

## Lead acetate induced oxidative stress and its possible reversal by *Tribulus terrestris* root extract in testes of Swiss albino mice

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**Abstract:** Lead is highly toxic naturally occurring element that affects numerous organ systems in humans. Present study was designed to investigate the preventive role of *T. terrestris* root extract and vitamin C against lead acetate induced testicular toxicity in Swiss albino mice. Animals exposed to lead acetate at a dose level of 10 mg/kg b.wt. subcutaneously showed significant decrease in protein, sialic acid, ascorbic acid, total thiols and ATPase activity but a significant increase was observed in cholesterol level. These modulatory effects of lead acetate were prevented by concurrent daily administration of *T. terrestris* root extract. The antioxidant and chemoprotective activity of *T. terrestris* root extract was compared with vitamin C.

**Key Words:** Biochemical assays, Lead acetate, Testes, *Tribulus terrestris*, Vitamin C.

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### I. Introduction

The diverse deleterious health effects upon exposure to heavy metals in the environment are a matter of serious concern and a global issue. Lead is the most abundant toxic metal in the environment [1]. Lead does not have any detectable beneficial biological role however on the contrary its detrimental effect on physiological, biochemical and behavioral dysfunctions have been documented in animals and humans by several investigators [2,3]. Lead is a male reproductive toxicant [4]. Toxicity is manifested in male reproductive function by deposition of lead in testes, epididymis, vas deferens, seminal vesicle and seminal ejaculate. Lead has an adverse effect on sperm count, sperm motility and retarded the activity of alive sperm [5]. Clinical and animal studies indicate that abnormalities of spermatogenesis result from toxic exposure [6]. The mechanism behind lead toxicity is the oxidative stress and it develops when there is an imbalance between the generation of reactive oxygen species and the scavenging capacity of antioxidants in the reproductive tract. Reactive oxygen species (ROS) have been shown to have an important role in the normal functioning of a reproductive system and in the pathogenesis of infertility [7].

*Tribulus terrestris* is a flowering plant in the family Zygophyllaceae and is native to warm temperate and tropical regions of the Old World in southern Europe, southern Asia, throughout Africa and Australia. It is commonly called Gokharu and it can also thrive even in desert climates and poor soil [8]. *Tribulus terrestris* is claimed to increase the body's natural testosterone levels and thereby improve male sexual performance and help build muscle. *Tribulus terrestris* has been shown to enhance sexual behaviour in an animal model [9]. The specific aim of the present study was to determine the efficacy of *Tribulus terrestris* root extract against the lead acetate induced testicular impairment.

### II. Materials and Methods

#### 2.1 Animals and Treatment

Random-bred, male Swiss albino mice (7-8 weeks) were used for the experiment. These animals were maintained in the animal house at a temperature of 24±3°C, relative humidity of 50%±15% and normal photoperiod (12 hr light and 12 hr dark). Animals were housed in polypropylene cages and fed standard mice feed (Hindustan Lever Ltd., India). Tap water was provided to the animals *ad libitum* and tetracycline was given as a preventive measure against infections once in a fortnight. The ethical committee of Department of Zoology, University of Rajasthan, Jaipur (India) has approved to carry out the experimental protocol.

#### 2.2 Chemicals

Lead acetate was procured from Central Drug House (New Delhi, India). All other chemicals used in the study were of analytical reagent and obtained from SD fine chemicals (Mumbai, India), HIMEDIA (Mumbai, India).

#### 2.3 Experimental Plant

The plant *Tribulus terrestris* (roots) were collected locally in the month of July and August and were identified in the herbarium of Botany Department, University of Rajasthan, Jaipur as an RUBL20825 variety. Shade dried *T. terrestris* roots were ground to a fine powder, the powder was then distilled in soxhlet apparatus

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(for 36 hours using Double Distilled Water) at 40°C. The remaining material was dried in oven at a temperature of 36°C and used for the study.

#### **2.4 Administration of Aqueous Extract of *T. terrestris* roots**

The animals were administered *T. terrestris* root extract dissolved in DDW orally by oral gavage up to 30 days (100, 400, 800 mg/kg body weight) and reduced glutathione (GSH) and lipid peroxidation (LPO) contents were measured in the liver. The optimum dose selection of *T. terrestris* root extract was decided on the basis of minimum LPO and maximum GSH level in the liver tissue. Among the doses 800 mg/kg b.wt. was selected for the study.

#### **2.5 Experimental Design**

Adult Swiss albino male mice were divided into four groups of 25 mice each and following experiments were designed.

**Group I (Normal Control):** Received DDW as a vehicle by oral gavage.

**Group II (Lead acetate treated group):** Freshly dissolved lead acetate in 0.1 ml double distilled water was given subcutaneously only once at a dose of 10 mg/kg body weight. This day was considered as day zero and the experiment was continued for 30 days.

