

Original Research Article

Toxic effects of lead-induced Damage in liver of rats male and the role of ethanolic ginseng extract as protective agents (histological and physiological)

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Abstract

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This study was to determine the hepato protective and activity effects of ethanolic extract of *Panax ginseng* roots on the biochemical and histology of liver of adult *Rattus norvegicus* rats following lead – induced hepatotoxicity. Twenty-Four male rats divided into four groups: Group T1 control; Group T2 PbAc (administrated orally with PbAc, 100 mg/kg); group T3 *P. ginseng* (100 mg/kg+PbAc); group T4 *P. ginseng* (200 mg/kg) daily administrated for 2 months. PbAc treatment alone induced hepatotoxicity, evidenced by significant increases in BBL and lipid peroxidation, malondialdehyde (MDA) Level. Histological results reveal alteration of liver structure including congestion and dilated of central vein, hepatic vacuolation, infiltration of inflammatory cells with necrosis of hepatocyte and hyperplasia. Treatment with *P. ginseng* in PbAc in toxicated male rats showed some ameliorative effect to PbAc toxicity in low dose, while showed clear ameliorative effects to PbAc toxicity in high dose. In conclusion, this study shows that *Panax ginseng* root extract has ratable ability to prevent liver damage caused by lead toxicity.

Keywords: Liver histology, Lead acetate, Toxicity, Antioxidants, Ginseng

INTRODUCTION

Lead, a toxic heavy metals as an effective short-long term particularly showing accumulation in organs as liver, testis, kidney, bones and brain (Al Naimi et al., 2011). Humans are exposed to these substances through ingestion, inhalation and dermal exposure for their whole life time since the intrauterine life, most cases of toxicity occur following exposure to very elevated Pb concentrations industrial plants and waste dumps (Hussein et al., 2014). The absorbed lead is joined in the liver and threaded to the kidney, where a little quantity is excreted in urine and the residue accumulates in many body and overlap with their function specially the liver as a target site for Pb toxicity (Abdul Kareem, 2014). Biochemical studies showed that induces increase in

hepatic lipid peroxidation, and the alterations in histological characterization in the hepatic tissues due to Pb in toxication (Jarrar and Taib, 2011).

Ginseng is one of the most consumed medicinal plants, the family araliaceae contains over 700 species that are cultivated worldwide. The genus *Panax ginseng* is one important economically most heavily traded medicinal plant well known in eastern Asia (Hosseini et al., 2012). Ginseng was slow-growing aromatic perennial plants that consist of alight-colored and fleshy root, with long stalk and green leaves with oral shapes. The root are varies among species, some roots man-shaped (Ramesh et al., 2012). The root of *Panax ginseng* used as antioxide, antitumor, antistress and anti-stress and

anti-inflammatory, it helps the restoration of homeostasis irrespective of the direction of altered physiological function (Omotoso et al., 2015). The present study was designed to investigate the potential protective of root ginseng extract against the hepatic toxicity in lead exposed male rats.

MATERIALS AND METHODS

Preparation of the extract

Panax ginseng roots were obtained from locally market shop. The dried roots were ground into fine powder, 60 grams of the ground powder was bottle flask in 500 ml of ethanol 70% for 24 hrs in 50°C, and the extract is distributed into clean sterile petri-dishes and left to dry at lab temperature under the shade. The extraction was used for the experiment.

Experimental animals

Thirty healthy male adult (*Rattus norvegicus*) rats, aged (8-10) weeks and weighing 200-205 gram were obtained from the animal house, biological department, college of sciences, Basrah University in district hygienic and standard management condition at temperature 20-25°C and photo period was 12 hours. The animal given normal food and distal water during the experiment.

Experimental protocol

The rats were divided into 4 groups of 6 rats each group and animal were treated as follows;

- Group T1 (normal control): they were given distilled water only of orally administration daily for 2 months.
- Group T2 (PbAc only): the rats treated with PbAc (100mg/kg B. W.) daily for 2 months.
- Group T3 (PbAc + P.G): the rats treated PbAc (100mg/kg B. W.) with ethanolic ginseng extract (100mg/kg B. W.), orally daily for 2 months.
- Group T4 (PbAc): the rats treated PbAc (100mg/kg B. W.) with ethanolic ginseng extract (200mg/kg B. W.) orally daily for 2 months.

