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The Protective Effect of Ethanolic Extract of Leaves of *Vitex Negundo* Linn., Against Hepato-Toxicity Induced By Lead- Acetate In Swiss Albino Mice

K. Gautam*, P. Agrawal
Department of Zoology, IIS deemed to be university, Jaipur-302020, India
E-mail- gautamkeerti11@gmail.com

ABSTRACT

The present study was carried out to evaluate the hepatoprotective role of ethanolic extract of the leaves of Vitex negundo Linn., a well-known medicinal plant, against lead acetate induced toxicity in swiss albino male mice. Lead acetate was orally administered at a dose of 30mg/kg body weight/animal, according to the lethal dose (1/75th of LD 50) to the mice of experimental groups for the period of 15 days.Lead intoxication resulted in significant increase in the levels of lipid peroxidation, hepatic asprartate aminotransferase (SGOT), alanine aminotransferase (SGPT), tissue glycogen and cholesterol levels. Lead acetate exposure also produced deleterious effects on the redox status of the liver indicated by significant decline in the levels of hepatic antioxidants such as catalase, superoxide dismutase and glutathione. In addition, hepatic total protein levels were also found to be depleted in lead intoxicated group. The treated group that received the ethanolic extract dose i.e 175mg/kg body weight [1/10th of LD50 of Vitex negundo leaves] one hour prior to the administration of lead acetate, showed significant restoration of the altered hepatic parameters, suggesting its hepatoprotective role. Histological examination of theliver conjointly disclosed pathophysiological changes in lead acetate-exposed cluster and treatment with Vitex leaf extract improved the liver microscopic anatomy considerably. Thus, this study clearly advocates the abundance of phytoantioxidants in the leaves of Vitex negundo Linn., and also first time reports its prominent hepatoprotective role against lead acetate toxicity.
Keywords: hepatoprotective, *Vitex negundo*, lethal dose, phytoantioxidants, lead acetate, redox

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INTRODUCTION

Metals play a vital role in the functioning of enzymes, cell-signaling and gene regulation processes. Lead is a hazardous metal, present in both organic and inorganic forms in the environment [1]. Lead poisoning is one of the oldest and the most widely considered occupational and environmental threat [2]. Lead exposure can occur from a multiple source such as soil, air, water and industrial pollutants.It has been widely used in medicines, paintings, pipes, ammunition and in alloys for welding storage materials for chemical reagents [3]. There are worldwide, six categories of products considered as source of lead exposure ,that is gasoline additives, food cane soldering, lead based paints,ceramic glazes, drinking water systems and folk remedies [4].Several studies have reported that lead can cause neurological, hematological, gastrointestinal, reproductive, circulatory and immunological pathologies, based on the duration of time of lead exposure [5, 6]. The centers for Disease Control (UK) have set threshold level of lead in the blood. This is 10µg/dl. for adults and 5µg/dl. for children. Lead may be a non- threshold multi-targeted poisonous that causes alterations in several organs of the body [7,8,9]. It causes corpuscular necrosis and amiloidosis in kidney, also alveolar emphysema, and peribronchitis in lungs [10]. The absorbed lead is conjugated in the liver and passed to the kidney, where a small quantity is excreted in urine and the rest accumulates in various body organs and interferes with their functioning [11, 12]. Liver plays a vital role in detoxification and excretion of many endogenous and exogenous substances [13]. Continuous exposure and intoxication of liver to various kinds of exogenous compounds on a routine might cause viscous pathology [14].

The currently approved treatment for lead intoxication is 'Chelation Therapy', by giving chelating agents such as meso-2,3-dimercapto- succinic acid (DMSA) and monoisoamyl DMSA (MiADMSA), which form an insoluble complex with lead and protect it from biological targets, thereby reducing its toxicity. However, these chelators are potentially toxic and often fail to remove lead from all body tissues. Moreover, because they are hydrophilic or lipophobic, they cannot cross the cell membrane to capture intracellular

lead. Thus, drugs with lipophilic properties are needed. Recent trends in dominant and treating diseases favor natural antioxidants.