**Group III (*T. terrestris* root extract + Lead acetate + *T. terrestris* root extract):** *T. terrestris* root extract was given by oral gavage at a selected dose level (800 mg/kg body weight) for 7 days and on the 7<sup>th</sup> day just after 30 minutes of the *T. terrestris* root extract administration, lead acetate was given only once. Then from the next day (considered as day 1<sup>st</sup>) *T. terrestris* root extract was given continuously for 30 days. The total experimental period was about 37 days.

**Group IV (Vitamin C + Lead acetate + Vitamin C):** Vitamin C was administered at a dose level of 100 mg/kg body weight for 7 days and on the 7<sup>th</sup> day just after 30 minutes of vitamin C administration, lead acetate was given only once. Then from the next day (considered as day 1<sup>st</sup>) vitamin C was given continuously for 30 days. The total experimental period was about 37 days.

**Autopsy intervals:** The animals from the above groups were sacrificed under light chloroform anesthesia at various intervals i.e. 1, 3, 7, 15 and 30 days.

#### **2.6 Biochemical Assays**

Cholesterol content in tissue was measured by Leiberman Burchard [10] method as given by King and Wolten. The phenanthrene ring of cholesterol reacts with FeCl<sub>3</sub> to give rise to a pinkish to brown color. The intensity of which is directly proportional to concentration of cholesterol present.

Total protein content in testes was estimated by Lowery et al [11] method. Here, protein is precipitated with trichloroacetic acid. When Folin Ciocattou reagent (Phosphatungstic phosphomolybdic acid) is added, a complex results, the intensity of which accounts for the amount of proteins present in tissue Folin Ciocattou reagent after 50 percent dilution in double distilled water. Reading was taken at 640 nm against blank solution. Sialic acid in testis was estimated by the method of Svennerholm [12] as given by Glick. Principle involved depends on the fact that sialic acid (neuraminic acids) exhibit purple color in an acidic medium with resorcinol. Reading was taken at 580 nm (Green Filter) against blank.

Ascorbic acid in testis was estimated by the method of Roe and Kuethers [13]. About 30 mg tissue in 0.5 ml acetate buffer was taken for homogenate preparation. 2 ml of 4% TCA was added to the homogenate and kept overnight and centrifuge. 2 ml supernatant was taken and 0.5 ml color reagent was added. Incubation was done at 37°C for 45 minutes. 3.5 ml 65% H<sub>2</sub>SO<sub>4</sub> was added. Mixed well and reading was taken at 540 nm in colorimeter against blank.

The total thiols (total sulfhydryl groups) content was measured according to the method of Sedlak and Lindsay [14]. About 20 µl Sample/Standard was mixed with 75 µl dilution buffer, 25 µl DTNB reagent and 400 µl Methanol. Centrifugation of the samples was done at 3000x g for 5 min at room temperature and absorbance was measured of the supernatant at 412 nm. The total thiol content was calculated by using a molar extinction Co-efficient of  $13.6 \times 10^3 \text{ M}^{-1} \text{ CM}^{-1}$ .

The adenosine tri phosphatase activity in testis was estimated by the method of Sickevitz and Potter [15]. 0.9 ml of 0.25 M sucrose solution was added to 0.1 ml tissue homogenate. To the above solution 0.3 ml ATP solution, 0.3 ml MgCl<sub>2</sub> and 0.3 ml CaCl<sub>2</sub> was added. The above solution was then kept in water bath at 37°C for 15 minutes. After 15 minutes, 1.6 ml of 1.5 M TCA was added and was centrifuged for 10 minutes. 1 ml of Ammonium molybdate reagent and 0.4 ml of ANSA was added to the 0.5 ml of supernatant and make up to 10 ml by 8.1 ml of 5% TCA. Absorbance was measured at 640/660 nm after 10 minutes against blank.

## 2.7 Statistical Analysis

The data are expressed as mean  $\pm$  SEM. The data were analyzed using the Statistical Package for Social Science program (SPSS 11). Statistical analysis was done using analysis of variance followed by Tukey's test. The values of P as 0.05, 0.01 and 0.001 were considered to be almost significant, significant and highly significant, respectively.

