[Downloaded free from http://www.toxicologyinternational.com on Wednesday, April 27, 2016, IP: 14.139.224.243]

Original Article

www.toxicologyinternational.com

Oxidative Stress Induced by Lead and Antioxidant Potential of Certain Adaptogens in Poultry

M. Ratan Kumar, A. Gopala Reddy, Y. Anjaneyulu[1], G. Dilip Reddy

Department of Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, [1]Department of Veterinary Pathology, College of Veterinary Science, Rajendranagar, Hyderabad - 500030, India

ABSTRACT

Effect of lead was studied for its action on antioxidant defense in broilers. A total of 225 one-day-old male broiler chicks (Vencobb strain) were divided randomly into 15 groups consisting of 15 chicks in each group. Group 1 was maintained on basal diet, group 2 on polyherbal formulation (PHF; stressroak), group 3 on shilajit, group 4 on amla, and group 5 on vitamin E + selenium (Se). Group 6 was maintained on lead for 42 days (6 weeks) and group 7 on lead for 28 days and subsequently on basal diet without lead for the remaining two weeks. Groups 8, 9, 10, and 11 were given lead along with PHF, shilajit, amla, and vitamin E + Se, respectively throughout the experiment for 6 weeks. Groups 12, 13, 14, and 15 were given lead containing diet for the first four weeks (28 days) and subsequently treated with PHF, shilajit, amla, and vitamin E + Se, respectively for the remaining two weeks. Antioxidant status of the birds was analyzed by assaying blood samples for glutathione (GSH) peroxidase, GSH reductase, and catalase at the end of fourth and sixth weeks, whereas Thiobarbituric acid reacting substances (TBARS) and GSH concentrations were estimated in liver homogenate at the end of the sixth week. The antioxidant defense parameters were significantly altered in toxic control groups indicating the possible oxidative damage caused by lead, whereas the parameters were normal in control groups 1 to 5 and other groups that were given the drugs in test, indicating their good ameliorating activity in oxidative stress.

Key words: Amelioration by adaptogens, lead, oxidative stress

DOI: 10.4103/0971-6580.72668

INTRODUCTION

The evidence of involvement of free radicals and reactive oxygen species (ROS) in the pathogenesis of number of diseases and toxicities is increasing constantly. ROS are involved in the biological damage induced by a number of therapeutic molecules, poisonous chemicals, and toxins.[1] Studies have reported that lead has a potential of inducing oxidative stress and acts as a catalyst in the oxidative reactions of biological molecules by producing free radicals ROS. Therefore, the toxicities to several organ systems associated with this metal might be due to oxidative tissue damage. To prevent peroxidative tissue damage, there are protective mechanisms *in vivo*, such as an enzymatic (antioxidant enzymes) and nonenzymatic (GSH) defenses. Lead interferes with the activities of different antioxidant defenses, and reduced amounts of antioxidants may contribute to damage to organ systems including liver, kidney, and nervous system.[2] Oxidative damage by free radicals can be prevented by the use of antioxidants.[3] Keeping the above facts in view, an experimental study was planned to evaluate the mechanisms of lead-induced oxidative stress and injury to the biological system, and to evaluate the prophylactic and therapeutic potential of polyherbal formulation (PHF; stressroak), shilajit, amla, and vitamin E + selenium (Se) against experimental lead toxicosis in broilers.

