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Protective Role of Vitamin E and Selenium On the Acute Hepatotoxicity of Lead Acetate in Albino Rats.

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ABSTRACT

Among heavy metals, lead is the most common pervasive environmental pollutant having diverse and deleterious effects on man and domestic animals health causing severe organ damage. Mature female Albino rats were divided in 4 groups. Group I: control group given distilled water,Group II: treated group administrated orallysublethal dose of (10mg/kg bwt) lead acetate trihydrate, Group III:regenerated group given lead acetate trihydrate plus vitamin E and selenium1ml/liter D.W and Group IV:orally administrated vitamin E and selenium in drinking water. The structural change in the liver was investigated histologically,histochemically,ultrastructurally, immunohistochemically and biochemical assay of liver enzymes and liver albumin. The results in treated rats showed dilation of central vein and hepatic sinusoids, decreased glycogen granules,swollen mitochondria and immunohistochemical examination revealed positive expression of PCNA,increase in liver enzymes compared to control group. Liver specimens of regenerated group revealed narrower sinusoids, increase in glycogen granules, regenerated mitochondria and moderate PCNA expression, decrease in liver enzymes level in serum compared to treated group.

Keywords: Lead acetate; Liver; Histology; Histochemical study; Electron microscopy; immunohistochemistry: Biochemical assay.

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INTRODUCTION

Lead (Pb) had been a toxic problem for human beings from the earliest time **(Hurst and Martin, 2004)**. Despite its recognized hazards, lead continue to have widespread commercial applications as in plumbing, paints, manufacture of lead acid batteries, soldering because it unique properties like softness, high malleability, ductility, low melting point and resistance to corrosion**(Kosnett, 2004** and **Flora et al.,2012).**

Lead may enter foods through glazed pottery or ceramic dishes, the use of lead solder in the food canning industry and soft drink cans. Lead also can leach into drinking water from leaded pipes in water distribution systems of individual house **(Loghman, 1997; Gidlow, 2004** and**Astdr, 2005)**

Lead primarily affecting the central nervous, hematopoietic, hepatic and renalsystems producing g serious disorders in animals and human **(Kalia& Flora**, **2005;Kasten-Jolly et al., 2010; El-Neweshy and El-Sayed, 2011** and**Dewanjee et al., 2013).**

Oxidative stress reported as the major mechanism of lead toxicity **(Flora et al.,2012).** One of the most important vitamins for the body is vitamin E which comprises eight natural fat- soluble compounds, the most active of which is alpha-tocopherol (**Malafa et al.,2002;Songthaveesin et al.,2004** and**Ramanathan et al., 2005).**

Vitamin E is one of the primary antioxidants**(Vaya and Aviram,2000)** which capable of scavenging free radicals result in initiation of chain breaking mechanism by donating free electron to ROS and converting them into stable molecules (**Kalender et al., 2005;Cemek et al., 2010**and **Flora et al.,2012).**

Also selenium is a mineral antioxidant which is an essential constituent of the enzyme glutathione peroxidase which protects membrane lipids and other cell constituents from oxidative damage by free radicals**(Al-Bideri,2011).**

Little information is available in the literature regarding the antioxidant activity of vitamin E and Selenium together. So, the aim of the present study was designed to evaluate the role of low dose (1ml/L D.W.) of vitamin E and Selenium combination on theacutehistological alterations in albino rat liver exposed to sub-lethal dose of lead acetate.

MATERIALS AND METHODS

Experimental animals

The present study was conducted using 40 mature Albino rats of both sexesweighing 120-250 g. They were bred in 8 plastic cages of 5 rats each in a room with optimum temperature of 24±2 Cº and 55-60% humidity.Rats were kept on a 12-14hr/day light program and had access to commercial food and distilled water.

Chemicals

Lead acetate trihydrate powder $(CH_3COO)_2Pb.3H_2O$, Lupa, Indian supplied from El-Mekawy company.

Vitamin E and Selenium liquid obtained from AL-Shark company.

Experimental design

The rats were divided into 4 groups. **Group I:** control albino rats given distilled water. **Group II:** treated rats orally administrated sub-lethal dose of (10mg/Kg bwt) lead acetate by gavage needle. **Group III:** regenerated rats administrated orally lead acetate plus vitamin E and Selenium in drinking water as 1ml/liter D.W. and the last group **Group IV:**that administrated vitamin E and selenium in drinking water during 4 weeks of experiment.

Histological and Histochemical examination:

Albino rats from each group were sacrificed under ethical protocol approved by the animal experimental local ethics comitte at Cairo University.The liver dissected out and portion from the right and left lobe of liver removed, sectioned and fixed in 10% buffered neutral formalin, dehydrated in ascending grades of alcohol, embedded in paraffin and 3µ thick sections were obtained by using rotary microtome and stained by Hematoxylin and Eosin (H&E), PAS and Best's Carmine stain as outlined by **(Bancroft and Gamble,2008).**Histological sections were examined by light microscope with full HD microscopic camera (Leica Microsystems, Germany).

Transmission Electron Microscope:

Small tissue blocks from the different parts of liver tissues were fixed in paraformaldehyde-glutaraldehyde in phosphate buffer**(Karnovsky,1965).** Specimens were post- fixed in 1% osmium tetraoxide for one hour, washed in 0.1 M phosphate buffer (pH 7.3), then dehydrated in gradual ethanol and embedded in open araldite mixture **(Mollenhaur, 1964)**. Semithin sections (1µm) were cut, stained with Toluidine blue **(Richardson et al., 1960)** and examined with light microscope. Ultra-thin sections were cut and stained with uranyl acetate and lead citrate and examined under Transmission Electron Microscope TEM -109 of SEO Company in Military Veterinary Hospital.

