GREEN TEA EXTRACT INHIBITED LEAD ACETATE INDUCED HEMOLYTIC CHANGES IN RAT ERYTHROCYTES

Manuj Kr Bharali and Rijumoni Saikia

Cell and Molecular Biology Section, Dept of Zoology, Rajiv Gandhi University, Rono Hills, Doimukh: 791112, Arunachal Pradesh, India.

ABSTRACT

Lead (Pb^{++}) is known to cause hemolytic changes in the erythrocytes. The objectives of the present study was to evaluate the protective effect of Green Tea extract on lead acetate induced erythrocyte hemolysis both in vitro and in vivo condition. Isolated erythrocytes from rat were incubated with lead acetate alone or in combination with Green Tea extract (GTE) for 90 mins at 37° C. Hemolysis was evaluated by estimating the % of haemoglobin in the supernatant. For in vivo experiment rats were randomly divided into four groups (n=5) and given only water, 0.1% lead acetate in drinking water and 0.1% Lead acetate + GTE (50 or 100 mgKg^{-1}) continuously for 4 weeks. At the end of 4th week, blood was collected and analyzed. Spleens from all animal groups were processed for histopathological and biochemical analysis. Exposure of erythrocytes to lead acetate caused dose dependent increases in the extent of hemolysis and significant alteration in hematological profile in rats, whereas GTE supplementation minimized this effect. Spleen from lead treated rats exhibited severe histopathological lesions, whereas in GTE treated rats the severity of splenic lesion was less. Lipid peroxidation, a marker of oxidative stress was significantly higher in eryhthrocytes and splenic tissue of lead acetate treated rats and GTE abrogated this effect.The findings of the present study explain the beneficial effect of GTE supplementation in lead acetate induced hemolytic anaemia and subsequent histopathological effect on spleen of rat.

Keywords:Lead acetate, erythrocytes, hemolysis, anaemia, spleen, lipid peroxidation.

INTRODUCTION

Lead ($Pb^{++)}$ has been recognised as one of the most common environmental toxicants for several years. The principal sources of Pb^{++} pollution are due to anthropogenic activities which include factories [1], leaded paints, gasoline and lead-contaminated drinking water [2]. Low level of Pb^{++} exposure is known to be associated with behavioral abnormalities, learning impairment, decreased hearing, and impaired cognitive functions in humans and in experimental animals [3]. Chemical-induced anemia may be caused by impaired erythrocyte production in the bone marrow or excessive erythrocyte destruction in the spleen or liver. Exposure to Pb^{++} known to causes intravascular hemolysis and is known to be a factor behind lead induced anemia. Exposure to Pb^{++} decreased life span of circulating erythrocytes due to intravascular hemolysis [4].

Reduction in RBC antioxidant enzyme activities in lead-exposed workers [5,6] and animals [7,8] has been recently reported to be another cause of intravascular hemolysis and subsequent anaemia in human and laboratory animals. Oxidative stress has been reported as a mechanism behind lead induced hemolytic changes both in human and laboratory animals. Lead is known to generate free radicals and hence reduces the antioxidant defence system in erythrocytes [9].Therefore supplementation of antioxidant might be beneficial in reducing hemolytic changes in lead exposed organism. Green tea (*Camellia sinensis*, Theaceae) is a popular beverage in many Asian countries and known to contain polyphenols such as catechin, epicatechin, epigallocatechin [10]. Catechins are known as strong scavangers of free radicals and chelator of metal ions [11].

Corresponding Author:-**M. K. Bharali** Email:-manuj_rb@yahoo.co.in

Hence the present study investigated the role of GreenTea Extract (GTE) in lead acetate induced haemolytic changes in rat erythrocytes.

MATERIALS AND METHODS
Chemicals and reagents

Lead acetate was procured from Sigma (St. Louis, MO, USA). All other reagents used in the study were laboratory grade and purchased from commercial sources. Green tea was purchased from local market and solutions were prepared in deionized water.

In vitro assay protocol

Heparinized blood was obtained from healthy male Sprague-dawley rats. Plasma and the buffy coat were removed after 5 min centrifugation at 700g and the RBC washed three times with saline.1% RBC suspension was prepared with 150 mM NaCl, 10 mM sodium phosphate at the moment of its use. Stock solution of lead acetate was prepared using the same buffer at the moment of its use. The incubation of RBC suspension with lead acetate in the presence or absence of the green tea extract at different concentrations was performed in a gentle-shaking 37° C water bath. After incubation, the samples were centrifuged for 5 min at 5000 rpm, supernatant and whole packed RBC was used to investigate the extent of hemolysis. The level of hemolysis was determined by the absorbance of hemoglobin at 540 nm. Total hemolysis (100%) was established with a 0.25% RBC suspension indistilled water incubated at 37° C for 10 min [12].

