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Research article

IN VIVO EVALUATION OF ANTIOXIDANT ACTIVITY OF ALCOHOLIC EXTRACT OF *RUBIA CORDIFOLIA* AND ITS INFLUENCE ON LEAD NITRATE INDUCED DAMAGE TO TISSUE

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ABSTRACT: To evaluate the in vivo antioxidant activity of alcoholic extract of the roots of *Rubia cordifolia* (RC). Male mice, were exposed, either to lead nitrate at a dose containing 40mg/kg body weight or combined with *Rubia cordifolia* for the first period of 40 days, where the animals were sacrificed for oxidative studies and biochemical studies. Lead has induced a significant increase in LPO, where as significant depletion SOD, CAT and GSH in liver and testis tissues. *Rubia cordifolia* alone had moderate effect on mice, where as co-administration of lead nitrate with *Rubia cordifolia* reversed the effect of lead. Ingestion of Pb $(NO_3)_2$ showed significant elevation in AST, ALT, ACP, ALP, and total cholesterol, level in tissue homogenate whereas Total protein content decreased significantly in comparison to control animals. On the other hand Alcoholic extract of *Rubia cordifolia* (low and high dose), along with lead nitrate decreased, elevated levels of AST, ALT, ACP, ALP, and total cholesterol, as compared to lead nitrate intoxicated mice. A significant rise in the level of total protein was also noticed. Preliminary analysis has revealed that *Rubia cordifolia* has significant amount of GSH, Vitamin C, other important antioxidants and polyphenols. In addition it also contains important trace elements like Zn, Cu, Vd, Se and Mo. These contribute to its antioxidant properties

Key words: *Rubia cordifolia*, Antioxidant, liver, testies and Lead Nitrate.

INTRODUCTION

Lead is known to induce a broad range of physiological, biochemical, and behavioural dysfunctions in laboratory animals and humans (Flora *et al*., 2006), including central and peripheral nervous systems, haemopoietic system (Bressler *et al*., 1999), cardiovascular system (Lanphear *et al*., 2000), kidneys (Khalil-Manesh *et al*., 1993), liver (Damek-Poprawa *et al*., 2004), and male (Sharma and Street *et al*., 1980), and female reproductive systems (Lancranjan *et al*., 1975). Lead, however, was reported to have no pro-oxidant catalytic activity with respect to lipid peroxidation (LPO). Yiin and Lin (1995) demonstrated a significant enhancement of malondialdehyde (MDA) when lead was incubated with linoic, linolenic and arachidonic acid. These initial studies for the first time and subsequent studies demonstrated that lead exposed animals showed increased lipid peroxidation or decrease in antioxidant defense mechanism (Bokara *et al*., 2008). Similar effects were shown by Sandhir and Gill (1995) in liver of lead exposed rats. Although the mechanism by which lead induces oxidative stress is not fully understood, a large number of evidences indicate that multiple mechanisms may be involved.

The antioxidant enzymes SOD, catalase and GPx are potential targets of lead. Selenium is essential for GPx activity, and lead forms a complex with selenium, thereby decreases its activity (Whanger *et al*., 1992). Inhibition of heme synthesis by lead is well reported and since CAT is a heme-containing enzyme, its activity decreases (Mylroie *et al*., 1984). SOD requires copper and zinc for its activity. Copper ions play functional role in the reaction by undergoing alternate oxidation whereas zinc ions seem to stabilize the enzyme (Halliwell and Gutteridge, 1989). Both the metal ions are replaced by lead, which decreases the activity of SOD. Overall, these inhibitory effects of lead on various enzymes would probably result in impaired antioxidant defense by cells and render cells more vulnerable to oxidative attacks.

Several chelating agents have been used to reduce the burden of the toxic effect of lead, but these have also produced a toxic potential themselves. This has necessitated researches into the therapeutic potential of various medicinal plants and herbs (Baldwin *et al*., 1999).

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Rubia cordifolia (Rubiaceae) also known as 'manjistha' is an important medicinal plant, which is used for treatment of various ailments such as anti-tumor (Karita *et al*., 2005), anti-inflammatory (Pande *et al*., 2002), urinary disorders (Senapati *et al*., 2001), antistress antimicrobial (Singh *et al*., 2005), Hepatoprotective (Rao *et al*., 2006), radio protective (Tripathi *et al*., 2007) and anticancer (Son *et al*., 2008).This plant has also been listed officially as herbal medicine in the Chinese Pharmacopeia for the treatment of arthritis, dysmenorrhea, hematorrhea and hemostasis, which are free radical related diseases. Apart from its medicinal value, this plant has also been used as natural food colorants and as natural hair dye (Gao *et al*., 2000).The interest in the isolation of natural and coloring matters is increasing due to their use in food, drugs and other human consumptions.
The objective of this study, therefore, is to evaluate the protective role of manjistha supplemented against lead toxicity in male mice.

