

Efficacy of Myrrh Extract "Mirazid®" to Reduce Lead Acetate Toxicity in Albino Rats with Special Reference to Cerebellum and Testes

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Abstract: Lead is a ubiquitous environmental and industrial pollutant with a worldwide health problems. A total fifty adult male albino rats were equally divided into five groups to evaluate the toxic effects of lead acetate on liver, kidneys, brain and testes of albino rats, besides assessing the efficacy of myrrh extract in reducing these toxic changes. Group (1) was left without treatment. Each rats in group (2) was orally given 2 ml distilled water. Each rats in group (3) was given 20 mg lead acetate / kg B.wt. Each rats in group (4) was given 500 mg myrrh-extract / kg B.wt. Each rats in group (5) was simultaneously given similar doses of lead acetate and myrrh-extract as those given to groups (3&4). Treatments of groups (1-5) were administered daily by stomach tube for 3 months. At the end of the experimental period, all rats were sacrificed, necropsied and the gross lesions were recorded. Moreover, specimens were collected from the liver, kidneys, brain and testes of groups(1-5) and prepared for histopathological examination and morphometrical analysis of cerebellum and testes.. Histopathological examination of group (3) showed degenerative and necrotic changes in the hepatic and renal cells. Interstitial and perivascular aggregations of lymphocytes and hyperplasia of epithelial lining of some bile ducts with newly formed bile ductules were detected. Some hepatocytes and renal cells showed karyomegaly and cytomegaly with presence of eosinophilic intranuclear inclusion bodies. The brain revealed congestion, degenerated neurons, satellitosis, neuronophagia, encephalomalacia and coagulative necrosis of Purkinje cell. The testes showed interstitial edema among degenerated seminiferous tubules. The previous changes in cerebellum and testes were supported by the morphometric results which revealed a significant reduction of the mean thickness of the Purkinje cell layer when compared with the control rats. A significant decrease ($p < 0.001$) of the width of germinal epithelial layer of the affected seminiferous tubules and number of Sertoli cells, spermatogonia and primary spermatocytes was detected. Group (5) showed marked amelioration of the encountered lesions in group (3). It could be concluded that, the adverse effects, induced by lead acetate, were markedly ameliorated by co-treatment with myrrh extract.

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1. Introduction

The environmental contamination by heavy metals has increased drastically along with the rapid development of modern industry. Among these metals is lead, of which its levels have increased substantially during the last few years (Bilandz'ic et al. 2009). It is still mined and added to many commercial products including paints, eye cosmetics, gasoline, enamels and water pipes (Fischbein et al. 1992, Sajitha et al. 2010 and Sansar et al. 2011).

Exposure to low levels of lead has been associated with functional and structural impairments in both human and experimental animals (Reza et al. 2008). The main targets of lead are the hematopoietic, nervous and renal tissues. Moreover, it hinders the efficacy of the hepatic, reproductive and immune function (Teijón et al. 2006 and Durgut et al. 2008). Many studies have shown that reproductive toxicosis is an important feature of lead toxicity. Lead toxicity induces apoptosis of the germinal epithelium with disruption of

spermatogenesis (Adhikari et al. 2001 and Batra et al. 2001). Furthermore, it has been shown that chronic lead exposure produces central nervous system impairments (Antonio et al. 2003 and Sansar et al. 2010).

Calcium Disodium EDTA is a traditional synthetic lead expellant but it is toxic to the liver and kidneys. Therefore recently there have been many studies on the use of natural products such as vitamins and herbal drugs to expel lead (Xu et al. 2005). There has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical-induced tissue injury (Siddique et al. 2000 and Koleva et al. 2002). Numerous plant products have antioxidant activity as they scavenge free radicals and inhibit of lipid peroxidation (Scartezzini and Speroni, 2000 and Tapiero et al. 2002).

