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Protective effect of ginger against lead toxicity on renal antioxidant enzymes in male albino rats

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ABSTRACT

The present study aimed to evaluate the protective effect of *Zingiber officinale* on lead acetate induced nephrotoxicity in albino wistar rats were investigated by analyzing various biochemical parameters. Lead acetate induced kidney damage was well manifested by significant decrease in renal parameters like Glutathione peroxidase, Glutathione reductase, Glutathione-S-transferase, Catalase and Superoxide dismutase. The oral administration of ethanol extract 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 are responsible for the nephroprotective activity of *Zingiber officinale*. Further extensive studies are required for its potential uses in clinical practice.

Keywords: Lead acetate, *Zingiber officinale*, Antioxidants, Oxidative stress.

1. INTRODUCTION

Lead (Pb) is one of the environmental pollutants that can threaten the life of living creatures in many ways. Various industries producing batteries, paints and pigments make use of lead extensively and as such, the population may get exposed to lead by food and water contamination as well as air pollution caused by industrial emission and gasoline containing lead compounds [1].

Lead intoxication is one of the leading occupational health problems. This metal causes a broad range of biochemical, physiological and behavioral dysfunctions. Lead is recognized as a dangerous neurotoxin, even at low levels of exposure [2]. Lead can affect individuals of any age, but it has a disproportionate effect on children because their behavioral patterns place them at higher risk for exposure to lead. Their bodies absorb a larger percentage of the lead that they ingest and they exhibit lead toxicity at lower level for exposure than adults do. Accumulation of lead produces damaging effects in the hematopoetical, hematic, renal and gastrointestinal systems [3]. Many authors propose that the formation of free radicals is the most important molecular mechanism of the lead toxicity [4]. Only one hour after intestinal absorption, lead accumulates in bone, kidney, liver and other organs [4].

Kidney damage occurs with exposure to high levels of lead, and evidence suggests that lower levels can damage kidneys as well [5]. Effect of lead on renal system is characterized by dysfunction of proximal renal tubules manifested by glycosuria, generalized amino aciduria, hyperphosphaturia, hyperphosphataemia and rickets are noted in acute lead poisoning. Long-term exposure to lead is known to cause irreversible functional and morphological changes, which include interstitial, tubular atrophy, and ultra structural changes in renal tubule mitochondria [6]. Lead poisoning inhibits excretion of the waste product urate and causes a predisposition for gout, in which urate builds up. This condition is known as saturnine gout. Chronic lead toxicity is caused by the change of renal function parameters.

Animal tissues are constantly coping with high reactive oxygen species, such as super oxide anion, hydroxyl radicals, hydrogen peroxides and other radicals generation during numerous metabolic reactions [7]. The generation of small amount of free radicals appears to have an important biological function, but oxidative stress is caused by excess production of reactive oxygen species [8,9]. To protect cell organ system of the body against reactive oxygen species mammal cells are well equipped with a highly sophisticated and complex defense mechanism known both enzymatic and non enzymatic antioxidants. The

enzymatic mechanism includes Superoxide dismutase(SOD), Catalase(CAT), Glutathione peroxidase(GPx), Glutathione reductase(GR), Glutathione-S-transferase(GST) etc., [10-12], where as non-enzymatic mechanism includes a variety of compounds as ascorbic acid, tocopherol [13] and glutathione(GSH) etc., When the production of reactive oxygen species exceeds the ability of the antioxidant system, it results in oxidative stress. To prevent cellular damage by free radicals, free radicals mediated lipid peroxidation and tissue antioxidants are essential.

Medicinal plants have continued providing valuable therapeutic agents, both in modern and in traditional medicine [14]. With the associated side effects of modern medicine, traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions [15].

Ginger, the rhizomes of the plant *Zingiber officinale*, is arguably one of the most widely used culinary agent and spice in the world [16]. In addition to its culinary use, ginger also possess medicinal properties, and has been used since antiquity to treat ailments like cold,diarrhea, digestive, headaches, nausea, stomach upset, gastrointestinal disturbances, rheumatic complaints, asthma, diarrhea, nausea, parasitic infections, arthritis and muscular discomfort in the various alternative and folk systems of medicine in the world [17]. Phytochemical studies have shown that the unique culinary and medicinal properties of ginger are due to the presence of phytochemicals like zingerone, shogaols, gingerols, pardols, β-phellandrene, curcumene, cineole, geranyl acetate, terphineol, terpenes, borneol, geraniol, limonene, β-elemene, zingiberol, linalool, α-zingiberene, β-sesquiphellandrene, β-bisabolene, zingiberenol and α-farmesene [18]. Scientific studies carried out in accordance to the principles of modern system of medicine have convincingly shown that ginger possesses numerous health benefits like antimicrobial, antiviral, gastroprotective, antidiabetic, anti-hypertensive, cardioprotective, anticancer, chemopreventive and immunomodulatory effects [19].