MATERIALS AND METHODS

A total of 225 male broiler chicks (Cobb strain) of one-

Address for correspondence: Dr. A. Gopala Reddy, Department of Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar, Hyderabad - 500030, India. E-mail: gopalareddy123@rediffmail.com

[Downloaded free from http://www.toxicologyinternational.com on Wednesday, April 27, 2016, IP: 14.139.224.243]

day-old were randomly divided into 15 groups of 15 chicks in each group. Feed and water were provided *ad libitum* throughout the experiment. Groups 1, 2, 3, 4, and 5 were maintained on basal diet control, PHF (stressroak; 100 ppm in feed), shilajit (100 ppm in feed), amla control (500 ppm in feed), and vitamin E + Se (300 + 0.3 ppm in feed), respectively, and groups 6 and 7 were the toxic controls that were kept on lead for 42 and 28 days, respectively. Groups 8, 9, 10, and 11 were given lead along with PHF, shilajit, amla, and vitamin E + Se, respectively for six weeks (1 – 42 days). Groups 12, 13, 14, and 15 were maintained on lead for the first four weeks and on PHF, shilajit, amla, and vitamin E + Se for the subsequent two weeks.

Birds of all the groups were vaccinated with New castle disease vaccine on 7th and 28th day and infectious bursal disease vaccine on 10th day. Blood samples were drawn from the wing vein on 28th and 42nd day from the birds (identified by wing band numbers) in each group for assay of glutathione peroxidase (GSH-PX),[4] glutathione reductase (GSH-R),[5] and catalase.[6] The TBARS[7] and glutathione (GSH)[8] concentrations were estimated by using liver homogenate at the end of the sixth week. All the chemicals used in the study are of analytical grade and were procured from Qualigens Pvt. Ltd., Mumbai, India. The data were subjected to statistical analysis by applying ANOVA as per the standard methods of Snedecor and Cochran.[9] Differences between means were tested using Duncan's multiple comparison test, and significance was set at $P<0.05$.

RESULTS AND DISCUSSION

The results of the study are presented in Table 1. The activities of GSH-PX, GSH-R, and catalase and the concentration of TBARS were significantly ($P<0.05$) elevated, whereas the concentration of GSH was significantly ($P<0.05$) reduced in lead toxic control groups 6, 7, 12, 13, 14, and 15 at the end of fourth week. Groups 12, 13, 14, and 15

