Research Article

LEAD INDUCED HEPATOTOXICITY IN MALE SWISS ALBINO MICE: THE PROTECTIVE POTENTIAL OF THE HYDROMETHANOLIC EXTRACT OF *WITHANIA SOMNIFERA*

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ABSTRACT

The study was planned to evaluate the efficacy of *Withania somnifera* (WS) root extract in preventing lead induced oxidative stress in liver tissue of mice. Mice randomly divided into 6 groups were treated with and without lead nitrate and WS alone or in combination for 42 days. The oxidative stress was measured by LPO level, reduced glutathione content, total protein level and by enzymatic activities of SOD, CAT, GSH and GST in liver tissue homogenate. These biochemical observations were supplemented by histopathology/histological examinations of liver sections. Lead nitrate enhanced lipid peroxidation with concomitant reduction in SOD, CAT, GST, GSH and total protein content. In addition, lead nitrate produced hepatic histopathology evidenced by histological alterations in liver histology. Treatment of mice with WS resulted in marked improvement in most of the studied parameters. On the basis of above results it can be hypothesized that WS, a natural product can protect lead nitrate mediated hepatic toxicity.

Keywords: *Withania somnifera*, hepatotoxicity, oxidative stress, lead.

INTRODUCTION

Lead (Pb) is a ubiquitous heavy metal. Its exposure mainly occurs through the respiratory and gastrointestinal systems. Absorbed Pb (whether inhaled or ingested) is stored in soft tissues. Autopsy studies of Pb-exposed humans indicate that liver tissue is the largest repository (33%) of Pb among the soft tissues followed by kidney cortex and medulla[1]. Pb can cause liver damage and may disturb the normal biochemical process. Several antioxidant molecules such as glutathione (GSH) and glutathione disulphide (GSSG) and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) are the most common parameters used to evaluate lead induced oxidative damage[2].

Medicinal plants are commonly used for the treatment of various ailments, as they are considered to have advantages over the conventionally used drugs that are much expensive and known to have harmful side effects. *Withania somnifera* (WS), commonly known as Ashwagandha (Solanaceae), is an important herb in Ayurvedic and indigenous medical systems for centuries in India[3]. The roots are the main portion of the plant and are categorized as rasayanas, which promote health and longevity by augmenting defense against disease, arresting the ageing process, increasing the capability of the individual to resist adverse environmental factors and by creating a sense of mental wellbeing[3].

Despite the fact that Ashwagandha has myriad medicinal properties, very few reports of its use in metal detoxification are available. Hence, there is a strong demand for its use in metal detoxification especially lead elimination from body tissues. Keeping this perspective in mind and the above mentioned properties of Ashwagandha, the present investigation was undertaken to determine, if the oral intake of a hydro methanolic root extract of *Withania somnifera* could modify lead induced biochemical changes in hepatic tissue of male mice.

MATERIALS AND METHODS

Chemicals

Lead nitrate was purchased from Central Drug House (India). All other chemicals used in the study were of analytical reagent and obtained from Sisco Research Laboratories (India). Qualigens (India/Germany), SD fine chemicals (India), HIMEDIA (India) and Central Drug House (India).

Experimental plant

The experimental plant WS was collected from the university medicinal plant garden, Banasthali, India. It was recognized and authenticated by a botanist of our institute (Department of Bioscience and Biotechnology, Banasthali University) as a local variety.

Preparation of hydromethanolic WS root extract

The dried and powered WS roots (50g) were extracted successively with 80% methanol and 20% H_2O in a soxhlet extractor for 48 h at 60^0C. After extraction; the solvent was evaporated to dryness at 40^0C by using a rotary evaporator. The yield was 5g/kg and was stored at 4^0C. It was dissolved in distilled water whenever needed for experiment.