Nowadays, there is an increasing interest in discovering the protective biological function of natural compounds contained in plants due to safe use, their antioxidative properties and their possible roles in intra and extracellular defense against oxygen radicals and liquid peroxides in response to oxidative stress [15].

Vitex negundo Linn. belongs to family Verbenaceae (which comprises seventy-five genera and nearly 2500 species), commonly known as 'Five leaved chaste tree (Eng)'. Although, all parts of *V. negundo* are used as drugs within the native system of medicine, the leaves are the foremost potent for healthful use. The decoction of leaves is used for treatment of inflammation, eye-disease, toothache, leucoderma, enlargement of the spleen, ulcers, cancers, catarrhal fever, rheumatoid arthritis, gonorrhea, sinuses, scrofulous sores, bronchitis and as tonics, vermifuge, lactagogue, emmenagogue, antibacterial, antipyretic, antihistaminic, analgesic, insecticidal, ovicidal, feeding deterrence, growth inhibition and morphogenetic agents.

The objective of this study was to evaluate the hepatoprotective potential of ethanolic extracts of leaves of the *Vitex negundo* against lead toxicity in Swiss albino mice.

MATERIAL AND METHODS

Plant collection and authentication:

Leaves of *Vitex negundo* Linn. were collected in the month of September and October 2018 from the western parts of India (Jaipur, Rajasthan). Plant was identified by senior taxonomist at department of Botany, university of Rajasthan and (voucher specimen no: RUBL20838) was submitted to the herbarium, Botany department, university of Rajasthan.

Preparation of Extracts:

Fresh leaves of *Vitex negundo* Linn. plant were collected washed under running tap water and dried under shade then those were grounded to fine powder, and homogenized in 95% ethanol at a ratio of 1:10 of plant to ethanol. The mixture was left to soak for four days at 25ºC with occasional shaking and stirring. Subsequently, the mixture was filtered using a filter paper, and the filtrate was concentrated in a reduced pressure at 45ºC to obtain the extract.

MAINTENANCE OF ANIMAL MODEL:

The adult male swiss albino mice (25-30gm.) were obtained from animal house unit in the Faculty of Zoology, The IIS University, Jaipur. The animals were kept in cages, five mice per cage. Floors of cages were covered with soft crushed wood shaving; all cages were washed two times per week with 70% alcohol throughout the period of the study. The animals were provided with tap water *ad libitum* and were fed with the standard commercial chow. The animals were kept in the animal house of Faculty of Lifescience, The IIS University, Jaipur [IAEC NO.-1689/PO/a/113/CPCSEA] in an air conditioned room with an optimum temperature of 25±2 º C, humidity (60-70%) and light/dark condition (12/12). The animal procedures were performed in accordance with Guide Lines for Ethical Conduct in the care and use of animals.

Experimental Design: After one week of acclimation the animals were randomized and divided into following groups (5 mice each group):

Group A (Control group): was provided with tap water and were fed with normal diet by oral gavage.

Group B (Lead Acetate experimental group): The animals received lead acetate dose according to LD50 i.e. 30mg/kg body weight (1/75th of LD50) by oral gavage once daily for 15 days.

Group C (Lead Acetate treated group co administered by Ethanolic extract of leaves of *Vitex negundo* Linn.) The animals received ethanolic extract i.e 175mg/kg body weight [1/10th of LD50 of *V. negundo*], one hour prior to the administration of lead acetate at the dose of 30mg/kg body weight (1/75th of LD50) by oral gavage once daily for 15 days.

At the end of the experimentation and 24 hrs. after the last dose, all animals were weighed and then were sacrificed under light ether anesthesia. Livers were removed immediately, weighed, rinsed in ice-cold saline, blotted, and used for various biochemical assays and histological studies.

Biochemical assays: Liver was minced and homogenized (10% w/v) in chilled 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 1520 min at 48^{0}C twice to get the enzyme fraction. The supernatant was used for biochemical assays.

Serum Glutamate Pyruvate Transaminase (SGPT)

The SGPT activity was measured by ACCUREX KIT METHOD. The serum and working solution were thoroughly mixed and the assay mixture was immediately transferred to the thermostated cuvette and

the stop watch was started simultaneously. The estimation was done using biochemical analyzer. OD was measured at 340 nm.