Mirazid is a new herbal anthelmintic drug. It is composed of purified myrrh extract. Myrrh is an oleo-gum resin extract from the stem of Commiphora

molmol plant (Hassan et al. 2003). The oleo-gum resin of myrrh contains 2– 8% volatile oils and contains terpenes, sesquiterpenes, and cuminic aldehyde (Chevallier, 1986). The various pharmacological activities of myrrh have been reported by many authors (Sheir et al. 1986, Massoud et al. 2001 and Al-Rowais, 2002). Myrrh extract (Mirazid) has been produced and marketed as an antischistosomal drug since 2001 (Barakat et al. 2005). Commiphora molmol was reported to have anti-inflammatory activity and antineoplastic activity equivalent to the standard cytotoxic drug cyclophosphamide (Qureshi et al. 1993). Moreover, it was reported to have a protective effect on gastric ulcer that was attributed to its free radical-scavenging and prostaglandin-inducing properties (Al-Harbi et al. 1997). However, up to our best knowledge, there is no previous studies that investigated the effects of myrrh extract upon the pathological changes induced by lead acetate, so the aim of this study was to evaluate the efficacy of myrrh extract in reducing the toxic effects of lead acetate in albino rats.

2. Material and Methods

Material:

1-Chemical:

A-Lead acetate ($C_4H_6O_4Pb_3H_2O$) in the form of white crystals, was purchased from ADWIC (A governmental Pharmaceuticals Company), Egypt.

B- Myrrh -extract was obtained from Pharco Pharmaceuticals Company, Egypt, in the form of soft gelatin capsules commercially named "Mirazid".

2- Experimental Animals:

Fifty adult male Wistar albino rats, weighting 200 ± 20 gm, were obtained from the Unit of Laboratory Animal, Faculty of Veterinary Medicine, Zagazig University. Animal care was performed according to the guidelines of the National Research Council and the American Association of Accreditation for Lab Animal Care (NRC, 1994). All animals were allowed 7 days for acclimatization to their new environment and to ascertain their physical well being. They were housed in separate well ventilated cages, under standard conditions, with free access to the standard diet and water ad libitum.

Methods:

1-Experimental design:

After 7 days adaptation period, rats were allocated randomly to five equal groups.

Group (1) was left without treatment. Each rats in group (2) was orally given 2 ml distilled water. Each rats in group (3) was given 20 mg lead acetate / kg B.wt. (Nehru and Kanwar, 2004). Each rats in group (4) was given 500mg myrrh-extract / kg B.wt. Each rats in group (5) was simultaneously given similar

doses of lead acetate and myrrh-extract as those given to groups (3&4).

Treatments of groups (1-5) were administered daily by stomach tube for 3 months. Myrrh extract solution (500mg /kg B.wt.) was given 1 hour before treatment with lead. The timing and dose of myrrh extract pretreatment have been selected on the basis of previous reports (Badria et al. 2000 and Farid and Attia, 2007) to build antioxidant pool in animal body before heavy metal exposure. At the end of the study, all the rats were sacrificed and necropsied to elucidate the lesions.

2- Pathological examination:

All animals were necropsied and the gross lesions were recorded. Moreover, specimens were collected from the liver, kidneys, brain and testes of groups (1-5) and fixed in 10% neutral buffered formalin. Five micron thick paraffin sections were prepared, stained with HE and examined microscopically (Bancroft and Stevens, 2002). The H&E stained sections of cerebellum and testes were subjected for morphometric analysis.

3- Morphometrical analysis of the cerebellum and testes:

The image analyzer computer system (Leica Qwin 500) was used for the morphological analysis of the cerebellar and testicular sections in the Histology Department, Faculty of Medicine, Cairo University. The hematoxylin and eosin stained sections were examined using light microscope equipped by eyepiece graticule for measuring the structural changes. For cerebellar sections the mean thickness of the Purkinje cell layer in each group was measured (Hoffer et al. 1987).

The number of germ cells per seminiferous tubules, spermatogonia, primary spermatocytes, Sertoli cells (Fawcett and Raviola, 1994) and the seminiferous tubules diameter (Batra et al. 2004) were recorded for testicular sections. The examination was done at 1200 X magnification in ten randomly chosen non-overlapping fields, from five animals for each group.

4- Statistical analysis:

Data were presented as means \pm SD. The differences were compared for statistical significance by student's t test. Difference was considered significant at $p < 0.05$. The statistical analysis was performed using Epi-Info version 6.1 (Dean et al. 2000).

3. Results

1-Pathological findings:

The liver, kidneys, brain and testes of groups (1,2&4) showed neither gross nor microscopical lesions, meanwhile group (3), revealed enlarged and

congested liver with small grayish-white foci. Microscopically, the liver was congested. The portal areas showed fibroblastic proliferation infiltrated with leukocytes and bile ducts with hyperplastic epithelial lining with newly formed bile ductules (fig.1). Diffuse vacuolation of hepatocytes (fig.2) and coagulative necrosis of hepatocytes, evidenced by pyknosis and karyolysis (fig. 3) were found. Individualization and disorganization of the hepatic cords were encountered. Focal replacement of the hepatic parenchyma by round cells, mainly lymphocytes was detected (fig.4). Some hepatocytes showed karyomegaly and cytomegaly with presence of eosinophilic intranuclear inclusion bodies.