MATERIALS AND METHODS

Material

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).
Animals: Male Swiss albino mice weighing 15–30 g (2–2.5 months) were obtained from Haryana 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). They were housed in polypropylene cages in an air-conditioned room at 253C, relative humidity of 505% and 12-h alternating light and dark cycles. The mice were provided with chow diet (Hindustan Lever Limited, India) and drinking water ad libitum.

Experimental Plant Material

Rubia cordifolia (Linn.) belonging to family Rubiaceae is a well known ayurvedic herb popularly known as Indian Madder (English), manjeshta (Marathi), majit or manjit (Hindi), manjishtha, aruna, chitra, raktaangi, manjusha (Sanskrit) manjeeth iraani (Unani), manjitti (Siddha) (Kirtikar *et al*., 1980; Khare *et al*., 2004). The roots of the plant *Rubia cordifolia* were procured from the local market of Jaipur and are identified by a plant taxonomist/ botanist of our department.

Preparation of Plant Extract

About 200 g of powdered roots (dry) were extracted with ethanol (95%) using Soxhlet apparatus for 4-6 hours. Alcohol removal carried out in vacuum oven (45 ^{O}C) afforded a semi solid mass with a yield of 9%.

Procedure and design of experiments

In the present study 36 adult male Swiss albino mice (Mus musculus L.) weighing 25-30 g (3 to 4 months old) was used for oxidative stress parameters and biochemical analysis.
For Oxidative stress studies, 36 mice were divided into 6 groups of 6 mice each.
The groups for each parameter were treated by oral gavage once, daily as follows:
Group 1, received 1 ml distilled water by oral gavage; served as control,
Group 2, received 40 mg/ kg body weight/ day lead nitrate dissolved in distilled water by o\ral gavage for 40 days.
Groups 3 and Group 4 were administered with alcoholic root extract of *Rubia cordifolia* at the doses of 50 mg/ kg body weight and 100 mg/ kg body weight, respectively, by oral gavage once daily for 40 days to the end of the experiment.
Groups 5 and Group 6 were administered with alcoholic root extract of *Rubia cordifolia* at the doses of 50 mg/ kg body weight and 100 mg/ kg body weight with lead nitrate at 40 mg/kg simultaneously, by oral gavage once daily for 40 days to the end of the experiment.
The dose for lead nitrate was decided on the basis of experiments conducted in our laboratory and the concentration of lead nitrate used in the experiment was 1/ 56 of LD_{50} (Plastunov and Zub, 2008). The plant doses were selected on the basis of experiments conducted in our laboratory and on the basis of earlier published report, suggesting that *Rubia cordifolia* is having prophylactic efficacy (Joharapurkar *et al*., 2004).

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After the administration of the last dose, the animals were given rest overnight and then on the next day, they were sacrificed under light ether anesthesia. The organs liver and testis were excised, cleaned and washed with ice cold saline (pH-7.4), blotted and used for some oxidative stress parameters and biochemical analysis.

Oxidative stress and antioxidant defense related parameters

Liver and testis were minced and homogenized (10 % w/ v) in ice-cold 0.1 M sodium phosphate buffer (pH-7.4). The homogenate was centrifuged at 10,000 rpm for 15-20 min at 4 °C twice to get enzyme fraction. The resultant supernatant was used for various oxidative related parameters.

Lipid peroxidation (LPO) was estimated colorimetrically by measuring malondialdehyde (MDA) as described by Nwanjo and Ojiako (2005). Superoxide dismutase (SOD) activity was measured by the method suggested by Marklund and Marklund (1974). Catalase activity in tissues was assayed following the procedure of Aebi (1983). Reduced glutathione (GSH) was determined by the method of Ellman (1959). Aspartate amino transaminase (AST) and Alanine aminotransferase (ALT) Activity was assayed by the method of Reitman and Frankel (1957). Activities of acid phosphatase (ACP) and alkaline phosphatase (ALP) were determined according to the protocol described in laboratory practical manual (Sadashivam, 1986). The protein content was determined by using bovine serum albumin (BSA) as a standard by the method of Lowry *et al.* (1951). The cholesterol level was determined by using cholesterol as a standard by the method of Zak's *et al.* (1977).

Statistical Analysis

Data 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. 11). The level of significance was set at $p<0.05$.