Serum Glutamic Oxaloacetate Transaminase (SGOT)

The estimation was done using ACCUREX KIT METHOD. The required amount of working solution was prewarmed at 37^0C before use. The serum and working solution were thoroughly mixed and the assay mixture was immediately transferred to the thermostated cuvette and the stop watch was started simultaneously. Readings was recorded at 340 nm.

GLYCOGEN

Glycogen estimation was done using method given by Rex Montgomery [16].

CHOLESTROL

Cholesterol estimation was done using Zaks method [17].

PROTEIN ESTIMATION

Protein estimation was done using method proposed by Lowry et al., [18].

LIPID PEROXIDATION (LPO)

LPO estimation was done using method given by Ohkawa et al., [19].

SUPEROXIDE DISMUTASE

Estimation was done using method proposed by Markland and Markland [20].

CATALASE

Estimation was done using method proposed by Luck et al., [21].

GSH (GLUTATLHIONE)

Estimation was done using method proposed by Moron et al.,[22]..

RESULTS

Table 1 shows the hepatotoxic effect of lead acetate in experimental group by significantly altered biochemical parameters. Lead acetate exposure was calamitous to the redox status of liver, as evident by a significant increase ($p<0.05$) in LPO level (34.69%), SGPT (141.85%), SGOT (649.57%) as compared to the normal control group(Figure 1). Deleterious effects were also evident with increased tissue glycogen (84.06%), and cholesterol levels (330.13%), in contrast to the control group(Figure 2).Compared to the untreated group total protein levels showed marked decrease (15.09%) within the statistical significant limit ($p<0.05$). Hepatotoxicity also showed determinant decline in the levels of antioxidants like superoxide dismutase (28.99%), catalase (42.53%) and glutathione (24.99%) unlikely to the control group(Figure 3).

In comparison to lead treated experimental group, *Vitex* ethanolic extract fed treated group showed significant restoration of the altered biochemical parameters. The notable decline ($p<0.05$) was observed in the levels of LPO (11.96%), SGPT (59.18%) and SGOT (42.25%) in correspondence with lead intoxicated group (Figure 1). Simultaneously the tissue glycogen and cholesterol levels were also found to be decreased 37.96% and 69.03% within the statistically significant limits($p<0.05$). In contrast to the lead experimental group total protein levels also showed considerable increase i.e. 11.44% suggesting the protective effect of the extract under study (Figure 2).

Presence of antioxidants in the selected plant extract was also confirmed by the significantly increasing levels of different hepatic antioxidant enzymes ($p<0.05$) like superoxide dismutase(20.59%), catalase (33.35%) and GSH (26.19%), analogous to the experimental group (Figure 3).

Table-1: Protective effects of *Vitex* ethanolic extract against lead acetate induced changes in values of different biochemical and oxidative stress parameters in all the three groups

PARAMETER	CONTROL GROUP (1)	EXPERIMENTAL GROUP (2)	TREATED GROUP (3)
	MEAN±SD [%]	MEAN±SD [%]	MEAN±SD [%]
SGPT [IU/L]	0.313 ± 0.045	0.757 ± 0.182 [141.85%]	0.309 ± 0.179 [-59.18%]
SGOT [IU/L]	0.1749 ± 0	1.311 ± 0.247 [649.57%]	0.757 ± 0.100 [-42.25%]
GLYCOGEN [mg/g]	2119.2 ± 189.793	3900.652 ± 549.893 [84.06%]	2419.72 ±404.729 [-37.96%]
CHOLESTEROL[mg/g]	1106.17 ± 42.416	4758.017 ± 217.725 [330.13]	1473.466 ± 457.16 [-69.03%]
PROTEIN [mg/g]	7270.692±717.692	6173.318±1244.137 [-15.09%]	6880.14 ± 1374.65 [11.44%]
LPO [nmol/g tissue]	75.582 ± 9.123	101.803 ± 2.561 [34.69%]	89.619 ± 18.354 [-11.96%]
SOD [U/mg protein]	115.21±3.00	81.81±2.56 [-28.99%]	98.66±6.62 [20.59%]
CATALASE [U/mg protein]	98.67±5.85	56.70±1.21[-42.53%]	75.61±3.31 [33.35%]
GSH [mg/g protein]	742.373 ± 73.391	556.797 ± 17.919 [-24.99%]	702.634 ± 140.331 [26.19%]