## III. Results & Discussion

In the present study, we observed that lead acetate administration caused testicular dysfunction by disturbing various biochemical parameters such as ATPase, cholesterol, protein, sialic acid, ascorbic acid and total thiols. This impairment of testicular function was prevented by the treatment of *T. terrestris* root extract and vitamin C. In the present study, administration of lead acetate to mice brought a highly significant ( $P < 0.001$ ) reduction in ATPase activity (Fig.1). Our result is in agreement with Chowdhury et al [16] who observed low activity of ATPase at the basement membrane of seminiferous tubules in rats exposed to lead at dose of 6 mg/kg i.p. over a period of 90 days. Liu et al [17] has also been observed that after exposure of lead acetate (300 mg/L) via drinking water at week 6, 8, 10, and 12 in male offspring rat pups, ATPase activity was decreased significantly compared with the control. Decreased activity of ATPase was also observed by other authors after lead exposure in mice which is in agreement of our study [18,19].

During this investigation, a highly significant ( $P < 0.001$ ) increase was observed in testicular cholesterol level in lead intoxicated mice (Fig.2). Cholesterol is the precursor for androgen synthesis in the testes and the conversion of cholesterol to pregnenolone is the most important step in steroidogenesis [20]. Cholesterol is present in Leydig cells and spermatogenic cells including spermatocytes [21]. Our result is in agreement with Nair et al [22] who reported that the elevated cholesterol level has been attributed to its decreased utilization for steroidogenesis which may be due to pituitary repression or a direct inhibitory action of target tissue. A significantly higher cholesterol level in testes of rats after feeding lead acetate at a concentration of 0.25, 0.50 and 1.0 gm/l over a period of 60 days was observed by Chowdhury et al [23]. Daily intraperitoneal administration of lead (8 mg/kg) as lead acetate in male rats from 21 till 120 days of age triggered disturbance in spermatogenesis and increase in cholesterol level [24]. The study revealed a highly significant ( $P < 0.001$ ) reduction in protein level in lead intoxicated mice (Fig.3). Lead is multifactorial and directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl proteins (interrupting structural protein synthesis), alters calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body [25]. Moreover, lead disturbs intracellular  $Ca^{2+}$  homeostasis and damages the endoplasmic reticulum, which in turn results in reduction of protein synthesis [26]. The inhibitory role of lead in protein synthesis may be due to its damaging effect on DNA and RNA [27].

In this investigation, a highly significant ( $P < 0.001$ ) decrease in sialic acid concentration was observed after lead intoxication (Fig. 4). According to Mann [28] sialic acid concentration in the testes declined with the decrease in the rate of spermatogenesis. Therefore, impaired spermatogenesis further contributes to the declined level of sialic acid in the lead exposed mice testes. The synthesis and or secretion of sialic acid is under androgenic control [29]. The alteration in the sialic acid level in reproductive tissues indicates change in the level of glycoproteins/FSH and LH needed for normal functioning of gonads and accessory reproductive organs [30].

A highly significant ( $P < 0.001$ ) reduction in ascorbic acid was observed in lead acetate treated mice (Fig.5). Ascorbic acid in semen has been shown to play an important role in preventing oxidative damage to the spermatozoa [31]. A decrease in the concentration of ascorbic acid in the testis may reflect a decrease in testicular steroidogenesis [32]. Ait Hamadouche et al [6] reported the reduced synthesis of ascorbic acid in lead acetate exposed male Wistar rats compared with the control. Significant lowering of reaction intensity in the localization of testicular  $\Delta^5$ - $\beta$ -hydroxysteroid dehydrogenase along with decrease in testicular ascorbic acid suggested the inhibition of steroidogenesis [5]. A highly significant ( $P < 0.001$ ) decrease in total thiols was observed after lead acetate intoxication in mice (Fig.6). Our result is in agreement with Patra and Swarup [33] who observed that male calves exposed to lead acetate for a period of 28 days orally at the dose level of 0.75% solution/kg body weight showed a declined level of total thiols (protein-bound and non protein-bound -SH content) of erythrocytes. Adult female goats orally exposed to lead acetate at a dose level of 5.46 mg/kg body weight daily for 2 weeks, showed a significantly decreased level of thiol groups on 14<sup>th</sup> day indicating that they are highly involved in chelating lead [34]. Decreased total antioxidant capacity and thiol group levels were also observed in rat submandibular gland and saliva after lead acetate-induced oxidative stress [35]. Our studies indicate that lead causes disturbances in metabolism of reproductive organs by alterations of biochemical parameters due to oxidative stress.