The kidneys were macroscopically enlarged. Microscopically, congestion and focal replacement of the renal parenchyma with mononuclears were noticed (fig.5). Cloudy swelling and vacuolation of the renal epithelium were seen in the convoluted tubules. Cystic dilation of some renal tubules with flattened epithelium was seen (fig.6). Some epithelial cells of renal tubules showed karyomegaly and cytomegaly (Fig .7) with presence of eosinophilic intranuclear inclusion bodies (fig.8). Other renal epithelium showed coagulative necroses, characterized by karyolysis. Focal replacement of renal parenchyma with mononuclears in presence of hyaline casts, besides necrotic renal epithelium and cystic renal tubules (fig.9).

The brain revealed congested and edematous meninges (fig.10). Brain edema was evidenced by dilated Virchow-Robin spaces (fig.11). Focal gliosis was seen partially replacing the midbrain parenchyma (fig.12.). Satellitosis and neuronophagia were seen in the gray matter of cerebrum. The cerebellar medulla exhibited encephalomalacia and the Purkinje cell layer was partially necrotic (fig.13). The necrotic Purkinje cells were surrounded with clear spaces, suggesting edema, and presented karyolysis (figs. 14). The third ventricle showed atrophic and necrotic epithelial covering of the choroid plexus (figs.15&16). Cerebral encephalomalacia was detected.

The testes revealed interstitial edema and atrophy of the Leydig cells (fig.17). Aspermatogenesis was associated with absence of spermatids and spermatozoa (fig.18), besides presence of necrotic debris in lumina of seminiferous tubules (fig.19). Necrotic debris were seen in lumens of some seminiferous tubules with palisading Sertoli cells (fig.20).

Group (5) showed marked amelioration of the encountered lesions in group (3), where no gross abnormalities were detected in the collected organs. Microscopically, the liver was almost normal and revealed mild congestion (fig.21), besides hydropic degeneration and few round cell infiltration in portal areas. The kidneys showed mild congestion and hemorrhage. Small eosinophilic intranuclear inclusion bodies were rarely found together with a slight

karyomegaly and cytomegaly. Few interstitial aggregations of round cells were observed. Regenerative changes were detected in the epithelial lining of some renal tubules evidenced by hyperchromatic nuclei (fig.22). The brain showed mild encephalomalacia of the white matter of cerebellum with almost normal Purkinje cell layer (fig.23). The meningeal blood vessels were moderately congested. The normal structure of the majority of the seminiferous tubules was restored and spermatogenesis was evident (fig.24). Mild testicular congestion and interstitial edema were still encountered.

2-Morphometric study:

2-1- Morphometric cerebellar changes:

A significant decrease ($p < 0.05$) in the mean thickness ($16.22 \pm 2.60 \mu\text{m}$) of the Purkinje cell layer of group (3) was observed, while the mean thickness of Purkinje cell layer of group (1) was ($19.61 \pm 3.16 \mu\text{m}$). There was a significant improvement in this measurement ($18.72 \pm 3.09 \mu\text{m}$) in group (5) compared with the control value (Table : 1).

2-2- Morphometric testicular changes:

The mean diameter of the seminiferous tubules was decreased significantly in group (3) ($83.2 \pm 18.1 \mu\text{m}$) when compared with group (1) ($114.7 \pm 20.7 \mu\text{m}$) ($p < 0.05$). A significant decrease ($p < 0.05$) in the mean number of Sertoli cells, spermatogonia and primary spermatocytes (8.9 ± 4.1 , 14.4 ± 9.3 , and 7.7 ± 12.6 respectively) was observed in group (3) when compared with group (1) (15.2 ± 4.3 , 31.4 ± 9.9 , and 42.8 ± 13.3 respectively). There was a significant improvement in these measurements (99.9 ± 22.3 , 11.9 ± 3.0 , 27.5 ± 10.0 , and 33.0 ± 9.3 for mean diameter of seminiferous tubule, number of Sertoli cells, spermatogonia and spermatocytes respectively) in group (5) that was still significantly lower than the control (Table :2).