Values are expressed as mean ± SD of five animals in each group. n=5SD: standard deviation

$p < 0.05$: significant difference from control% decrease and increase -Group 2 Vs Group 1 and Group 3 Vs Group 2

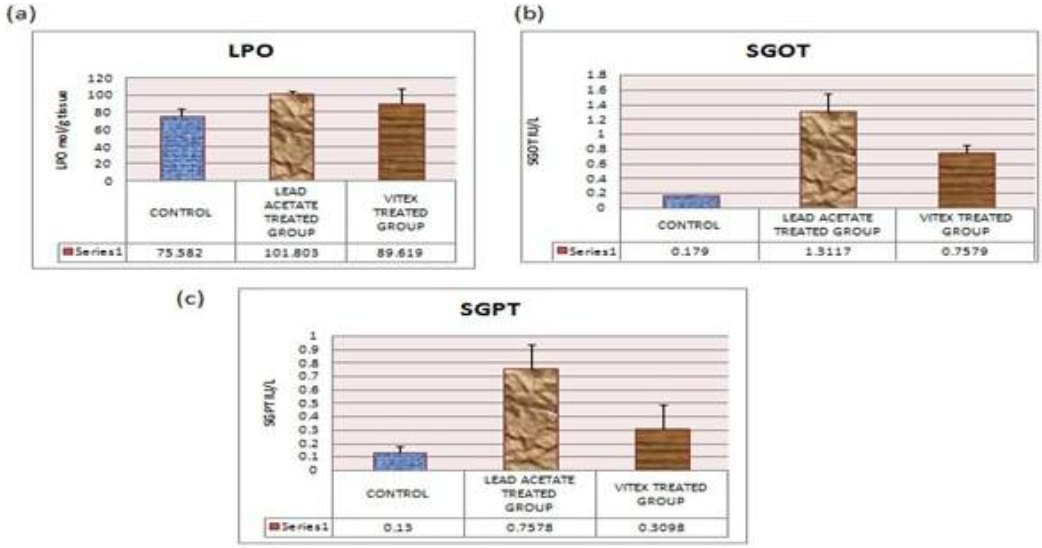

Figure:1 Protectiveeffectof*Vitex*extractsonhepaticoxidativestressrelatedparametersandinlead acetate exposed mice, LPO-lipid peroxidase (mg/g tissue), SGOT-serum glutamate oxaloacetate transaminases (IU/L), SGPT-serum glutamate pyruvate transaminases (IU/L)

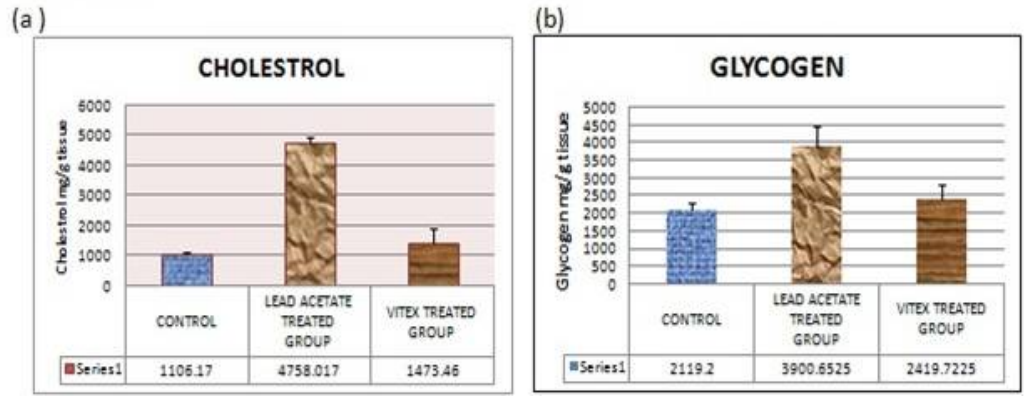

Figure: 2 Protective effect of *Vitex* extracts against lead acetate induced changes in some hepatic biochemical parameters in mice. Cholesterol (mg/g tissue), Glycogen (mg/g tissue)