Immunohistochemical examination:

Immunohistochemistry for detection of proliferating cell nuclear antigen (PCNA) was performed on paraffin – embedded liver sections from all groups and mounted on positively charged superhost glass slides. Antigen retrieval was performed in sodium citrate buffer (PH 6.0) (2 cycles of 15 minute each). Endogenous peroxidase activity was blocked using 0.05% hydrogen peroxide for 15 min. Non specific binding was blocked using ultra v Block (thermo Scientific, Cheshire,UK), then slides were incubated overnight at 4cº with primary antibodies against (PCNA) (thermo sci.). Biotinylated- secondary antibodies were used followed by streptavidin-biotin-peroxidase method. The immunological reaction was visualized using diaminobenzidine. All sections were counter-stained with hematoxyline. The sections were washed with phosphate buffered saline after each step. Negative controls were included using non – immune serum in place of the primary or secondary antibodies. The method used as outlined according to **Ramos-Vara (2005).**

Blood analysis:

Blood samples collected from orbital venous plexus of albino rats under total anaesthesia with diethyl ether. Blood collected in glass tubes and centrifugated at 3000 rpm for 20 min and the obtained serum was kept frozen at -20 Cº until used for determination of liver enzymes (ALT & AST) and liver albumin in 4 groups of experiment. Whichcarried out in biochemistry unit of Animal Health Research Institute, Dokki.

Oxidative stress parameter measurement

Specimens from liver tissue were weighted and homogenized with Teflon tissue homogenizer. The samples were homogenized in cold phosphate buffered saline (Ph 7.4) using Teflon homogenizer. The homogenates were centrifuged at 14,000 xg for 15 min at 4 Cº. The supernatant was used to measure the reduced glutathione (GSH) concentration **(Ellman, 1959**). Which carried out in biochemistry department, Faculty of Veterinary medicine, Cairo University.

Statistical analysis:

One- way analysis of variance (ANOVA) was used to compare multiple group means, followed by student's test to determine statistical significance ($p<0.05$) among the different groups. All statistical analysis was performed using SPSS version 16 package for Windows. Results are expressed as means ± SE.

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RESULTS

Histological Observations

Liver sections of control albino rats revealed normal histological architecture composed of hepatocytes arranged in cords that radiated from the central vein and separated by hepatic sinusoids. Hepatocytes were polygonal in shape had one or sometimes two spheroid nuclei. Hepatic sinusoids were lined by endothelial and kupffer cells. normal portal triad was present between hepatic lobules (Figs.1A&B).

Ultrastructurally, normal liver cell exhibited a polygonal or hexagonal. Hepatocytes contained prominant rounded nuclei, numerous ribosomal studded –endoplasmic reticulum that extended from a zone in close proximity to nuclear envelope to cell surface, cytoplasm appeared granular due to presence numerous free glycogen particles and contained numerous oval or rounded mitochondria and smooth endoplasmic reticulum (Figs. 2A&B).

However, hepatic sections of lead acetate treated albino rats exhibited many histological alterations such as disorganization of hepatic cords, some hepatocytes revealed loss of architecture, dilation of central vein with dislocation of it's wall. Hepatic sinusoids were congested and dilated. Some nuclei showed fragmented chromatin (karyolysis) and the cytoplasm contained many vacuoles (Figs.1C&D). Additionally inflammatorycellular infiltration around portal area observed.

Ultrastructurally, liver of lead acetate treated rats showed heavy infiltration with electron dense (lead) particles which appeared as dark spots, swollen mitochondria with short and marginated cristae if compared to well preserve parallel ones of normal rats. Also hepatocytes contained lipid droplets which showed dense particles in numbers that increased from the homogenous center to myelin –like periphery (Figs. 2C&D).

On the other hand, hepatic sections of regenerated group (lead acetate plus Vitamin E and Selenium) revealed disappearance of most alterations that represented by marked mitotic figures in hepatocytes , many binucleated hepatocytes were noted,the sinusoids became narrow and less congested. Central vein wall became continuous and the nuclear features were improved. Hepatocytes architecture was well defined (Fig. 1E). Decrease lymphocytic infiltration around portal area. Ultrastructurally, mitochondria regenerated, the electron dense particles disappeared, well developed golgi apparatus and rER are noted (Fig. 2E).

While by light and electron microscope,vitamin E and Selenium group simulated control group with an enhancement of hepatic cords arrangement and nuclear features (Figs. 1F&2F).

Histochemical Observations

There was a normal positive reactivity of hepatocytes with periodic acid Schiff (PAS) stain in livers of control rats. Weak PAS reactivity in lead acetate treated group. However, moderate reactivity of most hepatocytes in lead acetate plus Vitamin E and Selenium group. But intense reactivity observed in vitamin E and Selenium group (Fig.3).

The hepatocytes exhibited significant amount of cytoplasmic glycogen granules in control group. Strong decrease in cytoplasmic glycogen granules in hepatocytes of lead acetate treated albino rats. Moderate glycogen granules noticed in hepatocytes of regenerated group. While highly significant increase in cytoplasmic glycogen granules in hepatocytes of Vitamin E and Selenium group observed (Fig .4).

Immunohistochemical observations

The hepatic tissue of control rats revealed light brown staining PCNA of few nuclei of hepatocytes. Intense PCNA expression was localized in most nuclei of hepatocytes of lead acetate treated group that indicated by dark brown coloration. While moderate PCNA expression was localized in some nuclei of hepatocytes of lead acetate plus Vitamin E and Selenium group. Moreover slightly faint PCNA expression in some nuclei of hepatocytes of Vitamin E and Selenium group observed (Fig. 5).

