The ability of flavonoids to act as antioxidants depends on their molecular structure with multiple aromatic rings and hydroxyl groups[16,17]. Moreover, the hydroxyl groups together with the carbonyl groups donate electrons by performing resonance and scavenging free radicals to resolve oxidative stress[18].

Vitamin C (Vit. C), as a prominent antioxidant, is able to alleviate the oxidative-stress-related impairments in animal tissues[19]. In addition, Vit. C also increases the availability of the vitamins required in ameliorating oxidative damages. Consequently, Vit. C is considerably able to restore the activity of antioxidant enzymes[20]. Taken together, the antioxidant properties of Vit. C may attribute to its potential ability in scavenging free radicals as well as activating and generating other endogenous antioxidants[21]. Additionally, Vit C protects low-density lipoproteins from oxidation and reduces the harmful oxidants in cells[22]. Consistently, lead has been found to induce hepatotoxicity in terms of higher serum levels of AST, ALT and ALP enzymes, whereas Vit. C pre-treatment considerably protected against lead hepatotoxicity[23,24].

METHODS

Experimental animals: male adult Sprague Dawley rats (150-200 g) were kindly provided from NODCAR breeding center and kept for a week for acclimatization under normal conditions and constant temperature (25±1C°) with *ad libitum* water and food until starting the experiment. Rats were grouped and housed in a conventional clean facility according to the guidelines of the Institutional Animal Ethics Committee of NODCAR. All the experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals.

Chemicals

Lead acetate: lead acetate was purchased from El Gomhoureya For Drugs Trade & Medical Supplies (Zagazig, Egypt). Chemicals: All chemicals, unless specified otherwise, were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

Drug

Vit. C (ascorbic acid) tablets were purchased from the local pharmacy. It is manufactured by CID Company. Each tablet contains 1g ascorbic acid.

Ficus Carica leaf Extract preparation

The leaves of *Ficus carica* were washed with tap water and were shade dried for around one week and pulverized into coarse powder by a blender. The coarse powder was extracted with 70% aqueous EtOH by maceration at room temperature for 72 h maceration technique. The extract was subjected to filtration, and then the filtrate was concentrated in a rotary evaporator under reduced pressure[25]. The dried concentrate was kept at 4° Ctill use.

Animal Grouping

Rats were divided into seven groups (n = 18). G_1 (Control group) got refined water. G_2 (Lead acetate group) was administered with lead acetate (20 mg/kg of body weight) using gastric tube[26]. G_3 (*Ficus carica* group) was administered with *Ficus carica* leaf extract (200 mg/kg of body weight) using gastric tube[15]. G_4 (Ficus extract + Lead acetate group) was administered with *Ficus carica* leaf extract (200 mg/kg of body weight) followed by lead acetate (20 mg/kg of body weight) after 20 minutes by using gastric tube. G_5 (Vit. C group) was administered with Vit. C (200 mg/kg of body weight) using gastric tube[27]. G_6 (Vit. C + Lead acetate group) was administered with Vit. C (200 mg/kg of body weight) followed by lead acetate (20 mg/kg of body weight) after 20 minutes by using gastric tube. G_7 (Ficus carica leaf extract + Vit. C + Lead acetate group) was administered with *Ficus carica* leaf extract (200 mg/kg of body weight) and vitamin C (200 mg/kg of body weight) followed by lead acetate (20 mg/kg of body weight) after twenty minutes using gastric tube.

Blood sampling and tissue preparation

Blood samples were collected at an interval of two weeks, into clean glass tubes. The tubes were centrifuged at 3000 r.p.m to separate serum. Serum samples were transferred into Eppendorf tubes and stored at -70°c before measuring the level of AST, ALT, ALP, TP and DB.

Serum AST and ALT activities were measured by the colorimetric method[28]. Serum ALP activity was measured by the spectrophotometric method[29]. Total protein was measured by a commercial kit[30].Direct bilirubin concentration was measured by the colorimetric method[31].

Liver tissues were then homogenized in 10 ml of Phosphate buffer (pH7.4). The homogenates were centrifuged at 100 000× g at 4° C for 20 min. The clear supernatant was used to measure the level of MDA, SOD, and GSH. Then parts from liver were fixed in 10 % formalin for histopathological examination. Reduced and oxidized glutathione levels were measured by HPLC-UV method [32], MDA levels were measured by HPLC-UV method [33]. Superoxide dismutase (SOD) activity was measured by spectrophotometer method [34].

Histopathological examination

Finally, histopathological examination was carried out on liver tissues under different treatments [35].

Statistical analysis

Data presented as means ± SE. One-way ANOVA followed by LSD test were used to evaluate significant differences from the control and sleep deprived groups. $P< 0.05$ was considered to be statistically significant. Statistical processor system support (SPSS) for Windows software, release 10.0 (SPSS, Inc, Chicago, IL).

