

Hematobiochemical changes of lead Poisoning and amelioration with *Ocimum sanctum* in wistar albino rats

Sujatha K. Karamala, Sri latha ch, Anjaneyulu Y., Chandra Sekhara Rao T.S.,
Sreeni Vasulu D and Amravathi P. Pidugu

Department of Pathology,
College of Veterinary Science, Tirupati, AP, India.
* Corresponding author email : karamalasuajatha@gmail.com
Published online at www.veterinaryworld.org on 25-03-2011

Abstract

An experiment was carried out to study the hematobiochemical changes of chronic lead poisoning in adult male wistar albino rats for a period of twelve weeks. Adult 216 healthy rats were randomly divided into six groups viz. control (group I), higher dose of lead acetate @ 60mg/kg bwt (group II), Lower dose of lead acetate @ 30 mg/kg bwt (group III), Higher dose of lead + *Ocimum* @ 400 mg/kg bwt (group IV), lower dose of lead + *Ocimum* @ 400 mg/kg bwt (group V), *Ocimum* control (group VI). All lead treated and ameliorated groups given Lead acetate/ lead + *Ocimum* orally for three days in a week for a period of twelve weeks. The mean PCV, Hb, values were reduced significantly ($P < 0.05$) in lead treated rats as dose dependent manner. Where as significant improvement was noticed in *Ocimum* treated groups Increased TLC and PLC values as dose dependent manner. A significant reduction in PNC was noticed in *ocimum* treated groups. Significant ($P < 0.05$) decrease in serum total protein values, serum glucose and increased creatinine values were observed in lead treated groups as dose dependent. Increased protein & glucose and decreased creatinine values obtained in *Ocimum* treated groups. The alterations in hematological and biochemical parameters in the present study indicates decreased lifespan & fragility of RBC and damage to liver, kidney and Pancreas in lead poisoned wistar albino rats.

Key words: Lead poisoning, hematobiochemical alterations, *Ocimum* amelioration, wistar rats

Introduction

Lead is an abundant, ubiquitous, dangerous and important toxic environmental contaminant of global concern due to its significant role in modern industry and it is still being used recklessly (Valverde *et al.*, 2002). Lead occurs in a variety of organic and inorganic compounds with a multitude of additional uses in the manufacture of protective paints for iron and steel, explosives, rodenticide, batteries etc. The manipulation of lead for these uses has caused lead contamination of air, dust and soil. It can be present in air in the vicinity of factories and on the highways. Lead levels in air, water and soil have been increasing during the last 10 years and it is considered to be one of the most important environmental pollutants of both urban and semi urban areas. Vegetables are polluted by lead from the air and a considerable amount of lead contamination is found in cereals and broad leafed vegetables. Because of its persistence in the environment, exposure to lead has become a major public health concern (Vaglenov *et al.*, 2001).

Several authors tried various ameliorating

agents like Thiamine, vitamin E, selenium, zinc etc. There was a meager information was available regarding herbal products as ameliorating agent. Keeping in view, *Ocimum sanctum* was used as an ameliorating agent in present research. *Ocimum sanctum* (OS) commonly known as 'Tulasi' in Hindi is a medicinal plant commonly grown in India. The use of this herb has been reported in Indian traditional systems of Medicine and its modern applications are receiving wide spread attention day by day. Different parts of this plant have been claimed to be valuable in a wide spectrum of diseases. It has been observed that tulasi has antioxidant, antibiotic, antiatherogenic, immunomodulatory, anti-inflammatory, analgesic, antiulcer, chemopreventive and antipyretic properties (Surender Singh *et al.*, 2007).

Material and Methods

Procurement of experimental animals, lead acetate and *Ocimum sanctum* (OS)

Male Wistar albino rats with body weight around 150g were utilized for the present experiment. Rats were acclimatized to the experimental conditions for

Table-1: Different groups with no. of animals and treatments.

Group	No. of Animals	Treatment
Group I	36	0.2 ml of D.W
Group II	36	1/10th of LD50 (60mg of lead acetate /kg bwt / 3 days a week)
Group III	36	1/20th of LD50 (30mg of lead acetate / kg bwt /3 days a week)
Group IV	36	1/10th LD50 (60mg/kg bwt of lead acetate / 3 days a week and 400mg / kg bwt OS/3 days a week)
Group V	36	1/20th of LD50 (30mg/kg bwt of lead acetate / 3 days a week) and 400mg OS / kg bwt /3 days a week)
Group VI	36	400mg / kg bwt of OS / 3days a week

one week, after acclimatization, animals were grouped and housed in standard poly propylene rat cages (three rats 1 cage) during the experiment. They were maintained at $25^{\circ} \pm 1^{\circ} \text{C}$ and a 12:12 hour interval light / dark cycle through out the experimental period for 12 weeks(3months) by taking necessary precautions, standard laboratory hygienic conditions by providing laboratory animal feed and water *adlibitum*. The approval of the institutional animal ethical committee was obtained prior to commencement of the experiment.