Table 1: Results of oxidative stress

Group	GSH-PX activity (units/ml)		GSH-R activity (units/ml)		Catalase activity (moles/sec)		GSH (n moles/g protein	TBARS activity (nmoles/mg protein)
	4th week	6th week	4th week	6th week	4th week	6th week	6th week	6th week
Basal diet (1 – 42 d)	85.387 ± 1.805^{bA}	87.278 ± 1.446^{bA}	43.481 ± 1.292^{dA}	42.300 ± 0.998^{dB}	2.517 ± 0.100^{cdA}	3.042 ± 0.042^{efB}	107.478 ± 0.57^{h}	0.807 ± 0.03^{c}
PHF (stressroak) (1 – 42 d)	68.080 ± 1.681^{aA}	70.799 ± 1.285^{aB}	21.237 ± 0.682^{aA}	19.170 ± 0.601^{aB}	1.906 ± 0.020^{aA}	2.129 ± 0.096^{aB}	122.028 ± 0.72^{j}	2.010 ± 0.05^{h}
Shilajit (1 – 42 d)	69.013 ± 1.456^{aA}	71.147 ± 1.474^{aA}	24.188 ± 1.177^{bA}	20.107 ± 0.777^{abB}	2.109 ± 0.062^{bA}	2.408 ± 0.065^{bB}	120.039 ± 0.48^{j}	0.407 ± 0.01^{a}
Amla (1 – 42 d)	70.045 ± 1.440^{aA}	72.134 ± 1.498^{aA}	27.271 ± 0.922^{cA}	22.196 ± 0.974^{bB}	2.401 ± 0.098^{cA}	2.609 ± 0.083^{cB}	115.927 ± 0.68^{i}	0.502 ± 0.00^{ab}
Vitamin E + Se (1 – 42 d)	71.990 ± 1.495^{aA}	74.165 ± 1.268^{aA}	28.999 ± 0.587^{cA}	25.023 ± 0.803^{cB}	2.608 ± 0.114^{dA}	2.807 ± 0.134^{dB}	116.058 ± 0.74^{i}	0.604 ± 0.01^{b}
Lead (1 – 42 d)	130.523 ± 9.643^{fA}	139.503 ± 10.088^{hB}	76.801 ± 2.225^{hiA}	86.065 ± 2.497^{lB}	3.396 ± 0.097^{gA}	3.830 ± 0.049^{kB}	68.325 ± 0.81^{a}	1.202 ± 0.07^{fg}
Lead (1 – 28 d); basal diet (29 – 42 d)	128.924 ± 5.939^{fA}	106.496 ± 3.379^{dB}	79.637 ± 2.072^{jA}	54.194 ± 1.528^{hB}	3.407 ± 0.088^{gA}	3.204 ± 0.032^{fghB}	72.357 ± 1.22^{b}	1.105 ± 0.07^{ef}
Lead + PHF (stressroak) (1 – 42 d)	108.228 ± 1.060^{cA}	112.383 ± 1.236^{deB}	60.127 ± 1.260^{eA}	64.097 ± 1.543^{iB}	2.800 ± 0.127^{eA}	3.339 ± 0.072^{hiB}	95.953 ± 1.01^{g}	0.913 ± 0.07^{cd}
Lead + shilajit (1 – 42 d)	111.223 ± 1.265^{cdA}	115.294 ± 1.404^{efgB}	63.204 ± 1.257^{fA}	67.258 ± 1.4089^{jB}	3.112 ± 0.095^{fA}	3.508 ± 0.085^{jB}	92.089 ± 0.80^{f}	1.066 ± 0.04^{ef}
Lead +amla (1 – 42 d)	115.078 ± 1.662^{deA}	119.232 ± 1.279^{fgB}	65.109 ± 1.387^{fgA}	69.094 ± 0.663^{jkB}	3.315 ± 0.087^{gA}	3.701 ± 0.111^{kB}	87.989 ± 1.11^{de}	1.201 ± 0.04^{fg}
Lead +vitamin E + Se (1 – 42 d)	118.050 ± 0.920^{eA}	121.240 ± 1.299^{gB}	67.019 ± 1.450^{gA}	70.746 ± 1.616^{kB}	3.415 ± 0.070^{gA}	3.796 ± 0.091^{kB}	89.205 ± 0.95^{e}	1.318 ± 0.10^{g}
Lead (1 – 28 d); PHF (stressroak) (29 – 42 d)	129.326 ± 4.765^{fA}	89.007 ± 1.969^{bcB}	78.308 ± 1.945^{ijA}	44.114 ± 1.222^{deB}	3.301 ± 0.065^{gA}	2.912 ± 0.142^{deB}	85.290 ± 1.37^{c}	0.806 ± 0.03^{c}
Lead (1 – 28 d); shilajit (29 – 42 d)	111.081 ± 1.781^{cdA}	114.955 ± 1.476^{efB}	75.046 ± 0.905^{hA}	45.135 ± 1.150^{efB}	3.401 ± 0.076^{gA}	3.074 ± 0.138^{efgB}	88.326 ± 0.80^{de}	0.907 ± 0.04^{cd}
Lead (1 – 28 d); amla (29 – 42 d)	129.322 ± 3.335^{fA}	93.330 ± 1.175^{bcB}	77.397 ± 0.935^{hijA}	47.203 ± 1.300^{fgB}	3.409 ± 0.108^{gA}	3.208 ± 0.070^{ghB}	85.927 ± 0.51^{cd}	1.018 ± 0.06^{de}
Lead (1 – 28 d); vitamin E + Se (29 – 42 d)	130.597 ± 3.625^{fA}	94.911 ± 1.391^{cB}	78.331 ± 1.357^{ijA}	49.211 ± 1.863^{gB}	3.349 ± 0.088^{gA}	3.419 ± 0.059^{ijB}	84.992 ± 1.05^{c}	1.111 ± 0.07^{ef}

