



**PROTECTIVE EFFECTS OF GINGER (*Zingiber officinale*) EXTRACT
AGAINST LEAD INDUCED OXIDATIVE STRESS ON LIVER
ANTIOXIDANT ENZYMES IN MALE ALBINO RATS**

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ABSTRACT

In the present study, ethanolic extract of ginger on lead acetate induced toxicity was studied in male wistar albino rats. Seven groups of rats were used in the study. Glutathione peroxidase, Glutathione reductase, Glutathione-S-transferase, Catalase and Superoxide dismutase were decreased in lead acetate treated (200mg/kg body weight, once daily for eight weeks) group. However treatment with ethanolic extract of ginger-I(200mg/kg body weight, once daily for eight weeks) and ginger-II(300mg/kg body weight, once daily for eight weeks) these parameters came to normal showing the antioxidant effect of ginger. The antioxidant effects of ginger may modulate the oxidative stress parameters. The results confirm the hepato protective effects of ginger in lead acetate treated rats.

KEY WORDS: lead acetate, ginger, antioxidants, albino rats, hepatotoxicity



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INTRODUCTION

The environmental pollution by heavy metals has increased severely along with the rapid development of modern industry. Among these metals lead is one, whose levels have increased substantially during the last few years¹. It is still mined and added to many commercial products including paints, eye cosmetics, gasoline, enamels and water pipes²⁻⁴. Lead is a pervasive and persistent environmental pollutant that can be detected in almost all phases of environment and biological systems. Lead became popular because of its dense, ductile, malleable and corrosion resistant properties⁵ and it has poor electrical conductivity when compared to most other metals. Lead poisoning is one of the oldest and the most widely studied occupational and environmental hazards⁶. Exposure to low levels of lead has been associated with functional and structural impairments in both human and experimental animals⁷. The main targets of lead are the hematopoietic, nervous and renal tissues. Moreover, it hinders the efficacy of the hepatic, reproductive and immune function^{8,9}. Many heavy metals, including lead, are known to induce over production of Reactive Oxygen Species (ROS) and consequently enhance lipid peroxidation, decrease the saturated fatty acids and increase the unsaturated fatty acid contents of membranes¹⁰, which become a hindrance in membrane transport. Liver is the highest depository (33%) of lead in soft tissues followed by kidney¹¹. Lead binds to form a stable complex with mitochondria in both the liver and kidneys¹². Chronic ingestion of lead leads to a significant decrease in liver glutathione synthase (GSH) levels¹³. Other studies have indicated that an increase in reactive oxygen species (ROS) in the liver¹⁴. ROS play an important role in inducing apoptosis under physiological and pathological conditions and leads to a significant increase in DNA damage and apoptosis. Therefore recently there have been many studies on the use of natural products such as vitamins and herbal drugs to expel lead¹⁵. There has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing free radical-induced tissue injury^{16,17}. Numerous plant

products have antioxidant activity as they scavenge free radicals and inhibit of lipid peroxidation^{18,19}. The potential role of dietary antioxidants to reduce the activity of free radical-induced reactions has drawn increasing attention. Ginger (*Zingiber officinale*) is an example of botanicals which is gaining popularity amongst modern physicians and its underground rhizomes are the medicinally useful part²⁰. Ginger was found to be a relief for symptoms of nausea, vomiting associated with motion sickness, surgery and pregnancy²¹. The pharmacological effects of ginger and its pungent constituents, fresh and dried rhizome were investigated. Among the effects demonstrated are anti-platelet, antioxidant, anti-tumour, antirhinoviral, anti-hepatotoxicity, anti arthritic and antidiabetic effect²²⁻²⁵. Ginger was found to have hypocholesterolaemic effects and cause decrease in body weight, glucose in blood, serum total cholesterol and serum alkaline phosphatase in adult male rats²⁶. So far, limited information exists the concerning the beneficial effects of ginger against lead acetate induced hepatic injuries in albino rats. Hence, the present study has been under taken to evaluate the possible ameliorative effects of ginger ethanol extract in lead acetate treated albino rats.