In the present study, *Tribulus terrestris* root extract and vitamin C were found very effective in terms of protection from lead acetate induced toxicity in the testes of Swiss albino mice. *Tribulus terrestris* has long been used as a tonic and aphrodisiac in Unani system of medicine. It has been used in India and Pakistan as a

treatment for impotence and as a stimulant to enhance sexual drive and performance [36]. *T. terrestris* stimulates spermatogenesis and increase the activity of Sertoli cells [37]. *T. terrestris* contains biologically rich compounds as steroids, saponins, flavonoids, alkaloids which have numerous physiological responses [38]. Five steroidal saponins (terrestrosins A-E) together with five known steroidal saponins, desgalactotigonin, F-gitonin, desglucolanatigonin, gitonin and tigogenin were isolated from the fruits of *T. terrestris* by Yan et al [38]. The phytochemical investigation of the aerial parts of *T. terrestris* has resulted in the isolation of novel Furostanol saponin 1, named tribol, together with the known spirostanol [39]. Dried fruit contain semi-drying oil, peroxides, diastase and traces of glucosides, resins, protein and large amount of inorganic matters. From the roots, stem and leaves, sitosterol and stigmasterol were isolated [40].

After administration of *T. terrestris* root extract against lead acetate, all biochemical parameters such as ATPase, cholesterol, protein, sialic acid, ascorbic acid and total thiols were improved as compared to lead treated group. Our results are in agreement with the study of Jagadeesan et al [41] who observed that after *T. terrestris* fruit extract administration in mercury-intoxicated *Mus musculus* mice, the decreased levels of biochemical constituents were restored and total protein content was increased in the liver tissue. Lead causes an increase in cholesterol level by the activation of cholesterol biosynthetic enzyme and suppression of cholesterol catabolism which affects spermatogenesis. Cholesterol enrichment was shown to have an inhibitory effect on many membrane ATPases, as it may directly interact with the boundary lipids of ATPase and alter the intermolecular hydrogen bonds of the protein. A number of studies have shown that saponins from different sources lower serum cholesterol levels in a variety of animals including human subjects [42].

Antioxidants provide a defense mechanism through 3 levels of protection- prevention, interception and repair. In a normal situation, the cellular antioxidant mechanisms present in almost all tissues and their secretions are likely to quench those reactive oxygen species (ROS) and protect against oxidative damage [43]. In the present study, it was observed that vitamin C is a potent antioxidant or free radical scavenger which reduces the lead toxicity in Swiss albino mice testes. Autopsies were done on 1, 3, 7, 15 and 30 days, and at all autopsy intervals there was an improvement in all biochemical parameters. It has been found that vitamin C had protective role on spermatogenesis against lead-induced cell damage which is in agreement with Songthaveesin et al [44]. Pretreatment of spermatozoa with free radical scavengers has been shown to protect sperm DNA from damage by ROS as well as improvement of the spermatozoa performance in the zona binding site or in vitro fertilization [45,46]. In a study of silver refining (involving lead smelting) workers with mean blood lead levels of 32.84 µg/dL and symptoms of lead toxicity (anemia, muscle wasting, abdominal colic) were given vitamin C to evaluate the ability of this supplements to affect lead exposure. With continuous lead exposure and 250 mg vitamin C twice daily for 30 days, significantly lowered blood lead levels [47]. Lead-induced ROS production as examined by rat sperm chemiluminescence generation reduced by 40% with supplementation of 500 mg vitamin C/l drinking water [48].

#### IV. Conclusion

It is concluded from this study that lead acetate induced testicular toxicity may be alleviated by *T. terrestris* root extract and vitamin C, which is reflected by the decline of cholesterol and elevation of ATPase, protein, sialic acid, ascorbic acid and total thiols in Swiss albino mice testes.

#### References

- [1] R.C. Patra, A.K. Rautray, and D. Swarup, Oxidative stress in lead and cadmium toxicity and its amelioration, *Veterinary Medicine International*, 2011, 2011, 1-9.
- [2] R.A. Goyer, and M.G. Cherian, Ascorbic acid and EDTA treatment of lead toxicity in rats, *Life Sciences*, 24(5), 1979, 433-438.
- [3] H.A. Ruff, M.E. Markowitz, P.E. Bijur, and J.F. Rosen, Relationships among blood lead levels, iron deficiency, and cognitive development in two-year-old children, *Environmental Health Perspectives*, 104(2), 1996, 180-185.
- [4] C. Winder, Reproductive and chromosomal effect of occupational exposure to lead on the male, *Reproductive Toxicology*, 3(4), 1989, 221-233.
- [5] A.R. Chowdhury, Recent advances in heavy metals induced effect on male reproductive function- A retrospective, *Al Ameen Journal of Medical Sciences*, 2(2), 2009, 37-42.
- [6] N. Ait Hamadouche, M. Slimani, B. Merad-Boudia, and C. Zaoui, Reproductive toxicity of lead acetate in adult male rats, *American Journal of Scientific Research*, 1(3), 2009, 38-50.
- [7] M.M. El-Tohamy, and W.S. El-Nattat (2010), Effect of antioxidant on lead-induced oxidative damage and reproductive dysfunction in male rabbits, *Journal of American Science*, 6(11), 2010, 613-622.
- [8] Wikipedia, the free encyclopedia, *Tribulus terrestris*, 2013.
- [9] K. Gauthaman, P.G. Adaikan, and R.N. Prasad, Aphrodisiac properties of *Tribulus terrestris* extract (Protodioscin) in normal and castrated rats, *Life Sciences*, 71(12), 2002, 1385-1396.
- [10] B. Leiberman, Microanalysis, in E.J. Kind, and I. Wolten (Eds.), *Medical Biochemistry*, (London: Churchill, 1959) 42-57.
- [11] O.H. Lowery, N.J. Rosebrough, A.L. Farr, and R.J. Randall, Protein measurement with Folin phenol reagent, *The Journal of Biological Chemistry*, 193, 1951, 365.
- [12] L. Svennerholm, Sialic acid quoted by M.W. Whitehouse, and F. Ziliker, in B. Glick, (Ed.), *Methods of Biochemical Analysis*, 8, 1956, 214.
- [13] J.H. Roe, and C.A. Kuethers, Ascorbic acid, *The Journal of Biological Chemistry*, 147, 1943, 399-407.