RESULTS

Hepatic oxidative stress and antioxidant defense related parameters

Effect of Lead nitrate, *Rubia cordifolia alcoholic* root extract either individually or in combination on lipid peroxidation and antioxidant defense related parameters in the hepatic tissue were observed and are presented in Table 1.

Table 1- Lead induced changes in some hepatic oxidative stress related parameters and their response to administration of alcoholic root extract of *Rubia cordifolia* in mice.

Parameters	Control (Normal, Untreated) (Distill water) Group 1	Lead nitrate ($Pb(NO_3)_2$, 40mg/kg body weight) Group 2	Alcoholic root extract of *Rubia cordifolia* (RC)		Lead nitrate ($Pb(NO_3)_2$, 40 mg/kg body weight) + alcoholic root extract of *Rubia cordifolia*(RC)	
			RC (50 mg/kg body weight) Group 3	RC (100 mg/kg body weight) Group 4	Lead nitrate + RC (50 mg/kg body weight) Group 5	Lead nitrate + RC (100 mg/kg body weight) Group 6
LPO	111.06±0.56	181.003±0.51* (62.98%)	109.006±0.52 (-1.85%)	106.04±0.50 (-4.52%)	135.42±0.51[a] (-25.18%)	128.01±0.51[a] (-29.28%)
SOD	1.138±0.04a	0.72±0.007* (-36.73%)	1.16±0.05 (1.93%)	1.143±0.02 (0.44%)	0.84±0.003[b] (22.78%)	1.15±0.03[a] (59.72%)
CAT	78.02±0.50	34.53±0.52* (-55.74%)	78.03±0.55 (0.01%)	78.18±0.52 (0.21%)	66.07±0.56[a] (91.34%)	70.38±0.54[a] (103.82%)
GSH	7.40±0.52	1.26±0.53* (-82.97%)	7.415±0.53 (0.20%)	7.59±0.54 (2.57%)	5.48±0.52[a] (334.92%)	6.646±0.56[a] (427.96%)

Abbreviations- LPO: Lipid peroxidation (nmole of MDA formed/ g of tissue): **SOD:** Superoxide dismutase (unit/ ml of tissue homogenate): **CAT:** Catalase (µmoles of H_2O_2 degraded/ min/ mg protein): **GSH:** Reduced Glutathione (mg/ g of tissue)

Values are Mean ± S.E.M.: n= 6 [*]$P< 0.001$ compared to normal animals. [a]$P< 0.001$ compared to lead nitrate exposed animals.

[b]$P< 0.01$ compared to lead nitrate exposed animals

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Lead nitrate exposure produced detrimental effects on the redox status of liver, which is evidenced by a significant (p<0.001), increase in lipid peroxidation level, along with significant depletion (p<0.001), in superoxide dismutase, and catalase activity, and glutathione content, in group 2, when compared with untreated animals (control).

Alcoholic extract of roots of *Rubia cordifolia* at 50 and 100 mg/kg body weight had moderate (not significant), effect on LPO, SOD, CAT, and GSH, respectively, as compared to normal group mice i.e. group 1.

Co-administration of alcoholic root extract of *Rubia cordifolia* at a dose of 50 mg/ kg body weight and 100 mg/ kg body weight along with lead nitrate in group 5 and 6 significantly (p<0.001), decreased lipid peroxidation in liver, respectively, in comparison to lead control group. CAT, and GSH, activities were raised significantly (p<0.001), at low and high dose of alcoholic root extract of *Rubia cordifolia,* respectively, when compared with lead nitrate administered mice. Alcoholic root extract of *Rubia cordifolia administration* in group 5 and 6 also showed significant (p<0.001), augmentation in SOD, respectively, in comparison to lead nitrate treated group

Hepatic biochemical variables

Effect of Lead nitrate, *Rubia cordifolia alcoholic* root extract either individually or in combination on some hepatic biochemical variables was recorded or is reported in Table 2.

Table 2- Lead induced changes in some hepatic biochemical parameters and their response to administration of alcoholic root extract of *Rubia cordifolia* in mice.

