Research Paper

Volume : 3 | Issue : 5 | May 2014 • ISSN No 2277 - 8179

Lead Toxicity on Kidney Antioxidant Enzymes with The Protective Role of Ginger in Male Albino Rats

Biology

KEYWORDS : lead acetate, nephrotoxicity, catalase, Zingiber officinale

*** PRATAP MANNEM**

Department of Zoology, P.V.K.K.P.G.College, Anantapuramu, Andhra Pradesh, India
* Corresponding Author

ABSTRACT

The protective effect of ginger extract against nephrotoxicity induced by lead acetate was investigated in male albino rats. Seven equal groups, each of six rats were used. Lead acetate induced kidney damage was well manifested by significant decrease in renal parameters like glutathione, superoxide dismutase and catalase. The oral administration of ethanolic extracts of Zingiber officinale along with lead acetate reversed these altered parameters to normal level which indicating the nephroprotective efficacy of Zingiber officinale against lead acetate induced kidney injury. From this, we concluded the phytochemical constituents such as flavonoids, gingerols, shagogals, phytochemicals are responsible for the nephroprotective activity of Zingiber officinale. Further extensive studies are required for its potential uses in clinical practice.

INTRODUCTION:

Environmental pollution is the presence of a pollutant in environment such as air, water, soil and consequently in food which may be poisonous or toxic and will cause harm to living things in the polluted environment (Duruibe et al., 2007).

Heavy metals such as lead are a major environmental and occupational hazard. These non-essential elements are toxic at very low doses and non- biodegradable with a very long biological half life. Thus, expuser to heavy metals is potentially harmful (Babier, 2005). Lead (LA) is a naturally occurring systemic toxicant and found in pipes, drains and sol-dering materials (Ansari et al., 2013). It is a widespread environmental toxic metal that poses serious threats to human health and it is mainly conveyed to humans through dietary and occupational sources (Chang et al., 2012). Kidneys play a major role in the excretion of lead from the body (Dev et al, 1991) and higher content of lead has been estimated in renal tissue than in liver and brain of the lead intoxicated animals (Zmudski, 1983).

Oxidative stress has also been identified as a common molecular mechanism of toxicity of lead acetate in biological systems (Khalaf et al., 2012). Oxidative stress occurs when a perturbation in the balance between oxidants and antioxidants culminates in damage to critical biomolecules such as DNA, lipids and proteins (Yonar and Sakin, 2011). It has been shown that antioxidants prevent or reduce the oxidation of other molecules by reactive oxygen species (ROS) in living organisms, scavenge free radicals and attenuate their deleterious effects (Koivula and Eeva, 2010). It has been also reported that lead exposure has a dose response relationship with changes in antioxidant enzyme levels and their activities (Adonaylo and Oteiza 1999). Ponce-Canchihuamán et al. (2010) administered 25 mg/0.5 mL of lead acetate intraperitoneally to rats weekly. It was found that activities of SOD, CAT and GSH in rat kidney were significantly ($p<0.05$) decreased while level of MDA was significantly ($p<0.05$) increased with respect to the control.

Ginger posses various pharmacological activities including hypoglycemia, anticancer, anticardic, antirenal and hepatoprotective and antioxidant (Nicoll and Henein, 2009). Ginger has many antioxidant compounds; these compounds may either mitigate or prevent generation of free radicals in toxic conditions. The active ingredients of ginger include gingerols, shagogals, phytochemicals and other compounds show antioxidant activity in various models (Dugasani et al., 2010). The present study was design to explore the nephroprotective property of ginger ethonolic extract against lead induced kidney toxicity in male albino rats.

MATERIALS AND METHODS

Preparation of ethanolic extract of Zingiber officinale:

The ginger was collected from local market and cut into small pieces and dried under ceiling fan for 5 to 6 days. The dried ginger was ground in an electronic grinder and powder was collected. 50g of powder was extracted in 250ml ethanol for 18hrs in soxhlet apparatus. The extract was dried at reduced pressure, stored at 0-4oC and used for the experimentation.

Animal Ethical Clearance:

Local Institutional Animal Ethical Committee of our University, obtained ethical clearance for conducting experiments on animals from committee for the purpose of control and supervision of experiments on Animals (CPCSEA) (REGD.No.470/01/a/CPCSEA, DT.24th Aug 2001).

Procurement of Animals and maintenance:

Adult male albino rats wistar strain (Rattus norvegicus) weighing 150±30gms obtained from Sri Raghavendra Animal Supplier, Bangalore, K.A. They were kept in cages under standard laboratory conditions (25±2^{0}C, 12 hrs dark/light) and were fed with commercial rat feed supplied by Sai Durga Feeds and Foods, Bangalore and water adlibitum. They were allowed to laboratory conditions for seven days after arrival before use.

