IJPSR (2012), Vol. 3, Issue 07 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 14 March, 2012; received in revised form 19 June, 2012; accepted 26 June, 2012

ANTIOXIDANT ACTIVITY OF *RUBIA CORDIFOLIA* AGAINST LEAD TOXICITY

Shweta Lodia* and Leena Kansala

Department of Bioscience and Biotechnology, Banasthali University, Banasthali, Rajasthan, India

Keywords:
Rubia Cordifolia,
Antioxidant,
Lead Nitrate

Correspondence to Author:

Dr. Shweta Lodia

C44, Kiran Kunj, NTPC ANTA, Distt.: Baran 325209, India

ABSTRACT

To evaluate the *in vivo* antioxidant activity of alcoholic extract of the roots of *Rubia cordifolia* (RC). Male mice, were exposed, either to a diet containing 40mg/kg body weight of Pb nitrate or combined with *Rubia cordifolia* for the first period of 40 days, where the animals were sacrificed for oxidative studies. Ingestion of Pb $(NO_3)_2$ was significantly decreased RBC count, WBC count, Hb level and serum total protein contents in the mice. But, serum Alanine Transaminase, Aspartate Aminotransferase, and cholesterol level were significantly increased after implication of this metal. However, oral administration of *Rubia cordifolia*, to lead treated mice led to marked improvement in both hematological and serum biochemical changes. *Rubia cordifolia* alone had moderate effect on mice, where as co-administration of lead nitrate with *Rubia cordifolia* reversed the effect of lead.

INTRODUCTION: Lead is known to induce a broad range of physiological, biochemical, and behavioural dysfunctions in laboratory animals and humans [1], including central and peripheral nervous systems, haemopoietic system [2], cardiovascular system [3], kidneys [4], liver [5], and male [6], and female reproductive systems[7]. Lead, however, was reported to have no pro-oxidant catalytic activity with respect to lipid peroxidation (LPO).

Yiin and Lin [8] 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 [9]. Similar effects were shown by Sandhir and Gill [10] 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.

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 [11].

Rubia cordifolia **(Rubiaceae)** also known as *'manjistha'* is an important medicinal plant, which is used for treatment of various ailments such as anti-tumor [12], anti-inflammatory [13], urinary disorders [14], antistress antimicrobial [15], Hepatoprotective [16], radio protective [17] and anticancer [18]. 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 [19] interest in the isolation of natural and coloring matters is increasing due to their use in food, drugs and other human consumptions.

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 [20].

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 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) was 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 further studies.

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:

a. Group 1, received 1 ml distilled water by oral gavage; served as control,

b. Group 2, received 40 mg/ kg body weight/ day lead nitrate dissolved in distilled water by oral gavage for 40 days,

c. 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.

d. 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} [21]. 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 [22]. 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. Blood was collected by heart puncture in eppendroff tubes rinsed with and without anticoagulant for hematological, serological.

Body weight: The body weight of animals recorded at the end of the experiment was nearest to gram. The absolute weight of organ (kidney, liver and testis) for different groups of experimental animals was recorded to the nearest milligram on a microbalance. The relative weight of different organ was calculated by using the following formula:

$$\text{Relative weight} = \frac{\text{Absolute weight}}{\text{Final body weight}} \times 100$$

Hematological parameters: For hematological parameters, blood was collected in heparinized tubes. Total Leukocyte Count (TLC), Hemoglobin estimation, Differential leukocyte count (DLC) content of sample was determined by using slandered laboratory manual. Activity of AST Aspartate amino transaminase (AST) was assayed by the method of Reitman and Frankel[23], Alanine aminotransferase (ALT) was assayed by the method of Reitman and Frankel [23], The protein content was determined by using bovine serum albumin (BSA) as a standard by the method of Lowry *et al.* [24] and The cholesterol level was determined by using cholesterol as a standard by the method of Zak's *et al* [25].

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:

Body Weight: Effect in the change in the body weight of animals was recorded at the end of the experiment in different groups and is presented in **Table 1**.

The initial body weight of mice in group 1, group 2, group 3, group 4, group 5 and group 6 (25.4±0.43, 26.8±0.51, 25.5±0.28, 25.4±0.45, 27.2±47 and 27.4±0.64 g, respectively), when compared with their final body weight (27.5±0.32, 28.01±0.40, 27.5±0.50, 27.2±0.43, 28.5±0.56 and 28.9±0.71 g, respectively), gave the percentage body weight change of 8.26, 4.51, 7.84, 6.61, 4.77 and 5.47%, respectively. From these results, it is clear that exposure of lead nitrate to male mice produced a significant decrease (3.75%, $p<0.001$), in percentage change in body weight when compared with control, indicating a condition of weight loss.

