Lead has been recognized as an ubiquitous environmental pollutant. Exposure to lead occurs via ingestion, inhalation and dermal contact. Once absorbed it is distributed particularly to the liver and kidney and is then stored in the bones and causes damage to the organs including liver, kidneys, heart, brain, gonads as well as affects the immune system\textsuperscript{1-3}. Generation of ROS such as hydroxyl radical, hydrogen peroxide, superoxide anion and lipid peroxides during Lead exposure might result in systemic mobilization and depletion of the antioxidant defense system of the cell\textsuperscript{4}. The role of lipid peroxidation in living tissues has recently received considerable attention as a potential health hazards due to generation of ROS by lead. ROS are known to damage various cellular components and have been associated with disease processes such as carcinogenesis, atherosclerosis, and hypertension\textsuperscript{5}.

Considering that lead toxicity is one of the serious problem worldwide, there is still no specific, reliable and safe treatment. Until now the studies regarding treatment of lead toxicity are restricted mainly to some sulfhydryl containing chelating agents such as meso 2,3-dimercapto Succinic acid (DMSA), 2,3-dimercaptopropane-1-sulfonate (DMPS) or British Anti Lewisite (BAL)\textsuperscript{6}, few antioxidants such as Vitamin C, Vitamin A\textsuperscript{7} or Cysteine derivatives such as N-acetyl cysteine\textsuperscript{8} and some essential micronutrients like Copper, Selenium, Zinc, Magnesium and Manganese\textsuperscript{9,10}. Most of the conventional metal chelating agents have been reported to possess toxic side effects\textsuperscript{11,12}. Thus, there have been an increased interest in the therapeutic potential of plant products or medicinal plants having antioxidant properties in reducing free radical induced tissue damage\textsuperscript{13, 14}.

\textit{Rubia cordifolia} also known as Manjistha is a perennial climber belonging to the family Rubiaceae. It is beneficial for almost all type of human disorders. The root has been reported to be a rich source of Rubiadin, a dihydroxy anthraquinone\textsuperscript{15}. Despite the fact that \textit{Rubia cordifolia} is an important medicinal plant, its efficacy in relation to regulation of lead poisoning if any, has not been previously studied. Also, known antioxidants such as Vitamin E and C are known to decrease free radical generation by directly quenching the lipid peroxyl radical and by increasing SOD and CAT activities\textsuperscript{2, 3, 16, 17}. These findings suggest potential role of antioxidants to ameliorate lead toxicity.

**PROTECTIVE EFFECT OF \textit{RUBIA CORDIFOLIA} ROOT EXTRACT IN THE REGULATION OF LEAD NITRATE INDUCED OXIDATIVE STRESS IN MICE**

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The present study was undertaken to evaluate antioxidant activity of roots of \textit{Rubia cordifolia} (RC) in male mice treated with Lead nitrate. Lead nitrate treated (2 mg/ kg b. wt., i. p., once daily for 30 days) male albino mice concurrently received \textit{Rubia cordifolia} root extract (50 and 100 mg/ kg b. wt. by gastric intubation, once daily) for the same period. The oxidative stress parameters such as lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT) levels were assessed in hepatic and renal tissues. Lead concentration in soft tissues was also measured by an atomic absorption spectrophotometer. Mice exposed to lead nitrate showed increase in hepatic and renal lead concentration. Increase in LPO and decrease in SOD and CAT levels were also noticed. These influences of lead were prevented by concurrent daily administration of \textit{Rubia cordifolia} root extract to some extent. The results lead us to conclude that simultaneous supplementation of \textit{Rubia cordifolia} protects against lead intoxication to some extent.
Therefore the present study was performed to evaluate preventive effects of the alcoholic root extract of Rubia cordifolia and Vitamin C on lead induced tissue oxidative stress and concentration of lead in male mice.

MATERIALS AND METHOD

Chemicals: Lead nitrate Pb(NO₃)² and all other chemicals used in this study were of analytical grade and were purchased from reliable firms like SRL and BDH chemical (India), MERCK (Germany) and were of highest purity.

Preparation of hydro-alcoholic root extract of Rubia cordifolia: The plant was collected from the medicinal garden of Banasthali University, Rajasthan, India. It was identified as Rubia cordifolia by a plant taxonomist of our Institute. The plant material (roots) were thoroughly washed with distilled water, shade dried and cut into small pieces and powdered using laboratory homogenizer. Known quantity of powdered materials was extracted using alcohol by Soxhlet apparatus as a solvent. The alcoholic extract thus obtained was concentrated on water bath (65°C). Finally after complete evaporation of the solvent, the residue was weighed and stored at 4ºC and used to treat the animals as needed.

Animals: Male Swiss albino mice weighing approximately 25-30 g were obtained from Haryana Agricultural University, Hissar, India. The animals were acclimatized for 7 days prior to experiment. The institutional ethics committee approved the experimental protocols. All the animals used in this study were placed in stainless steel cages in an air conditioned room maintained at temperature of 25±2ºC and 12 hrs. light and dark schedule.

Experimental design: Thirty six male Swiss albino mice (25-30 g) were randomly divided into six groups, with six mice in each group. The groups were as follows: Group I-Control (untreated); Group II-Lead (2 mg/ kg b. wt., i.p.); Group III-Lead + RC root extract I (50 mg/ kg b. wt., orally); Group IV-Lead + RC root extract II (100 mg/ kg b. wt., orally); Group V-Lead + Vitamin C (50 mg/ kg b. wt., orally); Group VI-Lead + Vitamin C (100 mg/ kg b. wt., orally). The entire study was carried out for 30 days. The tissue samples (Liver and Kidney) were collected at the end of the experimental period for oxidative stress parameters.