In vivo assay protocol

In order to investigate the effect of lead acetate alone or with GTE on hematology, rats were divided into four groups (n=5). Group 1 received no treatment and allowed free access to distilled water and food ad libitum. Group 2 received 0.1% lead acetate dissolved in double distilled water as the sole source of drinking water. Group 3 and 4 received 0.1% lead acetate in drinking water + GTE either 25 or 50 mgKg^{-1} through oral route dissolved in distilled water daily. The treatment schedule continues for 30 days. After that the animals were sacrificed by exsanguinations under light ether anaesthesia. Blood was collected in heparinised tubes and processed for hematological parameters (TEC, Hb). Spleens from all animals were weighed and fixed in bouins fixatives for minimum 24 hrs. Paraffin sections were prepared and stained with H & E. The sections were observed under light microscope and representative photographs were taken with a digital image recorder.

Statistical analysis

All the data were presented as mean+SEM of the number of animals (n=5). Statistical analysis was performed by ANOVA and post hoc tukey tests. Value was considered significant for p value<0.05.

Figure 1: (A) In vitro exposure of erythrocytes to lead acetate caused a dose dependent release of haemoglobin, *p<0.05, **p<0.001; (B) Correlation analysis between lead acetate doses and extent of hemolysis; (C) Effect of GTE supplementation on lead acetate induced hemolysis in red blood cells of mice. 1% erythrocyte preparation was incubated with lead acetate (50 μM) alone or with lead acetate (50 μM) + GTE (10 or 25 μgml^{-1}) for 90 mins at 37° C in an orbital shaker. Hemolysis was estimated in terms of % of hemoglobin released from the erythrocytes at specific time interval. GTE supplementation caused 50% reduction in haemoglobin release from the erythrocytes treated with lead acetate. *P<0.05, **P<0.001.
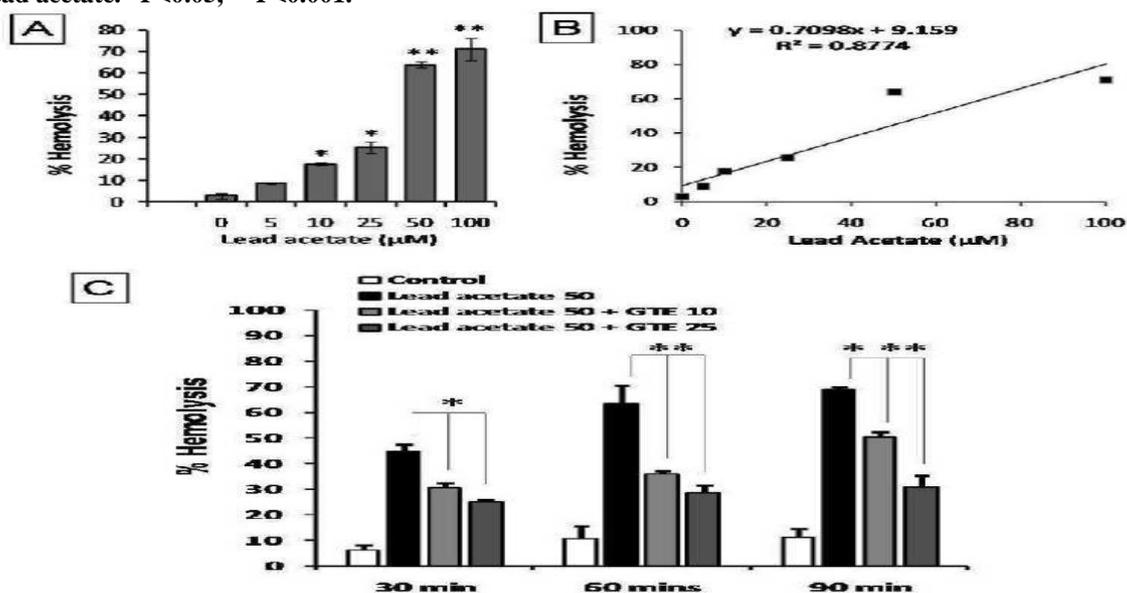
Figure 2: Effect of in vivo exposure to lead acetate alone or with GTE on hematology of SD rats. Light microscopic observation of lead acetate alone treated (A) or with GTE (B). lead acetate caused increased in the no. of acanthocytes (arrowhead), hemolysis (arrow) and changes in the central pallor (blockarrow) of otherwise normal erythrocytes. Supplementation with GTE attenuated this morphological alteration in the erythrocytes of mice.
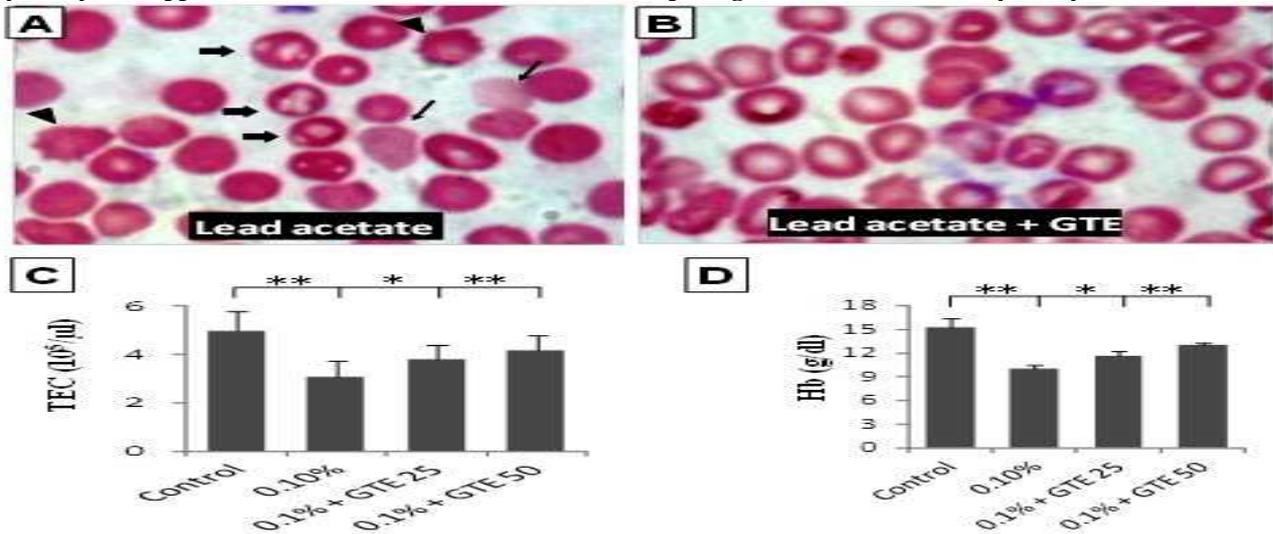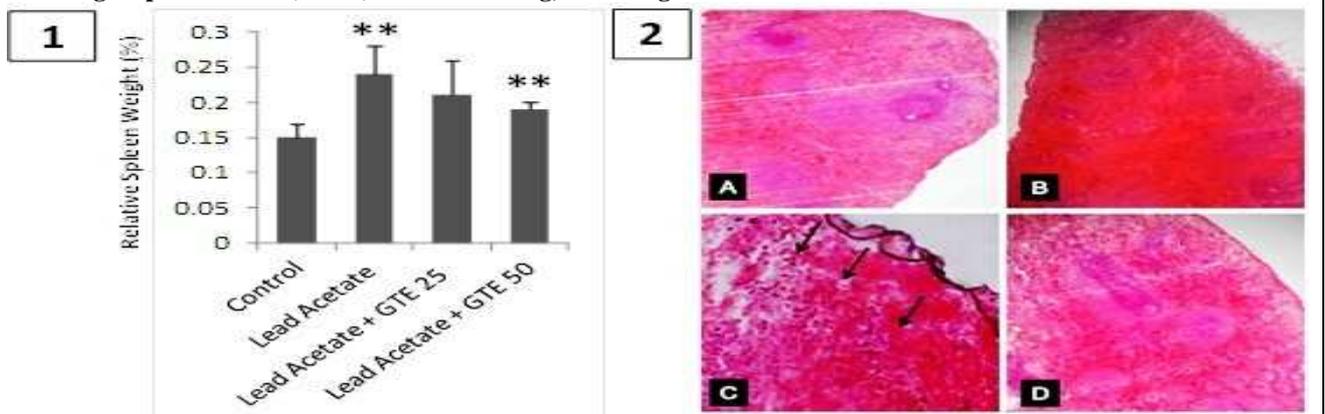

Figure 3: Relative spleen weight in control and treated group of animals (1). Histopatholigcal findings in control and treated group of animals (2 A-D). H & E staining, X 20 magnification.

