Protective effect of *Withania somnifera* roots extract on hematoserological profiles against lead nitrate-induced toxicity in mice

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The *in vivo* protective role of hydro-methanolic root extract of *Withania somnifera* (WS) was evaluated in alleviating lead nitrate (LN)-induced toxicity in male Swiss albino mice by measuring hematoserological profiles. The lead-treated (20 mg/kg body wt, p.o.) albino mice (25-30 g) concurrently received the root extract (200 and 500 mg/kg body wt, p.o.) once daily for the duration of six weeks. Animals exposed to LN showed significant (P<0.001) decline in haemoglobin content, red blood cell count, white blood cell count, packed cell volume and insignificant decrease in mean corpuscular haemoglobin and mean corpuscular haemoglobin content, while mean corpuscular volume and platelet count were increased. A significant elevation (P<0.001) in serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, acid phosphatase and total cholesterol were also observed, when compared with control mice. Thus, the study demonstrated that the concurrent daily administration of root extract of WS protected the adverse effects of LN intoxication in mice.

**Keywords:** *Withania somnifera*, Lead nitrate, Albino mice, Blood profiles

Lead is the most common environmental pollutant and has been shown to present health problems following exposure. It is potentially dangerous for everyone regardless of age, but infants and very young children are particularly vulnerable to its neuro-toxic effects\(^1\). Although lead poisoning has been studied for years, some of the toxic effects still have not been explained\(^2\).

The use of chelating agents and few antioxidants such as vitamin C and E\(^3\) can enhance lead excretion in lead poisoning, but these cannot be routinely recommended due to their many side effects\(^4\). Plants have also been investigated for their medicinal properties due to their potent pharmacological activities, low toxicity and economic viability\(^5-7\). Thus, there has been increased interest in the therapeutic potential of plant products or medicinal plants for their role in reducing lead poisoning.

*Withania somnifera* (Solanaceae), popularly known as “Ashwagandha” in Hindi and “Winter cherry” in English has a long history of use in herbal medicine and indigenous medical systems in India\(^8,9\). The roots of plant constitute the main drug Ashwagandha which is used therapeutically and categorized as Rasayanas, which promote health and longevity by augmenting defense against disease, arresting the ageing process, increasing the capability of the individual to resist adverse environmental factors and by creating a sense of mental wellbeing. The root has been used for all age groups and both sexes and even during pregnancy without any side effects\(^10\). However, despite various medicinal properties, reports are lacking on use of Ashwagandha in metal detoxification\(^11-13\).

In the present investigation, the protective effect of hydro-methanolic root extract of *W. somnifera* is evaluated against lead nitrate-induced toxicity in male Swiss albino mice by estimation of hematological parameters.

**Materials and Methods**

**Chemicals**

Lead nitrate [Pb(NO\(_3\))]\(_2\)] was purchased from Central Drug House (India). All other chemicals used were of analytical reagent grade and obtained from SISCO Research Laboratories (India), Qualigens.
Plant material and preparation of hydro-methanolic extract

*Withania somnifera* (WS) was collected from the University Medicinal Plant Garden, Banasthali, India. It was identified and authenticated by a botanist of our institute (Department of Bioscience and Biotechnology, Banasthali University) as a local variety. Dried powdered roots (50 g) were extracted successively with 80% methanol and 20% water in a soxhlet extractor for 48 h at 60°C. The hydro-methanolic root extract thus obtained was dried under reduced pressure at a room temperature not exceeding 40°C using a rotary evaporator to obtain a yield of 7% from the crude and stored at 4°C. It was dissolved in distilled water prior to use.

