

PRELIMINARY EVALUATION OF THE HEPATIC PROTECTION BY PHARMACOLOGICAL PROPERTIES OF THE AQUEOUS EXTRACT OF *ASPARAGUS RACEMOSUS* IN LEAD LOADED SWISS ALBINO MICE

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ABSTRACT

The aqueous extract from the roots of *Asparagus racemosus* (ARRE) was evaluated for lead detoxification from the hepatic tissue by the oral administration route in mice. The toxic effects of lead were studied simultaneously on hepatic biochemical and also on histopathological parameters. The experiment design with thirty-six male Swiss albino mice with the average weight 29 g, were divided into six experimental Groups and each group with six mice. Group I - (Control; Without any treatment), Group II- (Lead nitrate; 20 mg/Kg body weight, orally) Group III- (ARRE; 50mg/Kg body weight, orally) Group IV- (ARRE; 150mg/Kg body weight, orally) Group V- (Lead nitrate; 20 mg/Kg body weight, orally + ARRE; 50mg/Kg body weight, orally) Group VI- (Lead nitrate; 20 mg/Kg body weight, orally + ARRE; 150mg/Kg body weight, orally) respectively treated for 45 days. The effect of long-term administration of lead nitrate and ARRE independently and both in combination on lipid peroxidation (LPO) and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GSH), and glutathione-S-transferase (GST) was studied in hepatic system of Swiss albino mice. The study also concerns on effect of ARRE in lead loaded mice in hepatic enzymes (AST, ALT, and ALP), total and direct bilirubin and albumin level as a marker of liver damage. The results showed the significant hepatoprotective effects of ARRE on lead loaded mice as compared with control to some extent. Lead nitrate administration caused significant depletion of the level of SOD, CAT and reduced GSH, along with the increased level of Lipid peroxidation where as ARRE administration caused significant recovery in all the parameters. AST, ALT, ALP and bilirubin level were also negatively affected in lead treated group and significantly increased in ARRE treated groups. The total protein and albumin level revealed significantly increased in ARRE treated groups and decreased in lead treatment. The hepatic system showed hepatocyte pycnosis, vacuolation, blood congestion and high lymphocytic infiltration around the central vein. Results suggest that beneficial effect of aqueous ARRE may be, probably due to its antioxidant properties.

Keywords: Lead Nitrate, *Asparagus racemosus*, Hepatic system, Oxidative enzymes, Liver markers, Mice.

INTRODUCTION

Lead poisoning is a medical condition, also known as saturnism, plumbism or painter's colic, caused by increased of lead in blood levels, and may cause irreversible neurological damage, hepatic, renal, cardiovascular and reproductive toxicity. The systemic toxic effects of lead in humans have been well-documented by the EPA¹⁻³ and ATSDR⁴. The evidence shows that lead is a multi-targeted toxicant, causing harmful effects on various organ systems in human body. Hepatic damage caused by chronic iron overload⁵ is attributed to heightened production of reactive oxygen species. Normally, the free radical level in the body is low because healthy organisms can neutralize, metabolize, or subtract the toxic effects by free radical scavengers such as superoxide dismutase and catalase⁶. Liver diseases remain as one of the serious health problems. However we do not have satisfactory liver protective drugs in allopathic medical practice for serious liver disorders. Herbal drugs play a role in the management of various liver disorders most of which speed up the natural healing processes of the liver^{7, 8}. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India⁹.

Asparagus racemosus Wild (Asparagaceae), commonly known as Satavari (Hindi) is a perennial shrub, with a tuberous root-stock, stems covered with recurved spines, linear leaves arranged in a tuft, white flowers, sweet-scented appear in October¹⁰. *Asparagus racemosus* is recommended in Ayurvedic texts for prevention and treatment of various human ailments. The decoction of root has been used in blood diseases, diarrhoea, dysentery, cough, bronchitis and general debility¹¹⁻¹³.

Reports indicate that the pharmacological activities of root extracts include antiulcer¹⁴, anti-tussive¹⁵, antioxidant¹⁶ and antibacterial activities¹⁷.

However, till date no work has been reported on the hepatoprotective properties of this plant. Keeping this perspective, the present study was undertaken to investigate hepatoprotective

activity and antioxidant role of the aqueous extract of *Asparagus racemosus* on lead nitrate loaded male mice.

MATERIAL AND METHODS

Chemicals

Lead nitrate was purchased from Central Drug House (India). All other chemicals were of analytical grade and obtained from Sisco Research Laboratories (India), Qualigens (India/Germany), SD Fine Chemicals (India), HIMEDIA (India), and Central Drug House (India).