Lead acetate was dissolved in distilled water by dissolved (1g/L) according to Uzkeser et al. (2012), also the dose of ginseng extract was prepared by dissolving (1g) into 1000 ml of distilled water according to (Ramah et al., 2015). At the end of experiment periods, all experimental rats by anaesthetizing them, and placing rat in closed glass container with chloroform as an esthesia, abdominal cavity was opened by midline incision and take samples as following:

Biochemical parameter study: blood samples

Blood samples were collected via cardiac puncture, Blood were centrifuged at (3000) cycle/min for 10 min to obtain serum which then transferred Eppendorf tubes, serum was separated and biochemical parameters of Level. Pb. Serum was measured by using an atomic absorption spectrophotometer (Uzkeser et al., 2012). Also estimation of serum malondialdehyde (MDA) as one of the main endo product of lipid peroxidation was measured according to the method originally described by (Lukaszewicz-Hussain et al., 2007).

Histological Study

Fresh portion of the tissues from each rat were cut rapidly with fixed in formalin (10%) , were then dehydrated with grades of ethanol (70%, 80%, 90%, and 100%). Samples were processed for paraffin wax embedding. Section were cut on a rotary microtome at (4-5Mm) thickness were stained with routine haematoxylin and eosin (H –E), stained section of control and treated rat were examined for alterations in the structure, portal triads, sinusoids, hepatocytes and for the presence of necrosis, portal fibrosis.

Statistical Analysis

The data were expressed as mean standard deviation (S D). All groups were compared by analysis of one – way of variance (ANOVA) followed by post HOCLSD test. Significant difference was set at (P >0.05).

RESULTS

Biochemical parameters results

Result show that lead acetate levels is significantly increase following pb only treatment after 2 months administration compared to control group. P. ginseng extract in dose T3/T4. Caused significant reduced serum lead level more than the other treatments in comparison to T2 table and as seen table (1), the results show that administration pb Ac alone has effect on serum MDA. That caused significant increase (p>0.05) in parameters during 2month compared to the control groups, where, the animals treated with ethanolic *p.ginseng* extract (100mg/kg, 200mg/kg) with pb Ac. Administration caused significant reduction in serum MDA concentration compared with groups that treated with pb Ac.

Table 1. Serum lead and MDA concentration in experimental groups.

Periods	Groups	Serum lead concentration ppm	MDA ($\mu\text{m/L}$)
2 months	Control	$0.002 \pm 0.001^*a$	4.22 ± 0.06
	PbAc (100mg/kg)	$0.1639 \pm 0.0029^*b$	8.31 ± 0.04
	PbAc+PG(100mg/kg)	$0.1223 \pm 0.0013^*c$	6.63 ± 0.08
	PbAc+PG(200mg/kg)	$0.1021 \pm 0.0089^*d$	5.92 ± 0.05

Values represent mean $\pm$ SD (n=6). Represents a significant in comparison between treated groups and control ($p < 0.05$) a,b,c the different letters means there was significant ($p < 0.05$) between groups.

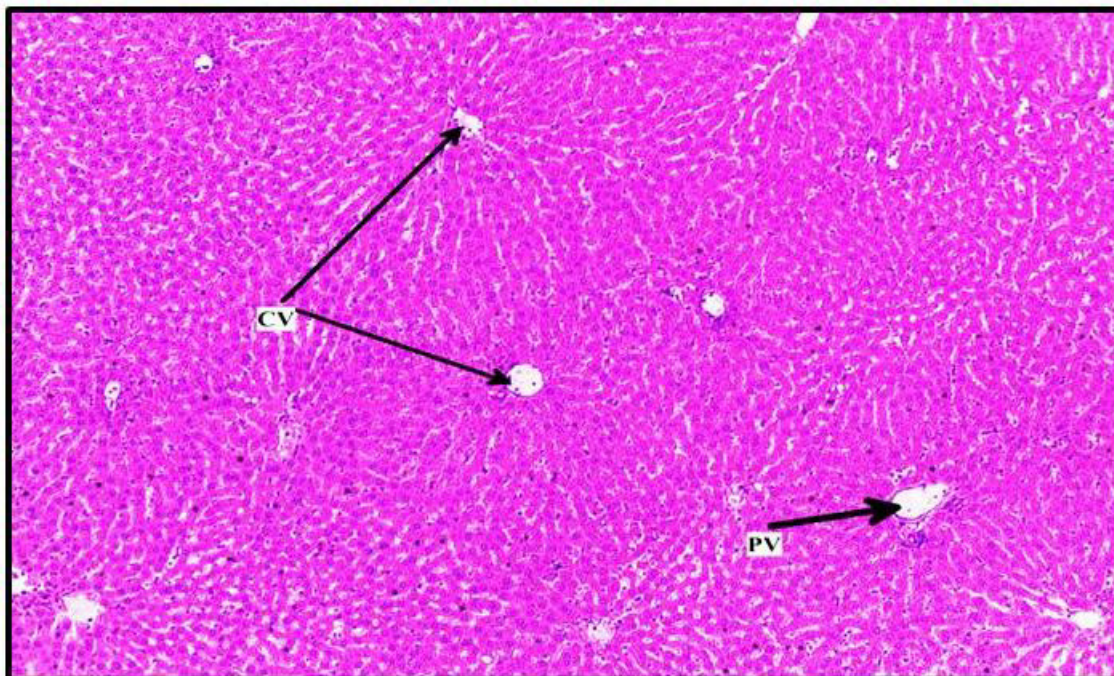


Figure 1. Photomicrograph of the liver section in control group post 2 months showing: the normal structure of the hepatic lobule with normal central vein (CV) and portal vein (PV). H&E. X100.