RESULT AND DISCUSSION

Lead acetate caused a dose dependent haemolysis in isolated erythrocytes of mice when incubated in isotonic solution at 37°C for 90 mins (Figure 1.A). It was observed that hemolytic curve was linear upto 50 µM concentrations and hence this was selected as the final dose for further haemolysis study induced by lead acetate exposure. Correlation study exhibited a positive correlation between dose of lead acetated exposure and percentage of haemolysis in the in vitro experiment (Figure 1. B). In order to observe the protective effect of GTE, dried green tea extracts were added in different concentration (10 & 25 µgml^{-1}) to theerythrocyte preparation alone or with lead acetate (50 µM). The highest dose of GTE (25 µgml^{-1}) selected for the study has been shown to reduce haemolysis up to 50%in lead acetate treated erythrocytes compared to lead

acetate alone treated erythrocytes (Figure 1.C).Lead acetate administration caused significant changes in the cellular morpholigcal features of erythrocytes. The normal discoytes RBC were converted to acanthocytes, with hyperchromasis, a prominent features of hemolysis in the circulating erythcocytes (Figure 2A). GTE supplementation attenuated this alteration in cellular morphological features of erythrocytes (Figure 2B). Lead acetate (0.1%) when given in drinking water also caused significant (p<0.001) reduction in the total no of erythrocytes and haemoglobin content of erythrocytes in the peripheral blood of treated animals (figure 2C). GTE supplementation on the other hand significantly (p<0.01, 0.001) inhibited the adverse effect of lead acetate on hematological parameters of mice (figure 2D). The spleen from lead acetate exposed group of mice exhibit

hypertrophy, congestion and hyperpigmentation of the red pulp (Figure B-C) when compared to the control group (Figure 3 A). Treatment with GTE significantly reduces hypertrophy and congestion in the red pulp of spleen (Figure D).Erythrocyte toxicity is often manifested in the form of cell shape changes leading to cellular deformability [13]. In vitro and in vivo exposure to lead acetate also found to have similar effect on erythrocyte morphological alteration and intravascular hemolysis. Lead acetate induced hemolysis has been observed to be mediated by damages to erythrocytes membrane structure, as confirmed by microscopic observation of lead exposed erythrocytes during present study. Conversion of normal discocytes to echinocytes makes erythrocytes also might be related to the reorientation of the phosphatidyl serine (PS) molecules present on inner leaflets of erythrocytes to be displayed on the surface [14]. The exposure of PS at the cell surface provides a signal for their recognition by macrophages, which stimulates 'eryptosis', an apoptotic like process present in erythrocytes [15]. Macrophages are equipped with PS receptor, which mediate the engulfment of the eryptotic/apoptotic cells and trigger their removal from circulation [16,17].Thus lead acetate induced hemolytic changes in the erythrocytes makes them more vulnerable to be removed prematurely from circulation, causing anemia in the long run. Spleen is one of the target organs of erythrocytes toxicity. Increased peripheral destruction of RBCs caused enhanced sequestration of damaged RBCs in the spleen which finally caused histopathological changes of splenic architechture as reported earlier [18].

Lead acetate exposure has reported to reduced natural antioxidant defence system present within the erythrocytes during laboratory investigation[7] and during occupational exposure. Lead is known to increase oxidative stress in different cell culture based study model as reported previously [19]. Since oxidative stress is a major pathway of lead acetate induced hemolysis in erythrocytes, antioxidant treatment must be effective in ameliorative the toxicological effects of lead. In line with this observation, several investigators have reported the efficacy of antioxidant treatment in ameliorating lead ($Pb2^{++}$) toxicity [20]. The polyphenols in GTE are known to possess proven antioxidant properties that can inhibit oxidative stress in many animal models. The antioxidant potential of GT polypehonls are reported to be equally as effective as vitamin C and E. The result of the present study indicated the protective role of GTE on lead acetate induced hemolysis both in vitro and in vivo condition using mice model.

Conflict of interest statement
The authors declare that there is no conflict of interest.