Plant collection and extraction

The plant *Asparagus racemosus* (Family: Asparagaceae) was collected in the month of October from Krishi Vigyan Kendra of Banasthali University, Rajasthan, India. The plant material was taxonomically identified by a plant taxonomist of our Institute. The roots of the whole plant were thoroughly washed with distilled water, shade dried to get constant weight and cut into small pieces and powdered with a mechanical grinder to obtain a coarse powder. Known quantity (5 g) of the powdered material was extracted using distilled water (500ml) as a solvent. The extract was then filtered through filter paper and concentrated on a water bath at 40-50°C. Finally after complete evaporation of the solvent, the residue (3.089 g) was weighed and stored at 4°C and was used to treat the animals as needed.

Experimental animals

Male Swiss albino mice weighing approximately 15-30 g (2-2.5 months old) were obtained from Harayana Agricultural University, Hissar, India. The Animal Ethics Committee of Banasthali University, Banasthali, India approved the study. All experiments were conducted on adult male albino mice when they weighed 25-35 g (3-4 months old). Thirty six adult male Swiss albino mice were left for 2 week before experimentation to adapt to laboratory conditions. The animals were grouped and housed in polyacrylic cages (38 x23 x10 cm) with not more than six animals per cage and maintained under

standard laboratory conditions (temperature $25^{\circ} \pm 20^{\circ}\text{C}$) with dark and light cycle (12/12 h). Throughout the experiment the animals were provided standard food pellet (Hindustan level Ltd.) and water *ad libitum*. Essential cleanliness and sterile conditions were also maintained.

Experimental design

In the present study 36 adult male Swiss albino mice (*Mus musculus*) weighing 25-30 g (3-4 month old) were used for the hepatic biochemical and histological studies. The groups for each parameter were treated by oral gavage once daily for 45 days as follows.

Group I - Control animals; Untreated or normal group, animals were given normal diet and distilled water.

Group II - Lead Nitrate: (LN); treated group, animals were treated with freshly dissolved $\text{Pb}(\text{NO}_3)_2$ in 1 ml distilled water at a dose of 20 mg/kg body weight/ day.

Group III- *Asparagus racemosus* aqueous root extract: (ARRE-1); treated group, animals were treated with freshly dissolved plant extract in 1 ml distilled water at a dose of 50 mg/ kg body weight/ day.

Group IV- *Asparagus racemosus* aqueous root extract: (ARRE-2); treated group, animals were treated with freshly dissolved plant extract in 1 ml distilled water at a dose of 150 mg/ kg body weight/ day.

Group V - (LN+ARRE-1): treated group, animals were treated with lead nitrate in 1 ml distilled water at a dose of 20 mg/kg body weight/ day + *Asparagus racemosus* aqueous root extract at a dose of 50 mg/ kg body weight/ day (after 60 min).

Group VI- (LN+ARRE-2); treated group, animals were treated with lead nitrate in 1 ml distilled water at a dose of 20 mg/kg body weight/ day + *Asparagus racemosus* aqueous root extract at a dose of 150 mg/ kg body weight/ day (after 60 min).

All these groups of mice i.e. group II to VI served as treated groups against group I as a control. The dose for lead nitrate was decided on the basis of experiments conducted in our own laboratory and the concentration of lead nitrate was decided according to Plastunov and Zub (2008)¹⁸. The plant doses were selected on the basis of experiments conducted in our own laboratory and on the basis of earlier published reports¹⁹.

After the administration of the last dose, animals were given overnight rest and then weighed on the next day. Later on these animals were sacrificed under chloroform anesthesia. The liver lobules were quickly removed, cleaned and washed twice in phosphate buffered saline (PBS, pH 7.4), blotted dried, weighed and stored at 4°C . Liver lobules (3/4th part) sample was subjected for biochemical parameters and remaining liver lobules were used for histological studies.

Preparation of liver homogenates

The weighed liver lobules were homogenized using pestle mortar in 10% ice cold 0.1M potassium phosphate buffer containing 1 mM EDTA, pH 7.4 and centrifuged at 12,000 rpm for 30 minutes at 4°C . The supernatant was collected and immediately used for following biochemical assays.

Biochemical assay

LPO and Antioxidant activity enzymes assay

Estimation of Lipid peroxidation (LPO) level

Lipid peroxidation in the fraction of hepatic tissue was estimated spectrophotometrically by Utley (1967)²⁰ method. In this process 1.0 ml sample was placed in a water bath for 1h at 37°C . After this incubation, added 1 ml TCA (10%) and then 1 ml TBA (0.67%), shaken well and centrifuged at room temperature for 20 min at 5000 rpm. Supernatant was taken and boiled on water bath for 20 min, pink color appeared, cooled in ice for 2 min and then OD was

taken. The absorbance of clear supernatant was measured against reference blank of distilled water at 532nm and 600nm in a spectrometer.

Estimation of SOD level

Hepatic SOD activity was assayed according to the method of Marklund and Marklund (1974)²¹.