Figure 3:

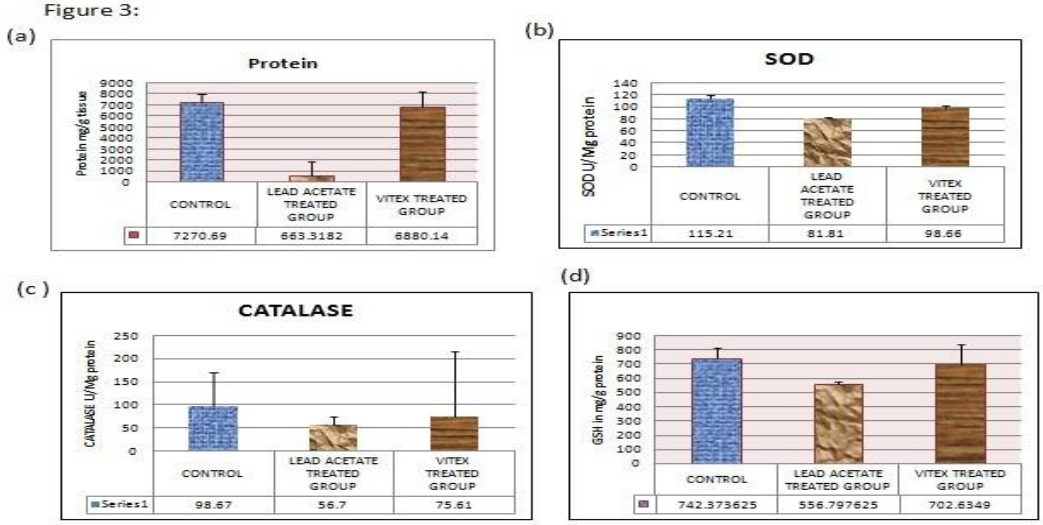

Figure 3: Protective effect of Vitex extracts against lead acetate induced changes in some hepatic biochemical and oxidative stress parameters in mice. Protein (mg/g tissue), SOD (IU/mg protein), Catalase (IU/mg protein), GSH (mg/g protein)

Histological results:

The liver of control mice showed normal hexagonal or pentagonal lobules with semicircular central veins and radially arranged hepatic cords, blood sinusoids in between the hepatic cells and portal triad at the periphery of hepatic lobules (Figure 4).

Lead acetate treated mice showed distortion of the arrangement of parenchyma of the liver, loss of radial arrangement of sinusoids from the central vein of the liver. Marked necrosis of hepatocytes that appeared deeply eosinophilic, binuclear cells and some with pyknotic nuclei when compared with the control. The hepatocytes appeared large with light and foamy cytoplasm filled with numerous vacuole-like spaces. Many hepatic cells were damaged and lost their characteristic appearance. Others showed severe cytoplasmic vacuolation which is so extensive in some cells to the extent that only slight remnants of the cytoplasmic mass were left. Hyper activation of Kupffer cells was observed. There were severe dilatations and congestions of central veins, sinus and portal blood vessels. Some areas showed multifocal to diffuse type of coagulative necrosis. Most of the portal veins appeared congested with inflammatory cells infiltrations (Figure 5).

The liver of lead acetate treated albino mice co-administered with ethanolic extract of *Vitex negundo* Linn. showed marked improvement in its histological structure in comparison to the group treated with lead acetate alone. The central vein appears more or less normal. The liver sections showed the hepatocytes regained their normal organization and architecture with significant decline in fibrosis, congestion, inflammatory cells infiltrations, hepatocytes swelling and hemorrhagic clots (Figure 6).