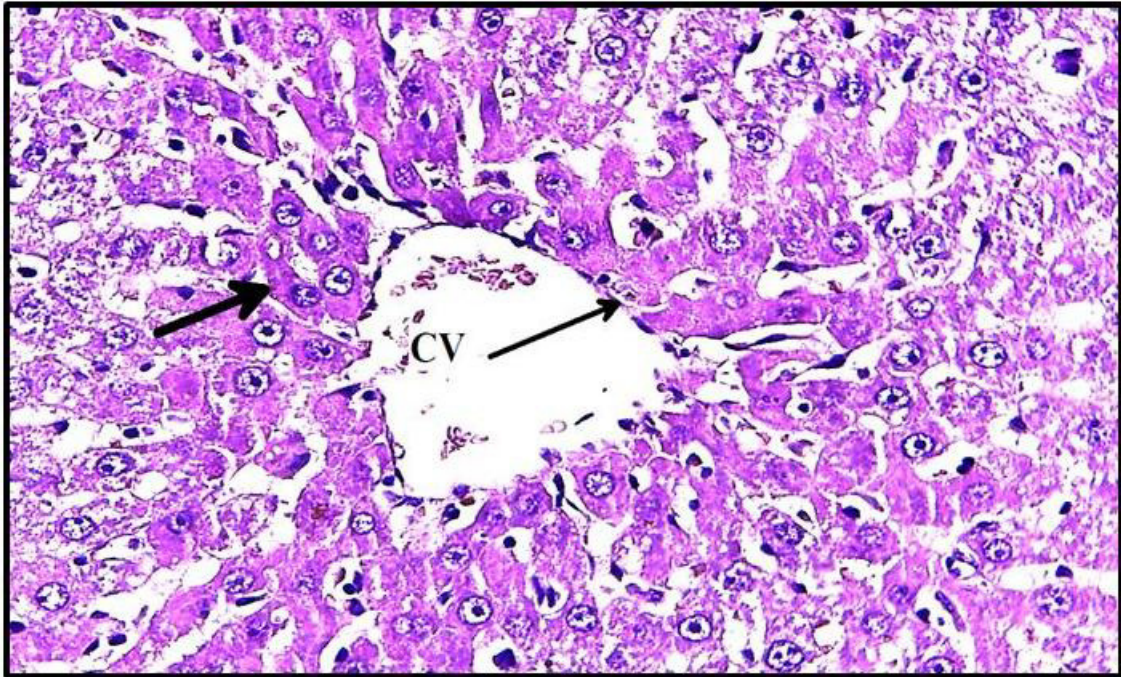


Figure 2. Photomicrograph of the liver section in control group for 2 months showing: the central vein (CV) in hepatic lobule lining by an endothelial cell (thin arrow), that is continuous with those lining with sinusoid (thick arrow), hepatocytes arranged radially from the central vein, and separated from each other by normal irregular blood sinusoid. H&E. X400.