Chemicals:

Lead acetate was purchased from MERK India Ltd., Silymarin suspension purchased from Micro labs, Bangalore. All other chemicals used were of technical grade.

EXPERIMENTAL DESIGN:

The animals were divided into 7 groups of 6 rats each and treated as follows:

Group- I:

Normal control (Nc): This group of rats received vehicle solution (5% Tween 80).

Group-II:

Ginger treatment (Gt1): Rats received ethanolic extract of ginger (200mg/Kg body weight) orally for 8 weeks.

Group-III:

Ginger treatment (Gt2): Rats received ethanolic extract of ginger (300mg/Kg body weight) orally for 8 weeks.

Group-IV:

Lead treatment (Lt): Rats received lead acetate orally at a dose of (200mg/Kg body weight) orally for 8 weeks.

Group-V:

Lead treatment + Ginger treatment (Lt+Gt1): This group of rats received both lead acetate and ginger as described in group II and group IV for 8 weeks.

Group-VI:

Lead treatment + Ginger treatment (Lt+Gt2): This group of rats received both lead acetate and ginger as described in group III and group IV for 8 weeks.

Group-VII:
Lead treatment + Silymarin treatment (Lt+St): This group of rats received both lead acetate and silymarin. Lead as described in group IV and silymarin (100mg/Kg body weight) orally for 8 weeks.

Lead acetate was dissolved in distilled water before administration. Food was withdrawn 12hr before Lead acetate administration. Ginger was suspended in 5% Tween 80.

Analytical procedures:
After completion of 8 weeks treatment the animals were sacrificed by cervical dislocation and immediately kidney tissue was excised at 4°C. The tissue was washed thoroughly with ice-cold 0.9% sodium chloride solution (saline). Kidney tissue of every animal was suspended in 0.15 M potassium chloride in polypropylene containers, sealed with parafilm, labelled carefully and stored at -20°C until assays were carried out. Levels of glutathione and the activities of superoxide dismutase and catalase were measured.

RESULTS:
In the present study the protective role of ginger and silymarin treatment against lead toxicity on the levels of kidney GSH, SOD and CAT were carried for 8 weeks. Table: 1. shows the levels of kidney GSH, SOD and CAT in all experimental groups.

Table:1. Antioxidant molecule and antioxidant enzymes levels in kidney of all experimental groups
Oral administration of lead acetate toxicity, protective effect of ginger-I, ginger-II and action of Standard drug silymarin on levels of GSH, SOD and CAT were measured in kidney tissue of all experimental groups. The activities of GSH, SOD and CAT in control rats were found to be 11.6933µg/mg, 7.1517U/mg/min and 25.03µM of H_2O_2/min respectively. In group-IV (lead control), these parameters were significantly decreased to 6.6933µg/mg, 2.9733U/mg/min and 12.2033µM of H_2O_2/min respectively.

DISCUSSION:
In the present study male albino rats treated with lead acetate once daily for 8 weeks. The GSH activity was very significantly decreased in group-IV (lead control). The kidney is highly vulnerable to damage caused by reactive oxygen species (ROS), likely due to the abundance of polyunsaturated fatty acids in the composition of renal lipids (Harve et al., 1997). Glutathione (GSH) is a multifunctional intracellular non-enzymatic antioxidant. It is considered to be the major thiol-disulfide redox buffer of the cell. Glutathione is highly abundant in the cytosol, nuclei and mitochondria, and is the major soluble antioxidant in these cell compartments. Lead binds to the –SH group of GSH, and interfere with the antioxidant activity of GSH thus decreases its level (Bechara, 2004). The sulfhydryl complex of glutathione also directly binds to toxic metals that have a high affinity for sulfhydryl groups. Jurczuk et al (2006) have revealed that Pb may induce lipid peroxidation by affecting the activity of antioxidative enzymes and GSH concentration in kidney. Lead effectively inactivates the glutathione molecule. So, it is unavailable as an antioxidant or as a substrate in kidney metabolism (Christie NT and Costa M, 1984).

Group V (lead+ginger-I) and group-VI (lead+ginger-II) showed recovered levels of GSH activity when compared to lead controlled rats. Ginger compounds like gingerols, shagols and other pharmacological compounds of ginger as they have the capacity to reduce the free radical capacity. As the GSH is the substrate for GPx, GST and several enzymes, it is believed that GSH dependent antioxidants activity is increased by ginger extracts (Kikuzaki and Nakatani, 1993).Group-VII (silymarin treated) showed recovered levels of GSH when compared to lead controlled, but there is no significant difference over normal controlled ones and slightly increased levels when compared to ginger controlled ones.