Experimental animal

Male Swiss albino mice weighing approximately 15–30 g (2-2.5 months) were obtained from Haryana Agricultural University Hissar (India) for experimental purpose. The Animal Ethics Committee of Banasthali University, Banasthali, India has approved experimental protocol. All experiments were conducted on adult male albino mice (Mus musculus L) weighing 25-30g (3-4 month old). They were housed in polypropylene cages in an air-conditioned room with temperature maintained at $25^0C \pm 3^0C$, relative humidity of 50%± 5% and 12h alternating light and dark cycles. The mice were provided with a nutritionally adequate chow diet (Hindustan lever Limited, India) and drinking water ad libitum throughout the study.

Experimental design

In the present study 36 male Swiss albino mice (Mus musculus) weighing 25-30g (3-4 months old) were used for hepatic biochemical parameters. For these parameters 6 groups with 6 mice in each group were taken and are treated by oral gavage once daily as follows:

Group-1: received 1ml distilled water; served as control.

Group-2: received lead nitrate (20mg/ kg body weight/day) dissolved in distilled water

Group-3 and 4: received hydromethanolic WS root extract at a dose of 200 & 500 mg/kg, body weight/per day, respectively.

Group-5 and 6: received lead nitrate at a dose of 20mgkg-1, body weight/per day along with a dose of hydromethanolic WS root extract at a dose of 200 and 500 mg/kg, body weight/per day, respectively.

The dose for lead and plant was decided and selected on the basis of previously published reports[4, 5].

Hepatic oxidative stress Parameters

After 42 days of duration the mice were fasted overnight and then sacrificed under light ether anesthesia. Liver lobules were dissected out, washed immediately with ice-cold saline to remove blood, and the wet weight was noted and then stored at -80°C for various biochemical assays, and histological studies. Half of each liver was processed for biochemical analysis and the other half was used for Histopathological/histological examination.

Biochemical analysis

Organ (liver) was sliced into pieces and homogenized with a blender in ice-cold 0.1 M sodium phosphate buffer (pH-7.4) at $1\text{-}4^0C$ to give 10% homogenate (w/v). The homogenate was centrifuged at 10,000 rpm for 15-20 min at 4^0C twice to get enzyme traction. The resulting supernatant was separated and used for various biochemical estimations. For biochemical assays, liver was dissected out, cleaned, washed and used to determine. Lipid peroxidation (LPO)[6], Superoxide dismutase (SOD)[7], Catalase (CAT)[8], Glutathione S-Transferase (GST)[9], Reduced Glutathione (GSH)[10], and total Protein content[11] in various groups of mice.

Histological examination

Histological analysis of liver was done according to the method of Mc Manus Mowry[12]. Liver fragments removed from the mice were fixed in Bovins solution, dehydrated in an ethanol series, and embedded in paraffin wax for histological procedure. Liver was cut to obtain representative section of all liver lobules.

Statistical analysis

The data was analyzed using the statistical package for social science program (S.P.S.S.11). The results were expressed as Mean ± S.E.M. (standard error of mean) and % of change- Level of significance between groups were set at P <0.05. For comparison between different experimental groups, one way analysis of variation (ANOVA) was used followed by post hoc Tukey's test.

RESULTS

Histobiochemical assays

Table 1 shows the effect of lead nitrate and *Withania somnifera* root extract either alone or in combination on hepatic biochemical variables.

Table 1: Effect of Lead nitrate and *Withania somnifera* root extract either alone or in combination on some hepatic biochemical variables.