RESULTS

Lead acetate treatment significantly (P<0.05) increased AST, ALT and ALP levels in rats as compared with control group, after 2, 4 and 6 weeks. Both Ficus and vitamin C pre-treatment significantly (p<0.05) decreased the elevating effect of lead on AST, ALT and ALP levels after 2 , 4 and 6 weeks. Combined treatment with *Ficus carica* and Vitamin C remarkably abolished the effect lead on AST, ALT and ALP levels (**Tables 1, 2 and 3**).

Table 4 demonstrated that lead administration induced a significant reduction in TP concentration after two, four and six weeks compared to the control group. Meanwhile, administration of *Ficus carica* or vitamin C or *Ficus carica* and vitamin C caused a significant elevation in TP concentration compared to the lead-treated group.

Table 5 demonstrated that lead administration caused a significant elevation in DB level after two, four and six weeks, compared to the control group. Pre-treatment with *Ficus carica* or vitamin C or the combined treatment significantly minimized the elevating effect of lead on DB level when compared to the control group after two and four weeks.

Table 6 showed that administration of lead caused a significant elevation in MDA level after two, four and six weeks compared to the control group. Pre-treatment with *Ficus carica* or vitamin C or the combined treatment significantly minimized the elevating effect of lead on MDA level when compared to the control group after two and four weeks.

Table 7 demonstrated that lead administration caused a significant decrease in GSH level after two, four and six weeks, compared to the control group. Pre-treatment with *Ficus carica* or vitamin C or the combined treatment significantly minimized the decreasing effect of lead on GSH level compared to the control group after two and four weeks.

Table 8 demonstrated that lead administration induced a significant reduction in SOD activity level after two, four and six weeks, compared to the control group. Pre-treatment with *Ficus carica* or vitamin C or the combined treatment significantly minimized the decreasing effect of lead on SOD activity compared to the control group after two and four weeks.

Histopathological examination

Histological examination of liver tissues of control group showed normal hepatocytes of hepatic lobule (**Figure 1A**, **Figure 2A** and **Figure 3A**). Lead acetate-treated animals exhibited parenchymatous degeneration of hepatocytes with severe necrosis and severe leucocytes infiltration throughout the experiment (**Figure 1B**, **Figure 2B** and **Figure 3B**). Treatment of Ficus carica extract and vit. C to control animals maintain the normal liver tissues (**Figure 1C,E**; **Figure 2C,E** and **Figure 3C,E**) Pre-treatment with Ficus carica extract and vit C alone or in combination remarkably provided a potential protective action against the histopatological effect of lead acetate in rat liver (**Figure 1D,F,G**; **Figure 2D,F,G** and **Figure 3D,F,G**).

DISCUSSION

The liver performs three principal functions which are essential to the body: detoxification of many toxins, synthesis of proteins and bile, and storage of vitamins (A, D, E, and K) and glycogen [36,37]. The liver is a target organ of lead toxicity [38]. The present study showed that lead caused a remarkable elevation in the enzymatic activity of ALT, AST, ALP and bilirubin levels. Elevation of liver enzymes in the serum may indicate inflammation or damage to liver cells which leak higher than normal amounts of liver enzymes, into the bloodstream, which can result in higher level of liver enzymes in the blood. The elevated MDA and decreased GSH levels might indicate an increase in lipid peroxidation and oxidative stress. This effect

Table 1: AST activity in different treated groups after two, four and six weeks (mean ± S.E)

Group	AST (U/L) after 2 weeks	AST (U/L) after 4 weeks	AST (U/L) after 6 weeks
Control group (C)	58.03 ± 0.225 b	58.37 ± 0.166 b	58.56 ± 0.049 b
Lead acetate treated group (L)	90.94 ± 0.369 a	98.78 ± 0.477 a	106.89 ±0.588 a
% of change of control	56.7%	69.2%	82.5%
Ficus carica treated group (Fc)	55.58 ± 0.201 a, b	57.22 ± 0.301 a, b	58.36 ± 0.151 b
% of change of control	-4.2%	-1.9%	- 0.34%
Ficus + Lead treated group (Fc+ L)	75 ± 0.365 a, b	68.83 ± 0.477 a, b	61.61 ± 0.201 a, b
% of change of control	29.2%	17.9%	5.2%
Vitamin C treated Group (Vit. C)	54.67 ± 0.422 a, b	56.44 ± 0.382 a, b	57.28 ± 0.315 a, b
% of change of control	-5.8%	-3.3%	-2.2%
Vit. C + Lead treated group (Vit. C+ L)	79.61 ± 0.203 a, b	70.39 ± 0.416 a, b	62.72 ± 0.315 a, b
% of change of control	37.2%	20.6%	7.1%
Ficus + Vit. C + Lead treated group (FC+ Vit. C + L)	76.5 ± 0.428 a, b	69.17 ± 0.307 a, b	58.5 ± 0.428 b
% of change of control	31.8%	18.5%	- 0.10%

(a) significant difference compared to the control group ($P \leq 0.05$), (b) significant difference compared to the lead-treated group ($P \leq 0.05$)