Group IV (lead acetate treated alone) showed significant ($p<0.05$) decrease in the activity of SOD and CAT when compared with normal control group, whereas, group V, VI and VII showed significant ($p<0.05$) increase in the antioxidant activity over the group IV. Group VI (ginger-II) showed a significant increase in the activity of SOD and CAT over group V (ginger-I) and not significantly different when compare with group VII (silymarin treated group). Group V, VI, and VII rats showed near results with normal control. In group-VII (silymarin treated) showed significantly recovered levels of SOD and CAT when compared with lead treated(group-IV) ones and the results were nearer to ginger treated(group-V and group-VI), normal control(group-I), ginger control(group-II and group-III) groups.

Cervello et al., (1992) suggested that GST enzyme catalyzes the reaction via the thiol (-SH) group of glutathione, thereby neutralizing and rendering the products more water-soluble. Taking into account mutual relations between GST and GSH in the redox system, the simultaneous decrease in both GST activity and GSH concentration may suggest that the decrease in renal GSH concentration might result, at least partly from the decrease in GST activity (Newairy and Abdou, 2009). The decrease in GST activity after exposure to lead could be caused by lead-induced changes in the enzyme structure as well as by the lack or insufficient amount of GSH, being a substrate for this enzyme (Neal et al., 1999) and GST contain sulfhydryl groups at their active site hence become inactive due to direct binding of lead to sulfhydryl group (Quig, 1998). Catalase is an efficient decomposer of H_2O_2 and known to be susceptible to lead toxicity (Sandhir and Gill, 1995). Inhibition of heme synthesis by lead is well reported and since CAT is a heme-containing enzyme, its activity decreases (Mylroie, Umbles, Kyle, 1984).

The current study results are in consonance with the reports of earlier authors Ansari et al., (2006) showed that the ethanolic Zingiber officinale extract pretreatment for 20 days in isoproternol treated rats induced oxidative myocardial necrosis in rats, enhances the antioxidant defense (catalase, superoxide dismutase and tissue glutathione) and exhibites cardioprotection property. In support of this authors reported previously Flavonoids, sterols, triterpenes and alkaloids as antioxidative compounds are rich and high content of these compounds was recorded in the ethanol extract of ginger. In support of our work Ajith et al., (2007) reported treatment of ginger ethanol extract 250mg/kg body weight showed protective effect in cis-platin induced nephropathy in rats by enhancing the antioxidant enzyme activities including GSH levels in kidney. In support of our work Karimi et al., (2005) reported that silymarin has antinephrotoxic activity against cisplatin induced nephrotoxicity in albino rats.

CONCLUSION:
In conclusion, the results generated from this study is suggestive of the fact that lead acetate has adverse effects on the kidney antioxidant molecule (GSH) and antioxidant enzymes (SOD & CAT) of rats which could lead to initiation of kidney diseases and that ginger (Zingiber officinale) has nephro-protective effect on lead acetate induced nephrotoxicity and this may due to the antioxidant properties possessed by ginger.

Volume : 3 | Issue : 5 | May 2014 • ISSN No 2277 - 8179

Table 1.

Sl. No.	Parameter		Group-I (Normal control)	Group-II (Ginger control-I)	Group-III (Ginger control-II)	Group-IV (Lead control)	Group-V (Lead+ Ginger-I)	Group-VI (Lead+ Ginger-II)	Group-VII (Lead+ Silymarin)
1.	GSH (µg/mg)	Mean S.D	11.6933^{a} ±0.4424	11.795^{a} ±0.4503	11.993^{a} ±0.5693	6.69333^{d} ±0.4244	8.7667^{c} ±0.3661	9.8883^{b} ±0.3052	10.07^{b} ±0.3272
2.	SOD (U/ mg/ min)	Mean S.D	7.1517^{a} ±0.2415	7.1133^{a} ±0.2453	7.1300^{a} ±0.2911	2.9733^{d} ±0.1494	5.2300^{c} ±0.1628	6.0650^{b} ±0.1526	6.1600^{b} ±0.0868
3	CAT (µM H_2O_2/ min)	Mean S.D	25.0300^{a} ±0.2262	24.7300^{a} ±0.3162	24.9533^{a} ±0.0965	12.2033^{d} ±0.1820	19.9700^{c} ±0.5911	21.3450^{b} ±0.3881	21.2183^{b} ±0.3083