Groups	Treatments (mg/kg body wt)	LPO (n mole MDA/g fresh wet tissue)	SOD (Units/mg protein)	CAT ($\mu mol.H_2O_2$/min/ mg protein)	GST (nmol CDNB formed/min/ mg/ protein	GSH (nmol GSH/gm tissue)	Protein (mg/g fresh wt of tissue)
Control	**-**	98.26±1.33	7.52±0.61	48.99±1.66	202..00±2.39	243.41±2.41	43.24±1.918
$Pb(NO_3)_2$	**20**	196.63±1.48[a]	3.61±0.25[a]	20.42±0.99[a]	111.67±2.44[a]	113.64±1.24[a]	24.01±0.77[b]
WS	**200**	98.98±0.87	7.74±0.20	51.23±0.71	203.96±3.36	265.56±1.14[a]	38.68±0.35
WS	**500**	97.37±1.45	8.12±0.187	51.57±0.95	204.92±3.00	250.28±1.44	40.43±0.40
LN+WS	**20+200**	97.73±1.21*	5.16±0.25****	33.66±0.96*	169.02±1.70*	205.04±1.48*	30.85±0.07**
LN+WS	**20+500**	96.42±1.78*	6.83±0.22 *	43.84±1.28*	188.67±0.97*	262.175±0.89*	38.67±1.47 *

Values are Mean±S.E.M; n= 6; **a***P<0.001 compared to normal animal;* **b***P<0.01 compared to normal animals;* **c***P<0.02 compared to normal animals; *P<0.001 compared to lead exposed animals; **P<0.01 compared to lead exposed animals; ***8P<0.05 compared to lead exposed animals.* ***Abbreviations-*** *LN: Lead nitrate; WS:* Withania somnifera *TBARS: Thiobarbituric acid reactive substances ((n mole MDA/g fresh wet tissue); SOD: Superoxide dismutase ((Units/mg protein)); Catalase ($\mu mol.H2O2/min/mg\ protein$); GST: Glutathione S-transferase (nmol CDNB formed/min/mg/ protein ; GSH: Reduced Glutathione (nmol GSH/gm tissue); Protein (mg/g fresh wt of tissue); Values are Mean± S.E; n= 6.*

Lead exposure induced a significant increase (P<0.001) in hepatic LPO in comparison to untreated animals. However, WS root extract treatment significantly reduced the lead induced increase in LPO level when compared to lead subjected animals. We have also determined some major components involved in the deregulation of substances formed during oxidative stress such as SOD, catalase, GST, and GSH respectively. Conspicuously, (P<0.001) these substances were significantly down regulated by lead treatment, and these effects were largely prevented by WS root extract treatment as compared to group 2 animals.

Histological Results

Histological examination of hepatic tissue section reveals that lead caused a severe inflammatory response of the liver, as indicated by inflammatory cellular infiltration as well as cytoplasmic vacuolation and degeneration of hepatocytes. In addition, the hepatic sinusoids were dilated and apparently contained more kupffer cells as compared to control liver of mice (figure 1).

Treatment of mice with WS largely prevented (figure 2) lead induced histopathological alterations in the liver as indicated by a reduction in inflammatory cellular infiltration and hepatocytic damages (figure 3, 4) when compared to lead treated mice group 2 (figure 5, 6).

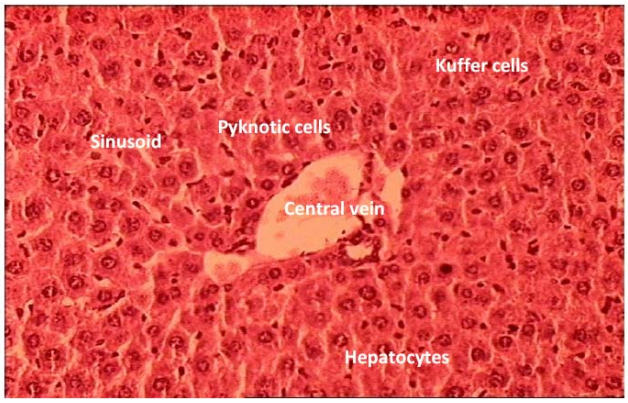

Figure 1: T.S. of the control mice showing radially arranged hepatic cords, sinusoid, pyknotic cells, kuffer cells and normal hepatocytes with centrally located nuclei around the central vein, (400X).