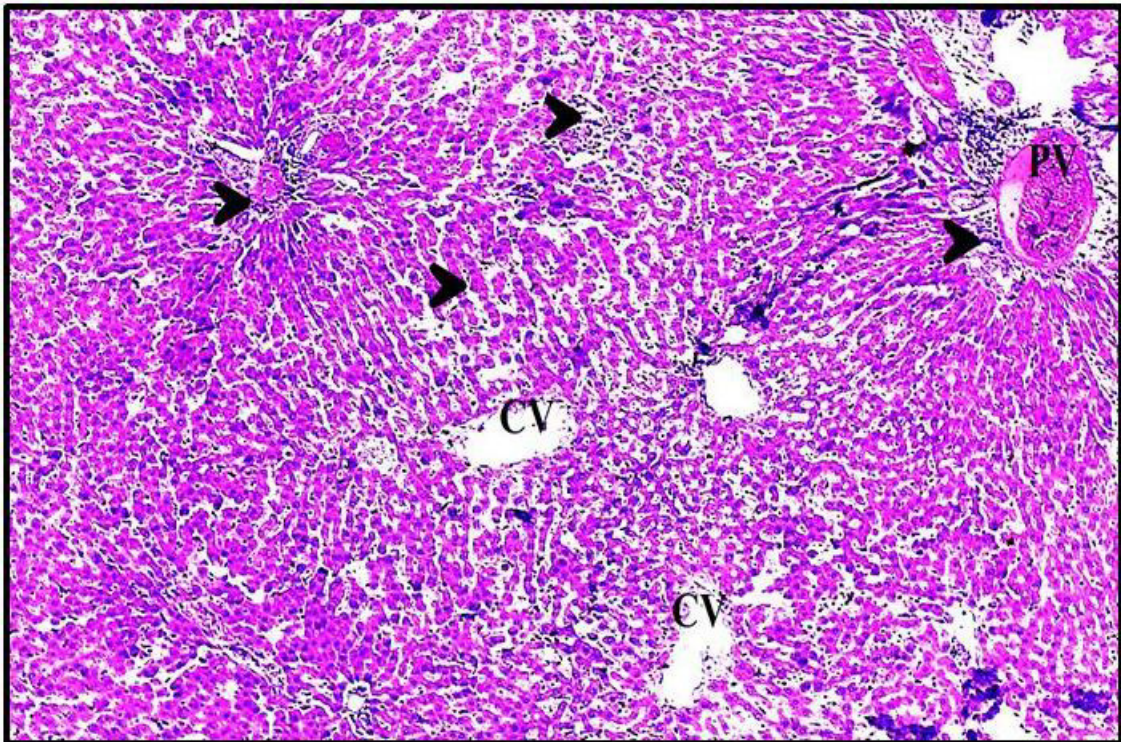


Figure 3. Histopathological observation in the liver rat section treated with Pb Ac post2 months; more degeneration of hepatocyte with inflammatory cells infiltrated around bile duct (head arrows) and congested and dilated in portal vein (PV). H&E. 100X.

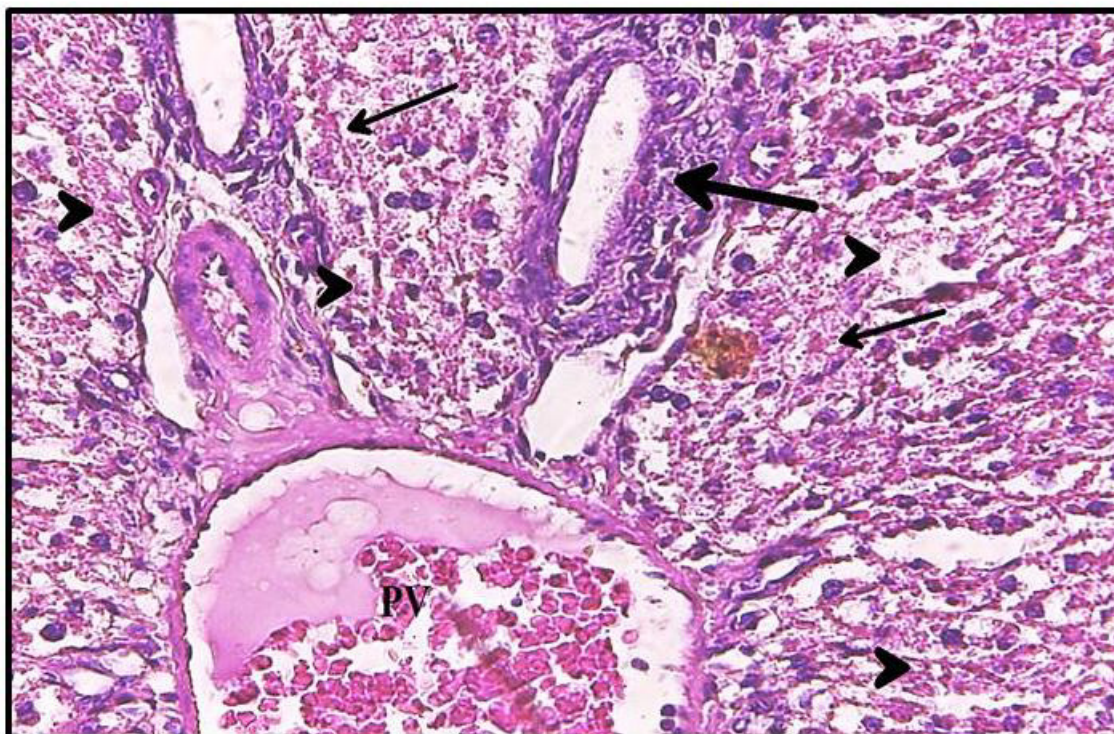


Figure 4. Histopathological observation in the liver rat section treated with Pb Ac post 2 months; degeneration of most hepatocytes (head arrows), bile duct hyperplasia (BD), deposition of collagenous fibers (thick arrow) and karyolysis of most nuclei (thin arrows). H&E. 400X.