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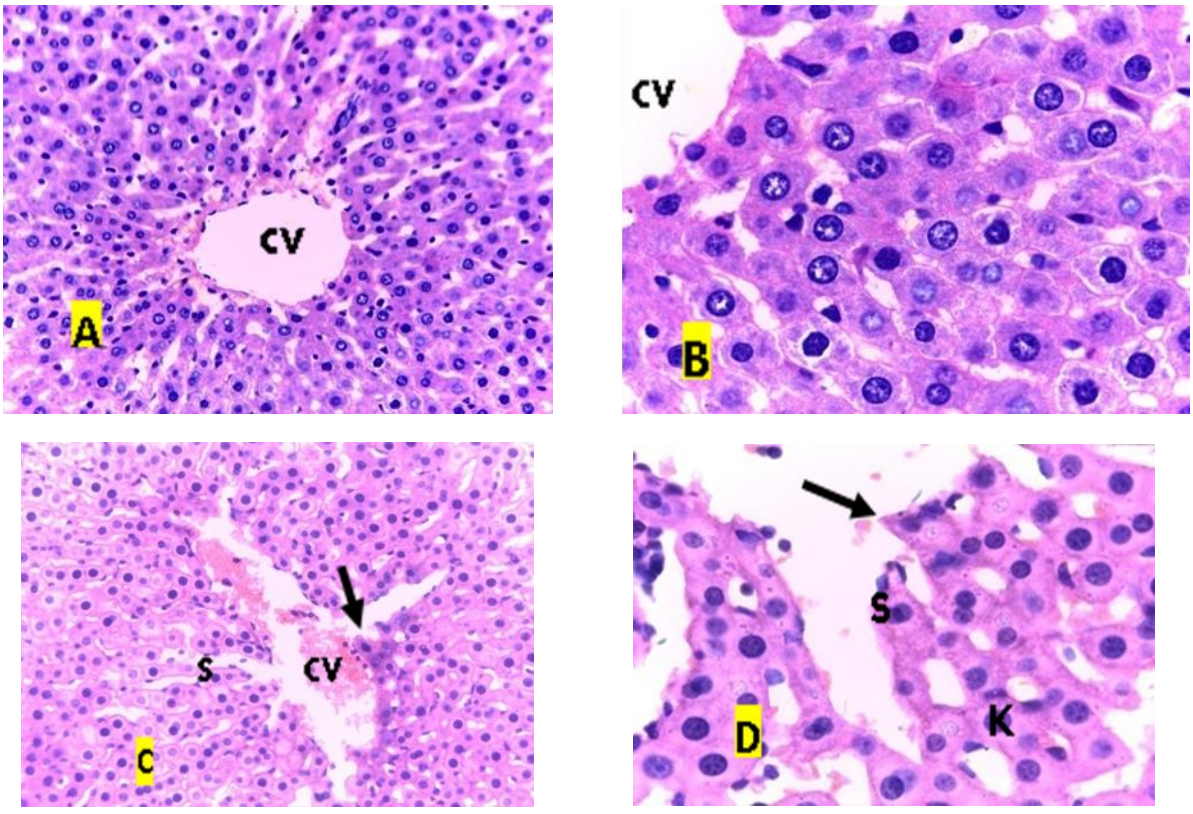
Oxidative stress parameter

The concentration of reduced glutathione (GSH), the non-enzymatic antioxidant,was significantly decreased in hepatic tissue of lead acetate treated albino rats compared to control. But in vitamin E and Selenium plus lead treated group, the concentration of GSH showed significant elevation compared to treated group. While in vitamin E and Selenium group, the concentration of GSH significantly increased compared to control, treated and regenerated groups (Fig. 6)

Liver enzymes and albumin

There was significant decrease in albumin level in serum of lead acetate treated albino rats compared to control. But in vitamin E and Selenium plus lead acetate treated albino rats, the level of serum albumin showed significant elevation compared to treated group. While the serum albumin level in vitamin E and Selenium group showed marked elevation compared to other groups (Fig.7).The AST and ALT level in serum of lead acetate treated albino rats showed significant elevation compared to control. But in regenerated group, the serum AST and ALT level showed significant decrease compared to treated group. While in vitamin E and Selenium group, the serum AST and ALT levelshowed significant decrease compared to regenerated group. AST serum level showed insignificant alteration compared to control but ALT revealed significant decrease compared to control (Figs. 8&9).



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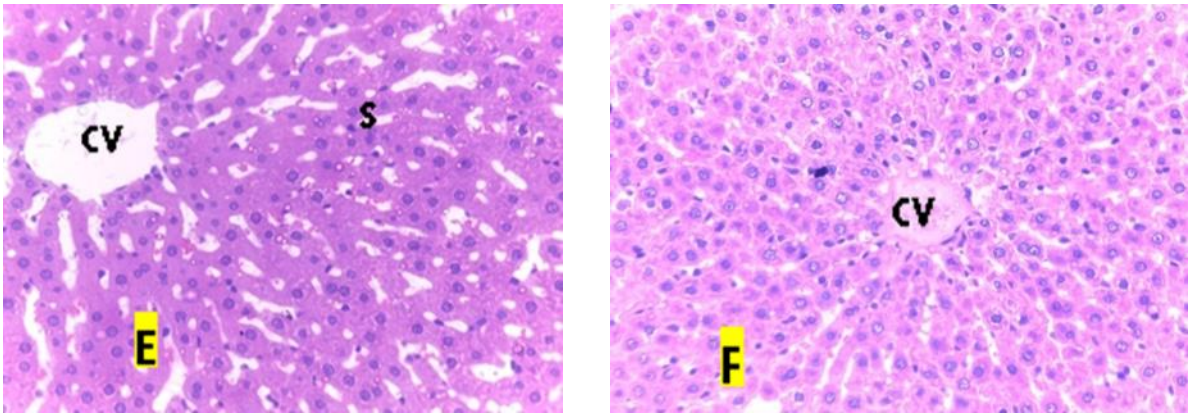
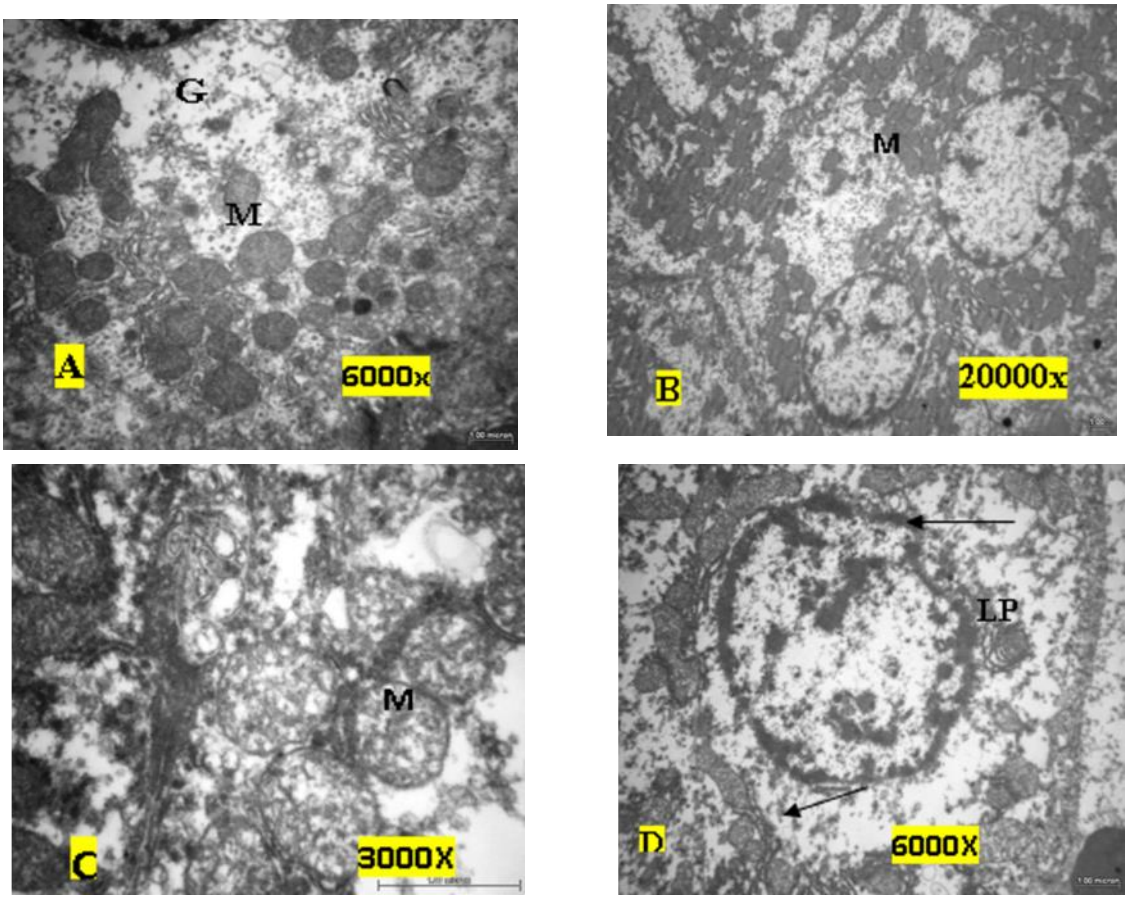