- [14] J. Sedlak, and R.H. Lindsay, Estimation of total, protein-bound and non protein sulfhydryl groups in tissue with Ellman's reagent, *Analytical Biochemistry*, (24-25), 1958, 192-205.
- [15] P. Sickevitz, and V.R. Potter, Intramitochondrial regulation of oxidative rate, *The Journal of Biological Chemistry*, 201(1), 1953, 1-13.
- [16] A.R. Chowdhury, N.J. Chinoy, A.K. Gautam, R.V. Rao, D.J. Parikh, G.M. Shah, H.N. Highland, K.G. Patel, and B.B. Chatterjee, Effect of lead on human semen, *Contraceptive Delivery System*, 2, 1986, 208-210.
- [17] H. Liu, R. Niu, J. Wang, Y. He, J. Wang, and S. China, Changes caused by fluoride and lead in energy metabolic enzyme activities in the reproductive system of male offspring rats, *Research Report on Fluoride*, 41(3), 2008, 184-191.
- [18] M.L. Vani, and K.P. Reddy, Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice, *Fluoride*, 33(1), 2000, 17-26.
- [19] N.J. Chinoy, S.B. Nair, and D.D. Jhala, Arsenic and fluoride induced toxicity in gastrocnemius muscle of mice and its reversal by therapeutic agents, *Fluoride*, 37(4), 2004, 243-248.
- [20] B.R. Witt, and L.H. Thorneycroft, Reproductive steroid hormone: Generation degeneration, reception and action, *Clinical Obstetrics and Gynecology*, 33, 1990, 563-575.
- [21] R.S. Gupta, R. Sharma, A. Sharma, A.K. Bhatnagar, M.P. Dobhal, and Y.C. Joshi, Effect of *Alstonia scholaris* bark extract on testicular function of Wistar rats, *Asian Journal of Andrology*, 4, 2002, 175-178.
- [22] N. Nair, M.S. Edwards, R.S. Bedwal, and R.S. Mathur, Effect of adrenalectomy and adrenalectomy hydrocortisone treatment of zinc, biochemical parameters and histology of testes of rats, *Indian Journal of Experimental Biology*, 25, 1987, 651-659.
- [23] A.R. Chowdhury, A. Dewan, and D.N. Gandhi, Toxic effect of lead on the testes of rat, *Europe PubMed Central*, 43(1), 1984, 95-100.
- [24] D.K. Saxena, R.S. Srivastava, B. Lal, and S.V. Chandra, The effect of lead exposure on the testes of growing rats, *Experimental Pathology*, 31(4), 1987, 249-252.
- [25] V. Sharma, L. Kansal, A. Sharma, Prophylactic efficacy of *Coriandrum sativum* (Coriander) on testes of lead-induced mice, *Biological Trace Elements Research*, 136(3), 2009, 337-354.
- [26] T. Simons, Lead-calcium interactions in cellular lead toxicity, *Neurotoxicology*, 14, 1993, 77-86.
- [27] M.G. Shalan, M.S. Mostafa, M.M. Hassouna, S.E. El-Nabi, and A. El-Refaei, Amelioration of lead toxicity on rat liver with vitamin C and silymarin supplements, *Toxicology*, 206, 2005, 1-15.
- [28] T. Mann, *Biochemistry of the semen and the male reproductive tract*, (London: John Wiley and Sons, Inc., 1964) 391-392.
- [29] T. Bohmer, S.C. Weddington, and V. Hansson, Effect of testosterone propionate on levels of carnitine and testicular androgen binding protein (ABP) in rat epididymis, *Endocrinology*, 100(3), 1977, 835-838.
- [30] S.I. Manawadi, and B.B. Kaliwal, Methomyl induced gonadal dysfunction, biochemical contents and enzyme activities in male albino mice, *International Journal Biotechnology Applications*, 2(2), 2010, 20-32.
- [31] C. Latchoumycandane, and P.P. Mathur, Effects of hyperthyroidism on the physiological status of pubertal rat testis, *Biomedical Letters*, 59, 1999, 33-41.
- [32] K. Dabrowski, and A. Ciereszko, Ascorbic acid protects against male infertility in a teleost fish, *Experientia*, 52, 1996, 97-100.