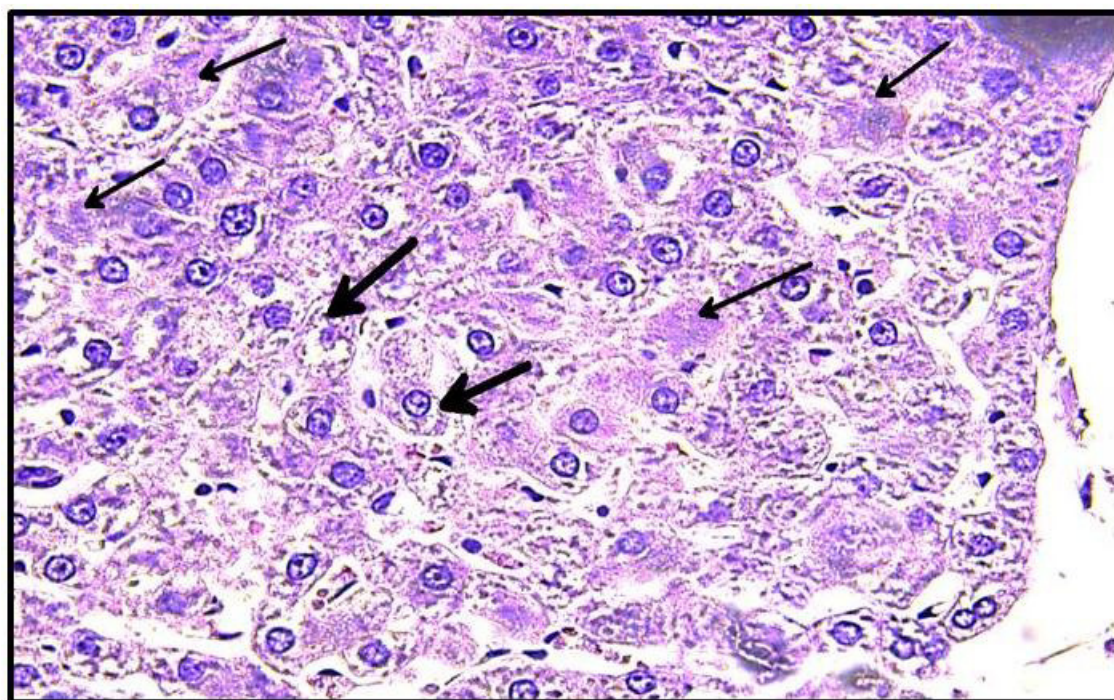


Figure 5. Photomicrograph of liver rat section treated group with (100mg/kg) lead acetate post 2 months showing, necrotic hepatocyte (thin arrows), degenerated and other vacuolated hepatocyte (thick arrows). H&E. X400.