Parameters	Control (Normal, Untreated) (Distill water) Group 1	Lead nitrate ($Pb(NO_3)_2$, 40mg/kg body weight) Group 2	Alcoholic root extract of *Rubia cordifolia* (RC)		ad nitrate ($Pb(NO_3)_2$, 40 mg/kg body weight) + alcoholic root extract of *Rubia cordifolia*(RC)	
			RC (50 mg/kg body weight) Group 3	RC (100 mg/kg body weight) Group 4	Lead nitrate + RC (50 mg/kg body weight) Group 5	Lead nitrate + RC (100 mg/kg body weight) Group 6
AST	33.11±0.46	66.47±0.52* (100.76%)	32.94±0.51 (-0.51%)	29.92±0.54 (-9.63%)	41.63±0.64ª (-25.73%)	38.001±0.49ª (-14.77%)
ALT	48.46±0.54	71.63±2.86* (47.81%)	48.17±0.65 (-0.60%)	47.87±0.77 (-1.22%)	61.52±0.89ª (-14.11%)	52.18±0.64ª (-27.15%)
ACP	12.41±0.60	37.07±0.53* (198.71%)	11.97±0.53 (-3.55%)	11.85±0.56 (-4.51%)	23.77±0.67ª (-35.88%)	14.09±0.69ª (-61.99%)
ALP	32.04±0.53	68.83±0.41* (114.83%)	32.45±0.55 (+1.28%)	31.44±0.57 (-1.87%)	47.43±0.57ª (-31.09%)	42.03±0.52ª (-38.94%)
TC	24.07±0.46	49.02±0.46* (103.66%)	24.1±0.58 (+0.12%)	23.30±0.56 (-3.20%)	31.13±0.34ª (-36.50%)	29.03±0.53ª (-40.78%)
TP	8.52±0.52	5.30±0.59* (-37.79%)	8.54±0.51 (0.23%)	8.56±0.51 (0.47%)	6.16±0.72 (16.23%)	7.35±0.52 (38.68%)

Abbreviations- AST: Aspartate transaminase (IU/ L): **ALT:** Alanine transaminase (IU/ L): **ACP:** Acid phosphatase (µmoles of PNP formed/min/g tissue): **ALP:** Alkaline phosphatase (µmoles of PNP formed/min/g tissue): **Total protein** (g/ dl): **Total cholesterol** (mg/ g of tissue)

Values are Mean ± S.E.M.: n= 6 *P< 0.001 compared to normal animals. ªP< 0.001 compared to lead nitrate exposed animals.

In the present study, administration of lead nitrate (40 mg/ kg body weight), alone significantly increased (p<0.001), AST, ALT, ACP, ALP, and total cholesterol, activities in the mice as compared to control animals. However, lead nitrate administration significantly decreased (p<0.01), total protein content, when compared with control group. *Rubia cordifolia* root extract at both low and high dose produced moderate but insignificant effect on AST, ALT, ACP, and on total protein content. No effect of *Rubia cordifolia root* extract at low dose was seen on ALP and total cholesterol, but plants high dose caused slight insignificant increase in ALP and total cholesterol level as compared to control.

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Co-administration of alcoholic root extract of *Rubia cordifolia* at a dose of 50 mg/ kg body weight and 100 mg/ kg body weight, significantly (p<0.001), prevented the augmentation of AST, ALT, ACP, ALP, and total cholesterol, respectively, as compared to lead nitrate exposed mice. Protein content in liver was insignificantly increased, as a result of administration of 50 mg/ kg body weight and 100 mg/ kg body weight alcoholic extract of *Rubia cordifolia with* lead nitrate, respectively, when compared with lead nitrated exposed animals.

Testis oxidative stress and antioxidant defense parameters

Effect of Lead nitrate, *Rubia cordifolia* alcoholic root extract either individually or in combination on lipid peroxidation and antioxidant defense related parameters in the testis tissue were noticed and are shown in Table 3.

Table 3- Lead induced changes in some testicular oxidative stress related parameters and their response to administration of alcoholic root extract of *Rubia cordifolia* in mice.

Parameters	Control (Normal, Untreated) (Distill water) Group 1	Lead nitrate ($Pb(NO_3)_2$, 40mg/kg body weight) Group 2	Alcoholic root extract of *Rubia cordifolia* (RC)		Lead nitrate ($Pb(NO_3)_2$, 40 mg/kg body weight) + alcoholic root extract of *Rubia cordifolia*(RC)	
			RC (50 mg/kg body weight) Group 3	RC (100 mg/kg body weight) Group 4	Lead nitrate + RC (50 mg/kg body weight) Group 5	Lead nitrate + RC (100 mg/kg body weight) Group 6
LPO	87.04±0.47	134.03±0.51* (53.99%)	86.91±0.52 (-0.15%)	87.25±0.55 (+0.24%)	124.01±0.40[a] (-7.48%)	99.02±2.33[a] (-26.12%)
SOD	1.12±0.03	0.89±0.02* (-20.54%)	1.17±0.05 (4.46%)	1.129±0.03 (5.13%)	0.99±0.0006 (11.24%)	0.99±0.001 (11.24%)
CAT	62.01±0.54	20.41±0.55* (-67.09%)	62.02±0.48 (0.02%)	62.13±0.52 (0.19%)	36.38±0.59[a] (78.25%)	30.11±0.52[a] (47.53%)
GSH	2.39±0.36	1.34±0.57 (-43.93%)	2.43±0.48 (1.67%)	2.56±0.57 (7.11%)	1.67±0.42 (24.63%)	2.03±0.47 (51.49%)