Table 2: ALT activity in different treated groups after two, four and six weeks (mean ± S.E)

Group	ALT (U/L) after 2 weeks	ALT (U/L) after 4 weeks	ALT (U/L) after 6 weeks
Control group (C)	44.61 ± 0.417 b	44.63 ± 0.306 b	44.68 ± 0.295 b
Lead acetate treated group (L)	98.87 ± 0.233 a	106.03 ±0.141 a	112.94 ± 0.245 a
% of change of control	121.6%	137.6%	152.8%
Ficus carica treated group (Fc)	41.17 ± 0.307 a, b	42.93 ± 0.267 a, b	44.57 ± 0.578 b
% of change of control	-7.7%	-3.8%	-0.25%
Ficus + Lead treated group (Fc + L)	69.24 ± 0.112 a, b	56.72 ± 0.185 a, b	47.33 ± 0.128 a, b
% of change of control	55.2%	27.1%	5.9%
Vitamin C treated Group (Vit. C)	40.44 ± 0.419 a, b	43.02 ± 0.344 a, b	44.32 ± 0.248 b
% of change of control	-9.3%	-3.6%	-0.8%
Vit C + Lead treated group (Vit. C+ L)	70.16 ± 0.201 a, b	58.77 ±0.186 a, b	48.18 ± 0.108 a, b
% of change of control	57.3%	31.7%	7.8%
Ficus + Vit. C + Lead treated group (Fc+ Vit. C + L	62.92 ± 0.233 a, b	53.51 ± 0.174 a, b	44.91± 0.072 b
% of change of control	41%	19.9%	0.52%

(a) significant difference compared to the control group ($P \leq 0.05$), (b) significant difference compared to the lead-treated group ($P \leq 0.05$)

Table 3: ALP activity in different treated groups after two, four and six weeks (mean ± S.E)

Group	ALP (IU/ L) after 2 weeks	ALP (IU/ L) after 4 weeks	ALP (IU/ L) after 6 weeks
Normal control group (C)	115.52 ± 0.079 b	115.54 ± 0.162 b	115.54 ± 0.204 b
Lead acetate treated group (L)	140.21 ± 0.154 a	153.83 ± 0.112 a	162.35 ± 0.208 a
% of change of control	21.4%	33.1%	40.5%
Ficus carica treated group (Fc)	110.77 ± 0.105 a, b	113.27 ± 0.209 a, b	115.01 ± 0.069 b
% of change of control	- 4.1%	- 1.9%	- 0.46%
Ficus + Lead treated group (Fc+ L)	128.96 ± 0.249 a, b	121.69 ± 0.094 a, b	116.61 ± 0.139 a, b
% of change of control	11.6%	5.3%	0.93%
Vitamin C treated Group (Vit. C)	109.32 ± 0.179 a, b	112.72 ± 0.145 a, b	114.65 ± 0.106 b
% of change of control	- 5.4%	- 2.4%	- 0.77%
Vit. C +Lead treated group (Vit. C+ L)	131.21 ± 0.188 a, b	123.72 ± 0.111 a, b	117.83 ± 0.106 a, b
% of change of control	13.6%	7.1%	1.9%
Ficus + Vit. C +Lead treated group (Fc+ Vit. C +L)	126.8 ± 0.129 a, b	120.12 ± 0.175 a, b	115.01 ± 0.126 b
% of change of control	9.8%	3.9%	- 0.46%

Table 4: TP concentration in different treated groups after two, four and six weeks (mean ± S.E)

Group	TP (g/ dl) after 2 weeks	TP (g/dl) after 4 weeks	TP (g/dl) after 6 weeks
Normal control group (C)	10.68 ± 0.011 b	10.67 ± 0.025 b	10.68 ± 0.029 b
Lead acetate treated group (L)	5.95 ± 0.013 a	4.81 ± 0.010 a	3.95 ± 0.050 a
% of change of control	- 44.3%	-54.9%	-63%
Ficus carica treated group (Fc)	9.83 ± 0.012 a, b	10.29 ± 0.009 a, b	10.56 ± 0.025 a, b
% of change of control	- 8%	-3.6%	-1.1%
Ficus + Lead treated group (Fc+ L)	8.66 ± 0.013 a, b	9.51 ± 0.014 a, b	10.31± 0.012 a, b
% of change of control	-18.9%	-10.9%	-3.5%
Vitamin C treated Group (Vit. C)	9.80 ± 0.017 a, b	10.26 ± 0.014 a, b	10.47 ± 0.009 a, b
% of change of control	-8.2%	-3.9%	-1.9%
Vit. C + Lead treated group (Vit. C + L)	8.42 ± 0.012 a, b	9.22 ± 0.015 a, b	10.05 ± 0.050 a, b
% of change of control	-21.2%	-13.6%	-5.9%
Ficus + Vit. C + Lead treated group (Fc+ Vit. C + L)	9.12 ± 0.029 a, b	10.09 ± 0.038 a, b	10.56 ± 0.007 a, b
% of change of control	-14.6%	-5.4%	-1.1%