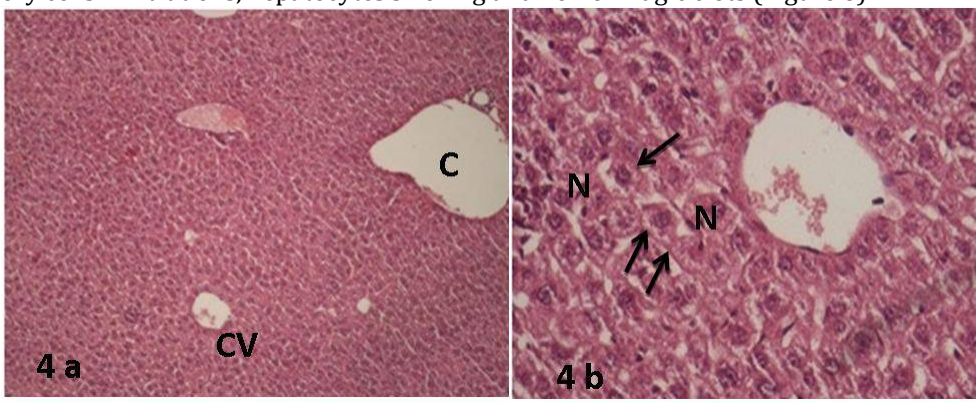

Figure 4: Transverse section of liver showing normal central vein [CV], normal nuclei [N], pentagonal structure of hepatocytes maintained [arrow].4a [x100], 4b [x400]

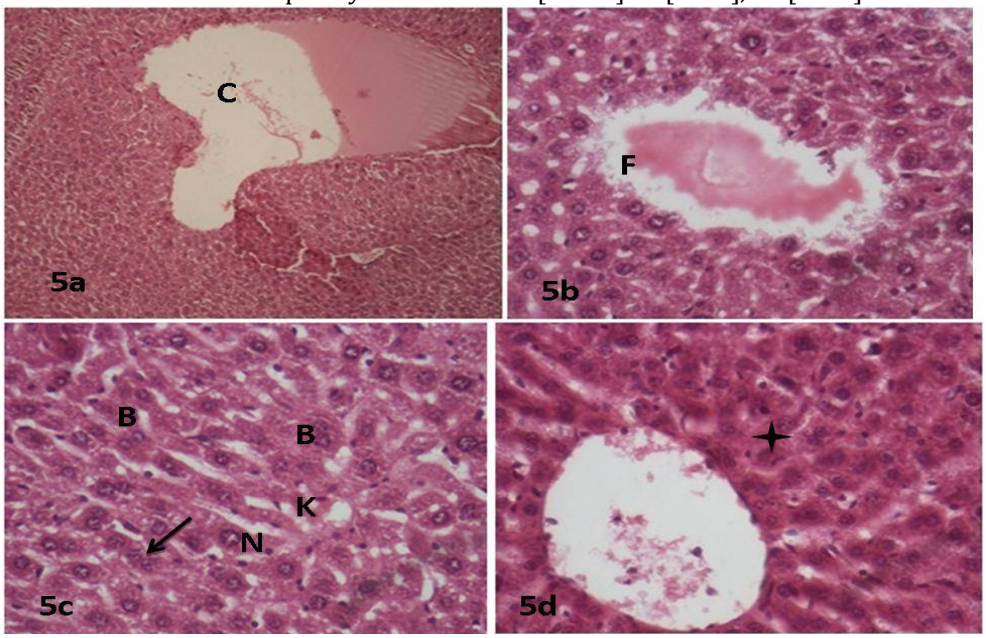

Figure 5:Transverse sections of Liver of mice received lead acetate showed dilated central vein [CV], fibrous tissue infiltration [F], pyknotic nuclei of hepatocytes [N], nuclear degeneration [long arrow], degenerated cells [star], binucleated cell [B], dilated blood sinusoids- Kupffer cells [K] and leucocytes infiltration. Fig 5a [x100], Fig 5b, 5c, 5d [x400].