Abbreviations- LPO: Lipid peroxidation (nmole of MDA formed/ g of tissue): **SOD:** Superoxide dismutase (unit/ ml of tissue homogenate): **CAT:** Catalase (µmoles of H_2O_2 degraded/ min/ mg protein): **GSH:** Reduced Glutathione (mg/ g of tissue)
Values are Mean ± S.E.M.: n= 6.
[*] P< 0.001 compared to normal animals.
[a] P< 0.001 compared to lead nitrate exposed animals.

A significant (p<0.001), elevation in lipid peroxidation level, and signification depletion in SOD, and CAT, activities were evident in mice ingested lead nitrate at a dose of 40 mg/ kg body weight, compared to untreated mice. A slight fall in GSH activity was also observed in lead treated group, as compared to normal control values. Alcoholic extract of roots of *Rubia cordifolia at* 50 and 100 mg/kg body weight had moderate but insignificant, effect on SOD, CAT, and GSH, respectively, as compared to normal group mice i.e. group 1. Slight but insignificant decline in LPO level was also observed with low dose of root extract of *Rubia cordifolia where* as plants high dose was found to be ineffective in regulating the LPO level compared to control animal.
Administration of alcoholic extract of *Rubia cordifolia* at low and high dose along with Pb $(NO_3)_2$ resulted in significant reduction (p<0.001), in the TBA reactive product, and significant elevation (p<0.001), in the activities of CAT. However in group 5 and 6, GSH and SOD content increased insignificantly, respectively, as compared to control animal.

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Testis biochemical variables

Effect of Lead nitrate, *Rubia cordifolia alcoholic* root extract either individually or in combination on testis biochemical variables were observed and are reported in Table 4.

In comparison to normal control mice, significant (p<0.001), increase in the activities of marker enzymes such as AST, ALT, ACP, ALP, and total cholesterol, were recorded in lead nitrate exposed mice. Also insignificant decrease in the total protein level, followed by lead nitrate exposure was noticed in group 2 when compared with control animals.

Table 4- Lead induced changes in some Testicular biochemical parameters and their response to administration of alcoholic root extract of *Rubia cordifolia* in mice.

Parameters	Control (Normal, Untreated) (Distill water) Group 1	Lead nitrate ($Pb(NO_3)_2$, 40mg/kg body weight) Group 2	Alcoholic root extract of *Rubia cordifolia* (RC)		Lead nitrate ($Pb(NO_3)_2$, 40 mg/kg body weight) + alcoholic root extract of *Rubia cordifolia*(RC)	
			RC (50 mg/kg body weight) Group 3	RC (100 mg/kg body weight) Group 4	Lead nitrate + RC (50 mg/kg body weight) Group 5	Lead nitrate + RC (100 mg/kg body weight) Group 6
AST	15.23±0.50	48.16±0.53* (216.22%)	15.29±0.52 (+0.39%)	15.20±0.43 (-0.20%)	37.05±0.56ª (-23.07%)	30.55±0.73ª (-36.57%)
ALT	10.59±0.57	24.001±0.76* (126.64%)	8.50±0.54 (-19.74%)	9.82±0.54 (-7.27%)	16.10±0.54ª (-32.92%)	13.003±0.52ª (-45.82%
ACP	10.26±0.65	32.16±0.61* (213.45%)	10.23±0.52 (-0.29%)	10.13±0.38 (-1.27%)	24.22±0.60ª (-24.69%)	15.05±0.58ª (-53.20%)
ALP	11.5±0.71	28.19±0.54* (145.13%)	11.55±0.62 (+0.43%)	11.44±0.65 (+0.52%)	24.14±0.41ª (-14.37%)	19.95±0.40ª (-29.23%)
TC	32.53±0.56	41.87±0.64* (28.71%)	33.67±0.55 (+3.50%)	33.09±0.55 (+1.72%)	38.91±0.57ª (-7.07%)	36.72±0.47ª (-12.30%)
TP	1.310±0.46	0.76±0.51 (-41.98%)	1.30±0.44 (-0.76%)	1.32±0.45 (+0.76%)	1.06±0.44 (39.47%)	1.13±0.45 (48.68%)

Abbreviations- AST: Aspartate transaminase (IU/ L): **ALT:** Alanine transaminase (IU/ L): **ACP:** Acid phosphatase (µmoles of PNP formed/min/g tissue): **ALP:** Alkaline phosphatase (µmoles of PNP formed/min/g tissue): **Total cholesterol** (mg/ g of tissue)**:Total protein** (g/ dl):

Values are Mean ± S.E.M.: n= 6

*P< 0.001 compared to normal animals.