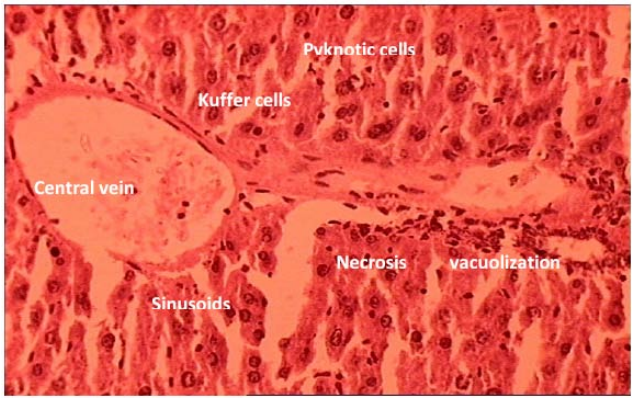

Figure 2: T.S. of the liver of the mice treated with lead nitrate showing congestion of central vein, vacuolization, leucocyti infilteration, pyknotic cells and loss of radial arrangement of hepatocytes (400X).

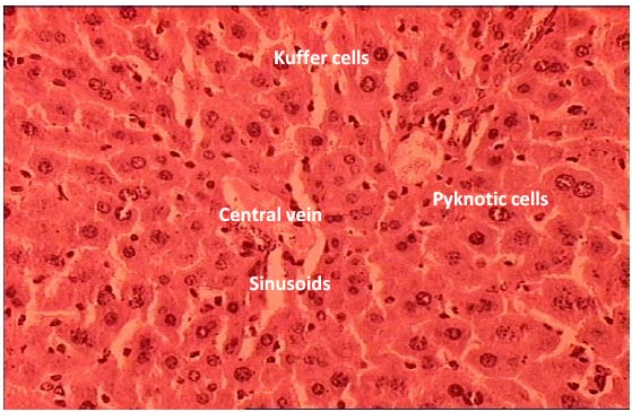

Figure 3: T.S. of liver section obtained from mice treated with *Withania somnifera* root extract (low dose) showing restoration of normal hepatic arrangement of the hepatocytes but central vein appeared congested (400X).

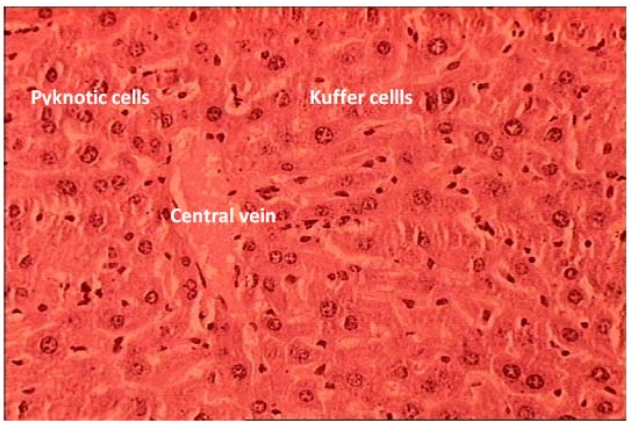

Figure 4: T.S. of liver section obtained from mice treated with *Withania somnifera* root extract (high dose) showing improvement of tissue (400X).

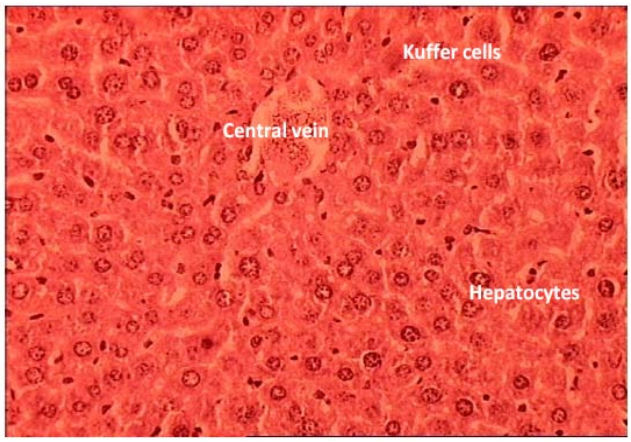

Figure 5: T.S .of liver section obtained from mice treated with lead plus *Withania somnifera* root extract (low dose) showing diminished fibrosis, congestion, incidence of inflammatory cells infiltration, centrolobular hepatocyte swelling, hepatocyte vacuolation, fatty changes and hemorrhagic clots (400X).