For control: Pyrogallol solution (0.1 ml of 20 mM) was added to 2.9 ml of Tris buffer, mixed, and reading was taken at 420 nm after 1.5 and 3.5 mins. The absorbance difference for 2 min was recorded and the concentration of pyrogallol was adjusted in such a way that the rate in change of absorbance per 2 min was approximately 0.020-0.023 optical density units. Liver extract (200 μl) was treated with 10 ml of 25% triton X-100 and kept at 4°C for 30 min.

For sample: Tris buffer (2.8 ml), 0.1 ml of treated sample was added and mixed, and the reaction was initiated by adding 0.1 ml of adjusted pyrogallol solution (as control). Reading was taken at 420 nm after 1.5 and 3.5 mins and the difference in absorbance was recorded. The enzymatic activity was expressed as U/ml of liver extract and 1 U of enzyme is defined as the enzyme activity that inhibits auto-oxidation of pyrogallol by 50%.

Estimation of CAT level

Catalase (CAT) activity was estimated following the method of Aebi (1993)²². Liver extract (100 μl) was treated with ethanol (10 μl) and placed on an ice bath for 30 min. To this, 10 μl of 25% triton X-100 was added and again kept for 30 min on ice. To 200 μl phosphate buffer (0.1 M), 50 μl of treated liver extract and 250 μl of 0.066 M H_2O_2 (prepared in 0.1 M phosphate buffer, pH 7.0) was added in a cuvette. The decrease in optical density was measured at 240 nm for 60 s. The molar extinction coefficient of 43.6 cm^{-1} was used to determine CAT activity. One unit of activity is equal to the moles of H_2O_2 degraded/min/mg protein

Estimation of GSH level

GSH was estimated by a colorimetric method of Jollow (1974)²³. The known homogenate suspension (1ml) was deproteinized by addition of an equal volume of 4% sulphosalicylic acid, kept it at 4°C for 1 h. After centrifugation at 8000 rpm for 15 min at 4°C , resulting supernatant (100 μl) was added in phosphate buffer for appropriate dilution. To this 200 μl Ellman's reagent was added. Without sample test tube and rest assay mixture test tube was used as blank. GSH was preposition to the absorbance at 412nm. The GSH activity is calculated with extinction coefficient of Ellman's reagent.

Estimation of GST level

In brief, GST activity of hepatic tissue was determined by the method of Habig (1974)²⁴. The total reaction mixture contained 1.65ml phosphate buffer, 0.2ml 1mM GSH (in PB), and 50 μl 1mM CDNB. The reaction was initiated by the addition of 100 μl of sample. The absorbance was read at 30s, 1 min, 2min, 3min interval at 340nm. The reaction mixture without the enzyme was used as blank. The specific activity of GST is expressed as μmol of GSH-CDNB conjugate formed $\text{min}^{-1}\text{ mg protein}^{-1}$ using an extinction coefficient of $9.6\text{ mM}^{-1}\text{ cm}^{-1}$.

Cellular metabolic enzymes assay

All cellular enzymatic activities ALT, AST²⁵ and ALP^{26, 27} were estimated with the help of Erba kits, Mannheim, transasia. The ACP level was estimated by the protocol given in practical book of Sadhashivam (1996)²⁸.

Total Protein estimation

The protein content was determined using bovine serum albumin as a standard by the method of Lowry²⁹.

Assessment of Markers of Hepatotoxicity

The other hepatic damage markers (Albumin³⁰ & Bilirubin (Direct and Total)³¹ were also studied with the help of Erba, Mannheim, transasia Kits.

Histopathological/ Histological Study

Microtomy of hepatic tissue was done according to the method of Mc Manus and Mowry (1965)³². Tissue was processed by routine histological techniques. Tissue was sectioned (3-4mm) and stained with hematoxylin & eosin (H & E). Finally, stained sections were examined under the light microscope and subsequently micrographs were taken.

Statistical analysis

Results are expressed as the mean +SEM. The data were analyzed by analysis of variance ANOVA followed by Tukey test using the Statistical Package for the Social Sciences (S.P.S.S. 16). The level of significance was set at $p < 0.05$.

RESULTS

Effect on body weight and organ weight

The total body weight of the mice was increased almost in all groups, except lead treated mice. This increased level was significant ($P < 0.001$) as compared to lead treated mice but this significance was higher in individual administration of plant extract doses (ARRE), rather than co-current treatment. Lead also influenced the weight of liver organ respectively ($P < 0.001$). On the other hand plant treatment improved the hepatic weight in comparison to group II animals (Table-1)

Table 1: Effect of Lead Nitrate on Body weight & hepatic weight (g)