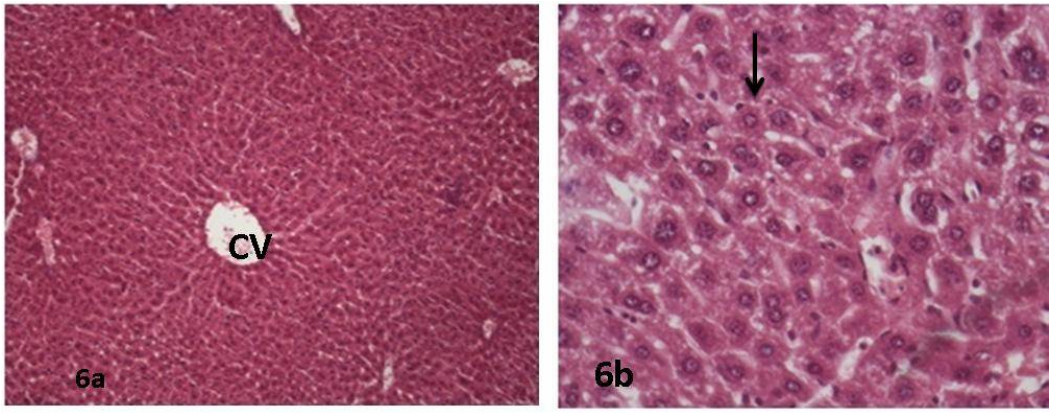

Figure 6: Transverse sections of liver of mice received *Vitex* extract co-administered with lead acetate showed normal central vein [CV], normal nuclei [arrow] and to some extent normal shape of hepatocytes is restored as compared to lead acetate i.e. experimental group. Fig 6a[x100], 6b[x400].

DISCUSSION

One of the most widely occurring elements of earth's crust is Lead. It can show deleterious effects in the form of anemia, hypertension, renal disorders, neurological and developmental defects. In current times, herbal drugs isolated from different medicinal plants having antioxidant properties are attracting the researchers. It has already been found that herbal products can minimize the damaging effects of various free radicals at the cellular levels. The present study is an effort to validate the antioxidant efficacy of*Vitex negundo* against hepatotoxicity of lead.

Present study clearly establishes a significant change in the peroxidation following lead acetate exposure. The increased LPO and decreased cellular antioxidant enzymes CAT, GSH and SOD are rational to the earlier reports [23, 24]. Cells are always ready to combat ROS-induced cellular damage by various antioxidants with different mechanisms. These free radicals produced by lead toxicity damage the cells by either producing singlet oxygen, H_2O_2 or hydroxide radicals or by depleting the antioxidant reserves. The main target of oxidative stress is the cell membrane, which is made up of variety of polyunsaturated fatty acids as components of phospholipids. Lead toxicity majorly results in peroxidation of these membrane constituents resulting in increased LPO levels. Lipid peroxides are produced by chain reaction which later on converts into lethal alkanes, alkenes and ketones etc. LPO also found to negatively affect the cell surface or integral membrane proteins. Previous studies also justify the toxic effects of lead on the various membrane components [25], established a marked increase in MDA concentration after incubation of essential polyunsaturated fatty acids with lead [26]. As per lead can directly or indirectly result in increased LPO levels.

In the present study the activities of SOD, CAT and GSH antioxidants were found to be reduced significantly following the lead acetate exposure that is also evident by increasing levels of lipid peroxidation (LPO). Catalase and superoxide dismutase are the metelloenzymes, which needs specific trace elements as prosthetic groups for their enzymatic activities to encounter the free radicals. Lead pathogenicity can result by multiple mechanisms by either complete enzyme inactivation, competitive inhibition or can bind with free sulfhydryl groups present at the active site of an enzyme. Lead toxicity also divulge GSH depletion that is a tripeptide having reductive sulfhydryl group that can either act non enzymatically by direct interaction of free –SH group with reactive oxygen species, or by enzymatic detoxification of ROS as a coenzyme. Lead decrease the antioxidant activity of GSH by interfering the access of reductive –SH group at the active site by the ROS.

Liver specific transaminases such as SGOT and SGPT are the marker enzymes for hepatic functional integrity. These elevated enzyme levels indicates acute or chronic hepatotoxicity or moderate hepatic injury which is in accordance with [27]. This same factor was also evident in our present study by significant increase in the levels of SGOT and SGPT, followed by lead intoxication which is in agreement with [28]. Lead causes the hepatic cell lysis by inactivating the Ca^{2+} and K^{+} channels, which can disturb the cytoplasmic integrity of the liver resulting in elevation of AST and ALP levels.