Values are mean + SE of eight observations; Means with different alphabets as superscripts differ significantly ($P<0.05$) ANOVA; Capital alphabets (horizontal comparison); small alphabets (vertical comparison); GSH-PX - glutathione peroxidase; GSH-R - glutathione reductase; TBARS - Thiobarbituric acid reacting substances; PHF - polyherbal formulation; Se – selenium; SE - Standard error

[Downloaded free from http://www.toxicologyinternational.com on Wednesday, April 27, 2016, IP: 14.139.224.243]

that were supplemented respectively with PHF (stressroak), shilajit, amla, and vitamin E + Se during the last two weeks following discontinuation of lead revealed a significant ($P<0.05$) improvement in these parameters at the end of sixth week as compared with groups 6 and 7.

The concentration of GSH in liver was assessed, as it is the major organ involved in xenobiotic metabolism. TBARS in liver tissue were analyzed to determine the extent of peroxidative stress, and the activities of GSH-PX, GSH-R, and catalase were assessed, as they form the major components of antioxidant defense system in the living. The activities of GSH-PX, GSH-R, and catalase in blood and TBARS activity in liver were significantly increased in toxic groups, whereas the concentration of GSH was reduced suggesting the ongoing peroxidative stress. Recent studies have shown that lead causes oxidative stress by inducing the generation of ROS, reducing the antioxidant defense system of cells by depleting GSH, inhibiting sulfhydryl-dependent enzymes, interfering with certain essential metals needed for antioxidant enzyme activities, and/or increasing susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition. Consequently, it is possible that impaired oxidant/antioxidant balance could be partially responsible for the toxic effects of lead. Enhanced oxidative stress contributes to lead-induced toxicity, where restoration of a cell's antioxidant capacity appears to provide a partial remedy. Several studies are underway to determine the effect of antioxidant supplementation following lead exposure. Data suggest that antioxidants may play an important role in abating some hazards of lead.[10] All the changes in the antioxidant defense profile were significantly reversed when treated with PHF (stressroak), shilajit, amla, and vitamin E + Se. The beneficial effects of PHF (stressroak) are attributed to antioxidant and antistress principles namely, *Withania somnifera*,[11] *Ocimum sanctum*,[12,13] *Phyllanthus emblica*,[14] *Mangifera indica*,[15] and shilajit.[16] The withanolides present in *Withania somnifera* are known to inhibit lipid peroxidation by their antioxidant properties that break the chain reaction.[17] The *Ocimum sanctum* has been reported to reduce lipid peroxidation and increase the GSH concentration in blood.[18] Gallic acid and geraniin, which are the active principles of *Phyllanthus emblica*, have been reported to possess strong nitric oxide (free radical) scavenging activity.[19] In a study, Hernandez *et al*.[20] reported that *M. indica* extract attenuated accumulation of ROS. Se is considered as an essential component of GSH-PX, which is the major intracellular antioxidative enzyme that catalyses the reduction of hydrogen peroxide and organic hydroperoxides to nontoxic compounds. Vitamin E, which is abundant in several natural sources, has free radical quenching activity.[13] Several reports have demonstrated an antioxidant synergism between vitamin E and Se in counteracting free radical-induced oxidative stress in the biological system.[21]

From this study, it can be concluded that lead-induced damage to the biological system is attributed to the excess generation of free radicals and impairment of antioxidant defenses, and supplementation of PHF (stressroak), shilajit, amla, and vitamin E + Se either prophylactically or therapeutically could significantly reverse the toxic effects of lead.