ªP< 0.001 compared to lead nitrate exposed animals.

Rubia cordifolia root extract at both low and high dose produced moderate (not significant), effect on ALT and ACP. No effect of *Rubia cordifolia root* extract low dose was observed on AST and ALP whereas on other hand plants high dose resulted in insignificant decrease in both parameters AST and ALP. It was also noticed that administration of *Rubia cordifolia* root extract at both dose caused no effect on total cholesterol level, but total protein content increased insignificant at high dose and decreased insignificantly at low dose of *Rubia cordifolia* root extract as compared to normal animals.

Simultaneous administration of lead nitrate with low and high dose of alcoholic root extract of *Rubia cordifolia significantly* (p<0.001), reduced the levels of AST, ALP, ACP, ALT, total cholesterol, respectively, as compared to lead nitrate control group. Total protein level recovered insignificantly in response to 50 and 100 mg/ kg body weight of alcoholic extract of *Rubia cordifolia,* when compared with lead administered mice.

DISCUSSION

Effect of lead on oxidative stress in Liver and Testis

Changes in oxidative stress parameters by lead nitrate and *Rubia cordifolia* either individually or in combination are presented in Table 1 and 3.

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Result indicates a marked alteration in the peroxidative process following lead nitrate exposure. The increase in LPO and decrease in SOD, CAT, and GSH in various tissues by lead nitrate are observed in the present study.

These results are in agreement with earlier reports (Shukla *et al*., 2003).

Lead treatment result in enhancement of lipid peroxidation in mice, which is directly related to free radical mediated toxicity. The target of oxidative damage is usually critical biomolecules such as nucleic acid, proteins, and lipids (Gutteridge and Halliwell 1990). Lead absorbed to blood and tissues produces highly reactive species, such as superoxide radical ($^2O-\bullet$), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH) and lipid peroxides (LPO). Lipid peroxidation (LPO) is a deleterious process that involves continuous fragmentation of membrane lipids (Comporti *et al*., 1985) and, very often, heavy metal toxicity has been associated with this process (Chaurasia *et al*., 1996b). The lipid peroxides, in turn, degrade to a variety of products, including alkanals, hydroxyl alkanals, ketones etc. (Halliwell and Gutteridge, 1985). All of these products inactivate cell constituents by oxidation or cause oxidative stress by undergoing radical chain reaction. Such changes may lead to disintegration of membrane structure (Maiti *et al*., 1995) and irreversible cell damage (Vandervliet and Bast, 1992). However, endogenous enzyme such as SOD and CAT can protect against peroxidation damage to the biomembranes (Sies, 1993). Both the enzymes are important against oxygen metabolism (Saxena and Flora, 2004). Radical scavenger may directly react with quench peroxide radicals to terminate the peroxidation chain reaction and improves the quality and stability of food products (Bran Williams *et al*., 1995). In fact much less is known that all oxygen atoms are life supporting. Some are actually quite destructive for our cells. These unhealthy oxygen atoms are unbalanced and constitute the most common "Free Radical" known. The "Oxygen Free Radical" is characterized by having an unpaired electron in its molecular structure called "superoxide", it is quite capable of causing cell damage. The first line of defense that the body has against superoxide free radicals is the enzyme known as "Superoxide dismutase" or (SOD), which is considered the most effective antioxidant. The importance of SOD is so paramount for the protection of our cells, that it represents a substantial proportion of the proteins manufactured by the body. In brief, SOD keeps oxygen under control. The augmented activity of metalloenzyme, SOD accelerates dismutation of superoxide radicals to H_2O_2, which is removed by catalase (Aebi, 1984). Depletion of SOD activity was observed during lead exposure. Decrease in SOD activity can be attributed to an enhanced superoxide production during lead metabolism. CAT is a tetrameric hemoprotein that undergoes reduction at its active site in the presence of its substrate, H_2O_2, and catalyses the dismutation of H_2O_2 to water and molecular oxygen (Mates *et al*., 1999). In the present study superoxide radical also inhibits the activity of Catalase. It is also well established that reduced glutathione (GSH), plays a pivotal role in protecting cell against ROS (Mitchell *et al*., 1973). GSH is an important cellular antioxidant defense system against free radical overproduction and decreasing of its cellular concentration impairs cellular defense against oxidative stress (Dickinson *et al*., 2003).The depletion of GSH content in the present work promotes generation of ROS and oxidative stress with cascade of effects thereby affecting function as well as structural integrity of cell and organelle membrane. It can act as a non-enzymatic antioxidant by direct interaction of the –SH group with ROS, or it can be involved in the enzymatic detoxification reactions for ROS, as a cofactor or a coenzyme (Gurer *et al*., 1998). It possesses carboxylic acid groups, an amino group, a –SH group, and two peptide linkages as sites for reactions of metals. Lead binds exclusively to the –SH group, which decreases the GSH levels and can interfere with the antioxidant activity of GSH (Bechara, 2004). Overall, lead disturbs pro-and anti- oxidative balance in the tissues causing oxidative stress which might explain the toxic nature of lead.