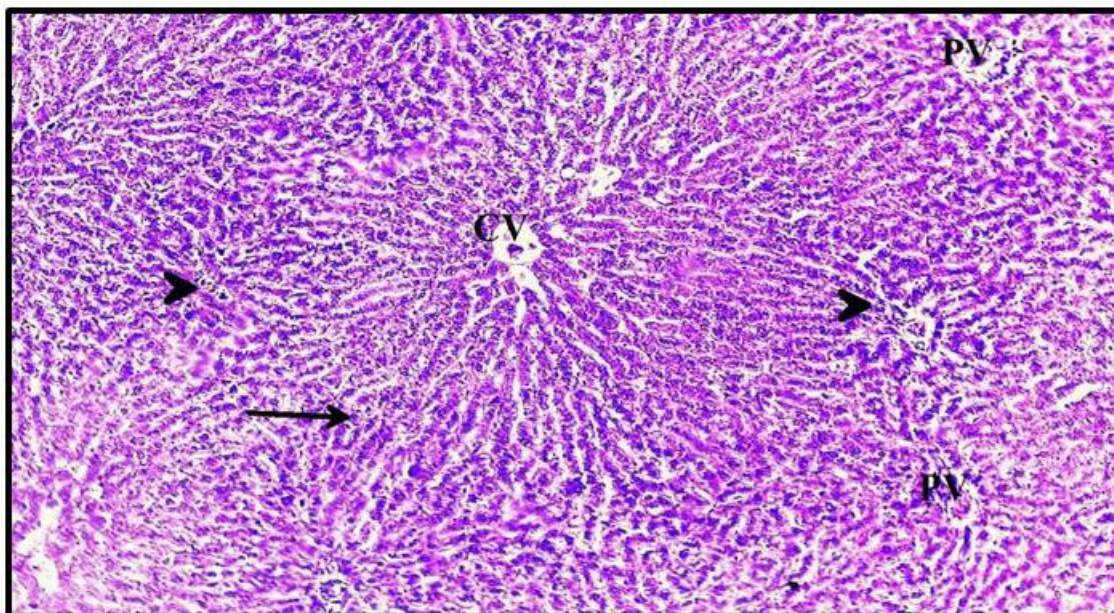


Figure 6. Liver section of rat treated with PbAc combination with (100 mg/kg) ginseng extract post 2 month showing; more restoration of hepatocyte (arrow) with infiltration of inflammatory cells (head arrows), reduced dilated of central vein (CV) and reduced dilated and congested of portal triad (PV). H&E. 400X.

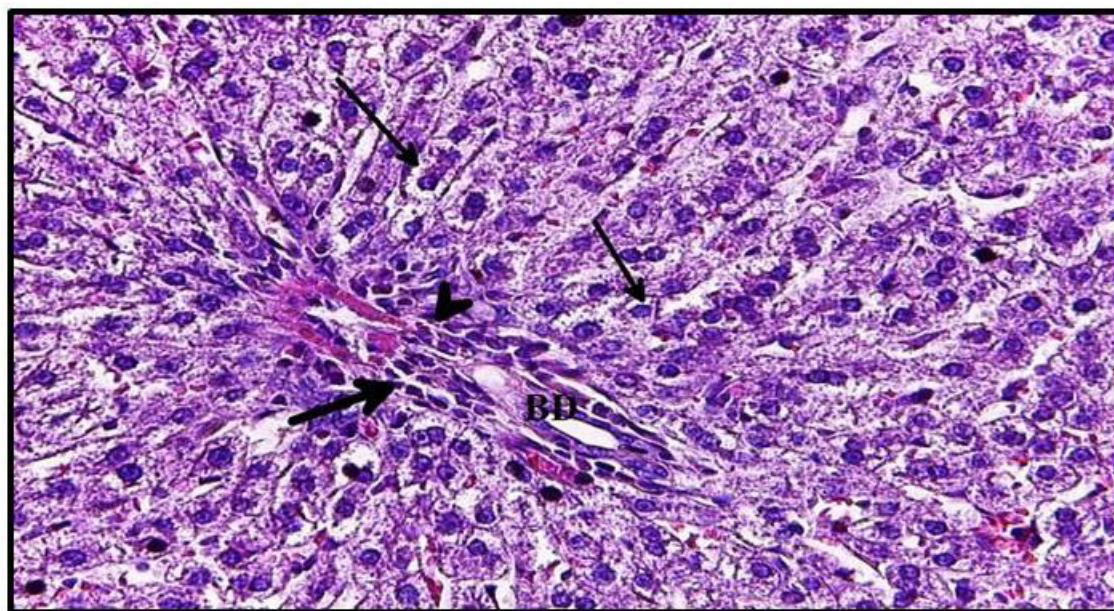


Figure 7. Liver section of rat treated with PbAc combination and (100 mg/kg) *P. ginseng* extract post 2 months showing; less infiltrated inflammatory cells around portal triad (thick arrow), with bile duct clear (BD), hepatocyte regenerated in most area with radical shape arrangement (thin arrow) and less deposition of collagen fiber around portal triad (head arrow). H&E. 400X.