Groups	Initial Body weight (g)	After treatment Final body weight (g)	Hepatic tissue weight (g)
Group I- (Control)	24.37 + 1.32	35.06 + 0.94 ^{†d}	1.97 + 0.04
Group II- (Lead Nitrate); (LN)	25.67 + 0.44	25.55 + 1.37 ^{ns}	1.05 + 0.17 ^{*d}
Group III- (ARRE-1)	24.99 + 0.64	32.53 + 3.76 ^{†d}	1.80 + 0.25 ^{*ns/†d}
Group IV- (ARRE-2)	24.69 + 1.52	32.68 + 2.27 ^{†d}	1.87 + 0.18 ^{*ns/†d}
Group V- (LN+ARRE-1)	24.60 + 0.51	29.11 + 2.19 ^{†d}	1.59 + 0.03 ^{†d}
Group VI- (LN+ARRE-2)	24.53 + 0.16	30.01 + 6.77 ^{†d}	1.76 + 0.21 ^{†d}

Abbreviations: Values are expressed as the mean + SEM for n=6 mice per group. *change with respect to control group I, †change with respect to lead treated group II, ‡change with respect to initial weight. b & d; used to show different levels of statistically significant; d= $p < 0.001$, *ns= not significant change with respect to control group I, †ns= not significant change with respect to toxicant treated group II, ns= not significant change with respect to both group, control group I and toxicant treated group II.

Effect on Lipid peroxidation level, Antioxidant enzymes, glutathione and total protein contents

Lead was also found to significantly ($p < 0.001$) increase the lipid peroxidation (LPO) level in hepatic tissue. Both dose (low & high) of aqueous ARRE significantly ($P < 0.001$) reduced the LPO level in groups III & IV and group V & VI. The difference between both doses was insignificant. The LPO level was comparatively lower in the groups (G IV and G VI), which consumed higher concentration of ARRE in comparison to lower concentration of ARRE dose.

Results as shown in Table 2 indicate significant ($P < 0.001$) decrease in the SOD and CAT levels after administration of lead nitrate in comparison to control group I but the level of both the oxidative enzymes was higher in *Asparagus racemosus* different dose (ARRE 1 and ARRE 2) treated groups (G III, G IV). Interestingly, the enhanced activity of SOD was observed in group III in comparison to group IV and increased CAT activity was observed in group IV rather than

group III, ARRE lower dose treated animals. SOD and CAT activities were also noticed in group V & VI, administered with low & high dose of aqueous ARRE along with lead nitrate. The amplified CAT level was significant ($p < 0.001$) in comparison to lead treated animals. SOD alteration level in group V was observed insignificant whereas group VI achieved significant ($p < 0.02$) alteration in comparison to lead treated animals of group II.

While the GSH and GST contents of hepatic tissues were found to be significantly ($P < 0.001$) decreased in lead treated group, when compared with the control group I. The simultaneous administration of ARRE extract with lead, tried to lower the toxicity values. For the GSH and GST level, ARRE higher concentration of the extract showed much alternative results than lower dose in comparison to lead exposed animals. ARRE was found incapable to change the GST level after simultaneous exposure with lead but both the doses of plant significantly ($P < 0.001$) ameliorated the toxic effects of lead nitrate in reducing the GST level.

Table 2: Effect of aqueous ARRE on hepatic oxidative stress, antioxidant enzymes and reduced glutathione on lead induced toxic damage in Swiss albino male mice

Groups	LPO (nmol/g wet tissue)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (nmol/g wet tissue)	GST (µmol GSH-CDNB conjugate formed/min/mg protein)
Control	101.17 + 0.85	6.84 + 0.34	94.40 + 0.80	4.47 + 0.32	122.74 + 0.99
Lead Nitrate (LN)	241.62 + 1.74 ^{*d}	3.62 + 0.13 ^{*d}	43.61 + 1.77 ^{*d}	2.24 + 0.21 ^{*d}	66.38 + 1.85 ^{*d}
ARRE-1	65.08 + 1.34 ^{†d}	6.18 + 0.29 ^{†d}	88.07 + 0.42 ^{*a/†d}	4.95 + 0.25 ^{†d}	105.02 + 0.50 ^{†d}
ARRE-2	60.63 + 0.70 ^{†d}	5.50 + 0.14 ^{†d}	96.68 + 0.76 ^{†d}	5.10 + 0.14 ^{†d}	108.40 + 0.57 ^{†d}
LN+ ARRE-1	134.57 + 1.51 ^{†d}	4.36 + 0.35 ^{†ns}	68.11 + 2.02 ^{†d}	2.20 + 0.12 ^{*d/†ns}	83.18 + 2.31 ^{†d}
LN+ARRE-2	105.01 + 1.31 ^{†d}	2.44 + 0.14 ^{†b}	73.38 + 0.85 ^{†d}	2.34 + 0.24 ^{*d/†ns}	84.40 + 1.9 ^{†d}

Abbreviations: Values are expressed as the mean + SEM for n=6 mice per group. *change with respect to control group I, †Change with respect to toxicant treated group II, * ns= not significant change with respect to control group I, †ns= not significant change with respect to toxicant treated group II, ns= change with respect to both control group I and toxicant treated group II; a, b, c, d; different letters are used to show different levels of statistically significant. a= $p < 0.01$, b= $p < 0.02$, c= $p < 0.05$, d= $p < 0.001$.