However, administration of *Rubia cordifolia* extract alone had moderate but not significant effect on renal, hepatic and testicular oxidative stress parameters, where as in the later part of the study, it was observed that daily administration of alcoholic extract of *Rubia cordifolia* (50 and 100 mg/ kg) with lead nitrate significantly prevented the influence of lead on antioxidative system. It decreased LPO and concomitantly increased the activities of SOD, CAT and GSH levels in various tissues. The in vivo protection by *Rubia cordifolia* extract against lead induced oxidative damage may be because of its free radical scavenging potential.

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It could also because of direct scavenging/neutralization of the free radical or by induction of the endogenous antioxidant enzymes such as CAT and SOD. *Rubia cordifolia* extract possesses polyhydroxyl substituted anthraqunions, which may be responsible for these protective properties (Singh *et al*., 2005; Maliakel *et al*., 2008). Protective effect of *Rubia cordifolia* on LPO in isolated rat homogenate was also studied by pandey *et al*., 1994. Besides, *Rubia cordifolia* extract significantly chelates iron (Tripathi and Sharma, 1999) maintains the level of reduced Glutathione content and Vitamin C (Tripathi and Sharma, 1997) and also scavenges the hydroxyl radicals. Thus present results demonstrate the free radical scavenging important antioxidant properties of the alcoholic root extract of the plant.

Effect of lead on Biochemical parameters in tissues Liver and Testies

Lead nitrate treatment increased levels of AST, ALT, total cholesterol and decreased total protein content in serum and various tissues; and these parameters were partially normalized on treatment with alcoholic root extract of *Rubia cordifolia* (Table 2 and 4). ACP and ALP were only assessed in tissues and they increased after lead nitrate administration, while treatment with alcoholic root extract of *Rubia cordifolia* decreased the altered levels (Table 2 and 4).

Lead has been known to be environmental pollutant and its toxicity has also been associated with some health hazards. Enzymes such as ALT, AST, ACP and ALP are marker enzymes for tissue function and integrity (Adaramoye *et al.,* 2008). These enzymes are usually raised in acute or mild tissue cellular injury, but tend to decrease with prolonged intoxication due to damage to the tissues particularly liver (Jens and Hanne, 2002). In hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage (Nkosi *et al.,* 2005). Heavy metals have also been found to increase AST and ALT levels (Bersenyi *et al*., 2003). It is postulated that administration of lead nitrate could cause cell lysis, resulting in the release of cytoplasmic enzymes into the blood circulation, leading to their increase levels in serum, and this property is often implicated to assess the extent of lead nitrate induced cellular damage. Lead causes cell lysis by affecting the K^{+}–Ca^{2+} channels. A cytoskeleton alteration was found to induce membrane susceptibility to lysis (Raquel *et al*., 1997).

Administration of lead nitrate also causes assimilation of fat in the liver leading to the increased ACP activity. The increase in ACP activity may be due to the lysosomal imbalance resulting in the destruction of the intact membranes (Abraham and Wilfred, 2000). ALP has been reported to be the marker enzyme for plasma membrane (Wright and Plummer, 1974) and is required in certain amounts for proper functioning of organs (Brain and Kay, 1927). Increase in the ACP and ALP activities in the present work indicated the increased permeability, damage and/or necrosis of cells.

Rich sources of alkaline phosphatase are the bile canaliculli of the liver, osteoblasts in the bone, proximal tubules in the kidney and mucosal cells of the small intestine (Verley, 1967). Damage to any of these organs or tissues would lead to elevated activity of its isoform of ALP in the serum (Ngaha *et al*, 1989). Moderate exposure to lead is known to cause the leakage of IALP. The significantly high alkaline phosphatase activity detected from the renal tissues enumerated above, which are established target organs of heavy metal toxicity (Kido *et al*, 1995). It seems, therefore, that lead induces the biosynthesis of alkaline phosphatase in the bone and kidney before the disruption of cellular integrity, if any, and this usually occurs by way of lipid peroxidation (Sarkar *et al.*, 1995). Moreover, the increased activity of testicular acid phosphatase and alkaline phosphatase in lead nitrate treated mice reflects testicular degeneration, which may likely be a consequence of suppressed testosterone and indicative of lytic activity (Kaur *et al.*, 1999). Total protein level is rough measure of protein states but reflects major functional changes in liver and kidney function.