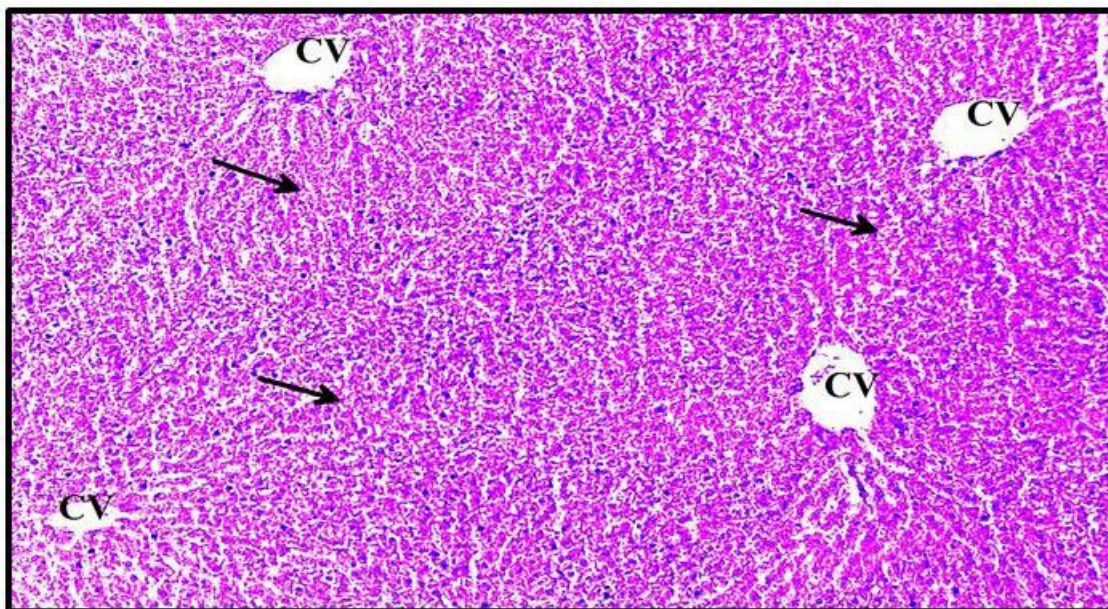


Figure 8. Section of the liver rat treated with lead acetate and (200 mg/kg) *P.ginseng* extract post 2 months clarified liver structure, more clear of central vein (CV) with narrow sinusoid (arrows), also clear radiating the hepatocytes arranges as hepatic cords. H&E. X100.

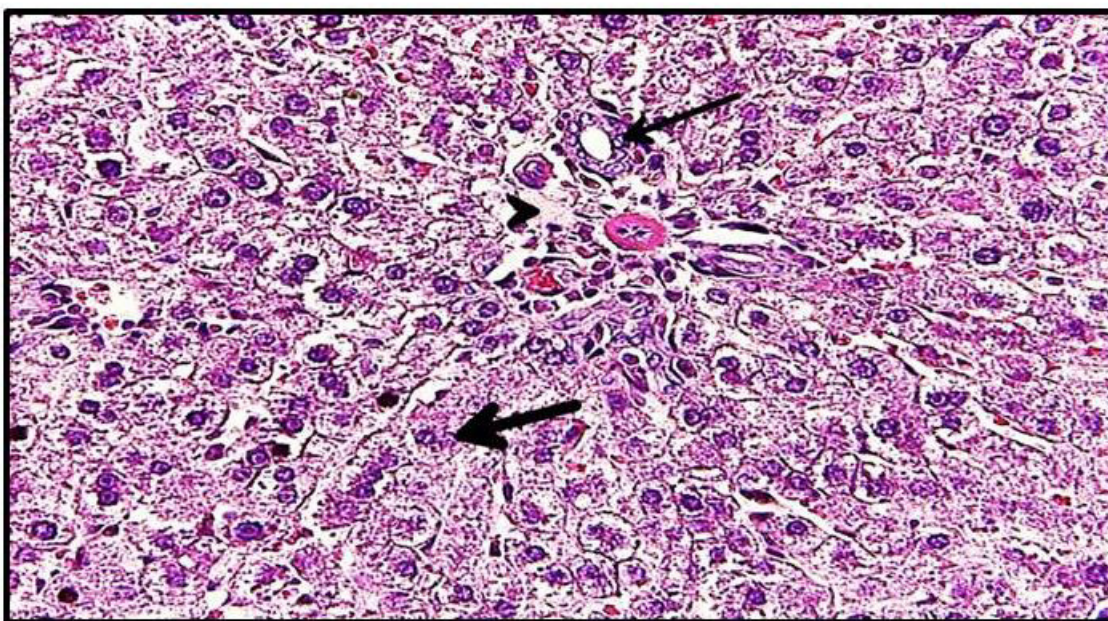


Figure 9. Liver section of rat treated with PbAc combination and (200 mg/kg) *P. ginseng* extract post 2 months showing; marked clear in branch of bile duct (thin arrow), with regenerated of hepatocyte (thick arrow), with weak of inflammatory cells and very less deposition of collagen fiber around bile duct (head arrow). H&E. 400X.

Histological Results

The control group T1: light microscopic examination of the stained section of liver illustrates the normal histological structure of the hepatocytes are arranged in

cords radiating from the central vein and separated by blood sinusoids, portal tract with its structures from branches of portal vein, hepatic artery and bile duct, the sinusoids are separating the hepatic cords that lined by endothelial cell. (fig 1,2). Treated group with pb only (T2):

the liver of the treated rats showed many histopathological changes represented as disorganization of hepatic lobules, dilation and congestion of central vein and sinusoid, which containing the portal triad to the portal triad could be revealed to the formation of fibrosis, infiltration of mononuclear inflammatory cells in portal area and central vein, thickening in the lining endothelial layer, many nuclei chromatin was fragmented and cytoplasm contained many vacuoles and necrotic in many area, Also hyperplasia around portal triad and deposition of collagen fiber was observed in some tissue (fig 3,4,5)

The liver of rats treated with pb Ac and T3 after 2 months showed signs of restoring normal structure were seen in some sections, clear central and portal veins were filled with erythrocytes, some hepatocytes with eosinophilic cytoplasm and hyperchromatic nuclei, little deposition of collagen fiber around portal triad and reduced of hyperplasia (fig 6.7). Light microscopic examination of the treated rat's group T4 post 2 month showed more regenerated of liver structure. Central vein and the lamellar pattern of hepatocytes was more restored with narrow sinusoid space which few infiltrated with inflammatory cells ground portal triad and very less by hyperplasia (fig 8,9).