Effect on hepatic indicators

Lead nitrate showed a significant ($p < 0.001$) increment in ALT, AST, ALP, ACP and bilirubin (total & direct) in hepatic markers with the significant ($p < 0.001$) declined level in total protein and albumin levels in comparison to control animals group I. The aqueous root extract of *Asparagus racemosus* significantly ($p < 0.001$) declined the level of AST & ALT in groups III & IV when

compared with control group I and lead treated group II. ALP reduction significant level ($p < 0.01$) was differ from all these alteration in group III in comparison to control animals but found equally significantly reduced ($P < 0.001$) in comparison to lead treated animals of group II.

The administration of ARRE along with lead nitrate significantly ($p < 0.001$) controlled the level of AST, ALT, ALP and ACP activities in

hepatic tissue of animals of groups V & VI when compared to lead exposed animals.

ARRE extract showed significant ($P < 0.001$) increased level of total protein and albumin in group III & IV in comparison to lead treated animals of group II. Only the albumin level was significantly ($P < 0.001$) enhanced after simultaneous exposure with lead in groups V & VI. Lower dose of plant was found ineffectual to recover the protein level with lead simultaneous exposure in group V animals in comparison to both control animals and lead treated animals. The higher dose of plant was found significantly ($P < 0.001$) effective to recover the protein level in simultaneously lead exposed animals of group VI in comparison to lead animals.

The bilirubin (Total and direct) levels were also significantly ($p < 0.001$) reduced in ARRE treated groups III & IV in comparison to group II. The simultaneous administration of ARRE was also found significant ($p < 0.001$) to decline the level of bilirubin (total & direct) in lead exposed animals in groups V & VI.

These observations of significant amendment in the levels of the liver enzymes may indicate that the extract of *A. racemosus* has hepatoprotective effects. This is also corroborated by significant decrease in the levels of total and direct bilirubin. *Asparagus racemosus* showed significant results with all types of assays and tried to make it up to control level to some extent.

Table 3: Effect of aqueous ARRE on hepatic enzymes (AST, ALT and ALP), albumin, and Total & Direct bilirubin on lead induced toxic damage in Swiss albino male mice.

Groups	AST (U/L)	ALT(IU/L)	ALP(U/L)	ACP (U/L)
Control	205.36 + 1.04	175.28 + 0.86	23.62 + 0.59	15.91 + 0.68
Lead Nitrate (LN)	295.02 + 1.39 ^{*d}	262.63 + 0.82 ^{*d}	61.03 + 0.83 ^{*d}	31.55 + 1.96 ^{*d}
ARRE-1	181.62 + 1.63 ^{*d/†d}	163.08 + 0.54 ^{*d/†d}	20.6 + 0.39 ^{*a/†d}	18.45 + 0.33 ^{†d}
ARRE-2	176.81 + 0.75 ^{*d/†d}	154.93 + 0.79 ^{*d/†d}	14.99 + 0.39 ^{*d/†d}	15.40 + 0.13 ^{†d}
LN+ ARRE-1	285.41 + 1.29 ^{†d}	236.06 + 1.23 ^{†d}	46.79 + 0.65 ^{†d}	23.66 + 0.54 ^{†d}
LN+ARRE-2	233.95 + 1.04 ^{†d}	203.86 + 0.71 ^{†d}	41.94 + 0.64 ^{†d}	22.1 + 0.31 ^{†d}

Abbreviations: Values are expressed as the mean + SEM for n=6 mice per group. *change with respect to control group I, †Change with respect to toxicant treated group II. a, b, c, d; different letters are used to show different levels of statistically significant, a= $p < 0.01$, b= $p < 0.02$, c= $p < 0.05$, d= $p < 0.001$, ns= not significant.

Table 4: Effect of aqueous ARRE on some markers of hepatic tissue and total protein content in lead induced toxic damage in Swiss albino male mice.