MATERIALS AND METHODS

Animals

Adult male albino rats wistar strain (*Rattus norvegicus*) weighing 150±30gms obtained from Sri Raghavendra Animal Supplier, Bangalore, Karnataka. The rats were housed in clean polypropylene cages having 6 rats per cage and maintained under temperature controlled room (25±2°C) with 12 hrs dark/light photoperiod. The rats were given standard pellets diet supplied by Sai Durga Feeds and Foods, Bangalore and water *ad libitum* throughout the experimental period. They were allowed to laboratory conditions for seven days after arrival before use.

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).

Preparation of ethanolic extract of rhizome 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 0-4°C and used for the experimentation.

Treatment

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

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

Group-II: Ginger treatment (Gt₁): Rats received an 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 liver tissue was excised at 4°C. The tissue was washed thoroughly with ice-cold 0.9% sodium chloride solution (saline). Liver 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. In the present investigation the effect of lead toxicity, protective activity of ginger-I, ginger-II and standard drug silymarin treatment for 8 weeks on levels of liver antioxidant enzymes like Glutathione peroxidase (GPx), Glutathione reductase (GR), Glutathione-S- transferase (GST), Catalase (CAT) and Superoxide dismutase (SOD) activities were measured in liver tissue of albino rats by methods of Ellman's²⁷, Pinto and Bartley²⁸, Habig et al²⁹, Beers and Seizer³⁰ and Soon and Tan³¹ respectively.

RESULTS

Liver antioxidant enzymes like Glutathione peroxidase, Glutathione reductase, Glutathione-S- transferase, Catalase and Superoxide dismutase activities were presented in table I.

Table I
Antioxidant enzymes level of liver in normal and all experimental rats.

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.	GPx(μg of GSH/ min)	Mean S.D	11.6817 ^a ± 1.0154	11.44 ^a ± 0.7784	11.77 ^a ± 0.9100	6.0383 ^d ± 0.2381	7.9483 ^c ± 0.4422	10.018 ^b ± 0.4176	10.6433 ^b ± 0.5866
2.	GR (μM / min)	Mean S.D	0.1295 ^a ± 0.033	0.1282 ^a ± 0.0026	0.1265 ^a ± 0.0042	0.0513 ^c ± 0.0024	0.1145 ^b ± 0.0055	0.1218 ^a ± 0.0036	0.1277 ^a ± 0.0032
3	GST (μM / min)	Mean S.D	5.9383 ^a ± 0.5893	6.2467 ^a ± 0.2279	6.2767 ^a ± 0.2313	3.6 ^d ± 0.2858	4.74 ^c ± 0.4008	5.1733 ^b ± 0.4752	5.33 ^b ± 0.6089
4	CAT(μM H ₂ O ₂ / min)	Mean S.D	61.7067 ^a ± 2.3223	61.9833 ^a ± 1.3298	62.0017 ^a ± 1.9410	40.5850 ^d ± 1.2043	53.728 ^c ± 1.2419	58.475 ^b ± 1.0453	59.6233 ^b ± 0.7495
5	SOD (U/mg/ min)	Mean S.D	11.4033 ^a ± 0.2459	11.2167 ^a ± 0.2453	11.2817 ^a ± 0.2911	3.9283 ^d ± 0.1494	8.7683 ^c ± 0.1628	9.9617 ^b ± 0.1526	10.2517 ^b ± 0.0868

Values are mean \pm S.E.M, Values with different superscripts with in the column are significantly different at $P < 0.05$ (Duncan's Multiple Range Test)

Oral administration of lead acetate toxicity on GPx, GR, GST, CAT, and SOD were studied in liver tissues of all experimental groups. The data presented in Table:I showed analysis of various antioxidant enzymes like GPx, GR, GST, CAT, and SOD activities. These antioxidant enzymes activity in normal control (group-I) rats were found to be 11.6817 μg of GSH/min, 0.1295 μM /min, 5.9383 μM /min, 61.7067 μM of H₂O₂/min and 11.4033U/mg/min respectively. In group-IV(lead control), these enzymes were significantly decreased to 6.0383 μg of GSH/min, 0.0513 μM /min, 3.6 μM /min, 40.585 μM of H₂O₂/min and 3.9283 U/mg/min respectively.