- [33] R.C. Patra, and D. Swarup, Effect of lead on erythrocytic antioxidant defence, lipid peroxide level and thiol groups in calves, *Research in Veterinary Science*, 68(1), 2000, 71-74.
- [34] H.M. Mousa, A.A. Al-Qarawi, B.H. Ali, H.A. Abdel Rahman, and S.A. El-Mougy, Effect of lead exposure on the erythrocytic antioxidant levels in Goats, *Journal of Veterinary Medicine*, 49, 2002, 531-534.
- [35] M. Abdollahi, F. Fooladian, B. Emami, K. Zafari, and A. Bahreini-Moghadam, Protection by sildenafil and theophylline of lead acetate-induced oxidative stress in rat submandibular gland and saliva, *Human & Experimental Toxicology*, 22(11), 2003, 587-592.
- [36] G.A. Brown, M.D. Vukovich, E.R. Martini, M.L. Kohut, W.D. Franke, D.A. Jackson, and D.S. King, Endocrine and lipid responses to chronic androstenediol-herbal supplementation in 30 to 58 year old men, *The Journal of the American College of Nutrition*, 20(5), 2001, 520-528.
- [37] D. Sangeeta, H. Sidhu, S.K. Thind, and R. Nath, Effect of *Tribulus terrestris* on oxalate metabolism in rats, *Journal of Ethnopharmacology*, 44(2), 1994, 61-66.
- [38] W. Yan, K. Ohtani, R. Kasai, and K. Yamasaki, Steroidal saponins from fruits of *Tribulus terrestris*, *Phytochemistry*, 45(5), 1996, 1417-1422.
- [39] B. Sun, W.J. Qu, and X.L. Zhang, Investigation on inhibitory and apoptosis-inducing effects of saponins from *Tribulus terrestris* on hepatoma cell line BEL-7402, *Zhongguo Zhongyao Zazhi*, 29(7), 2004, 681-684.
- [40] M. Akram, H.M. Asif, N. Akhtar, P.A. Shah, M. Uzair, G. Shaheen, T. Shamim, S.M. Ali Shah, and K. Ahmad, *Tribulus terrestris* Linn. A review article, *Journal of Medicinal Plants Research*, 5(16), 2011, 3601-3605.
- [41] G. Jagadeesan, A.V. Kavitha, and J. Subashini, FT-IR study of the influence of *Tribulus terrestris* on mercury intoxicated mice, *Mus musculus liver*, *Tropical Biomedicine*, 22(1), 2005, 15-22.
- [42] M. Matsuura, Saponins in garlic as modifiers of the risk of cardiovascular disease, *Journal of Nutrition*, 131, 2001, 1000-1005.
- [43] R. Jones, T. Mann, and R.J. Sherins, Peroxidation breakdown of phospholipids in human spermatozoa: Spermicidal effects of fatty acid peroxides and protective action of seminal plasma, *Fertility Sterility*, 31, 1979, 531-537.
- [44] C. Songthaveesin, J. Saikhun, Y. Kitiyanant, and K. Pavasuthipaisit, Radio-protective effect of vitamin E on spermatogenesis in mice exposed to gamma-irradiation: A flow cytometric study, *Asian Journal of Andrology*, 6(4), 2004, 331-336.
- [45] E. Kessopoulou, H.J. Powers, K.K. Sharma, M.J. Pearson, J.M. Russell, I.D. Cooke, and C.L. Barratt, A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin E to treat reactive oxygen species associated male infertility, *Fertility Sterility*, 64(4), 1995, 825-831.
- [46] S. Lopes, A. Jurisicova, J.G. Sun, and R.F. Casper, Reactive oxygen species: Potential cause for DNA fragmentation in human spermatozoa, *Human Reproduction*, 13(4), 1998, 896-900.
- [47] S.K. Tandon, M. Chatterjee, A. Bhargava, V. Shukla, and V. Bihari, Lead poisoning in Indian silver refiners, *Science of the Total Environment*, 281, 2001, 177-182.
- [48] P.C. Hsu, C.C. Hsu, M.Y. Liu, L.Y. Chen, and Y.L. Guo, Lead-induced changes in spermatozoa function and metabolism, *Journal of Toxicology and Environmental Health, Part A*, 55(1), 1998, 45-64.