REFERENCE

Adonaylo VN, Oteiza PI. Lead intoxication: Antioxidant defenses and oxidative damage in rat brain. Toxicology. 1999; 135:77-85 | | Ajith TA, Nivitha V and Usha S. Zingiber officinale Roscoe alone and in combination with alpha-tocopherol protect the kidney against cisplatininduced acute renal failure. Food Chem. Toxicol. 2007;45: 921-927. | | Ansari MA, Maayah ZH, Bakheet SA, El-Kadi AO et al. The role of aryl hydrocarbon receptor signalling pathway in cardiotoxicity of acute lead intoxication in vivo and in vitro rat model. Toxicology. 2013;306:40-49. | | Ansari MN, Bhandari U, Pillai KK. Ethanolic Zingiber officinale R. extract pretreatment alleviates isoproterenol -induced oxidative myocardial necrosis in rats. Indian J. Exp. Biol. 2006; 44(11): 892-897. | | Babier O, Jacquillet G, Tauc M et al. Effect of heavy metals on, and Handling by, the kidney. Nephron physiology. 2005; 99:p105. | | Bechara EJH. Lead poisoning and oxidative stress. Free Radic Biol Med. 2004;36: S22. | | Cervello I, Lafuente A, Giralt M et al. Enhanced glutathione S-transferase (GST) activity in pregnant rats treated with benzo(a)pyrene. Placenta. 1992;13:273-280. | | Chang BJ, Jang BJ, Son TG et al. Ascorbic acid ameliorates oxidative damage induced by maternal low-level lead exposure in the hippocampus of rat pups during gestation and lactation. Food. Chem. Toxicol. 2012;50(2):104-108. | Christie NT, Costa M. In vitro assessment of the toxicity of metal compounds. IV. Disposition of metals in cells: Interaction with membranes, glutathione, metallthionein, and DNA. Biol. Trace Elem. Res. 1984.6:139-158. | | Dev PR, Swarup D, Dwivedi SK. Some renal function tests in experimental lead toxicity in Goats. Indian Veterinary Journal. 1991;68: 1163-1167. | | Dugasani S, Pichika MR, Nadarajah VD et al. Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]- gingerol, [10]-gingerol and [6]-shogaol. J. Ethnopharmacol. 2010;127:515-520. | | Duruibe JO, Ogwuegbu MC, Egwurugwu GN. Heavy metal pollution and human biotoxic effects. Int J Phys Sci. 2007; 2(5):112-118. | | Harvey C. Gonick HC, Ding Y et al. Lead induced hypertension, interplay of nitric oxide and reactive oxygen species. Hypertension. 1997;30:1487-92. | | Jurczuk M, Moniuszko-Jakoniuk J, Brzoska MM. Involvement of some low molecular thiols in the peroxidative mechanisms of lead and ethanol action on rat liver and kidney. Toxicology. 2006;219:11-21. | | Karimi G, Ramezani M and Tahoonian Z. Cisplatin nephrotoxicity and protection by milk thistle extract in rats. Evid. Based Complement Alternat. Med. 2005;2:383-386. | | Khalaf AA, Moselhy WA, Abdel-Hamed MI. The protective effect of green tea extract on lead induced oxidative and DNA damage on rat brain. Neurotoxicol. 2012; 33(3):280-289. | | Kikuzaki H and Nakatani N. Antioxidant effect of some ginger Constituents, J. Food sci. 1993;58:1407-1410. | | Koivula MJ, Eeva T. Metal-related oxidative stress in birds. Environ. Pollut. 2010; 158(7):2359-2370. | | Mylroie AA, Umbles C, Kyle J. Effects of dietary copper supplementation on erythrocyte superoxide dismutase activity, ceruloplasmin and related parameters in rats ingesting lead acetate. In: Hemphill, editor. Trace substances in environ health. Columbia: University of Missouri Press. 1984;18:497-504. | | Neal R, Cooper K, Kellogg G et al. Effects of some sulfur containing antioxidants on lead-exposed lenses. Free Radic. Biol. Med. 1999;26:239-243. | | Newairy AS, Abdou HM. Protective role of flax lignans against lead acetate induced oxidative damage and hyperlipidemia in rats. Food Chem. Toxicol. 2009;47(4):813-818. | | Nicoll R and Henein MY. Ginger (Zingiber officinale Roscoe):a hot remedy for cardiovascular disease? Int. J. Cardiol. 2009;131:408-409. | | Ponce-Canchihuamán JC, Pérez-Méndez O, Hernández-Muñoz R et al. Protective effects of Spirulina maxima on hyperlipidemia and oxidative-stress induced by lead acetate in the liver and kidney. Lipids Health Dis. 2010;9:35. | Quig D. Cysteine metabolism and metal toxicity. Altern Med Rev. 1998;3:262-70. | | Sandhir R, Gill KD. Effect of lead on lipid peroxidation in liver of rats. Biol Trace Elem Res. 1995;48: 91-97. | | Yonar ME, Sakin F. Ameliorative effect of lycopene on antioxidant status in Cyprinus carpio during pyrethroid deltamethrin exposure. Pestic. Biochem. Phys. 2011;99(3):226-231. | | Zmudski J, Bratton GR, Womac C et al. Lead poisoning in cattle: Reassessment of the minimum toxic oral dose. Bulletin of Environmental contain. Toxicology. 1983;30:435-441. |