(a) significant difference compared to the control group ($P \leq 0.05$), (b) significant difference compared to the lead-treated group ($P \leq 0.05$)

Table 5: DB level in different treated groups after two, four and six weeks

Group	DB (mg/ dl) after 2 weeks	DB (mg/dl) after 4 weeks	DB (mg/dl) after 6 weeks
Normal control group (C)	0.35± 0.025 b	0.36 ± 0.019 b	0.37 ± 0.014 b
Lead acetate treated group (L)	0.59 ± 0.013 a	0.65 ± 0.008 a	0.760 ± 0.021 a
% of change of control	71.6%	88.3%	105.4%
Ficus carica treated group (Fc)	0.27 ± 0.003 a, b	0.31 ± 0.008 a, b	0.350 ± 0.004 b
% of change of control	-21.2%	-13.3%	- 5.4%
Ficus +Lead treated group (Fc+ L)	0.48 ± 0.011 a, b	0.42 ± 0.007 a, b	0.362 ± 0.004 b
% of change of control	38.8%	16.7%	- 2.2%
Vitamin C treated Group (Vit.C)	0.27 ± 0.002 a, b	0.30 ± 0.005 a, b	0.34 ± 0.001 b
% of change of control	-22.6%	-16.6%	-8.1%
Vit. C + Lead treated group (Vit. C+ L)	0.51 ± 0.015 a, b	0.44 ± 0.008 a, b	0.371 ± 0.002 b
% of change of control	46.1%	22.2%	0.27%
Ficus +Vit C +Lead treated group (Fc+ Vit. C +L)	0.43 ± 0.005 a, b	0.38± 0.004 a, b	0.35 ± 0.002 b
% of change of control	24.9%	5.6%	- 5.4%

(a) significant difference compared to the control group ($P \leq 0.05$), (b) significant difference compared to lead-treated group ($P \leq 0.05$)

Table 6: MDA level in liver tissue of different treated groups after two, four and six weeks

Group	MDA (nmol/g) after 2 weeks	MDA (nmol /g) after 4 weeks	MDA (nmol /g) after 6 weeks
Control group (C)	18.71 ± 1.66 b	18.73± 0.43 b	18.75 ± 1.66 b
Lead treated group (L)	37.32 ± 2.38 a	43.78 ± 1.95 a	47.53 ± 0.56 a
% of change of control	99.5%	133.7%	153.5%
Ficus carica treated group (Fc)	19.92± 0.37 b	19.03 ± 0.89 b	18.99 ± 0.31 b
% of change of control	6.5%	1.6%	1.28%
Ficus + Lead treated group (Fc+ L)	30.23±1.47a, b	25.56 ± 0.71a, b	19.68 ± 1.32 b
% of change of control	61.6%	36.5%	4.96%
Vitamin C treated group (Vit. C)	20.11 ± 0.64 b	19.56 ± 0.94 b	18.96 ± 1.19 b
% of change of control	7.5%	4.4%	1.1%
Vit. C + Lead treated group (Vit. C+ L)	32.68± 0.46 a, b	27.78 ± 0.77 a, b	20.64 ± 1.45 b
% of change of control	74.7%	48.3%	10.1%
Ficus +Vit. C +Lead treated group (Fc+ Vit. C +L)	27.15± 1.22 a, b	19.57 ± 1.44 b	18.34 ± 0.67 b
% of change of control	45.1%	4.5%	- 2.2%

(a) significant difference compared to the control group ($P \leq 0.05$), (b) significant difference compared to the lead-treated group ($P \leq 0.05$)

Table 7: GSH level in liver tissues of different treated groups after two, four and six weeks (mean ± S.E)

Group	GSH (μmole/g) after 2 weeks	GSH (μmole/g) after 4 weeks	GSH (μmole/g) after 6 weeks
Control group (C)	14.1±0.21 b	14.13+0.22 b	14.09+0.28 b
Lead treated group (L)	9.59±0.69 a	7.75+0.66 a	6.24+0.45 a
% of change of control	-31.9%	-45.2%	-55.7%
Ficus carica treated group (Fc)	12.7+0.24 b	13.53+0.62 b	14.05+0.59 b
% of change of control	-9.9%	-4.3%	-0.28%
Ficus + Lead treated group (Fc + L)	8.79+0.79 a	11.16+0.46 a, b	13.87+0.40 b
% of change of control	-37.7%	-21%	-1.6%
Vitamin C treated group (Vit. C)	13.28+0.57 b	13.8+0.21 b	14.01+0.52 b
% of change of control	-5.8%	-2.3	-0.66%
Vit.C + Lead treated group (Vit. C + L)	7.32+0.22 a, b	10.03+0.21 a, b	12.55+0.14 a, b
% of change of control	-48.1%	-29%	-10.9%
Ficus + Vit. C +Lead treated group (Fc+ Vit. C + L)	10.13+0.62 a	12.41+0.86 a, b	13.98+0.73 b
% of change of control	-28.2%	-12.2%	-0.78%