Alcoholic root extract of *Rubia cordifolia alone* at 50 and 100 mg/kg body weight had no significant effect on mice body weight (0.42% and 1.65%) when compared with normal animals, but, the weight loss was significantly ($p<0.001$), reversed by the co-administration of alcoholic root extract of *Rubia cordifolia along* with lead nitrate, (0.26% and 0.96%, respectively), when compared with group 2.

TABLE 1:- LEAD INDUCED CHANGES IN BODY WEIGHT AND ORGAN WEIGHT IN MICE AND THEIR RESPONSE TO ADMINISTRATION OF ALCOHOLIC ROOT EXTRACT OF *RUBIA CORDIFOLIA*

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 (50mg/kg body weight) Group 3	RC (100mg/kg body weight) Group 4	Lead nitrate + RC (50mg/kg body weight) Group 5	Lead nitrate + RC (100mg/kg body weight) Group 6
Initial body weight (g)	25.4±0.43	26.8±0.51	25.5±0.28	25.4±0.45	27.2±47	27.4±0.64
Final body weight (g)	27.5±0.32	28.01±0.40	27.5±0.50	27.2±0.43	28.5±0.56	28.9±0.71
% change in body weight (change in body weight × 100/initial weight)	8.26	4.51* (3.75%)	7.84 (0.42%)	6.61 (1.65 %)	4.77ª (0.26%)	5.47ª (0.96%)

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

Hematological Parameters: Effect of Lead nitrate, *Rubia cordifolia* alcoholic root extract either individually or in combination on some hematological profile was observed or are presented in **Table 2**.

Lead exposure led to insignificant fall in TLC, and significant fall in Hb, lymphocyte, and monocyte count, whereas neutrophil count increased insignificantly as compared to respective control values. Alcoholic extract of roots of *Rubia cordifolia at* 50 and 100 mg/kg body weight had moderate (not significant), effect on TLC, Hb and on lymphocyte count. No effect of *Rubia cordifolia root* extract was observed on monocyte, neutrophil count and on Hb, as compared to normal group mice i.e. group 1.

Co-administration of *Rubia cordifolia* at 50 and 100 mg/kg body weight with lead nitrate increased TLC, Hb, lymphocyte, monocyte insignificantly, and significantly ($p<0.001$), increased in group 6, and neutrophil showed insignificant effect, in comparison to lead nitrate treated group.

TABLE 2: LEAD INDUCED CHANGES IN SOME OF THE BLOOD 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 (50mg/kg body weight) Group 3	RC (100mg/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
TLC ($\times10^3$/ mm^3)	5.23±0.034	2.09±0.031 (-60.04%)	5.33±0.025 (1.91%)	5.48±0.049 (4.78%)	3.28±0.039 (56.94%)	4.28±0.055 (104.78%)
Hb (g/dl)	10.40±0.01	4.24±0.012 * (-59.23%)	10.21±0.026 (-1.83%)	10.51±0.11 (1.06%)	6.31±0.11 [c] (48.82%)	7.46±0.011[a] (75.94%)
Lymphocyte ($\times10^3$/ ml)	8.18 ± 0.29	4.42 ± 0.37* (-45.97 %)	8.45 ± 0.37 (3.30%)	8.45 ± 0.37 (3.30%)	7.72 ± 0.32[a] (74.66 %)	8.13 ± 0.23[a] (83.94 %)
Monocyte ($\times10^3$/ ml)	0.415±0.05	0.22 ± 0.03* (-46.99%)	0.39 ± 0.03 (-6.02%)	0.40 ± 0.04 (-3.61%)	0.28 ± 0.04 (27.27%)	0.30 ± 0.04[a] (36.36%)
Neutrophil ($\times10^3$/ ml)	4.70 ± 0.26	5.27 ± 0.23 (12.13%)	4.07 ± 0.19 (-13.40%)	4.62 ± 0.18 (-1.70%)	5.62 ± 0.20 (+6.64 %)	4.72 ± 0.25 (-10.44%)

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, [c]P< 0.02 compared to lead nitrate exposed animals

Serological Parameters: Effect of Lead nitrate, *Rubia cordifolia alcoholic* root extract either individually or in combination on some serum biochemical assays was noticed or are shown in **Table 3**.

Daily treatment with lead nitrate (40 mg/ kg body weight), for 40 days showed significant (p<0.001), increase in AST, ALT and total cholesterol level, and significantly decreased total protein content, as compared to normal mice.

Alcoholic root extract of *Rubia cordifolia at* 50 and 100 mg/kg body weight had moderate (not significant), effect on AST, ALT, total cholesterol, and total protein, as compared to normal group mice i.e. group 1.

Rubia cordifolia administration in group 5 and 6 showed significant (p<0.001), decrease in AST, ALT, and total cholesterol, in comparison to lead nitrate treated animals. Total protein at low dose showed insignificant effect and at high dose total protein increased significantly, in comparison to lead group 2.