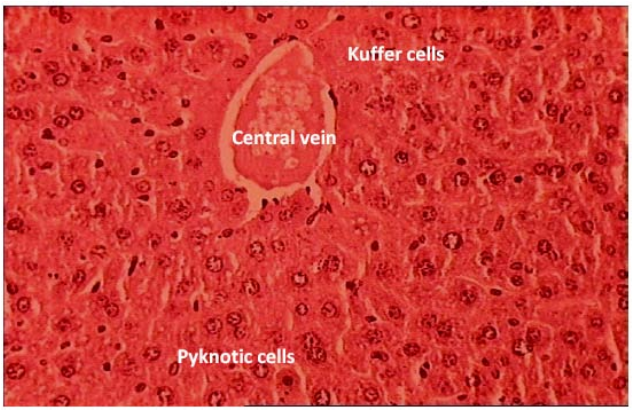

Figure 6: T.S of liver section obtained from mice treated with lead plus *Withania somnifera* root extract (high dose) retained the normal architecture of tissue (400X).

DISCUSSION

Stimulation of lipid peroxidation and depletion of antioxidant reservoirs have been postulated to be major contributors to hepatic tissue damage related to lead exposure[13-16]. In the present study, we showed the protective role of WS root extract on lead induced oxidative stress in hepatic tissue of male mice. Lipid peroxidation is one of the manifestations of oxidative damage and occurs readily in the tissues due to presence of membrane rich polyunsaturated highly oxidizable fatty acids. It was found that LPO induced by lead alter physiological and biochemical characterization of biological system[17].

The improper balance between Reactive oxygen species (ROS) metabolites and antioxidant defense results in "oxidative stress". Participation of iron in Fenton reaction, production of more reactive hydroxyl radicals from superoxide radicals and H_2O_2 results in increased lipid per oxidation[18]. This might be one of the reasons for significant alteration in LPO and significant changes in the activity of antioxidant enzymes.

Antioxidant enzyme levels are applied as markers of oxidative stress. Based on the present study lead induced toxicity might result in decreased tissue activities of enzymatic antioxidants SOD and CAT. The decrease of SOD and CAT activities might predispose the examined tissue of mice to oxidative stress, because these enzymes catalyze the decomposition of ROS. The levels of these antioxidants might provide a clear indication on the extent of cytotoxic damage that occurs in hepatic tissue.

Diminished or inhibition in the activities of these antioxidants upon Pb exposure may led to increased oxidative modifications of cellular membrane and intracellular molecules. The potential mechanism for lead-induced alterations in superoxide dismutase and glutathione peroxidase activities might be inhibition of their functional groups or through binding to their metal enzyme cofactors[19].

Reduced Glutathione (GSH) concentration in the present study suggests the utilization of glutathione by glutathione peroxidase. The GPx catalyses the oxidation of GSH to GSSG and this oxidation reaction occurs at the expense of (H_2O_2). Direct coupling of lead to GSH, which results in the formation of a GSH- lead complex that is subsequently excreted in the bile, has been demonstrated in vivo[20]. Indirect depletion of GSH may occur when lead catalyze the condensation of two molecules of d-amniolevulinic acid (δ-ALA) to prophobilinogen. When the activity of ALAD is impeded, the amount of δ-ALA increase due to the effect of lead exposure which has been confirmed experimentally by several authors[21]. Since δ-ALA itself is known to be a potent inducer of lipid peroxidation (LPO) and ROI formation both in vivo and in vitro, its accumulation may facilitate the depletion of GSH from lead burdened cells.