(a) significant difference compared to the control group ($P \leq 0.05$), (b) significant difference compared to the lead-treated group ($P \leq 0.05$)

Table 8: SOD activity in liver tissues of different treated groups after two, four and six weeks (mean ± S.E)

Group	SOD (U/g) after 2 weeks	SOD (U/g) after 4 weeks	SOD (U/g) after 6 weeks
Control group (C)	70.69 + 0.82 b	70.63 + 0.65 b	70.6 + 0.37 b
Lead treated group (L)	42.76 + 0.79 a	37.49 + 1.64 a	31.16 + 1.53 a
% of change of control	-39.5%	-46.9%	-55.9%
Ficus carica treated group (Fc)	68.7+0.44 b	68.82+0.58 b	69.99+0.59 b
% of change of control	-2.8%	-2.6%	-0.9%
Ficus + Lead treated group (Fc + L)	53.59+1.27 a, b	62.61+1.16 a, b	68.54+0.53 b
% of change of control	-24.2%	-11.4%	-2.9%
Vitamin C treated group (Vit. C)	66.56+0.56 a, b	67.93+0.61 a, b	68.71+0.32 b
% of change of control	-5.8%	-3.8%	-2.7%
Vit. C + Lead treated group (Vit.C + L)	52.62+0.81a, b	60.51+0.20 a, b	66.25+0.83 a, b
% of change of control	-25.6%	-14.3%	-6.2%
Ficus + Vit C + Lead treated group (Fc+ Vit.C + L)	66.60+0.54 a, b	69.83+0.27 b	71.13+0.23 b
% of change of control	-5.8%	-1.1%	0.8%

(a) significant difference compared to the control group ($P \leq 0.05$), (b) significant difference compared to the lead-treated group ($P \leq 0.05$)