Lead binds to plasmatic proteins, where it causes alterations in a high number of enzymes. It can perturb protein synthesis in hepatocytes (Georing *et al.,* 1993). The observed decrease in protein content of mice treated with Pb in the present study may be due to decreased in hepatic DNA and RNA (Shalan *et al.,* 2005). Hassanin, 1994; El-zayat *et al.,* 1996, also reported decrease in hepatic total protein content in response to lead intoxication. Proteinuria due to kidney impairment in lead toxicity may be a cause of protein loss among these animals because inhibitory role of lead in protein synthesis is not yet reported.

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Protein loss in lead toxicity might decrease the level of specific proteins such as albumin, hormones, hormone and metal binding proteins, drug binding proteins, enzymes etc. and thereby disturb the homeostasis and rate of metabolic activities. The decreased level of protein concentration in the testis could be the change in the androgen production (Nocenti *et al.*, 1968). The significant reduction in the levels of protein is due to the interference like atrophy of accessory organs in androgen production. Moreover, Pb^{2+} disturbs intracellular Ca^{2+} homeostasis (Simons, 1993) and damages the endoplasmic reticulum which in turn results in reduction of protein synthesis. In addition, lead has been shown to enter in cells through voltage dependent Ca^{+2} channels at a higher rate then Ca^{+2} as an intracellular secondary messenger. Interaction between lead and two second messenger mediators of Ca signals (calmodulin and protein kinase C) has been studied extensively. Calmodulin exhibits a higher affinity for lead than it does for Ca^{+2}, leading to an up regulation of the enzymes.
The increasing testicular cholesterol might regulate the process of spermatogenesis (Dixit, 1977), and it acts as precursor for androgen synthesis (Verma *et al.*, 1980). The assessment of a possible relationship between lead level and lipid is an important step in elucidating the mechanisms underlying the excess cardiovascular morbidity among lead-exposed subject. Our finding of elevated total cholesterol is in agreement with Moussa *et al.,* 2008 and Ademuyiwa *et al.,* 2009**.** Lead-induced hyperplasia involves alterations in hepatic cholesterol metabolism that results in simultaneous increase in both liver and serum total cholesterol levels. Contrary to the general trend of suppression of CYP-450s, lanosterol 14a-demethylase (CYP51), an essential enzyme for cholesterol biosynthesis, was found induced in Pb nitrate-mediated liver hyperplasia (Pillai *et al.,* 2005). Lead nitrate-mediate development of hepatic hypercholesterolaemia involves the activation of cholesterol biosynthetic enzymes (*i.e.*, 3-hydroxy- 3methyglutaryl-CoA reductase, farnesyl diphosphate synthase, squalene synthase, CYP51) and the simultaneous suppression of cholesterol-catabolic enzymes such as 7a-hydroxylase (Kojima *et al.,* 2004).
Rubia cordifolia alone showed slight protection in mice. It was observed that daily administration of alcoholic root extract of *Rubia cordifolia* (50 and 100 mg/ kg) with lead nitrate prevented the influence of lead on biochemical related parameters. Simultaneous treatment with *Rubia cordifolia* significantly reduced the activities of AST, ALT, ACP and ALP, when compared to the mice treated with lead nitrate alone. The reduced serum and tissue ALT and ALP activities may generally be attributed to decreased production of these enzymes from the therapeutic sources present in the plant (Pandey *et al*., 1994) hence denotes the reversing effect of lead toxicity in mice.
The decreased concentrations of ALT, AST, ALP and ACP as a result of plant root extract administration might also be due to the presence of Rubiadin and quinone derivatives, the major hepatoprotective constituent of *Rubia cordifolia.* (Rao *et al.,* 2006). In the current investigation decreased cholesterol level and increased protein content were also recorded. According to Indian Ayurvedic, *Rubia cordifolia* possesses hypolipidemic and hypocholesterogenic, properties (Nadkarni, 1976).

CONCLUSION

Lead exposure produced significant adverse effects on status of liver and testis enzyme activities, suggesting the lead induced hepatic and testis injury and oxidative stress. Simultaneous supplementation of plant extracts with lead prevented the appearance of signs of lead toxicity in liver and testis. Thus the above results led us to conclude that the influences of lead were prevented by concurrent daily administration of alcoholic root extract of *Rubia cordifolia*

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