The Lead acetate ($(\text{CCH}_3\text{COO}_2)_2\text{Pb} \cdot 3\text{H}_2\text{O}$, M.w = 379.33) with a laboratory reagent grade was procured from the Qualigens Fine chemicals, Bombay, India and *Ocimum sanctum* (OS) from the Natural Remedies Pvt. Ltd. Bangalore, India.

Experimental trial

Total of 216 healthy young male rats were randomly assigned to the control and treatment groups. Six groups of rats consisting of 36 rats in each group were used for the study. To know the LD_{50} value of lead acetate a separate study was conducted with 36 rats before starting of the experiment and according to that dose was calculated. Lead acetate and OS gavaged per orally by using distilled water as vehicle. The groups and the doses employed for the present study were shown in Table-1.

Six rats from each group were randomly sacrificed at every fortnight intervals after starting the experiment i.e. 2nd, 4th, 6th, 8th, 10th and 12th weeks.

Selection of Dose

The lead acetate dose was selected based on the pilot study and the OS dose was selected based on the observations of Manikandan *et al.* (2007) who

reported that 100, 200,300,400 mg /kg bwt of OS were administered to Wistar rats followed by intra peritoneal injections of DMBA (7, 12, Dimethyl Benz (a) Anthracene) (30mg /kg bwt) 90 minutes after the final dose of the extract. At this dose of DMBA induced the genotoxicity by formation of micronuclei. The animals pretreated with OS @ a concentration of 300mg and 400mg /kg bwt was significantly reduced micronuclei formation i.e. reduced genotoxic effect of DMBA. Hence the higher dose of OS i.e. 400mg /kg bwt was selected as dose.

Hematology

Blood collected in 10% EDTA solution from all the groups at each sacrifice was used for the estimation of total erythrocyte count (TEC), Total Leucocyte count (TLC) and Packed cell volume (PCV) by Microhematocrit method (Jain 1986) and Hemoglobin (Hb) by sahli's method (Coles, 1986). The blood smears were directly prepared and stained by leishman's stain for Differential Leucocyte count (DLC) by Battlement method (Jain, 1986).

Biochemical assay

Blood was collected from all groups at each sacrifice directly in to the sterile test tube and allowed to clot. The serum was collected and stored at 4°C i.e. in a refrigerator until use and was used for the estimation of Total serum proteins (Transasia biochemical Ltd), glucose (M/S Excel Diagnostics Ltd) and creatinine (kamineni life sciences Pvt. Ltd) using kits.

Results

The mean TEC, PCV, Hb and PLC values reduced significantly ($P < 0.05$) and significant

Table-2: Hematological parameters (mean and SE) in animals of different experimental groups

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
TEC	6.28a \pm 0.05	5.63d \pm 0.16	5.83cd \pm 0.11	5.88bcd \pm 0.08	6.04a \pm 0.13	6.02abc \pm 0.19
Hb	13.97a \pm 0.23	12.70d \pm 0.25	13.11bcd \pm 0.15	12.91cd \pm 0.30	13.40abcd \pm 0.19	14.07a \pm 0.42
PCV	35.25a \pm 1.69	28.67d \pm 0.76	32.17bc \pm 0.70	30.83cd \pm 0.70	35.42a \pm 0.61	35.83a \pm 1.19
TLC	7.48c \pm 0.34	9.35a \pm 0.79	8.93bc \pm 0.73	8.85a \pm 0.59	8.49abc \pm 0.54	7.54bc \pm 0.34
% Neutrophil	17.83d \pm 0.48	24.50a \pm 1.98	21.83abc \pm 1.28	23.00cd \pm 1.84	19.33bcd \pm 1.41	18.67cd \pm 0.33
% Lymphocyte	80.83a \pm 0.65	75.17c \pm 1.66	77.50abc \pm 1.48	76.00bc \pm 1.59	79.83a \pm 1.25	80.67a \pm 0.33

Mean values with different superscripts differ significantly ($p < 0.05$), ANOVA, S.E – Standard error

Table-3: Biochemical parameters (mean and SE) in animals of different experimental groups