Fig. 1: photomicrograph of Albino rat liver. (A x400&B x1000) the control group reveal normal histological structure of hepatic tissue with central vein (CV). (C X400&D X1000) the Albino rats treated with lead acetate showing dilation and congestion of sinusoids (S) and central vein (CV) with dislocation of it's wall (arrow) and karyolysis of nucleus (K). (E x400) the regenerated group with less dilated central vein (CV) and narrower sinusoids (S). (F x400) the vitamin E and Selenium groupshowed normal histological structure similar to control. H&E stain.



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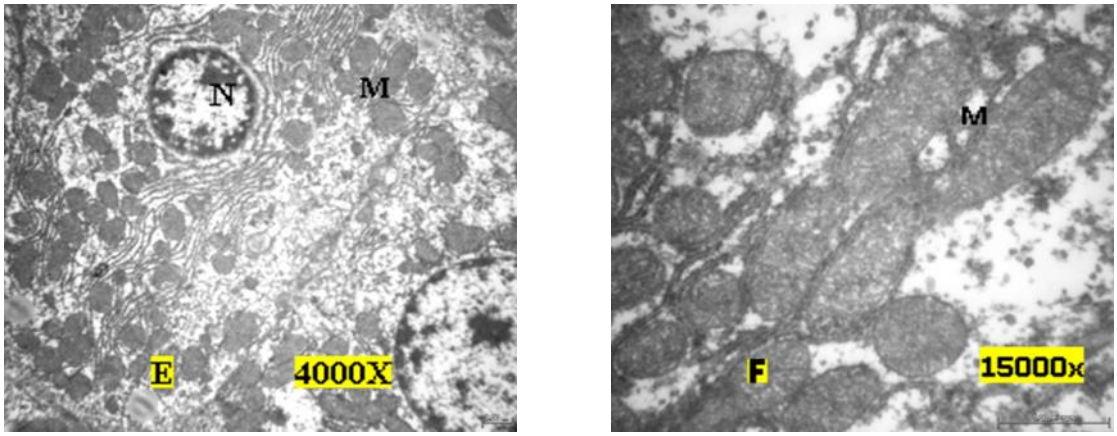

Fig. 2:Electromicrograph of Albino rat liver. A&B. the control group showed normal mitochondria (M) and glycogen granules (G). C&D. the treated group showed swollen mitochondria with fragmented cristae (M), lipid droplet (Lp) and electron dense particles (arrow). E. normal mitochondria (M) and normal nucleus (N) were noted in the regenerated group. F. the vitamin E and Selenium group revealed normal histological structures with normal mitochondria (M).Uranyl acetate & Lead citrate.