Groups	Total protein (mg/dl)	Albumin (mg/dl)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
Control	6.04 + 0.79	2.45 + 0.03	1.44 + 0.02	0.225 + 0.013
Lead Nitrate (LN)	3.90 + 0.47 ^{*d}	1.06 + 0.02 ^{*d}	5.85 + 0.03 ^{*d}	0.430 + 0.010 ^{*d}
ARRE-1	7.39 + 0.41 ^{†d}	2.64 + 0.04 ^{†d}	3.46 + 0.03 ^{†d}	0.243 + 0.012 ^{†d}
ARRE-2	8.61 + 0.23 ^{*a/†d}	2.87 + 0.02 ^{†d}	2.54 + 0.01 ^{†d}	0.263 + 0.013 ^{†d}
LN+ ARRE-1	5.66 + 0.46 ^{ns}	1.37 + 0.01 ^{†d}	1.60 + 0.08 ^{†d}	0.266 + 0.008 ^{†d}
LN+ARRE-2	6.65 + 0.37 ^{†d}	1.78 + 0.02 ^{†d}	1.57 + 0.01 ^{†d}	0.253 + 0.013 ^{†d}

Abbreviations: Values are expressed as the mean + SEM for n=6 mice per group. *change with respect to control group I, †Change with respect to toxicant treated group II; *ns= not significant change with respect to control group I, †ns= not significant change with respect to toxicant treated group II, ns= not significant change with respect to both groups control group I and toxicant treated group II; a, b, c, d; different letters are used to show different levels of statistically significant. a= $p < 0.01$, b= $p < 0.02$, c= $p < 0.05$, d= $p < 0.001$, ns= not significant.

Effect of Lead on Histopathological studies

Selected organ tissue sections of liver were prepared from both subjects of control and treated groups. All sections examined under the light microscope are represented by the fig (1-a-f).

The H & E stained sections of treated subject include the presence of more scattered cell and vacuolation in the liver cell texture. Lead also affects the hepatocytes by rupturing the cells. The hepatocytes showed dense lymphatic infiltration near the central vein and dark stained hepatocytic nuclei indicating cell pycnosis (fig-1b). Lead poisoning also showed some other abnormalities as compared to control subjects. The individual administration of ARRE displays mild necrosis and blood pooling near or in the central vein respectively (fig- c, d). Hepatocytes also showed the necrosis and congested blood vessels. The hepatic architecture showed a better potential to retain the normal lobular, artery and bile duct pattern with low blood pooling, recover necrosis, inflammation and hepatocyte vacuolation in simultaneous treatment ARRE doses with lead in group V & VI.(fig:-e-f)

DISCUSSION

Lead is classically a chronic or cumulative toxin. It is an environmentally persistent toxin that causes neurological, hematological, gastrointestinal, reproductive, circulatory, and immunological pathologies³³⁻³⁸. The hepatic and renal damages have been reported in some cases^{35,36}.

In the present study, mice consumed the given amount of lead nitrate showed a significant lower body weight and liver weight. Reduced adipose tissue may be the foremost cause of lower body weight. It may be due to the interruption in absorption and metabolism of feed nutrients essential for health^{37, 38}. The finding of this study with *Asparagus racemosus* on growth performance of body and organ weight revealed a considerable improvement in live body and organs (liver) weight. This study was similar to the observations of Sud³⁹ (1982); Jadhav⁴⁰ (1999) and Rekhate⁴¹ (2010) who recorded body weight gain in experimental model after *Asparagus racemosus* administration.

In the current investigation lead was found to be responsible for high MDA formation after the 45 days of lead exposure. MDA elevated values may be due to free radical generated by irradiation also react with unsaturated lipid generating hydroperoxides, which in turn can induce changes in the lipid bilayer thereby altering the membrane permeability and induced lipid peroxidation. *A. racemosus*, under our protocol, is an effective antioxidant, as it reduced the level of tissue MDA by inhibiting lipid peroxidation caused by free radicals. This observation is in conformation with Visavadiya and Narasimhacharya⁴², 2005; Jain and Agrawal⁴³, 2008. The function of ARRE antioxidant substance systems is not to remove oxidants entirely, but instead to keep the LPO level at an optimum level to some extent. The antimetaltotoxic like activity of aqueous extract of ARRE might be due to saponins like shatavarin I-V.