The decrease in GST activity after the exposure to Pb in the present study could be caused by Pb-induced changes in the enzyme structure as well as by the lack or insufficient amount of GSH, being a substrate for this enzyme[22]. Pb and EtOH-induced decrease in GSH concentration in the liver and kidney may be one of the mechanisms of peroxidative action of Pb and EtOH in these organs. Enzymes, such as GPx, GR and GST, take part in maintaining GSH homeostasis in tissues. The mutual relations between GST and GSH in the redox system, the simultaneous decrease in both GST activity and GSH concentration and the positive correlation between these parameters may suggest that the decrease in the hepatic GSH concentration might result, at least partly, from the decrease in GST activity.

The pathogenesis of lead toxicity is responsible for the depletion of protein observed in the present study. Lead is multifactorial and directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl proteins (interrupting structural protein synthesis), alters calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body.

Administration of Withania somnifera root extract alone had slight effect on LPO, SOD, CAT, GST and GSH activity but no effect of plant extract was seen on protein content. However, treatment with plant root extract in two different doses (200 and 500 mg/kg body weight) along with lead decreased the lipid peroxidation in hepatic tissue as compared with lead treated animals, thus indicating protective role of this plant extract in lead intoxication.

Moreover, elevated levels of the antioxidant enzymes (SOD, CAT and GST) and non-enzymatic potential (GSH), further support the antioxidant role of the root extract. Earlier studies showed that this plant helps in counteracting lead induced oxidative damage[23] and has antioxidant, antiperoxidative and free radical quenching posterities in various diseased conditions.

Hence, the mechanism by which the Withania somnifera exerts a hepatoprotective effect could be attributed to (i) presence of natural antioxidants, (ii) its free radical scavenging and antioxidant properties and (iii) excess removal of urea related compounds. The exact underlying mechanism is still unclear and further research is needed to clarify antioxidant role of this plant. It is thus concluded that hydromethanolic root extract of Withania somnifera may provide protective effect against lead intoxication.

Effects on histology of hepatic tissue

In the present investigation, lead exposure produced pronounced hepatic histopathology as evidenced by histological alternations in liver including focal necrosis with hepatocyte vacuolation, swelling, leucocytic infilteration, pykonotic nuclei, dilation of central vein and sinusoids. These observations are in conformation with Sharma et al[16].

Lead is well known for inducing hepatic injury. The pathological changes may lead to impaired liver function, which interferes with the secretion of plasma proteins. This leads to declined blood osmotic pressure, with subsequent decreased drainage of tissue fluids, which explains the odema and congestion observed in the tissue. Results also showed a remarkable cellular infiltration in the hepatic tissue. Cellular infiltration in the hepatic tissue suggest abundance of leucocytes, in general, and lymphocytes, in particular, that are a prominent response of body tissues facing any injurious impacts.

To some extent both doses of WS extract produced protective effects in this organ against lead toxicity. When WS extract along with lead administered, it retained hepatic architecture and was able to diminish the fibrosis, congestion, hepatocyte vacuolation, swelling, leucocytic infilteration, pykonotic nuclei, dilation of central vein and sinusoids. This might be due to the presence of flavonoids, withanolides and ascorbic acid. Antioxidant potential is claimed to be one of the mechanism of hepatoprotective drug[24].