The doses were decided on the basis of experiments conducted in the laboratory. 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 kidneys were removed, cleaned and washed with Phosphate buffer saline (pH-7.4) and used for various biochemical assays and for metal analysis.

Half portion of the tissues from each mouse was processed immediately for biochemical assays, and the remaining portion was stored at -20°C before wet acid digestion with HNO₃ for estimation of Lead contents.

Preparation of Tissue Homogenate: The liver and kidney tissues were homogenized in 10% ice cold Phosphate buffer (0.1 M, pH 7.4). The homogenate was centrifuged at 15,000g for 30 minutes at 4°C and the supernatant was used for subsequent analysis.

Determination of oxidative stress Parameters: LPO was measured by the method of Ohkawa et al. The tissue SOD activity was assayed according to the method of Marklund and Marklund. Catalase activity was estimated following the method of Aebi.

Metal analysis: Lead concentration in liver and kidney was measured after wet acid digestion using a Microwave Digestion System (model MDS-2100, CEM, Matthews, CT). Lead was estimated using a Hydride Vapour Generation System (model MHS-10, Perkin Elmer) fitted with an atomic absorption spectrophotometer (model A Analyst 100, Perkin Elmer).

Statistical analysis: Data are expressed as the mean± standard error of the mean values. Data comparison were carried out using one way analysis of variance followed by Tukey’s post test to compare mean between different treatment groups. A difference between unexposed and exposed groups with value of P< 0.05 was considered significant.

RESULTS AND DISCUSSION

Lead burden in liver and kidney: The concentration of lead in liver and kidney from mice exposed to lead nitrate was significantly higher than the respective control levels. Post exposure treatment with Rubia cordifolia root extract I (low dose) and Vitamin C (Low dose) did not significantly reduce the tissue lead level and the lead burden in these organs remained slightly higher or slightly lower (non-significant) than lead group. In contrast, Rubia cordifolia root extract II (high dose), significantly lowered the tissue lead concentration than lead treated group whereas Vitamin C (high dose, II) significantly reduced the lead content from both tissues than the lead exposed subjects.
Lipid peroxidation concentration in tissues: The lipid peroxide in liver and kidney from mice of lead group was significantly higher than the respective control values. Administration of *Rubia cordifolia* root extract I (low dose) and II (high dose) and Vitamin C I (low dose) and II (high dose) produced significant protective effect on lipid peroxidation in kidney as compared to lead treated mice. In the liver, although the plant extracts and Vitamin C treatment decreased the LPO but the effect did not reach statistical significance. Fig. 2 shows the effect of administration of *Rubia cordifolia* root extract on lipid peroxidation in the liver and kidney.

Activity of antioxidant enzymes: Figs. 3 and 4 shows the activity of antioxidant enzymes: superoxide dismutase (SOD) and catalase (CAT) in the liver and kidney. Significant decrease in SOD and CAT activities in liver and kidney were recorded in lead treated mice as compared with the controls. Treatment with *Rubia cordifolia* root extracts I (low dose) and Vitamin C I (low dose) significantly increase the tissue SOD but did not show any effect on the CAT levels whereas high dose of *Rubia cordifolia* root extract II and Vitamin C II (high dose) significantly elevated the liver and kidney SOD and CAT levels as compared to lead treated animals.

The higher concentration of lead in liver and kidney during experimental exposure was associated with increased oxidative reaction, which might be responsible for lead induced toxic effects. The results from this study indicated lead induced oxidative stress and the protective role of *Rubia cordifolia* root extract and ascorbic acid (Vit C) in reducing the lipid peroxidation as evidenced by decreasing MDA (malonaldehyde) concentration in liver and kidney. Lipid peroxidation, a basic cellular deteriorative change is one of the primary effects induced by oxidative stress and occurs readily in the tissues due to presence of membrane rich in polyunsaturated highly oxidizable fatty acids. Depletion of superoxide dismutase and catalase activity has also been observed during lead exposure. SOD and CAT are the most important enzymes against toxic effects of oxygen metabolism.

Interestingly, administration of root extracts of *Rubia cordifolia* in two different doses (50 & 100 mg/kg b.wt.) reduced lead burden and lipid per-oxidative process indicating their anti-peroxidative role in the liver and kidney tissues in a dose dependent manner. This was further supported by an increase in the activities of antioxidant enzymes (SOD and CAT) by the treatment. This was further supported by an increase in the activities of antioxidant enzymes (SOD and CAT) by the treatment.
plant extracts. Preliminary analysis has revealed that *Rubia cordifolia* has significant amount of GSH (glutathione), Vitamin C, other important antioxidants and polyphenols. In addition it also contains important trace elements like Zn, Cu, Vd, Se and Mo, which contribute to its antioxidant properties. It is also reported that *Rubia cordifolia* roots are rich source of Rubiadine, a hydroxyl anthraquinone. Studies have also shown that an extract of *Rubia cordifolia* scavenges superoxide radical and prevents lipid peroxidation, perhaps because of polyphenols in the extract.

However, treatment with Vit C in two different doses reduced lead burden in the investigated organs in a dose dependent manner. This might be due to direct interaction of ascorbic acid with oxidizing radicals and protects cells from ROS. Vitamin C inhibits lipid peroxidation by scavenging the aqueous ROS (reactive oxygen species) by rapid electron transfer and this activates Superoxide dismutase and catalase.

**CONCLUSION**

From the present study it is evident that simultaneous supplementation of *Rubia cordifolia* and Vitamin C is capable of scavenging lead-induced free radical generation. It thus appears that the drug may prove useful in treating/preventing lead toxicity to some extent.

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**REFERENCES**