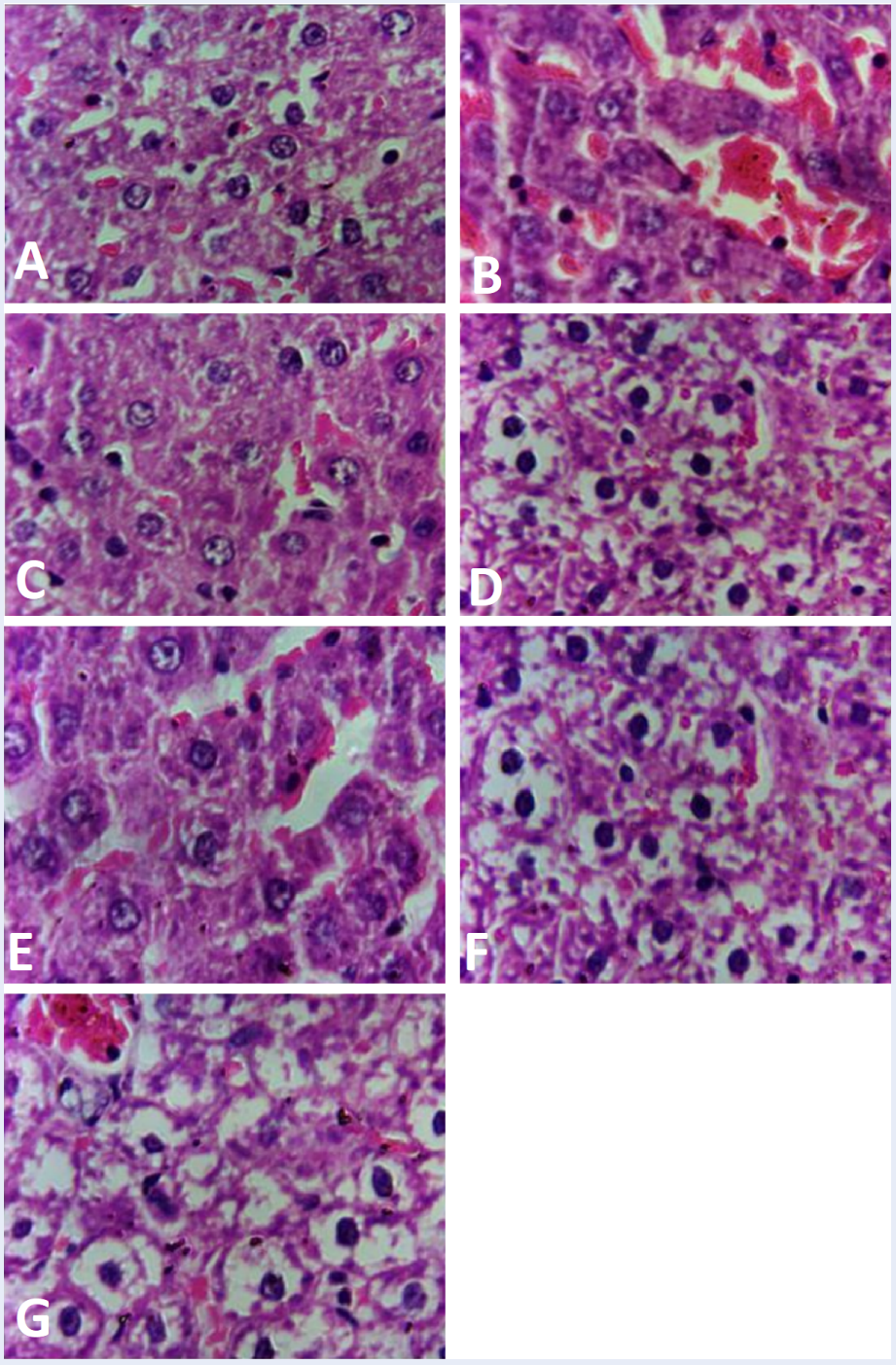

Figure 1: **Photomicrograph of murine liver sections in different groups after 2 wks stained with hematoxylin and eosin (H&E) (x400).** (**A**) Control rat after 2 wks showed normally arranged hepatocytes of hepatic lobule; (**B**) Rat treated with lead acetate after 2 wks showed parenchymatous degeneration of hepatocytes with severe necrosis, severe leucocytes infiltration, congested portal vein with thichkended hyalinized wall and a branch from the PV into the sinusoids. (**C**) Rat treated with Ficus carica after 2 wks showed normally arranged hepatocytes of hepatic lobule. (**D**) Rat treated with Ficus carica and lead acetate after 2 wks showed parenchymatous degeneration of hepatocytes, mild congested portal vein with thickened hyalinized wall and mild kupffer cells. (**E**) Rat treated with vitamin C after 2 wks showed normal central vein, normally arranged hepatocytes of heptic lobule mild congestion of central vein. (**F**) Rat treated with vitamin C and lead acetate showed parenchymatous degeneration of hepatocytes with mild necrosis, congested portal vein with thickened hyalinized wall and mild kupffer cells. (**G**) Rat treated with Ficus carica, vitamin C and lead acetate showed normal central vein and mild congested portal vein with thickened hyalinized wall.