DISCUSSION

According to the results that pb Ac caused a significant increase in blood lead level (BLL) and serum malondialdehyde (MDA) concentration in rats treated with pb Ac alone as compared to the normal control, and this results may be caused by effect of lead on liver structure damaged of Hepatocyte and disturbance in enzymes secretion, this result was discussed by other researchers (Repetto et al., 2010; Omobowal et al., 2014) who showed that oxidative stress is an important mechanism of lead-induced toxicity and imbalance between generation and removal of reactive oxygen species (ROS) in cellular structure causing damage to membrane, oxidation of DAN, protein and membrane lipid. Therefore, damage membrane associated with change in fatty acid composition which responsible for the enhanced lipid peroxidation membrane (Ahmed et al., 2010).

The peroxidation of polyunsaturated fatty acids lead to conjugated dienes formation on, followed by the cleavage of the fatty acid chain and subsequent release of the reactive Malondialdehyde (MDA), when, degradation products of such processes (MDA) may, therefore be used as makers of peroxidation (MDA) polyunsaturated fatty acid (Aziz et al., 2012). The present results that Ginseng may strengthen the antioxidant defense which reduced BLL and serum MDA by reducing lipid peroxidation. Similar results are also reported by (Abdelghaffar et al., 2015) concluded that ginsenosides

free phenolic fraction have been shown to induce the cytosolic antioxidant enzyme superoxide dismutase by enhanced nuclear protein binding to its gene regulatory sequences. Chen et al. (2011) stated that ginseng contains poly phenols, flavonoids, saponins, these properties effects might be due to the potential antioxidant and free radical scavenging activities, DAN protection, therefore, the extract had protected from lead in toxication as indicated by the significant restoration of BLL and MDA level. Histopathological alteration of tissue significant histological changes was observed in liver after 2 months post lead treated rat compared to normal control. The effect of pb Ac was very obvious and deleterious on the liver, showed degenerative with irregular hepatocytes, inflammatory cell in hepatic tissue. Mononuclear infiltration in the portal triad was seen, congestion and dilated of central vein and sinusoid. Also hepatocytes necrosis due to chronic lead exposure might indicate oxidative stress on these cells by MDA intension. Similar investigation has been reported by (El-Shafai et al., 2011; Abdul Kareem, 2014).

In current study hepatocyte showed diffuse vacuolation of hepatocyte and periportal fibrosis represent the final common path way of long period exposure liver diseases, which described by increase connective tissue sedimentation in extra cellular matrix (ECM), So ROS can activate fibrogenic gene expression and transforming growth factor (TGFB) which is known to play main role in the activation of hepatic stellate cell (HSCs) in liver fibrosis (Abdul Kareem, 2014).

After ginseng extract had been used post 2 months it clearly ameliorated the texture of liver which were annihilated by lead acetate, when administrated in dose (200mg/kg B.W) with lead were more amelioration. This is in agreement with (Uzkeser et al., 2012) observation which was ginseng extract to show hepato protective effect against lead induced liver damage in rats which inflammatory effect of root ginseng have been responsible for the hepatic cells, that included the production of chemokines and inflammatory cytokines. (IL-1B, TNF- α), that inhibit tumor necrosis factor alpha (TNF- α). Stimulated NF- κ B activation. The ameliorating effects of ginsenosides significantly inhibits liver fibrosis by inhibits activation of proliferation with expression of collagen fibers and inhibitor of metalloproteinase-1 in hepatic stellate cells, that main cause of tissue fibrosis (Tasanarong et al., 2011; Kim et al., 2015).

However, the root ginseng extract regulating inducible hepatic enzymes and might be associated with modulating liver cytochrome P450 activation and protein phosphorylation, similar investigation have also been reported (Ramah et al., 2015).

CONCLUSION

In conclusion our study has demonstrated that exposure

to lead acetate induces chronic inflammation and damage in the liver tissue, and root ginseng extract appears to be benefit protected against the pb Ac induced hepatopathy. Ginseng extract that has hepatoprotective effect can be attributed to its antioxidant properties by inhibition of BBL and lipid peroxidation and anti-inflammatory activities.

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