REFERENCES

1. Mudipalli A. Lead hepatotoxicity and potential health effects. Indian J Med Res 2007; 126: 518-527.
2. Patra RC, Swarup D, Dwidedi SK. Antioxidant effects of α- tocopherol, ascorbic acid and L-methionine on lead-induced oxidative stress of the liver, kidney and brain in rats. Toxicol 2001; 162: 81-8.
3. Sharma V, Sharma S, Pracheta, Paliwal R. *Withania somnifera*: A rejuvenating ayurvedic medicinal herb for the treatment of various human ailments. Int journal of Pharmtech Res2011; 3(1): 187-192.
4. Mishra LC, Singh BB, Dagenais S. Scientific Basis for the Therapeutic Use of *Withania somnifera* (Ashwagandha). Alt Medicine Rev 2000; 5: 4.
5. Plastunov B, Zub S. Lipid peroxidation processes and antioxidant defense under lead intoxication and iodinedeficient in experiment. Anales Universites mariae curie skolodowska Lublin- polonia 2008; 21: 215-17.
6. Utley HC, Bernhein F, Hachslein P. Effect of sulphahydryl reagent on peroxidation in microsome. Arch Biochem Biophys 1967; 260: 521-531.
7. Marklund S, Marklund G. Involvement of Superoxide anion radical in the autooxidation of pyrogallol and convenient assay for SOD. Eur J Biochem 1974; 47: 469-74.
8. Aebi H. Catalase, In. Methods in enzymatic analysis. Bergmeyer H ed. New York . Academic Press 1983; 2: 76-80.
9. Jollow, DJ, Mitchell JR, Zampaglione N, Gillete JR. Bromobenzen-induced liver necrosis. Protective role of gluthione and evidence for 3, 4- bromobenzen oxide as the hepatotoxic metabolite. Pharmacol 1974; 11: 151-169.
10. Habig WH, Pabst MJ, Jakoby WB. The first enzymatic step in mercapturic acid formation. The J Biolo Chem 1974; 219: 7130-39.
11. Lowry OH, Reserbroug N J, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951; 193-65.
12. Mc Manus FA, Mowry RW. Staining methods. In: Hober PB, editor. Histology and Histochemistry. New York: Harper and Brothers; 1965.
13. Sharma A, Sharma V, Kansal L. Therapeutic effects of *Allium sativum* on Lead-induced Biochemical changes in soft tissues of Swiss Albino Mice. Phcog Mag 2009; 5(20): 364-371.
14. Sharma V, Sharma A, Kansal L. The effect of oral administration of *Allium sativum* extracts on lead nitrate induced toxicity in male mice. Food Chem Toxicol 2010; 48: 928-936.
15. Sharma A, Sharma V, Kansal L. Amelioration of lead induced hepatotoxicity by *Allium sativum* extracts in Swiss albino mice. Libyan J Med 2010; 5: Article No. 091107.
16. Sharma V, Pandey D. Protective Role of *Tinospora Cordifolia* against Lead-induced Hepatotoxicity Taxicol Intern 2010;17(1):12-17.
17. Patrick L. Lead toxicity part II: the role of free radical damage and use of antioxidants in the pathology and treatment of lead toxicity. Altern Med Rev 2006; 11: 114-127.
18. Halliwell B. Free radicals, antioxidants and human disease: Curiosity, cause and consequence? Lancet 1994; 344: 721.

19. Hsu PC, Guo YL. Antioxidant nutrients and lead toxicity. Toxicology 2002; 180: 33-44.

20. Christie NJ, Costa M. In vitro assessment of the toxicity of metal compounds. IV. Disposition of metals in cells: interaction with membranes, glutathione, metallothionein, and DNA. Biol Trace Elem Res 1984; 6: 139–158.

21. Gibbs PNB, Gore MG, Jordan PM. Investigation of the effect of metal ions on the reactivity of thiol groups in human 5-aminolevulinic dehydratase. Biochem J 1991; 225: 573–580.

22. Sivaprasad R, Nagaraj M, Varalakshmi P. Combined efficacies of lipoic acid and 2,3-dimercaptosuccinic acid against lead-induced lipid peroxidation in rat liver. J Nutr Biochem 2004; 15: 18–23.

23. Harikrishan B, Subramanian P, Subash S. Effect of *Withania somnifera* Root powder on the levels of Circulatory Lipid peroxidation and liver Markerenzyme in chronic hyperammonemia. E J of Chem 2008; 5: 872-877.

24. Dhuley JN. Adaptogenic and cardioprotective action of ashwagandha in rats and frogs. J Ethanopharmocol 2000; 70(1): 57-63.

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