Table 1: Means and standard deviations (mean \pm SD) of the thickness of Purkinje cell layer in the studied groups.

	Groups				
	1 mean \pm SD	2 mean \pm SD	3 mean \pm SD	4 mean \pm SD	5 mean \pm SD
Purkinje cell layer thickness (μm)	19.61 \pm 3.16	19.15 \pm 3.40	16.22 \pm 2.60	19.58 \pm 3.08	18.72 \pm 3.09
P-value (compared with Group I)	-	0.5758	0.00019*	0.97004	0.27239

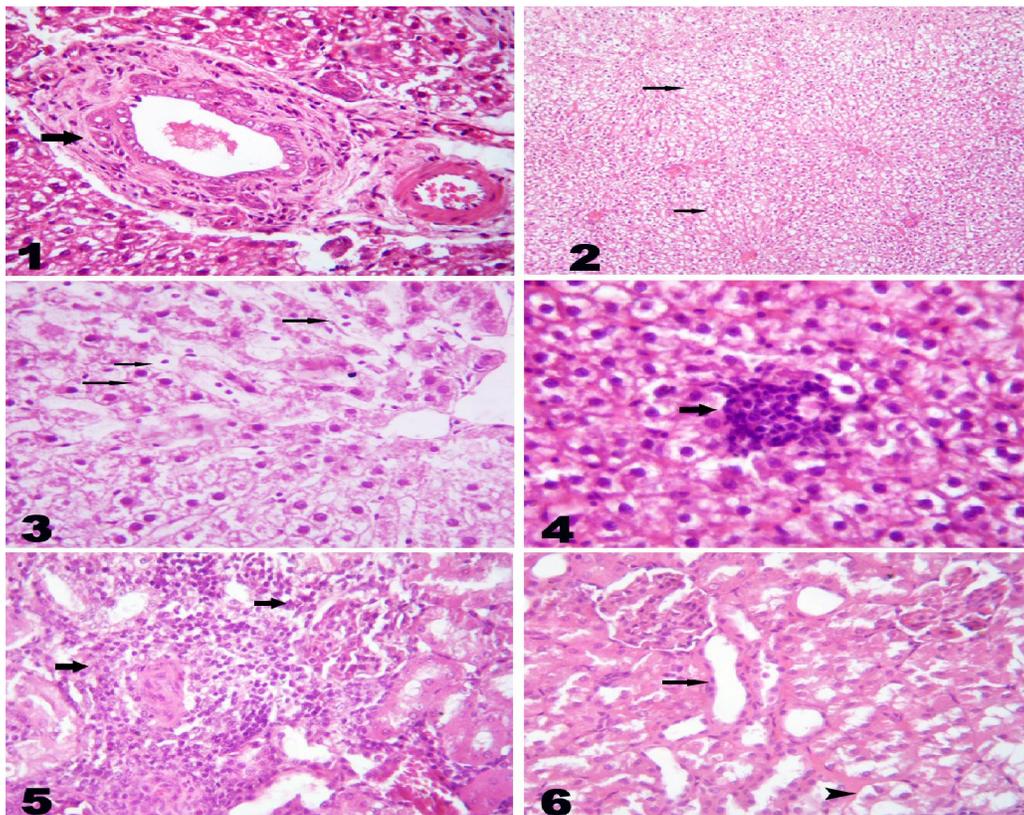
*Very highly significant ($P < 0.001$).

n=10 in all groups

Table (2): Means and standard deviations (mean \pm SD) of Morphometric parameters of testes in the studied groups .

Groups	No.	Semineferous tubule diameter (μm) mean \pm SD	Sertoli cells number mean \pm SD	Spermatogonia Number mean \pm SD	primary spermatocytes number mean \pm SD
1	10	114.7 \pm 20.7	15.2 \pm 4.3	31.4 \pm 9.95	42.8 \pm 13.3
2	10	112.4 \pm 21.6	14.2 \pm 3.8	32.1 \pm 10.15	40.9 \pm 11.4
3	10	83.2 \pm 18.1 * \diamond \neq	8.9 \pm 4.1 * \diamond \neq	14.4 \pm 9.36 * \diamond \neq	7.7 \pm 12.6 * \diamond \neq
4	10	113.8 \pm 23.3	13.0 \pm 4.7	33.2 \pm 9.67	43.4 \pm 10.5
5	10	99.9 \pm 22.3 * \diamond \neq \dagger	11.9 \pm 3.0 * \diamond \neq	27.5 \pm 10.00 * \diamond \neq	33.0 \pm 9.3 * \diamond \neq

*= Significant compared with control group($p < 0.05$), \diamond = significant compared with group(2)($p < 0.05$), \neq = significant compared with group (4)($p < 0.05$), \dagger = significant compared with group (5)($p < 0.05$).