TABLE 3: LEAD INDUCED CHANGES IN SOME OF THE BLOOD SERUM 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 (IU/ L)	34.61±0.44	68.27±0.51* (97.26%)	32.41±0.45 (-6.36%)	31.74±0.46 (-8.29%)	56.03±0.55[a] (-17.97%)	47.91±0.056 [a] (-29.82%)
ALT (IU/ L)	17.7±0.02	27.90±0.80* (57.63%)	17.01±0.1 (-3.90%)	16.89±0.3 (-4.58%)	22.92±0.1 [a] (-17.85%)	19.13±0.35 [a] (-31.43%)
TC (mg/ dl)	70.25±0.30	132.77±0.23* (89.00%)	69.01±0.26 (-1.77%)	68.89±29 (-1.94%)	90.138±0.50 [a] (-32.11%)	99.69±0.90 [a] (-24.92%)
TP (g/ dl)	6.72±0.01	2.14±0.02* (-68.15%)	6.73±0.01 (+0.15%)	6.80±0.01 (+1.19%)	3.46±0.02 (61.68%)	4.53±0.01[c] (111.68%)

Values are Mean ± S.E; n= 6, *P< 0.001 compared to normal animals, [a]P< 0.001 compared to lead nitrate exposed animals, [c]P< 0.02 compared to lead nitrate exposed animals

DISCUSSION:

Body Weight: *Rubia cordifolia* was investigated for their probable beneficial role in lead-induced loss of body weight and organ weight. It is observed in the present investigation that lead nitrate, given at 40 mg/ kg body weight/ day dose, has caused adverse effect on the body weight (Table 1) where as *Rubia cordifolia* on the other hand treatment improved lead induced alternations in body weight.

Lead exposure is known to provoke weight reduction [26]. The retardation in body weight growth could explain the effect of lead and loss of appetite and gastrointestinal disturbances [27]. Several previous reports also confirm these findings. Yokayama and Araki [28], in a study noted 15% decreased in final body weight gain, where lead acetate was administered via drinking water to Wistar rats. Later, Dieter *et al*., [29] reported 14-20% decrease in body weight of male rats.

Similar finding was observed by Freeman *et al*., [30] who noted that reduction in body weight was due to decreased in food intake. The weight reduction in lead toxicity in the present study was also similar to the findings of some other researchers such as Szymezak *et al*.[31], who reported reduced weight gain after intoxication with lead acetate in a dose of 400 mg/kg body weight. Haque[32] and Kamruzzaman[33] also noticed similar results. The reduction in body weight might also due to interruption in absorption and metabolism of feed nutrients essential for health[34].

It has been demonstrated that inorganic lead selectively bound to proteins in some tissues[35], disrupts protein function and results in weight loss. Further, Kaltreider *et al*[36] also demonstrated that exposure to metal alter hormonal function in the glucocorticoid system. The glucocorticoid hormones play an important role in glucose regulation, as well as carbohydrate, lipid and protein metabolism. Dysfunction in the glucocorticoid system has been linked to weight gain/loss. The glucocorticoid hormonal system plays an important role in protein metabolism.

The current investigation suggest that alcoholic root extract of *Rubia cordifolia* alone had slight insignificant effect on mice body and whereas, above effects of lead, were significantly reversed by co-administration of alcoholic root extract of *Rubia cordifolia* with lead nitrate. This protective effect might be due to presence of some therapeutic agents in the alcoholic root extract of *Rubia cordifolia*.

Hematopoietic System: Haematological parameters namely TLC, Hb and differential leucocytes were monitored during lead toxicity study in mice because of their important role in providing reliable information concerning haematological changes (**Table 3**). The interesting observation is that the *Rubia cordifolia* was able to improve the deranged blood parameters to some extent which were altered by lead nitrate administration.

The alterations in haematological changes serve as the earliest indicators of toxic effects on tissue [37]. Blood is the most important tissue, in which changes in metabolic processes are going on, therefore, abnormal alteration in blood parameters are the reliable indicator of toxic effects of drugs, chemicals and diseases.

Lead has been recognized as a biological toxicant. Absorbed lead following oral ingestion is carried via blood to soft tissues and 95 % of blood lead is transported by the erythrocytes as lead diphosphate [38]. This might be the reason of increase lead concentration in the blood following oral exposure to lead.

The results of the present work demonstrate decline in the Hb level and total lukocyte count in lead nitrate administered mice. The result obtained is in agreement with Kamruzzaman [39] who observed that lead acetate administration significantly decreased TEC, TLC and Hb content.