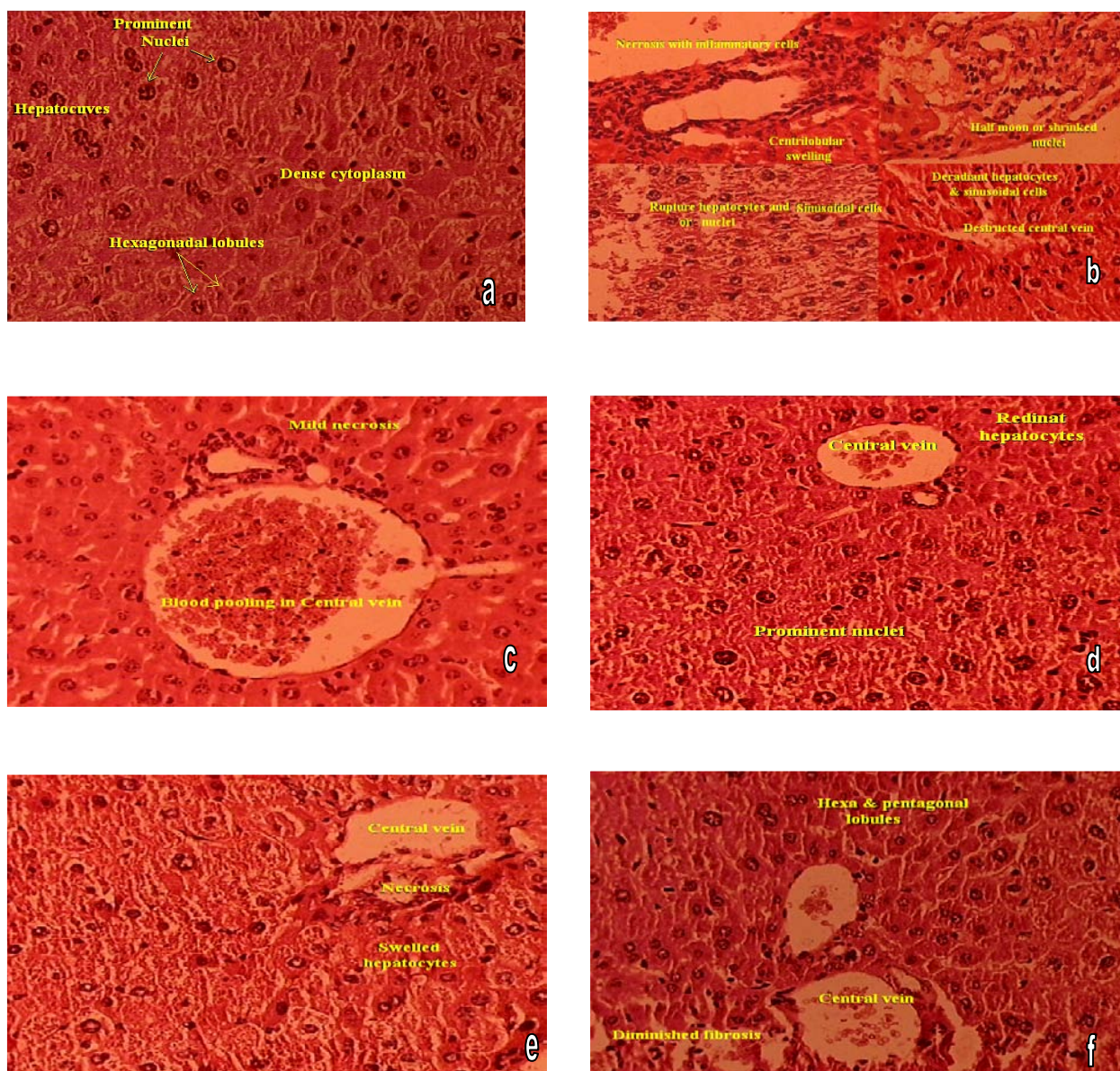


Fig. 1: (a)Control group I- Normal cells; (b) Lead nitrate group II-Rupture hepatocytes (RH) with vacuolation and Dead nuclei (DN), Congested blood vessels with mild infiltration; (c) ARRE-1 group III- Blood pooling and mild necrosis, but reorganized hepatocytes; (d) ARRE-2 group IV- extract produced normal arrangement of the hepatocytes and prominent nuclei but central vein was mild congested; (e) LN+ ARRE-1 group V- recovery in the hepatocyte arrangement and retain central vein and sinusoidal dilation with mild inflammation or disorganization of portal triad; (f) LN+ ARRE-2 group VI- retained hepatic architecture, diminished the fibrosis, necrosis, congestion, inflammatory cells, hepatocyte vacuolation and retain normal structure to some extent (H & E, 150X).

Reactive oxygen species (ROS) are well known suggested as the causative factors in the process of abnormalities and toxicity⁴⁴⁻⁴⁶. SOD and catalase are important enzymes, which project against the free radical injury mediated by O_2^- and H_2O_2 . Low levels of the antioxidant enzymes SOD and CAT in lead treated animals might be due to the overwhelming effects of free radicals, as evidenced by the elevated levels of lipid peroxidation.

According to our results the SOD, CAT, GSH and GST levels were found low as compared to control in lead treated mice. The low levels of the enzymatic antioxidants SOD and CAT in lead nitrate-induced toxic lesions of mice further confirm the state of oxidative stress⁴⁴⁻⁴⁶. The present work shows that the *Asparagus racemosus* treated groups showed higher levels of catalase and superoxide dismutase. These findings are in accordance with Visavadiya^{42, 2005}. The quantitative analysis of *Asparagus racemosus* root powder indicated the presence of flavonoids, polyphenols and ascorbic acid^{47, 48}. It is well known that flavonoids and polyphenols

are natural antioxidants but have also been reported to significantly increase SOD and catalase activities⁴⁷⁻⁵⁴. Further, it was shown that these compounds act as promoters for SOD and catalase⁵² and cause the expression of SOD and catalase⁵⁵. Impaired SOD and CAT activity together with decreased levels of GSH might make the tissue vulnerable to O_2^- toxicity and lipid peroxidation. Also, because GSH is a substrate for GST, it might hamper the detoxifying capabilities and make the system more prone to H_2O_2 toxicity. Oxidative damage produced by lead in mice has also been reported^{56, 57}.