DISCUSSION

The etiology of free radicals in lead acetate liver disease is well established. The results of the present study show that supplementation of ginger may protect the hepatic cells and reduce the severity of damage due to lead acetate toxicity. In the present study male albino rats treated with lead acetate once daily for 8 weeks. Group IV (lead acetate treated) showed significant ($p < 0.05$) decrease in the activity of GPx, GR, GST, CAT and SOD when compared to 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 GPx, GR, GST, CAT and SOD over group V

(ginger-I) and not significantly different with group VII (silymarin treated group). Group V, VI, and VII rats showed a slightly significant difference over normal control rats. Lead causes oxidative stress by inducing the generation of ROS, reducing the antioxidant defence system of cells via depleting glutathione, interfering with some essential metal, inhibiting sulfhydryl dependent enzymes or antioxidant enzymes activities or increasing susceptibility of cells to oxidative attack by altering membrane integrity and fatty acid composition³²⁻³⁴. Lead can cause liver damage and may disturb the normal biochemical process. Cellular damage and necrosis in liver could be due to reduction of some enzymes activities as the result of lead binding to sulfhydryl groups³⁵. It was also found that generation of oxidants may be involved in cellular damage mediated by lead intoxication³⁶. It was suggested that lead induced disruption of the prooxidant/antioxidant balance resulting in tissue injury via oxidative damage to critical biomolecules with accumulation of malondialdehyde, a by-product of lipid peroxidation^{37,38}. Superoxide dismutase (SOD) is the primary antioxidant enzyme in the cell and cellular defense against superoxide radicals. The SOD catalyzes the dismutation of two superoxide (O₂⁻) radicals in to hydrogen peroxide (H₂O₂) and oxygen. GPx, CAT, and SOD are potential targets for lead toxicity because these antioxidant enzymes depend on various essential trace

elements for proper molecular structure and activity³⁹. SOD requires copper and zinc for its activity. Copper ions play functional role in the reaction by undergoing alternate oxidation whereas zinc ions seem to stabilize the enzyme. Both the metal ions are replaced by lead, which decreases the activity of SOD. Catalase is a tetrameric peroxidative enzyme which catalysis the destruction of hydrogen peroxide into water and oxygen and whose gene expression is regulated by H₂O₂. Inhibition of heme synthesis by lead is well reported and since CAT is a heme-containing enzyme, its activity decreases⁴⁰. GPx is a seleno enzyme two third of which (in liver) is present in the cytosol and one third in the mitochondria. It catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. Selenium is essential for GPx activity, and lead forms a complex with selenium, thereby decreases its activity⁴¹. GR is concerned with the maintenance of cellular level of GSH (especially in the reduced state) by effecting fast reduction of oxidized glutathione to reduced form. GST plays an essential role in liver by eliminating toxic compounds by conjugating them with glutathione. The decrease in GST activity could be caused by Pb-induced changes in the enzyme structure as well as by the lack or insufficient amount of GSH, being a substrate for this enzyme⁴². Group V (lead+ginger-I), group-VI (lead+ginger-II) showed recovered levels of SOD, CAT, GPx, GR and GST when compared to lead controlled rats. In support of this authors Miller et al⁴³ and Ahmed et al⁴⁴

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reported previously ginger extracts have been extensively studied for a broad range of biological activities, especially antioxidant activities found that ginger significantly lowered lipid peroxidation by maintaining the activities of the antioxidant enzymes like superoxidedismutase, catalase and glutathione peroxides in rats. Group VII also showed recovered levels of these antioxidants when treated with standard drug silymarin over lead control and the results showed were as close to that of normal control and ginger treated ones. The present study exclusively carried out to know the impact of ginger on liver antioxidant defense system enzymes and protective role of ginger on liver. Kidney antioxidant enzymes like GPx, GR, GST, CAT, and SOD were also estimated. All these antioxidant enzymes level were decreased in lead acetate treated rats and with ginger treatment these parameters were reversed back to normal levels.

CONCLUSION

The results confirmed that lead acetate induced hepato toxic effects may be due to free radical mechanism and provide evidence that ginger significantly protect the hepatic cells and reduce the severity of damage caused by intoxication of lead acetate. Recovered levels of antioxidant enzymes in liver were higher in ginger-II when compared with ginger-I treated animals. However, further detailed studies are required to establish its clinical application.

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