REFERENCES

1. Loguerico C, De Girolamo V, De Sio I, Tuccillo C, Ascione A, Baldi F, *et al*. Non alcoholic fattyliver disease in an area of southern Italy: Main clinical, histological and patho physiological aspects. J Hepatol 2001;35:568-574.
2. Farrukh A, Iqbal A, Zafar M. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turk J Biol 2006;30:177-83.
3. Kamashi K, Reddy AG, Reddy KS, Reddy VR. Evaluation of zinc against salinomycin toxicity in broilers. Indian J Physiol Pharmacol 2004;48:89-95.
4. Paglia DE, Valentine WN. Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967;70:158-69.
5. Raghuramulu N. Manual of Laboratory Techniques. Hyderabad: National Institute of Nutrition (ICMR); 1983. p. 204-6.
6. Britton C, Machlly AC. Assay of catalase and peroxidases. Methods Enzymol 1956;136:765-8.
7. Balasubramanian KA, Manohar M, Mathan VI. An unidentified inhibitor of lipid peroxidation in intestinal mucosa. Biochim Biophys Acta 1988;962:51-8.
8. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione - s- transferase in rat lung and liver. Biochim Biophys Acta 1979;582:67-8.
9. Snedecor WG, Cochran GW. Statistical Methods. 8th ed. USA: Iowa State University Press; 1994.
10. Gurer H, Ercal N. Can antioxidants be beneficial in the treatment of lead poisoning? Free Radic Biol Med 2000;29:927-45.
11. Russo A, Izzo AA, Cardile V, Borrelli F, Vanella A. Indian medicinal plants as antiradicals and DNA cleavage protectors. Phytomedicine 2001;8:125-32.
12. Devi PU, Ganasoundari A. Modulation of glutathione and antioxidant enzymes by *Ocimum sanctum* and its role in protection against radiation injury. Indian J Exp Biol 1999;37:262-8.
13. Halim EM, Mukhopadhyay AK. Effect of O*cimum sanctum* (tulsi) and vitamin E on biochemical parameters and retinopathy in streptozotocin induced diabetic rats. Indian J Clin Biochem 2006;21:181-8.
14. Bhattacharya A, Ghosal S, Bhattacharya SK. Antioxidant activity of tannoid principles of *Emblica officinalis* (amla) in chronic stress induced changes in rat brain. Indian J Exp Biol 2000;38:877-80.
15. Martinez G, Delgado R, Perez G, Garrido G, Nunez Selles AJ, Leon OS. Evaluation of the *in vitro* antioxidant activity *of Mangifera indica* L. extract (Vimang). Phytother Res 2000;14:424-7.
16. Bhattacharya SK, Bhattachrya A, Chakrabarti A. Adaptogenic activity of Siotone, a polyherbal formulation of Ayurvedic rasayanas. Indian J Exp Biol 2000;38:119-28.
17. Devasagayam TP, Kamat JP, Nair S, Sreejayam N, Nesaretnam K, Packer L. Antioxidant action of curcumin. Micronutrients and health: Molecular Biological Mechanisms. In: Nesaretnam K, Packer L, Editors. USA: AOCS, Press Champaign, IL; 2001. p. 42-59.

[Downloaded free from http://www.toxicologyinternational.com on Wednesday, April 27, 2016, IP: 14.139.224.243]

18. Gupta S, Mediratte PK, Singh S, Sharma KK, Shukla R. Antidiabetic, antihypercholesterolaemic and antioxidant effect of *Ocimim sanctum* (Linn) seed oil. Indian J Exp Biol 2006;44:300-4.
19. Kumaran A, Karunakaran RJ. Nitric oxide radical scavenging active component from *Phyllanthus emblica* L. Plant Foods Hum Nutr 2006;61:1-5.
20. Hernández P, Delgado R, Walczak H. *Mangifera indica* L. extract protects T cells from activation-induced cell death. Int Immunopharmacol 2006;6:1496-505.
21. Bansal AK, Bansal M, Soni G, Bhatnagar D. Protective role of Vitamin E pre-treatment on N-nitrosodiethylamine induced oxidative stress in rat liver. Chem Biol Interact 2005;156:101-11.

Source of Support: Nil, **Conflict of Interest:** None declared.