In the present study, administration ARRE was found to significantly restore GSH and antioxidant enzymes suggestive of free radical scavenging activity of ARRE, which in turn may be associated with preventive activity as observed in this study. The currently noted elevated levels of SOD, catalase, GSH and GST with *Asparagus racemosus* root powder could be due to the influence of flavonoids and polyphenols.

Liver cell damage is characterized by a rise in plasma enzymes such as AST, ALT, ALP and ACP level⁵⁸⁻⁶⁰. Heavy metals are responsible to increase the level of both enzymes (ALT & AST)⁶¹. Along with ALT & AST enzymes, ALP and ACP are also used as marker enzymes for liver function and integrity^{59, 60}. The alteration in enzymes level may be due to the damage and dysfunction of the related tissues. ALT activity is only found in increased level of heavy metal toxicity, toxic hepatosis and muscular dystrophy⁶². Lead exposure is able to disturb the lipid-bilayer order of the membrane structure of related organ system. In the present study extract of *Asparagus racemosus* showed a protective effect on hepatic tissue against lead poisoning. AST and ALT content in the hepatic tissue, was decreased significantly after exposure to ARRE at two different aqueous doses. The present results showed that plant both doses have regulating effects on AST and ALT in lead loaded mice. These results are in agreement with the recent study of Jahan⁶³ (2009), in which AR is used in polyherbal formulation. The declined ACP level may be due to lysosomal alteration resulting in the modification of the intact membranes of related tissues⁶⁴. The adjusted level of liver enzymes may be due to the presence of Vitamin-C components in ARRE⁶⁵. Vitamin C has been shown to effectively reduce the hepatotoxicity effect of acute lead poisoning³⁵.

Total protein level is a frequent parameter of metal poisoning in any living organism. Albumin is the protein with the highest concentration in plasma. The present study showed that hepatic albumin and total protein were significantly decreased in mice exposed to lead alone, but on the other hand increased the level of bilirubin (total as well as direct) in the hepatic tissue. These results may be attributed to the great demands and cellular damage that occurred in the tissues of lead-toxicated mice and lead toxicity may be possible cause of protein and albumin breakdown. Bilirubin is a breakdown product of hemoglobin. Bilirubin formed in the reticulo-endothelial system is transported to the liver bound albumin. In the liver, bilirubin is conjugated to glucuronic acid to form direct bilirubin. Conjugated bilirubin is excreted via the biliary system into the intestine where it is metabolized. Total bilirubin is elevated in obstructive conditions of the bile duct, hepatitis, cirrhosis, in hemolytic disorders and several inherited enzyme deficiencies. The extract of *Asparagus racemosus* has a protective effect on hepatic tissue against lead poisoning. AR extract supplementation also caused elevation of albumin level in normal mice and reduced lead toxicity in lead loaded mice. The present work clearly showed that extract of AR has the capacity to reduce the metal ions stress, which may restrict the interaction of the metal ion with membrane lipids, thus avoiding oxidative damage to membrane lipids and proteins. *Asparagus racemosus* showed protective effects on the hepatic tissue, by down regulating the level of bilirubin near to control level in hepatic tissues. The mechanisms behind their action are thought to be the laxative effect of these extracts causing increased intestinal motility and also binding to bilirubin in the GI system which causes a decrease in the enterohepatic circulation, and finally excretion of bilirubin from the GI system and binding to bilirubin in the intestine. A protective effect on the liver and caused an increase in liver enzymes which is thought to be the mechanism behind their serum bilirubin lowering property. Since extract has been reported to contain saponin glycosides⁶⁶⁻⁶⁹ and new antioxidant compound named 'racemofuran' along with two known compounds asparagamine A and racemosol, worked as potential antioxidant⁷⁰. Antioxidants present in *Asparagus* help to protect against several disorders that result from the increased action of free radicals and other harmful substances in the body⁷⁰. This antioxidant effect of ARRE may be beneficial in plumbic detoxification.

CONCLUSION

The findings of present investigation conclude that ARRE treatment partly mitigates lead nitrate-induced changes in hepato-chemical parameters. This could be due to its antioxidant constituents, which combines free radical scavenging with metal chelating properties. The therapeutic effect of ARRE was also confirmed by histological remarks, which suggest that the extract is effective in bringing about functional improvement of hepatocytes. Therefore, it is recommended that the regular intake of *Asparagus racemosus* may

be beneficial in reducing the toxic effects of lead in the exposed populations.

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