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
TSP(g/dl)	5.98a±0.04	3.47e±0.67	3.88de±0.38	4.23cde±0.29	4.52bcde±0.32	6.72a±0.21
Serum glucose (mg %)	68.61bc±0.58	48.61f±4.58	58.87e±2.05	61.54de±0.58	68.32c±1.37	79.88a±0.70
Creatinine (mg/dl)	2.67e±0.12	5.39a±0.60	4.03 bc±0.37	5.08a±0.45	3.82cd±0.28	2.85de±0.07

Mean values with different superscripts differ significantly ($p < 0.05$), ANOVA, S.E – Standard error

increase in TLC, PNC were observed in lead fed rats (group II & III) when compared to control. Significant improvement was noticed in TEC, Hb, PCV, PLC values and significant reduction in the TLC, PNC values in *Ocimum* ameliorated groups (Group IV & V) when compared to corresponding lead fed groups. The overall mean Total erythrocyte count (TEC), packed cell volume (PCV), Hemoglobin (Hb), Total leucocyte count (TLC), Percent neutrophil count (PNC) and Percent lymphocyte count (PLC) were given in Table-2.

Total serum proteins and glucose levels decreased significantly ($P < 0.05$) and significant increase in serum creatinine values were observed throughout the experimental period in lead treated rats (group II & III) as dose dependent manner when compared to control. In *Ocimum* ameliorated groups (IV & V) the TSP and glucose values were improved and decreased serum creatinine levels were noticed in *Ocimum* ameliorated groups than lead treated groups as dose dependant manner (Table-3).

Discussion

The present study revealed that the mean TEC, PCV, Hb and PLC values were reduced significantly ($P < 0.05$) and significant increase in TLC, PNC were observed in lead fed rats (group II & III) when compared to control and suggesting that decreased values of TEC, Hb and PCV might be due to decreased life span of erythrocytes (Julian Chisolm 1971) and increased fragility of erythrocytes (Donavick, 1966) and inhibitory effect of lead on erythrocyte enzymes (GA3PD & G6PD) (Stone and Soares 1976). Similar observations were made by MuGahi et al. (2003) in adult rats. In contrary Suradkar et al. (2009) reported decrease in TLC and no significant change in neutrophil count in wistar rats fed with lead acetate @ 1ppm, 100ppm and 1000ppm orally for 28 days. The increased values of TLC, PNC might be due to lead inflammation in tissues (Yagminas et al. 1990). Lymphocytopenia might be due to pathological changes that were taken place in spleen and lymphnode.

Significant improvement in TEC, Hb, PCV, PLC values and significant reduction in the TLC, PNC

values in *Ocimum* ameliorated groups were noticed (Group IV & V) when compared to lead fed groups. This might be due to hemoprotective immune stimulatory and anti inflammatory effect and antioxidant property of *Ocimum* (Surendar singh, 2007).

Biochemically TSP values were decreased significantly in lead treated rats as dose dependent manner and the results are in accordance with Stowe et al. (1973) and Hameed et al. (2008). Decreased levels in TSP might be due to binding of lead to albumin (Stone and Soares, 1976), disturbance in the protein metabolism in the liver consequent to accumulation of lead leads to liver injury (Sharaf et al. 2008). Reduced glucose levels were observed in all lead treated groups (II & III) throughout the experimental period as dose dependant manner. The earlier authors reported hypoglycemia i.e. Ashour (2002) in rabbits and Ashour et al. (2007) in male albino rats. In the present study, the hypoglycemia might be due to dysfunction of liver, thyroid (Goud et al. 1985) and pancreatic damage that was evident microscopically. A significant improvement was observed in TSP and glucose levels of *Ocimum* ameliorated groups in dose dependant manner and this might be due to hepatoprotective effect of *Ocimum* (Razvi Syad Ubiad et al. 2000). Significant increase in serum creatinine values in all lead treated groups in a dose dependant manner when compared to control. Similar changes reported by Ashour et al. (2007) and Suradkar et al. (2009). The increase in creatinine values might be due to severe renal parenchymal damage as evidenced by microscopic examination of kidneys that might have lead to impaired glomerular infiltration (Dev et al. 1991). Decreased serum creatinine levels were noticed in *Ocimum* ameliorated groups than lead treated groups as dose dependant manner. This might be because of nephroprotective effect of *Ocimum* as reported by Mukesh Kumar Sharma et al. (2005).