Group (3): Figs. (1-4) Liver, Figs. (5&6) Kidneys. HE.

Fig.(1)- Hyperplastic epithelial lining of bile ducts with newly formed bile-ductules (arrow). x1200.

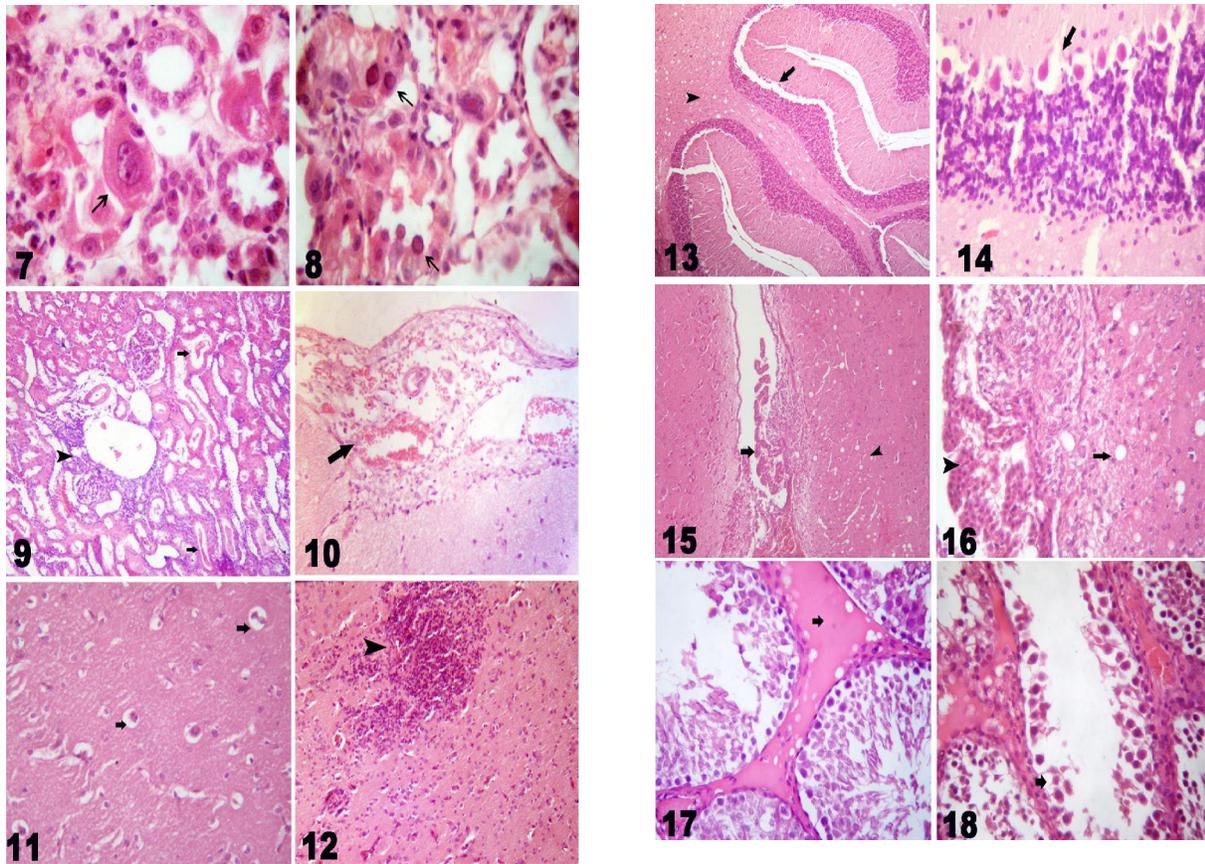
(2)- Diffuse vacuolation of hepatocytes (arrow). x 300 .

(3)-Coagulative necrosis of hepatocytes evidenced by pyknosis and karyolysis (arrow). x 1200.