Animals and treatment

Male Swiss albino mice (*Mus musculus*) weighing approx. 25–30 g (3-4 months) were obtained from Haryana Agricultural University, Hissar (India). The Animal Ethics Committee of Banasthali University, Banasthali, India approved experimental protocol. Animals were housed in polypropylene cages in an air-conditioned room with temperature maintained at 25 ± 3°C, relative humidity of 50 ± 5%, 12 h alternating light and dark cycles and fed with standard mice pellet diet (Hindustan Lever Ltd., India) and drinking water *ad libitum* throughout the study. Seventy-two Swiss albino male mice weighing 25-30 g (3-4 months old) were randomized into six groups comprising of six mice in each group and used for hematological and serological parameters. All these groups were treated by oral gavage, once daily for 42 days: Group I received 1 ml distilled water and served as control; Group II: received lead nitrate (LN, 20 mg/kg body wt/day) dissolved in distilled water; Groups III & IV received hydro-methanolic WS root extract at a dose of 200 & 500 mg/kg body wt, respectively; and Groups V and VI received lead nitrate (20 mg/kg body wt) along with hydro-methanolic WS root extract at a dose of 200 and 500 mg/kg body wt, respectively.

The doses for LN and plant were selected on the basis of experiments conducted in our own laboratory and other published reports\(^\text{11-15}\). WS root extract was given at an interval of 30 min of LN administration. After administration of last dose the animals were sacrificed after six weeks under light ether anesthesia and blood samples were collected from the retro-orbital venous plexus of all animals with and without anti-coagulant for hematological and serological parameters.

Hematological and serological profiles

The various blood profiles of each sample were determined by using standard laboratory procedures viz., hemoglobin (Hb) estimation was done using Sahl’s hemoglobin meter\(^\text{16}\), red blood cell count and total white blood cell count by haemocytometer\(^\text{17}\), and PCV by Wintrob method\(^\text{18}\). Total platelet count, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular Hb content were also estimated\(^\text{19,20}\).

Serum was prepared by centrifugation of blood samples at 860 × g for 20 min and stored at -20°C until used for analysis. The various serological profiles viz., serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT)\(^\text{21}\), alkaline phosphatase (ALP), acid phosphatase (ACP)\(^\text{22}\) and total cholesterol\(^\text{23}\) in mice were estimated.

Statistical analysis

The results were expressed as mean ± S.E.M. (standard error of mean). Statistical significance between the different experimental groups was determined by one-way analysis of variance (ANOVA) using the statistical package for social science program (S.P.S.S.11). Post-hoc test was performed for inter group comparisons using Tukey’s multiple comparison test. The level of significance between groups was set at P<0.05.

Results

Hematological parameters

Table 1 shows the effect of lead nitrate (LN) and hydro-methanolic root extract of WS either alone or in combination with LN on hematological profiles. A significant (P<0.001) decline in Hb content, TRBC, TWBC and PCV and significant increase (P<0.001) in platelet count was observed in LN-treated group, when compared with control animals. LN-treated group II also showed insignificant (P>0.001) decrease in MCH, MCHC and slight, but insignificant increase (P>0.001) in MCV, as compared to control animals. Oral administration of hydro-methanolic root extract of WS (200 and 500 mg/kg body wt, p.o) significantly (P<0.001) elevated the Hb content, PCV and total erythrocyte (P<0.02), when compared with control animals. Total WBC count, platelet count and other
blood indices i.e. MCV, MCH and MCHC changed insignificantly with WS extract (at both doses), when compared with normal animals. Administration of root extract at both doses along with LN significantly enhanced (P<0.001) the Hb content, TRBC, TWBC, PCV, when compared with LN-exposed subjects. Elevated values of MCH, MCHC and declined platelet count were also noticed.

Serological parameters

Table 2 shows the effects on LN and hydro-methanolic root extract of WS either alone or in combination on serological variables. The treatment with LN caused a significant elevation (P<0.001) in SGOT, SGPT, ALP, ACP and TC, as compared to control. Administration of root extract at both doses alone had no effect on SGOT, SGPT and ALP activities and TC, as compared to control animals. The values of SGOT, SGPT and ALP decreased significantly (p<0.001) at both doses of extract when given alone, but along with LN. The root extract at 200 mg/kg body wt insignificantly decreased the ACP activity, but a significant (P<0.02) decrease was observed when treated with 500 mg/kg body wt dose of root extract, when compared with LN-exposed animals.