Conclusion

The results in the present study suggest that the hematologically TEC, PCV, Hb and PLC values were significantly decreased and increased TLC & PNC and

biochemically TSP& glucose values were reduced and increased creatinine values in lead fed Wistar albino rats as dose dependent manner. In *Ocimum* ameliorated groups (IV & V) hematological & biochemical parameters were improved when compare to its lead treated groups. Further, suggest that *Ocimum* effectively minimizing the lead induced hematobiochemical alterations @ dose rate of 400 mgs/kg bwt. This might be due to its hemoprotective immune stimulatory and anti inflammatory effect and antioxidant property.

Acknowledgements

The authors thankful to Sri Venkateswara Veterinary University for providing various facilities to carryout PhD research work in the Department of Pathology, College of Veterinary Science, Tirupati for fulfillment of PhD degree of first author.

References

1. Ashour, A. E. (2002): Can Garlic lobes, Olive oil (or) Black seed oil offer protection for some serum biochemical constituents against lead toxicity in Rabbits. *AL-AQSA University J.* 6: 74-95.
2. Ashour, A. E. R. A., Yassin, M. M., Abuasis, N. M. and Ali, R. M. (2007): Blood, Serum Glucose and renal parameters in lead-loaded Albino rats and treatment with some chelating agents and natural oil. *Turkey J. Biolo.* 31:25-34.
3. Dev, P. R., Swarup, D. and Dwivedi, S. K. (1991): Some renal function tests in experimental lead toxicity in Goats. *Ind. Vet. J.* 68: 1163-1167.
4. Donawick, W. K. (1966): Chronic lead poisoning a cow. *JAVMA* 148: 655-661.
5. Gouda, I.M., Abdel-Aziz, S.A., Ahmed, A.A., Lotfic, M.M., Soliman, M.M. (1985): changes in some Liver function in experimentally lead poisoned goats. *Archive fur veterineri Medizine Leipzig*, 39: 257-67.
6. Hameed, A. R. A. E., Shalaby, S. I. A., Mohamed, A. H. and Sabra, H. A. (2008): Effect of oral administration of lead acetate on some biochemical and hormonal parameters during pregnancy in Baladi Goats. *Global Veterinaria* 2(6): 301-307.
7. Julian Chisolm, J. (1971) Lead poisoning. *Scientific American*, 224:15-24.
8. MuGahi, M. N., Heidar, Z., SaGheb, H. M. and Barbarestani, M. (2003): Effects of Chronic lead acetate intoxication on blood indices of male adult rat. *DARU* 11(4): 147-151.
9. Mukhesh Kumar Sharma, Madhu Kumar and Ashok Kumar, (2005): Protection against mercury induced renal damage in Swiss albino mice by *Ocimum sanctum*. *Environmental Toxicology and Pharmacology* 19(1): 161-167.
10. Razvi Syed, Uaid Kothekar Mudgal and Anant Rao, (2000): Effect of *Ocimum sanctum* leaf extract on hepatotoxicity induced by antitubercular drugs in rats. *Ind. J. physiology and Pharmacology.* 47(4): 465-470.
11. Sharaf, N.E. Zaki, M.S. Gomaa, W.R. Batrawy, N.E.I. and Fawzi, O.M. (2008): Some Clinicopathological and microbiological studies on lead toxicity in bull. *American Eurasian Journal of Agricultural and Environmental Science*, 3(2): 165-168.
12. Stone, C. L. and Soares, J.H. (1976): The effect of dietary selenium level on lead toxicity in the Japanese quail, *Poultry Science* 55: 341-349.
13. Stowe, H. D., Goyer, R. A., Krigman, M. M., Wilson, M. and Cates, M. (1973): Environmental Oral lead toxicity in young dogs. *Archie. Pathology* 95: 106-116.
14. Suradkar, S. G., Ghodasara, D. J., Pritivihol Jatin Patel, Vikas Jaiswal and Prajapati, K. S. (2009): Hemato biochemical Alterations induced by lead acetate toxicity in wistar rats. *Veterinary World* 2(11): 429-439.
15. Surender Singh, Manish Taneja, Dipak, K. and Majumdar (2007): Biological activities of *Ocimum sanctum* L fixed Oil-An overview. *Ind. J. Experi. Biology* 45: 403-412.
16. Yagminas, A.P., Franklin, C.A., Villeneuve, D.C., Gilman, A.P., Little, P.B. and Valli, V.E.O. (1990): Sub chronic oral toxicity of Triethyl lead in the male weanling Rat. Clinical Biochemical Hematological and Histopathological effects. *Fundamentals of Applied Toxicology* 15: 580-596.

* * * * *