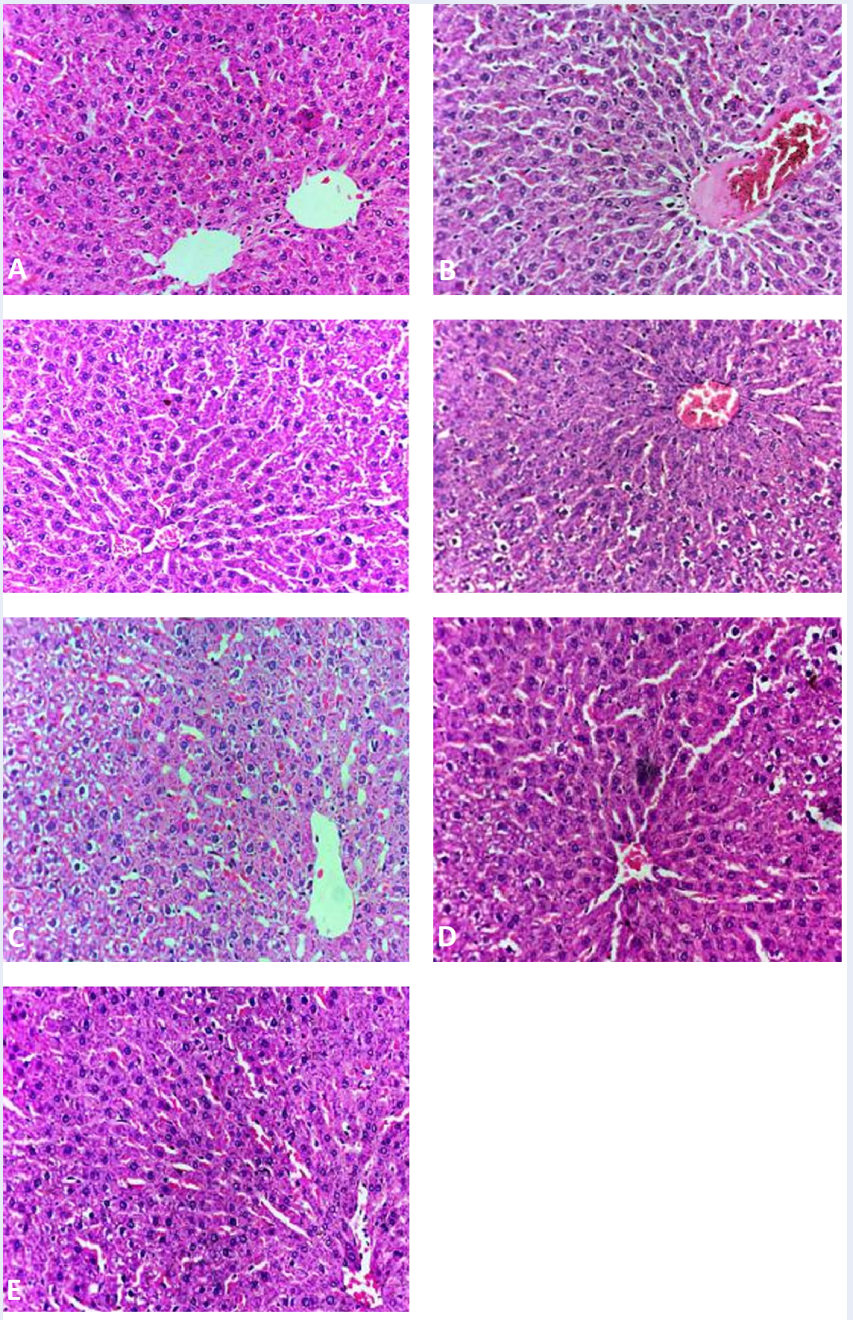

Figure 2: **Photomicrograph of murine liver sections in different groups after 4 wks stained with hematoxylin and eosin (H&E) (x400).** (**A**) Control rat showed normal central vein and normally arranged hepatocytes of hepatic lobule; (**B**) Rat treated with lead acetate showed parenchymatous degeneration of hepatocytes with severe congested portal vein with thichkended hyalinized wall. (**C**) Rat treated with *Ficus carica* showed normal central vein, normally arranged hepatocytes if hepatic lobule and mild congestion of central vein. (**D**) Rat treated with *Ficus carica* and lead acetate showed mild congestion of portal tract with inflammation and mild leucocytes infiltration. (**E**) Rat treated with vitamin C showed normal central vein, normally arranged hepatocytes of heptic lobule. (**F**) Rat treated with vitamin C and lead acetate showed congested dilated portal tract with inflammation and mild leucocytes infiltration. (**G**) Rat treated with *Ficus carica*, vitamin C and lead acetate showed normal central vein, normally arranged hepatocytes of hepatic lobule moderate congestion of central vein.