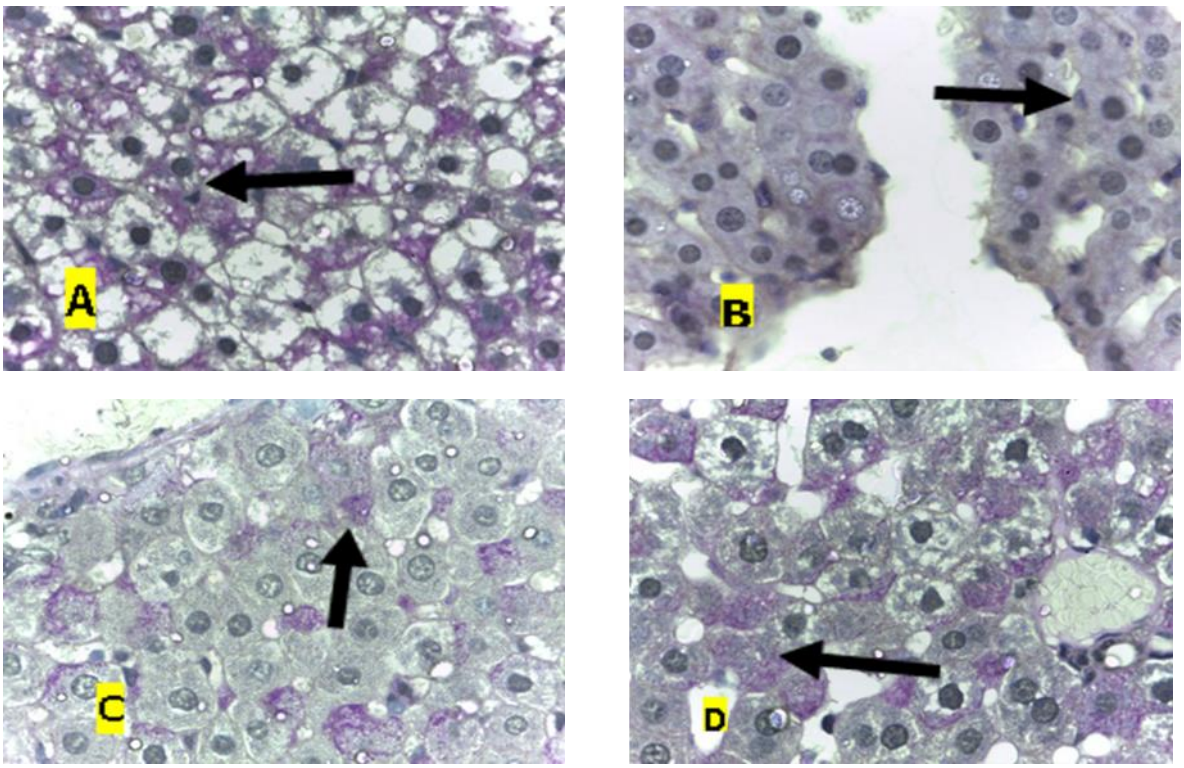

Fig. 3: Photomicrograph of hepatocytes reactivity for PAS stain of Albino rat liver showing. A. positive reactivity (arrow) of hepatocytes to PAS stain in control group. B. weak reactivity (arrow) in treated group. C. moderate reactivity (arrow) in regenerated group. D. intense reactivity (arrow) in vitamin E and Selenium group. Periodic acid Shiff stain x1000.

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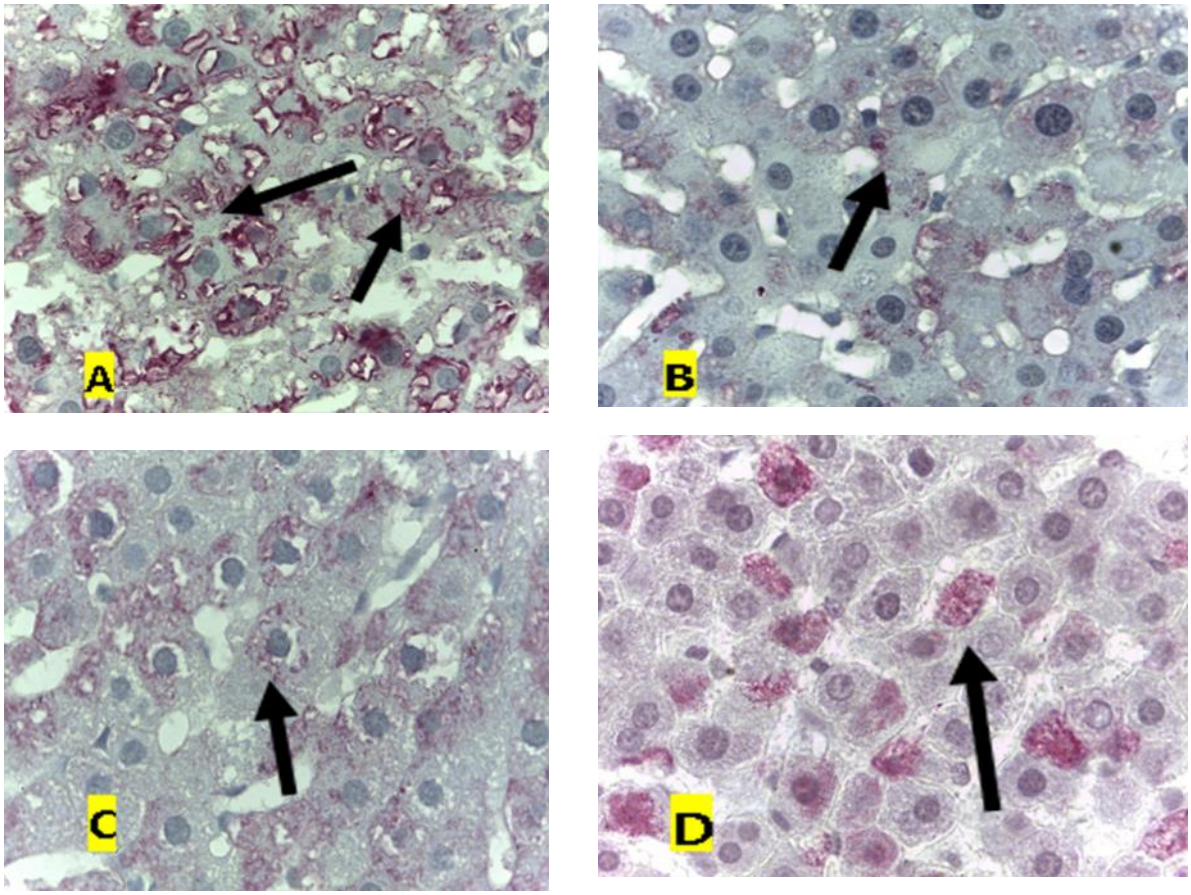
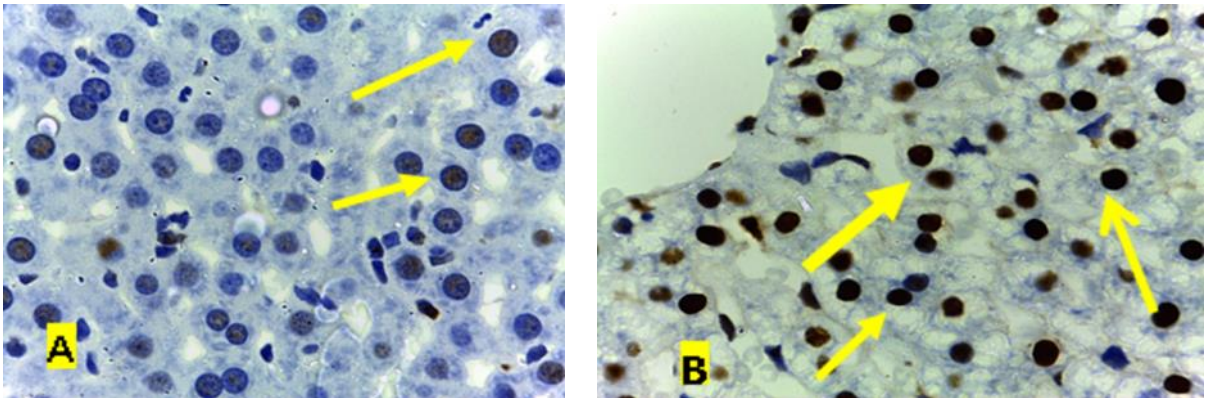


Fig. 4: Photomicrograph of Albino rat liver showing. A. significant amount of glycogen granules (arrows) in cytoplasm of hepatocytes in control group. B. depletion of glycogen granules (arrow) in cytoplasm of hepatocytes in treated group. C. moderate amount of glycogen (arrow) in cytoplasm of hepatocytes in regenerated group. D. highly significant increase in amount of cytoplasmic glycogen granules (arrow) in vitamin E and Selenium group. Best's carmine stain x1000.