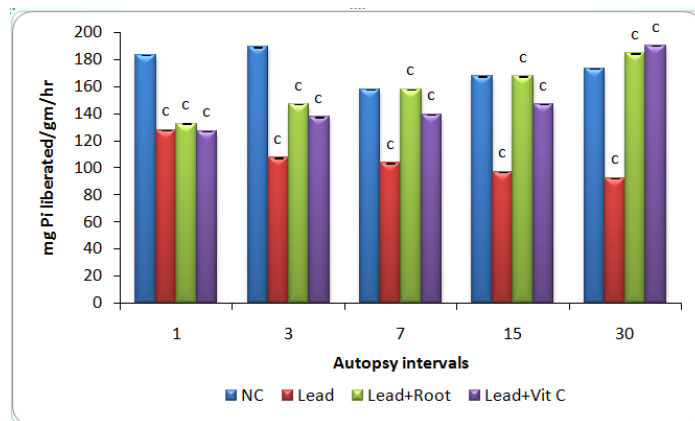


Fig.1. Variation in adenosine triphosphatase activity (mg Pi/gm/hr) in testes of male Swiss albino mice in different groups. Group II (Lead acetate) was compared with Group I (Normal control) and Group III (Lead + Root extract) and Group IV (Lead + Vitamin C) were compared with Group II statistically. Significance level was set as <sup>a</sup>P<0.05 = almost significant, <sup>b</sup>P<0.01 = significant, <sup>c</sup>P<0.001 = highly significant.

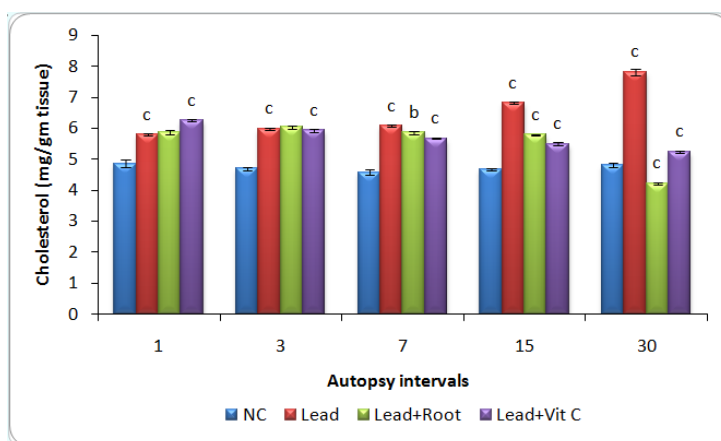


Fig.2. Variation in level of cholesterol (mg/gm tissue) in testes of male Swiss albino mice in different groups. Group II (Lead acetate) was compared with Group I (Normal control) and Group III (Lead + Root extract) and Group IV (Lead + Vitamin C) were compared with Group II statistically. Significance level was set as <sup>a</sup>P<0.05 = almost significant, <sup>b</sup>P<0.01 = significant, <sup>c</sup>P<0.001 = highly significant.

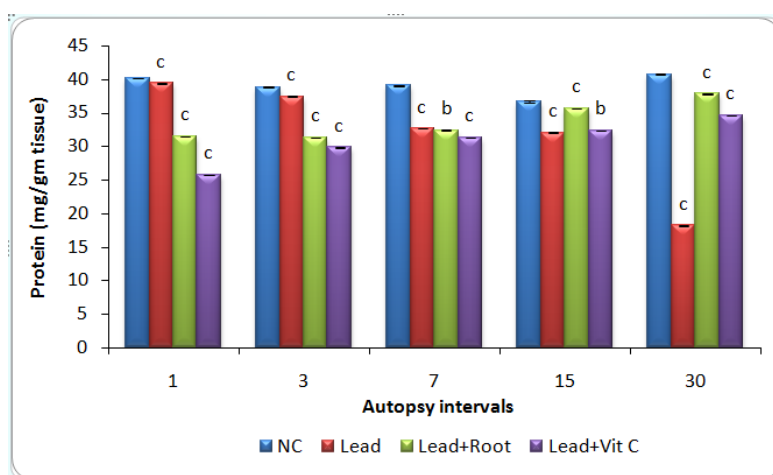


Fig.3. Variation in the amount of total protein (mg/gm tissue) in testes of male Swiss albino mice in different groups. Group II (Lead acetate) was compared with Group I (Normal control) and Group III (Lead + Root extract) and Group IV (Lead + Vitamin C) were compared with Group II statistically. Significance level was set as <sup>a</sup>P<0.05 = almost significant, <sup>b</sup>P<0.01 = significant, <sup>c</sup>P<0.001 = highly significant.