Discussion

Lead is used in various industries in high concentration and it is one of the three heavy metal pollutants of soil, water, air and bio-systems and cause tremendous health consequences. In the present study, LN toxicity caused significant alterations in hematological and serological parameters. The Hb content was decreased significantly in LN-treated animals at a dose of 20 mg/kg body wt. Previous reports have also observed decrease in Hb content in lead-treated animals. Lead might inhibit the body’s ability to make Hb by interfering with several enzymatic steps.
in the heme pathway. Decreased Hb content might be due to inhibition of enzymes δ-aminolevulinic acid dehydratase (ALAD) and ferrochelatase of the heme synthetic pathway that prevents the conversion of δ-aminolevulinic acid (ALA) to porphobilinogen and thus inhibits incorporation of iron into the protoporphyrin ring respectively. This results in reduced heme synthesis and elevated levels of the precursor ALA, which is a weak γ-aminobutyric acid (GABA) agonist that decreases GABA release by presynaptic inhibition.\(^\text{29,30}\)

The decreased RBC count in present study might be due to hemolysis and was in agreement with previous reports.\(^\text{27,28}\) The increase in erythrocyte destruction might be due to inhibition of pyrimidine 5-nucleotidase that resulted in an accumulation of pyrimidine nucleotides (cytidine and uridine phosphatase) in the erythrocyte or reticulocyte. Enzyme inhibition and nucleotide accumulation affect erythrocyte membrane stability and survival by alteration of cellular energetic. Decrease in WBCs in the present study might be due to decrease in lymphocyte count and was in agreement with earlier reports, which reported mild leucopenia in mice treated with lead. Elevated platelet count in current study was also consistent with earlier report, wherein hematological changes were noted in male weanling rat when treated with triethyl lead.

The increase in RBC count, Hb content and PCV value showed that WS probably enhanced erythropoiesis. Earlier, significant increase in Hb concentration and RBC has been reported in WS administered mice. Slight but not significant effect of root extract on WBC and platelet count and other blood indices suggested the anti-inflammatory potential of WS. We also observed slight increase in total WBC count at higher dose of WS. This result was in agreement with the findings of Agarwal et al., who reported that administration of WS increased total WBC count in cyclophosphamide induced myelosuppression in rat.

Several soluble enzymes of blood serum have been considered as indicators of hepatic dysfunction and damage. The results indicated that LN ingestion induced a significant elevation of SGOT, SGPT, ALP and ACP and total cholesterol level. Administration of LN caused assimilation of fat in the liver, leading to the increased ACP activity. This might be also due to the lysosomal imbalance resulting in the destruction of the intact membranes. ALP is a sensitive biomarker to metallic salts, since it is a membrane bound enzyme related to the transport of various metabolites. ALP has been reported to be the marker enzyme for plasma membrane and is required in certain amounts for proper functioning of organs. Increase in the ACP and ALP activities suggested the increased permeability, damage and necrosis of cells.

In the present study, LN ingestion significantly elevated the cholesterol level in serum. LN-mediated development of hypercholesterolemia involves the activation of cholesterol biosynthetic enzymes (i.e., 3-hydroxy-3-methylglutaryl-CoA reductase, farnesyl diphosphate synthase, squalene synthase, CYP51) and simultaneous suppression of cholesterol-catabolic enzymes such as 7α-hydroxylase. The increased cholesterol level could result in relative molecular ordering of residual phospholipids, resulting in a decrease in membrane fluidity. Treatment with root extract of WS alone (200 and 500 mg/kg body wt) showed insignificant effect on SGOT, SGPT, ALP and TC level, whereas ACP activity was enhanced with both doses, as compared to normal animals. The decreased cholesterol level in the present study might be due to hypolipidemic properties of WS.

It was also observed that daily administration of hydro-methanolic root extract of WS with LN significantly prevented the effects of LN on all the above-mentioned blood parameters in comparison to lead-exposed subjects, thus suggesting the ameliorating role of root extract in lead toxicity. The plant products like phenolic compounds and flavonoids have the ability to modulate the activities of various enzyme system due to their interaction with various biomolecules. Earlier, we have also reported the therapeutic efficacy of hydro-methanolic root extract of WS in the regulation of LN-induced nephrotoxicity, hepatotoxicity and oxidative damage in neurological parameters in Swiss albino mice.

In conclusion, the hematological and biochemical values were significantly affected by LN administration and the concurrent treatment of hydro-methanolic root extract of WS significantly recovered the adverse effects of lead nitrate.

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