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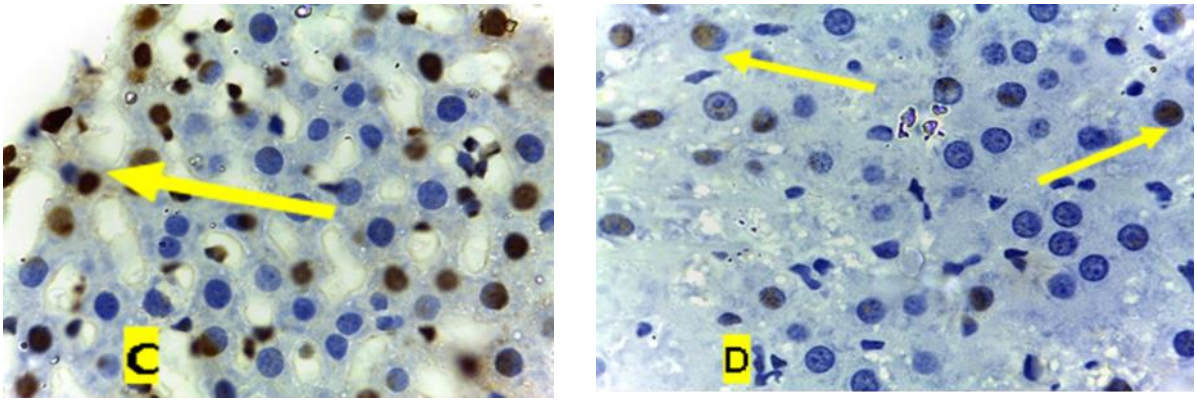

Fig. 5: Photomicrograph of Albino rats liver showing: A. low or little PCNA immunoreactivity (arrows) in one or two nuclei of hepatocytes in control group. B. intense positive response in most nuclei of hepatocytes in treated group. C. moderate immunoreactivity in nuclei of hepatocytes in regenerated group. D. low immunoreactivity in one or two nuclei of hepatocytes in vitamin E and Selenium group. PCNA immunohistochemistry x1000.

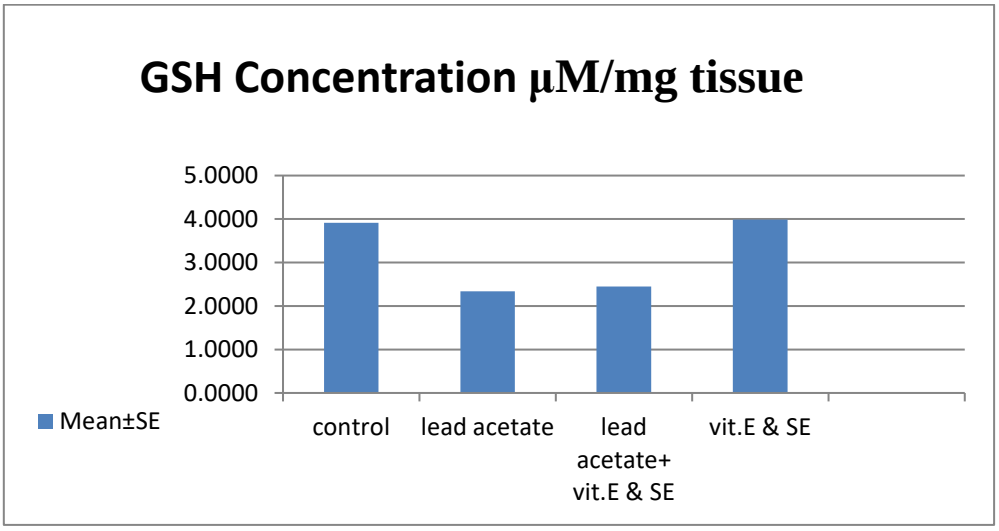

Fig. 6: Reduced glutathione (µM/mg tissue) in liver of control, lead acetate, lead acetate plus Vitamin E and Selenium and Vitamin E and Selenium groups for 4 weeks of experiment. Values are given as mean±SE for group 5 animals each. The significant differences between 4 groups at 0.005 level. GSH concentration in hepatic tissue of treated group significantly decreased compared to control. In regenerated group, the GSH concentration significantly increased compared to treated group. (p ≤0.05).

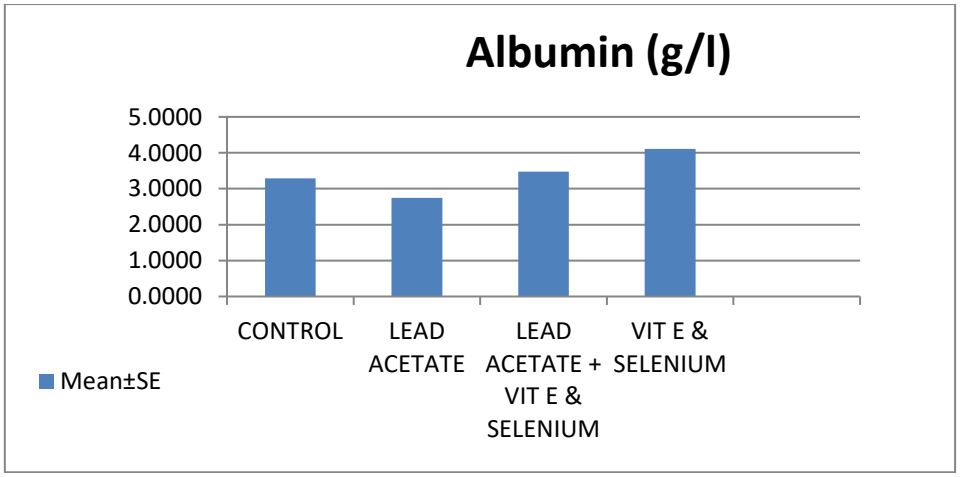

Fig.7: Albumin level(g/l) in serum of control, treated, vitamin E and Selenium plus lead acetate and vitamin E and Selenium group. Values are given as mean±SE for group 5 animals each. The significant differences between 4 groups at 0.0003 level. The serum albumin level significantly decreased compared to control. In regenerated group, albumin level significantly increased compared to treated group. (p≤0.05).