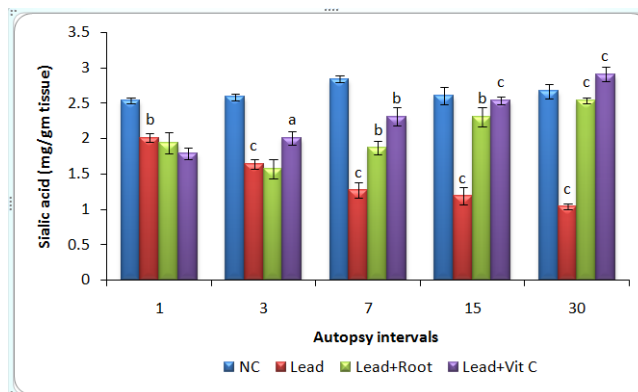


Fig.4. Variation in the amount of sialic acid (mg/gm tissue) in testes of male Swiss albino mice in different groups. Group II (Lead acetate) was compared with Group I (Normal control) and Group III (Lead + Root extract) and Group IV (Lead + Vitamin C) were compared with Group II statistically. Significance level was set as <sup>a</sup>P<0.05 = almost significant, <sup>b</sup>P<0.01 = significant, <sup>c</sup>P<0.001 = highly significant.

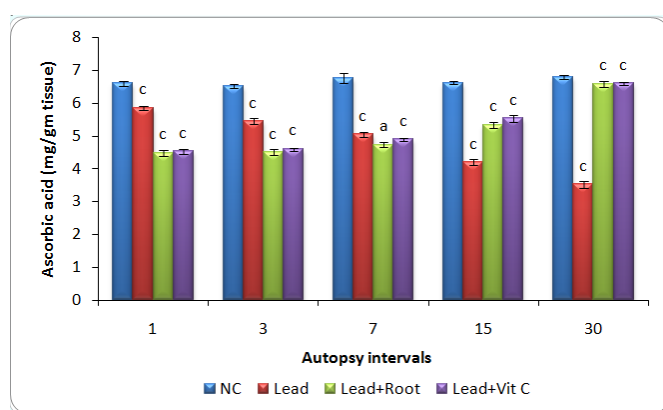


Fig.5. Variation in the amount of ascorbic acid (mg/gm tissue) in testes of male Swiss albino mice in different groups. Group II (Lead acetate) was compared with Group I (Normal control) and Group III (Lead + Root extract) and Group IV (Lead + Vitamin C) were compared with Group II statistically. Significance level was set as <sup>a</sup>P<0.05 = almost significant, <sup>b</sup>P<0.01 = significant, <sup>c</sup>P<0.001 = highly significant.

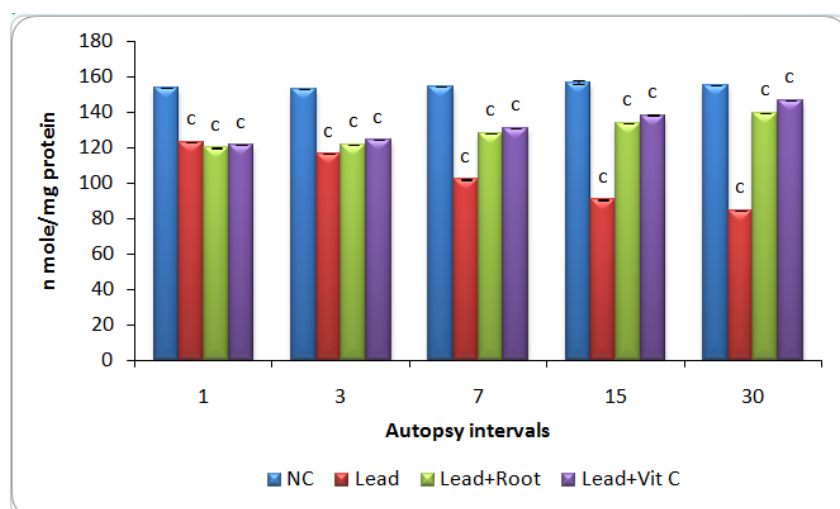


Fig.6. Variation in the amount of total thiols (n mole/mg protein) in testes of male Swiss albino mice in different groups. Group II (Lead acetate) was compared with Group I (Normal control) and Group III (Lead + Root extract) and Group IV (Lead + Vitamin C) were compared with Group II statistically. Significance level was set as <sup>a</sup>P<0.05 = almost significant, <sup>b</sup>P<0.01 = significant, <sup>c</sup>P<0.001 = highly significant.