Current study also resulted with decrease in total protein levels as a toxic effect of lead, which is in accordance with [29]. The deleterious effects of lead on total protein levels might be due to its effect on DNA and RNA by causing base pair mutation, deletion or free radical attack on the genetic material [1]. More than this lead also disturbs endoplasmic reticulum and its homeostasis, which can result in depletion in total protein levels [30].

The present work also advocates the toxic effects of lead acetate in terms of increased mean values of total hepatic cholesterol levels. This finding is also in agreement with the findings of other researchers like [27], who also demonstrated increase in plasma cholesterol with lead intake. This hepatic hypercholesterolemia may be due to the activation of crucial cholesterol biosynthetic enzyme HMG coA reductase or farnesyl and squalene synthase and simultaneous suppression of critical catabolic enzyme 7-alpha- hydroxylase.

An increase in glycogen content was also observed in lead intoxicated group that may be due to tissue destruction and its effect on enzymatic activities. The increase in glycogen level might be due to more available substrate as a result of tissue damage [31].

Histology:

Liver of the untreated mice showed normal architecture of hepatocytes having polygonal shape, intact cell membrane; normal nuclei with diffused chromatin, hepatic cords separated by sinusoids in between and normally placed hepatic cells.

Where as in lead intoxicated group pronounced histopathological symptoms were found showing necrosis with hepatic vacuolization and swelling, increase in number of binuclear heapatocytes showing cell membrane damage which was also evident by increased LPO levels, dilation of central vein and sinusoids, infiltration of leukocytes and tissue fibrosis. These findings are in support with[1, 32].

***Vitex negundo* leaves ethanolic extract:**

Vitex negundo Linn., is a widely used medicinal plant used for various diseases. It is rich in antioxidants like flavanoids, alkaloids, steroids but its protective effects have never been tested before against lead induced hepatotoxicity.

Results of the study clearly indicate that co administration of *Vitex negundo* leaf extract with lead had partly restored altered levels of antioxidant enzymes like SOD, Catalase and GSH. It had also reduced the levels of lipid peroxidation and serum transaminases AST and ALT, indicating that nirgundi can protect the hepatocellular integrity of the tissue from the toxicity induced by lead acetate.

Total restoration of protein levels of mice treated with *vitex* extract indicated the ability of this plant to stimulate the tissue regeneration. It had also reduced total liver cholesterol and glycogen, which is in agreement with [33], who indicated that possible mechanism of action would be that these extracts might inactivate the reactive thiol groups present at the active site of the crucial rate limiting enzymes for cholesterol biosynthesis.

When nirgundi extract was administered with lead, the liver regained its normal architecture and was able to reduce fibrosis, congestion, chromatin condensation, reduction in number of cells with binuclear stage, depletion in leukocyte infiltration and heapatocytes vacuolization. These results are in accordance with [34], to a great extent.

CONCLUSION

No signs of sickness and mortality were observed in all the three groups, and their food and water intake were found to be quite normal throughout the experiment. Body weight increased constantly from the beginning till the end of experimentation. Histological parameters clearly indicate lead induced necrosis of hepatocytes along with dilation of central vein and loss of radial arrangement of liver sinusoids and hepatic cords whereas, *Vitex negundo* extract was proved to be helpful in restoration of normal architecture of heapatocytes. Administration of lead acetate resulted in significant rise in LPO, SGOT, SGPT, cholesterol, glycogen& depletion in protein, GSH, catalase, SOD. The experimental results counsel the plant *Vitex negundo* Linn., as a hepatoprotectant. However, the mechanism of action and the active compound which is responsible for the actual hepatoprotecivity is not well known.

Vitex negundo extract could be used as an effective herbal product for the prevention of lead-induced hepatic damage. It is believed to be due to its flavanoids content and its antioxidant activity. In the near future, a further study is warranted to isolate, characterize and screen the active components of *Vitex negundo* that have the hepatoprotective activity.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship and publication of this article.

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