REFERENCES
1. Stohs SJ and Bagchi D. Oxidative mechanisms in the toxicity of metal ions. *Free Radic Biol Med*, 18(2), 1995, 321-36.
2. Zietz BP and Suchenwirth R. Lead in Drinking Water as a Public Health Challenge. Environ Health Perspect, 118(4), 2010, A154–A155.
3. Cory-Slechta DA and Pound JG. Lead neurotoxicity. In Handbook of Neurotoxicology (L.W. Chang and R.S. Dyer, Eds.), pp. 61–89. Dekker, New York. 1995.
4. Suplido ML and Ong CN. Lead Exposure among Small-Scale Battery Recyclers, Automobile Radiator Mechanics, and Their Children in Manila, the Philippines. *Environ Res*, 82(3),2000, 231-238
5. Gurer H and Ercal N. Can antioxidants be beneficial in the treatment of lead poisoning?. *Free Radic Biol Med*, 29, 2000, 927–945.
6. Kasperczyk S, Birkner E, Kasperzyk A, Zalejska-Fiolka J. Activity of superoxide dismutase and catalase in people protractedly exposed to lead compounds. *Ann Agric Environ Med*, 11, 2004, 291-296.
7. Patra RC and Swarup D. Effect of lead on erythrocytic antioxidant defense, lipid peroxide level and thiol groups in calves. *Res Vet Sci*, 68, 2000, 71–74.
8. Mousa HM, Al-Qarawi AA, Ali BH, Abdel Rahman HA and ElMougy SA. Effect of lead exposure on the erythrocytic antioxidant levels in goats. *J Vet Med A*, 49, 2002, 531–534.
9. Patil AJ, Bhagwat VR, Patil JA, Dongre NN, Ambekar JG, Jailkhani R, Das KK. Effect of lead (Pb) exposure on the activity of superoxide dismutase and catalase in battery manufacturing workers (BMW) of western Maharashtra (India) with reference to heme biosynthesis. *Int J Environ Res Public Health*, 3, 2006, 329–337
10. Campbell EL, Chebib M, Johnston GAR. The dietary flavonoids apigenin and (−)-epigallocatechin gallate enhance the positive modulation by diazepam of the activation by GABA of recombinant GABAA receptors. *Biochem Pharmacol*, 68, 2004, 1631–1638.
11. Guo Q, Zhao B, Li M, Shen S, Xin W. Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes. *Biochim Biophys Acta*, 1304, 1996, 210–222.
12. Sato Y, Takahashi T, Suzuki Y. Mechanism of free radicalinduced hemolysis of human RBC, hemolysis by water soluble radical initiator. *Biochemistry*, 34, 1995, 8940–8949.
13. Chabanel A, Reinhart W, Chien S. Increased resistance to membrane deformation of shape-transformed human red blood cells. *Blood*, 69, 1987, 739–743.

14. Henszen MM, Weske M, Schwarz S, Haest CW, Deuticke B. Electric field pulses induce reversible shape transformation of human erythrocytes. *Mol Membr Biol*, 14, 1997, 195–204.
15. Lang KS, Lang PA, Bauer C, Duranton C, Wieder T, Huber SM, Lang F. Mechanisms of suicidal erythrocyte death. *CellPhysiol Biochem*, 15, 2005, 195–202.
16. Boas FE, Forman L, Beutler E. Phosphatidylserine exposure and red cell viability in red cell aging and in hemolytic anemia. Proc Natl Acad Sci USA, 95, 1998, 3077–3081
17. Bratosin D, Mazurier J, Tissier JP, Slomianny C, Estaquier J, Russo-Marie F, Huart JJ, Freyssinet JM, Aminoff D, Ameisen JC, Montreuil J. (1997) Molecular mechanisms of erythrophagocytosis. Characterization of the senescent erythrocytes that are phagocytized by macrophages. *C R Acad Sci*, 1997, 320, 811–818.
18. Bharali MK and Dutta K (2012) Hematopathology in Sprague–Dawley Rats Following Sub-Chronic Topical Application of Para-Phenylenediamine.*Bull Environ Contam Toxicol*, 89, 712–717.
19. GurerH, OzgunesH, SayginE,ErcalN.Antioxidant effect of taurine against lead-induced oxidative stress. *Arch EnvironContam Toxicol*, 41, 2001,397–402
20. Aykin-Burns N, Franklin EA, Ercal N. Effects of N-acetylcysteine on lead-exposed PC-12 cells. *Arch Environ ContamToxicol*, 49 (1), 2005, 119–123.