(4)-Focal replacement of hepatic parenchyma with round cells (arrow). x 1200.

(5)- Focal replacement of renal parenchyma with mononuclears (arrow). x 1200.

(6)-Cystic dilation of some renal tubules (arrow) with necrotic renal epithelium (arrowhead). x1200.



Group (3): Figs. (7-9) Kidneys, Figs. (10-12) Brain. HE.

Fig. (7)- Karyomegaly and cytomegaly in some renal epithelium (arrow). x 1200.

(8)- Eosinophilic intranuclear inclusion bodies in renal epithelium (arrow) x 1200.

(9)-Focal replacement of renal parenchyma with mononuclears (arrowhead) besides hyaline casts (arrow) and cystic renal tubules. x 300 .

(10)-Congested (arrow) and edematous meninges . x 1200.

(11)- Edema evidenced by dilated Virchow-Robin spaces (arrow). x 1200.

(12)-Midbrain gliosis (arrowhead). x 1200.

Group (3): Figs. (13-16) Brain, Figs. (17&18) Testes . HE

Fig. (13)-Cerebellar medullary encephalomalacia (arrowhead) and necrotic Purkinje cells (arrow).x150.

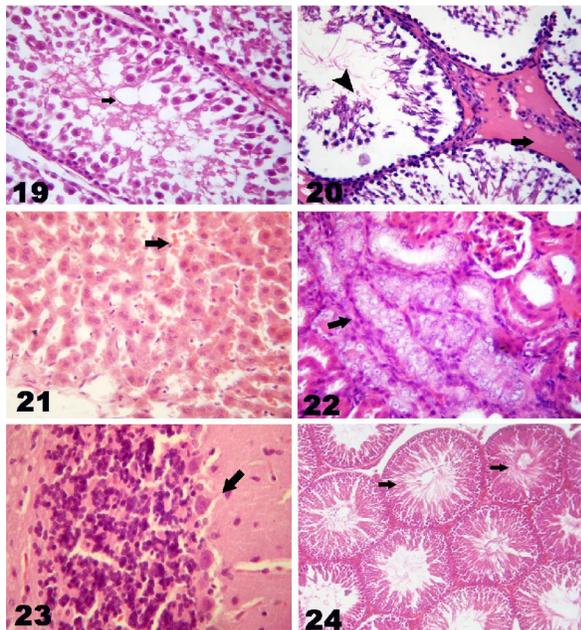
(14)-Magnification of fig. (13) to show necrotic and edematous Purkinje cell layer(arrow).x1200.

(15)-Midbrain displaying atrophic and necrotic choroids plexus (arrow) and vacuolated pons.x300.

(16)- Magnification of fig. (15) to show the atrophic and necrotic choroids plexus (arrowhead) and vacuolated pons. x1200.

(17)- Interstitial edema with atrophic leydig cells . x 1200.

(18)- Aspermatogenesis with absence of spermatids and spermatozoa .x 1200.



Group (3): Figs. (19&20) Testes. HE.

Fig. (19)- Aspermatogenesis with necrotic debris in lumen of seminiferous tubules (arrow).x1200.

(20)-Aspermatogenesis with desquamated germinal epithelium (arrowhead) and palisading Sertoli cells.x1200.

Group (5): Figs. (21-24). HE.

(21)- Liver: Almost normal liver with moderate congestion (arrow).x 1200.

(22)- Kidney: Regenerating renal cortical tubular epithelium evidenced by hyperchromatic nuclei (arrow).x 1200.

(23)- Brain: Almost normal Purkinje cell layer (arrow).x1200.

(24)- Testis: Most of seminiferous tubules displaying active spermatogenesis (arrow).x300.

4. Discussion

Both occupational and environmental exposure to xenobiotics such as lead, remains a serious problem in many developing and industrialized countries (Yücebilgiç et al. 2003). Our study was designed to evaluate the toxic effects induced by lead acetate treatment on liver, kidneys, brain and testes of adult male albino rats and to investigate the protective effect of myrrh extract against these toxic effects.