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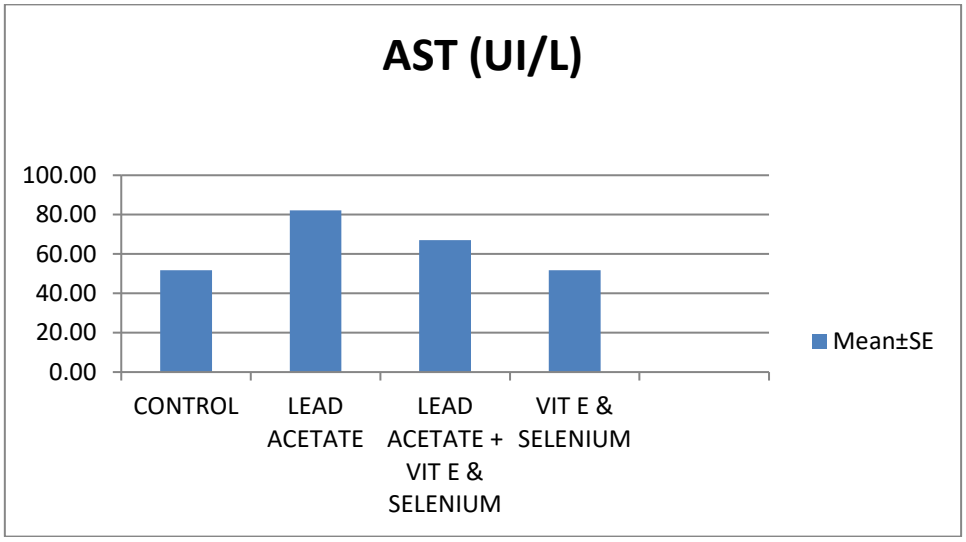

Fig. 8: The level of serum AST in 4 groups of experiment. Values are given as mean±SE for group 5 animals each. The significant differences between 4 groups at 0.016 level. Serum AST level significantly increased in treated group compared to control. In regenerated group, the AST level significantly decreased compared to treated group. (p≤0.05).

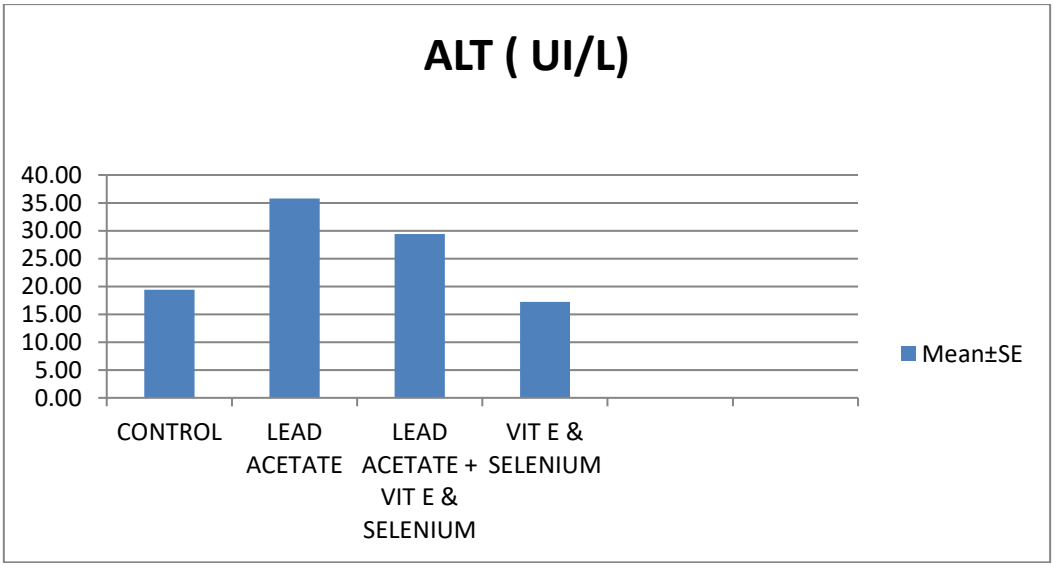

Fig. 9: The level of serum ALT in 4 groups of experiment. Values are given as mean±SE for group 5 animals each. The significant differences between 4 groups at 0.0004 level. Serum ALT level significantly increased in treated group compared to control. In regenerated group, the ALT level significantly decreased compared to treated group. (p ≤ 0.05)

DISCUSSION

Lead is a poison that affects virtually every system in the body and has a potent susceptibility tothe highly vascularized organs (**Srianujata, 1998).** The liver is considered as one of the target organs affected by lead toxicity owing to the storage, biotransformation and detoxification of toxic substances **(Herman andGeraldine, 2009)**. Absorbed lead is stored in soft tissues mainly liver **(Patrick,2006)** via portal vein, so that it is the first organ for which the histological analysis used to examine the morphological alterations that reflect lead effect on hepatic tissue.In the present study, liver specimens taken 4 weeks after exposure to lead acetate showed hepatic cords disorganization, loss of hepatic cells architecture and disintegration of hepatocytes that in line with **Al-Bideri(2011)** and **Suradkar et al.(2010)**who reported similar observations in rats receiving 1000 ppm lead for 28 days.Also, histological analysis of hepatic specimens obtained from lead acetate treated rats revealed inflammatory cellular infiltration around the portal area and cytoplasmic vacuolation of hepatocytes that is in agreement with **Gunawan and Kaplowiz (2004); Al-Attar(2011)** and