In the present study, total leukocyte count had decreased mainly due to decrease in lymphocyte and monocyte count. The lymphopenia may be attributed to decreased mean cellular life span and impaired proliferative capacity of cells [40], necrosis of leucopoietic tissues [41], impairment in hematopoetic cells in the kidney [42] and accumulation of lymphocytes in lymphoid tissues or destruction by corticosteroid hormones [43]. It is known that metal induces stimulation of kidney chromaffine cells and cortisol secretion during non specific stress reaction [44].

There was an increase in neutrophil count which was probably due to cortisol hormone, since this hormone prevents neutrophil migration into the tissues (inhibiting inflammatory response) and extends their life span by inhibition of apoptosis [45].

The current data showed that hemoglobin content was significantly decreased in experimental animals with lead nitrate inoculation at a dose of 40mg/ kg body weight. Some researchers have observed decrease in hemoglobin concentration in lead treated animals [46, 47, 48].

Lead may inhibit the body's ability to make hemoglobin by interfering with several enzymatic steps in the heme pathway. Studies have shown that lead inhibits the enzymes δ-aminolevulinic acid dehydratase (ALAD) and ferrochelatse of the heme synthetic pathway thus preventing conversion of ALA to porphobilinogen and inhibits incorporation of iron into the protoporphyrin ring respectively. This results in reduced heme synthesis and elevated levels of the precursor δ-aminolevulinic acid (ALA), which is a weak gamma-aminobutyric acid (GABA) agonist that decreases GABA release by presynaptic inhibition [49, 50, 51].

Szymezak [31] observed that Hb level was reduced after intoxication with lead acetate in dose of 400mg/ kg of the fodder. On the other hand, Berney [52] observed significant reduction in Hb, but increase in the TLC following lead acetate administration at different doses.

The present study indicates that administration of alcoholic root extract of *Rubia cordifolia* alone had moderate effect on almost all blood parameters. This protective role of *Rubia cordifolia* against lead toxicity could be attributed to the antioxidant action of it components. Plant root extract at the dose of 50 mg/kg body weight and 100 mg/kg body weight when given along with lead nitrate significantly ameliorates the hematological profile.

These results are in agreement with Pandey [53]; Tripathi [54]; Tripathi [55]; Joharapurkar [56]. Authentic reports are not available regarding its role as blood purifier or cardio tonic but ayurvedic texts claim it as blood purifier. Zlauddin [57] showed that *Withania somnifera* increases Hb concentration as well as red blood cell count. *Rubia cordifolia* has properties comparable to *Withania somnifera,* it could be inferred that *Rubia cordifolia* also has protective impact on these parameters. It was found that *Rubia cordifolia* chlorophyll has very similar structure to Hb. So it may have blood purifier effects

SEROLOGICAL PARAMETERS: Increasing the activities of AST and ALT in the blood sera was most likely a consequence of the hepatotoxic effect of lead, i.e. the occurrence of toxic hepatitis. It is true that the liver, among its numerous vital functions, has also a role in the process of accumulation and detoxification of foreign substances, and thus of toxic metals as well. The lead entering the body by oral gavages is delivered to the liver through the portal blood circulation where the greatest part of it remains stored. Only a smaller part of this toxic metal "breaks the liver barrier" and enters the body circulation.

The accumulated lead in the liver can act by directly damaging the hepatocytes, primarily by destroying the permeability of the cell membrane, which results in the increased release of cytosolic enzymes AST and ALT into the circulation. It has been reported that ALT elevated significantly more than AST on lead exposure which indicates liver damage and development of fibrosis [58].

Lead binds to plasmatic proteins, where it causes alterations in a high number of enzymes. It can perturb protein synthesis in hepatocytes [59]. 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 [58]. El-zayat [60], also reported decrease in hepatic total protein content in response to lead intoxication. 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 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 [61].

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 [62, 63].

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 [62].

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 [64] hence, denotes the reversing effect of lead toxicity in mice. Basker [65] reported that beneficial effect of *Rubia cordifolia* treatment might be due to different types of active principles, each with a single or a diverse range of biological activities.

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* [67, 68]. 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 [69].

SUMMARY: It could be concluded from the present study that *Rubia cordifolia* has potent antioxidant activity against lead sensitive Hematological, Sereological and also on effect on body weight. Root of Rubia cordifolia is rich in source of Rubiadine, a dihydroxy anthraquinone. Also contain GSH, Vit-C and important trace elements like Zn, Cu, Vd, Se and Mo. All this may be responsible for its antioxidant properties. Further work is needed to be done to isolate the particular compound responsible for particular properties of *Rubia cordifolia* agaist lead toxicity.

Conflict of interest: None.

ACKNOWLEDGMENTS: The authors are thankful to the authorities of Banasthali University, for providing support to the study.

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How to cite this article:
Lodia S and Kansala L: Antioxidant Activity of *Rubia cordifolia* against Lead Toxicity. *Int J Pharm Sci Res*, 2012; Vol. 3(7): 2224-2232.