The results of the current study clearly demonstrated that, lead acetate treatment at a concentration of 20 mg/kg B.wt. induced degenerative and necrotic changes in the hepatic and renal cells, besides interstitial and perivascular aggregation of mononuclears. Hyperplasia of epithelial lining of some bile ducts with newly formed bile ductules was detected. Intranuclear inclusion bodies in the renal and hepatic cells is considered diagnostic and pathognomonic for lead toxicosis (Weide et al. 2003). These inclusions were represented by dense eosinophilic homogenous cores surrounded by a membrane, chemically considered as protein containing lead (Jones et al. 1997). The hepatic and renal cells, containing these inclusions showed karyomegaly, and cytomegaly. Abdel-kalek et al. (2000), Ghorbe et al. (2001) and El-nattat et al. (2003) reported that oral dosing of experimental animals with lead induced necrosis, hemorrhage and degenerative changes which are in general agreement with the current study. Congestion, degenerated neurons, satellitosis, neuronophagia, encephalomalacia, coagulative necrosis of Purkinje cell of cerebellar white matter were observed. These changes were supported by the morphometric results which revealed a significant

reduction in the mean thickness of the Purkinje cell layer as compared to control rats. Similar results were previously described by Blood and Hinchcliff (2000), Sidhu and Nehru (2004) and Mc Gavin and Zachary (2007) who reported that lead is a neurotoxin that damages the white and gray matter of both the central and peripheral nervous system. Furthermore, Adonaylo and Oteiza (1999) recorded that lead treatment for 2 months disrupted the normal arrangement of the cellular layers of the cerebellum with large spaces between the Purkinje cell layer and the granular layer.

Regarding lead induced testicular toxicity, the present study showed obvious testicular degenerations and necrosis, where most seminiferous tubules were lined with few germ cells, composed mainly of spermatogonia, besides interstitial edema. The previous results were correlated with Hamir and Sullivan (2008), Al-Mansour (2009) and El-Sayed and El-Newshy (2009). Koizumi and Li (1992) attributed the lead induced testicular damage to its higher unsaturated fatty acids content which renders it more vulnerability to oxidative stress. An increased level of reactive oxygen species production in the male reproductive organs in rats was observed after lead exposure (Acharya et al. 2003 and Marchlewicz et al. 2007) and in human (Naha and Manna, 2007 and Kasperczyk et al. 2008). The lead induced cellular damage, in the current work, may be attributed to its ability to generate reactive oxygen species that damages tissues by enhancing lipid peroxidation as reported by El-Missiry (2000). The lipid peroxidation inactivates the cell constituents by oxidation and ultimately loss of its membrane integrity (Abdel-Wahhab, 2005 and El-Nekeety, 2009). Moreover,

lead depletes the antioxidant reserves as superoxide dismutase, catalase and glutathione peroxidase (Newairy and Abdou, 2009 and Ashry et al. 2010).

On the other hand, myrrh extract treated rats did not reveal any pathological or morphometric deviations from the control rats. The current findings are in agreement with several experimental and clinical studies on myrrh extract which proved to be safe (Al-Ashmawy et al. 2006 and Abdul-Ghani et al. 2009). The concurrent administration of myrrh extract with lead acetate ameliorated the lead acetate-induced lesions. Similar findings were described by Farid and Attia (2007) who found that myrrh extract at a dose of 500 mg/kg B.wt. significantly reduced the malondialdehyde and hepatic fibrosis induced by carbon tetrachloride toxicity. Interestingly, Al-Ashmawy et al (2006) reported that supplement of lead acetate with myrrh extract alleviated the lead induced lipid peroxidation in liver homogenate and genotoxicity in mice. The previous results may be attributed to the potent cytoprotective properties and antioxidant activity of Myrrh extract. The latter inhibits lipid peroxidation by increasing the activity of the antioxidant enzymes such as glutathione peroxidase and superoxide dismutase (Qureshi et al. 1993 and Al-Harbi et al. 1994).

Conversely, the ameliorating effect of myrrh extract against lead-induced toxicosis could be attributed to the various antioxidant compounds present in the extract. Previous reports revealed that myrrh has immunostimulatory, anti-inflammatory and antioxidant potential (Qureshi et al. 1993). Myrrh causes a significant reduction in production of Interleukin-1 β , IL-6, IL-8 (Tipton et al. 2003). IL-1 β is involved in lipid peroxidation induced by certain pathological conditions as cadmium toxicity and brain aging process (Murray et al. 1999).

It could be concluded that, lead acetate, at a dose of 20 mg/kg B.wt. induced lesions in the liver, kidneys, brain and testes of adult albino rats, meanwhile the concurrent administration of myrrh extract ameliorated and prevented the lead acetate-induced lesions.

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