Hegazy and Fouad(2014). Cytoplasmic vacuolation considered a cellular defense mechanism against injurious substances which segregated in vacuoles thus prevented from interfering with cellular metabolism (**Mollendorf, 1973)**.In this investigation, the liver of treated albino rats showed dilated and congested central vein and sinusoidal capillaries that indicator for liver alterations. Similar observations reported by **El-Sokary et al. (2005); Sharma et al. (2010); Al-Attar(2011)** and **Haous et al.(2014)**. The present study indicates clearly that the mitochondria are highly susceptible to lead toxicity and mitochondrial swelling was a frequent ultrastructural finding that in agreement with **Hasan(2011)**. This swelling may be due to change in osmolarity that lead to an influx of salts and water via the inner mitochondrial membrane which becomes distended **(Hegazy and Fouad,2014)**. Also, mitochondrial cristae showed fragmentation and disorientation that may indicate a special lead affinity for mitochondrial membranes which play a key role in functional integrity of this organelle (**Fowler, 1981** and **Shlan et al., 2005, 2006).** Another ultrastructural finding in lead acetate treated albino rats , there was lipid droplets noted in cytoplasm of hepatocytes which increase from homogenous center to myelin like periphery that in line with **Russo et al.(1988)**and**Hasan(2011)**.The accumulation of fat droplets in the cytoplasm of the affected hepatocytes may suggest lead interference with lipid removal from these cells through impairment of ATP-dependent fatty acids **(Piasek et al., 1989)**. But the administration of Vitamin E and selenium to lead acetate treated albino rats decreases the hepatotoxic effect of lead that indicated by narrowing of sinusoids, decreasing the congestion, the wall of central vein became continuous, less lymphatic infiltration around portal area and the hepatic cords disarrangement became mild that in agreement with **Al-Bideri(2011);Al-Attar(2011)**and **Boussekine et al.(2015).** By electron microscopy, mitochondria regeneratedand the electron dense particles disappeared.

In the present study, depletion of glycogen granules was noted in hepatocytes by using Best's carmine stain in treated albino rats compared to control that in line with **Abdul- Kareem(2014).** The depletion in glycogen granules may attribute to decreasing glycogen synthetase enzyme activity which leads to inhibition of glycogenesis **(Hakim et al., 1971)**.While in vitamin E and Selenium plus lead acetate treated group, glycogen granules increased than treated group that indicate the improvement of glycogen synthetase enzyme activity and formation of glycogen. In vitamin E and Selenium group, there was intense concentration of glycogen granules in hepatocytes. In this investigation, lead acetate treated group revealed low concentration of neutral muco-polysaccharides compared to control. The low concentration of neutral muco-polysaccharides may be due to degeneration of the hepatic cells accompanied by damage of mitochondria. Accordingly, reduction of mitochondrial content of the cell will reduce the amount of ATP, the matter which inhibits neutral mucopolysaccharides formation **(Robbins and Cotran 1999)**. The concentration of neutral mucopolysaccharides increases in regenerated group compared to treated group. In vitamin E and Selenium group, there was intense concentration of neutral mucopolysaccharides in hepatocytes compared to other groups.

According to current study, there was intense PCNA expression recorded in most of hepatocytes nuclei in lead acetate treated albino rats compared to control that agreed with **Singh (2006)**. This could be an indication for increase the proliferating rate as an attempt to repair or renew the damaged hepatocytes. **Puszati et al. (1993**) stated that the accelerated proliferation might indicate an increased mutagenic risk on cells. **Itall et al. (1990)** concluded that PCNA which were involved in cellular cycle could be identified in replicating cells of both benign and malignant lesions. While in regenerated group, there is less PCNA expression compared to treated group also there is no or slight PCNA expression in one or two nuclei of hepatocytes in vitamin E and Selenium group.

In this study, a significant decrease in GSH concentration noticed in hepatic tissue of lead acetate treated albino rats compared to control that in agreement with **Wang et al.(2007)** and **Boussekine et al. (2015).**GSH considered a primary biomarker of oxidative stress, it is a non-enzymatic antioxidant with SH group that can directly interact with reactive oxygen species (ROS) which resulted from lead toxicity (**Sivaprasad et al., 2002).** So the decrease in GSH concentration in hepatic tissue may be due to the ability of lead to bind with SH group which decrease the GSH concentration so interfering its antioxidant activity **(Patrick, 2006).** While the administration of vitamin E and Selenium to lead acetate treated albino rats markedly elevated the concentration of GSH in hepatic tissue that in line with **Al-Attar(2011)**and **Boussekine et al. (2015)**. So the elevation of GSH concentration in hepatic tissue of treated albino rats with vitamin E and Selenium administration indicate a protective effect of vitamin E and Selenium against lead induced hepatoxicity that evidenced by the ability of vitamin E to chain breaking and prevention of free radicals formation **(Flora et al., 2012)** and Selenium bind with lead- selenium complexes (**Flora et al., 1982)** subsequently increase GSH

concentration that scavenges free radicals and decrease the oxidative stress resulted from lead acetate. In the vitamin E and Selenium group, there was a significant elevation of GSH concentration compared to other groups.

According to current study, the level of serum albumin is decreased in lead acetate treated group compared to control that in line with **Abdou and Newairy(2006).** The decrease in albumin level may be due to interference of lead with protein synthesis or by binding of lead to some metal-binding proteins and their removal through detoxification processes **(Cawson et al., 1982)** or could be attributed to changes in protein and free amino acids metabolism and their synthesis in liver (**Rivarola and Balegno, 1991).** But in regenerated group elevation of albumin level in serum recorded compared to lead acetate group and control. While vitamin E and Selenium group revealed significant increase in albumin level compared to control, lead acetate and vitamin E and Selenium plus lead acetate groups.

Liver enzymes (ALT and AST) are considered as an important biomarker for detection of lead hepatotoxicity. According to current results, lead acetate caused a significant increase in serum ALT and AST levels of lead treated albino rats compared to control. Similar results have been found by **Sivaprasad et al., (2003); Abdel-Kader et al., (2011)** and **Haous et al.,(2014).** Former authorobserved an increase in serum transaminases level after exposure of male Wistar rats to 0.2% lead acetate in water for 5 weeks and in line with**.** Increasing levels of ALT and AST in serum is mainly due to leakage of these enzymes from liver cytosol into blood stream **(Concepción et al., 1993)**. So we can deduce that high level of transaminases in serum that normally located in hepatocytes cytosol are sign of hepatocytes damage leading to liver dysfunction that similar to **Haous et al. (2014)**. In regenerated group, vitamin E and Selenium markedly attenuated lead induced hepatotoxicity as indicated by the significant decrease in ALT and AST level in serum that in agreement with **Boussekine et al., 2015**. In the group administrated vitamin E and Selenium only, the level of AST slightly decreased than control and ALT revealed insignificant alteration compared to control.

CONCLUSION

In the present study, low dose (1ml/L D.W.) of vitamin E and Selenium combination could have a protective effect against acute lead induced hepatotoxicity.

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