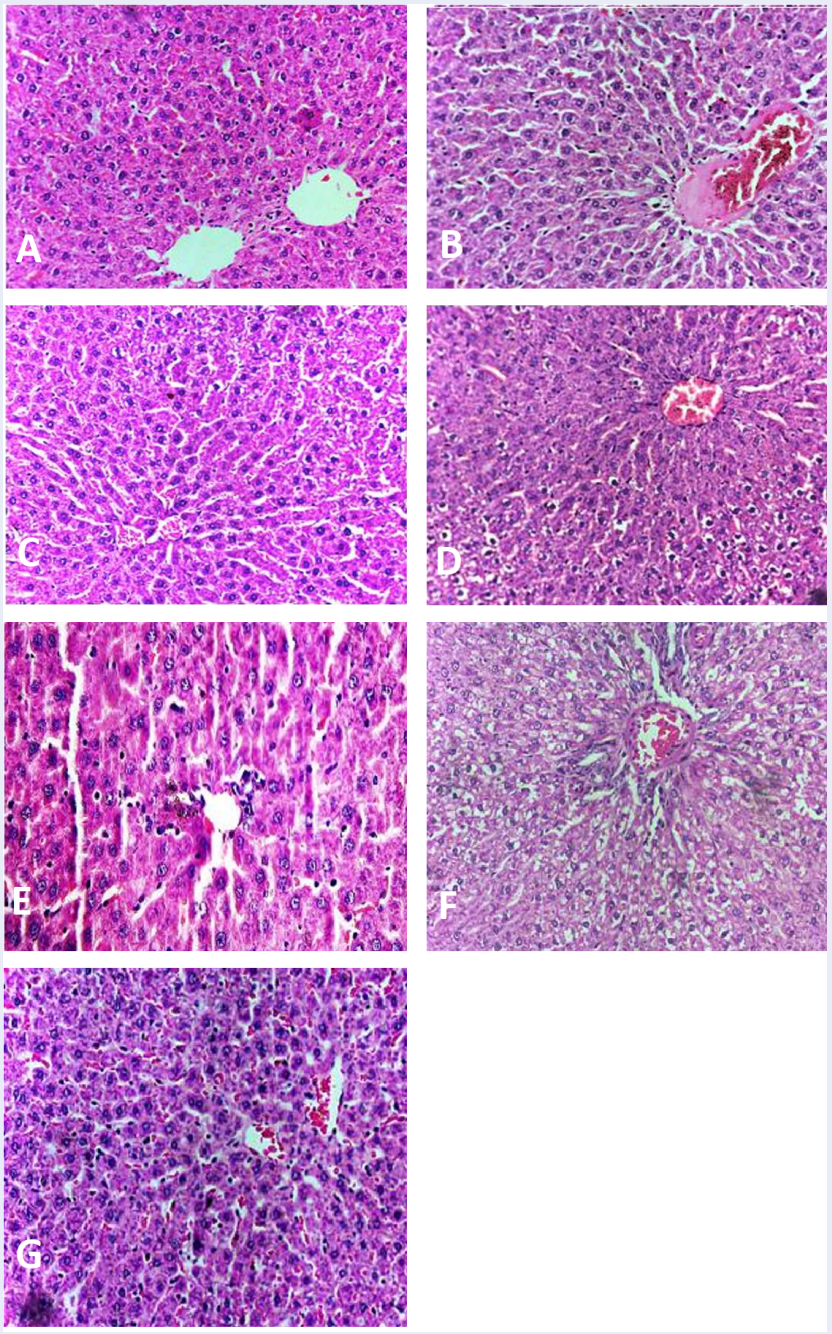

Figure 3: **Photomicrograph of murine liver sections in different groups after 6 wks stained with hematoxylin and eosin (H&E) (x400).** (**A**) Control rat showed normal central vein and normally arranged hepatocytes of hepatic lobule; (**B**) Rat treated with lead acetate showed parenchymatous degeneration of hepatocytes with severe congested portal vein with thichkended hyalinized wall, many mitotic cells and leucocytes infiltration. (**C**) Rat treated with *Ficus carica* showed normal central vein with mild dilatation, normally arranged hepatocytes of hepatic lobule slight congestion of central vein. (**D**) Rat treated with *Ficus carica* and lead acetate showed moderate parenchymatous degenderation of hepatocytes with mild congested portal vein, mild sinusoidal, pyknoti cells, and slight leucocytes infiltration. (**E**) Rat treated with vitamin C showed normal central vein, normally arranged hepatocytes of heptic lobule. (**F**) Rat treated with vitamin C and lead acetate showed mild parenchymatous degeneration of hepatocytes with mild congested portal vein, mild sinusoidal, pyknotic cells, and slight leucocytes infiltration. (**G**) Rat treated with *Ficus carica*, vitamin C and lead acetate showed slight parenchymatous degeneration of hepatocytes with mild congested portal vein, mild sinusoidal, pyknotic cells, and slight leucocytes infiltration.

might be interpreted that lead may induce metabolic dysfunction through inhibition of enzymatic activities and disturbance in the oxidant/antioxidant status. In accordance, a recent study indicated that oral administration of lead acetate increased the activity of blood enzymes: alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase, alkaline phosphatase, lactate dehydrogenase and a decrease of creatinine level in rats[39]. It has been suggested that lead exposure induced metabolic disorders and biochemical changes in the liver[40]. Consistently, previous studies showed that lead decreased blood glutathione (GSH), glutathione peroxidase, adenosine triphosphatase, and catalase but increased oxidized GSH and intracellular calcium in rat[3,41].

The pretreatment with *Ficus carica* leaf extract or Vit. C alone or the combined treatment noticeably prevented the effect of lead on serum AST, ALT, ALP activities, and direct bilirubin and total protein concentration in the serum compared to the lead acetate treated group after two, four and six weeks. The hepatoprotective effect of both *Ficuscarica* leaf extract and Vit. C might be attributed to their antioxidant properties. In accordance, previous studies showed that pretreatment with *Ficus carica* leaf extract normalized the levels of TP, ALP and the level of total serum bilirubin[15,42]. Moreover, *Ficus carica* was highly successful in attenuating lead hepatotoxicity due to its high total phenol and flavonoid contents, suggested three mechanisms for this attenuation: first: lowering the oxidative stress, second: increasing the oxidant enzymes level and third: acting as chelating agent for lead ions[43,44]. The observation that vitamin C caused suppression of increased ALT and AST activities induced by administration of lead acetate might be attributed to its ability to ameliorate the lipid peroxidation through the free radicals scavenging activity and restoring the liver capability[45,46]. Collectively, the results revealed that the pretreatment with *Ficus carica* leaf extract only or Vit. C only improve the hepatotoxicity while the pretreatment with the antioxidant mixture containing both *Ficus carica* leaf extract and Vit. C was the most effective treatment in alleviating hepatotoxicity.

In accordance with the biochemical data, the histopathological examination of the liver tissues of the animals treated with lead showed that lead acetate-induced liver hyperplasia and apoptosis, plausibly mediated by oxidative stress in Kupffer cells. Pretreatment with *Ficus carica* leaf extract and Vit. C provided effective protection to the liver against harmful effects induced by lead acetate.

CONCLUSION

Our data concluded that the combination of *Ficus carica* leaf extract and Vit. C had a critical protective action on lead acetate- induced oxidative damage in rats due to their antioxidant/anti-radical properties. The combined treatment of *Ficus carica* leaf extract and Vit. C offered more effective protection compared to the individual treatments.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

All authors contributed equally in the study design, interpretation of the data and writing of the final manuscript.

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ABBREVIATIONS

ALP: Alkaline phosphatase enzyme
ALT: Alanine aminotransferase
AST: Aspartate amino transferase
CAT: Catalase
DB: Direct bilirubin
HPLC: High-Performance Liquid Chromatography
MDA: Malondialdehyde
NODCAR: National Organization for Drug Control and Research
ROS: Reactive oxygen species
SOD: Superoxide dismutase
TP: Total protein

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