The present study was performed to examine the lead induced oxidative stress and nephrotoxicity as well as the nephroprotective efficacy of *Zingiber officinale* extract as an antioxidant in male albino rats.

2. MATERIALS AND METHODS

2.1. 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^{0}C) with 12 hrs dark/light photoperiod. The rats were given standard pellets diet supplied by Sai Durga Feeds and Foods, Bangalore and water adlibitum throughout the experimental period. They were allowed to laboratory conditions for seven days after arrival before use.

2.2. 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).

2.3. 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^{o}C and used for the experimentation.

2.4. 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 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 alcohol 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 alcohol 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.

2.5. 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.

In the present investigation the effect of lead toxicity, protective activity of ginger-I, ginger-II and std.drug silymarin treatment for 8 weeks on levels of kidney 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's [20], Pinto and Bartley [21], Habig et al [22], Beers and Seizer [23] and Soon and Tan [24] respectively.

3. RESULTS AND DISCUSSION

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

Table - 1: Antioxidant enzymes level of kidney in normal and all experimental rats.

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)
GPx (µg of GSH/ min)	Mean	8.2517^{a}	8.3700^{a}	8.3867^{a}	4.1483^{d}	6.5300^{c}	6.9017^{b}	8.0733^{b}
	S.D	±0.0844	±0.1849	±0.1722	±0.1408	±0.3595	±0.2506	±0.1194
GR (µM/ min)	Mean	0.0473^{a}	0.0466^{a}	0.0490^{a}	0.0198^{d}	0.0411^{c}	0.0432^{b}	0.0447^{b}
	S.D	±0.0038	±0.0020	±0.0003	±0.0013	±0.0010	±0.0005	±0.0004
GST (µM/ min)	Mean	1.7017^{a}	1.6983^{a}	1.6967^{a}	0.7000^{d}	1.1117^{c}	1.2983^{b}	1.3517^{b}
	S.D	±0.0195	±0.0430	±0.0553	±0.0173	±0.0195	±0.0157	±0.0267
CAT (µM H_2O_2/ min)	Mean	25.0300^{a}	24.7300^{a}	24.9533^{a}	12.2033^{d}	19.9700^{c}	21.3450^{b}	21.2183^{b}
	S.D	±0.2262	±0.3162	±0.0965	±0.1820	±0.5911	±0.3881	±0.3083
SOD (U/mg/ min)	Mean	7.1517^{a}	7.1133^{a}	7.1300^{a}	2.9733^{d}	5.2300^{c}	6.0650^{b}	6.1600^{b}
	S.D	±0.2415	±0.2453	±0.2911	±0.1494	±0.1628	±0.1526	±0.0868

Values are mean ± S.E.M

Values with different superscripts with in the column are significantly different at $P<0.05$ (Duncan's Multiple Range Test)

In the present study male albino rats treated with lead acetate once daily for 8 weeks. The data presented in table 1 showed analysis of various antioxidant enzymes like GPx, GR and GST, CAT and SOD activities. These antioxidant enzymes activity in normal control (group-I) rats were found to be 8.2517µg of GSH/min, 0.0473µM/min and 1.7017µM/min, 25.03µM of H_2O_2/min and 7.1517U/mg/min respectively. In group-IV (lead control), these enzymes were significantly decreased, 4.1483µg of GSH/min,

0.0198 µM/min and 0.7µM/min, 12.2033µM of H_2O_2/min and to 2.9733U/mg/min respectively.

As a pervasive environmental pollutant, Lead has been reported to induce a broad range of physiological, biochemical and behavioral dysfunctions in man and laboratory animals [25]. The present study demonstrated some ameliorative effects of *Zingiber officinale* extract on enzymatic activities of some antioxidant parameters after eight weeks of lead exposure.

In the present study male albino rats treated with lead acetate once daily for 8 weeks. SOD, CAT, GPx, GR, and GST activities were more significantly decreased in group-IV (lead control). Lead is a non-threshold multi-targeted toxicant that causes alterations in different organs of the body including the kidney [26,27]. The absorbed lead is conjugated in the liver and passed to the kidney, where a small quantity is excreted in urine and the rest accumulates in various body organs and interferes with their function, specially the kidney as a target site for lead toxicity [28]. An elevation of mean blood pressure was found in rats treated with low lead (0.01% lead acetate) for 3 months which was considered to be related due to an increase in endothelin-3 and reactive oxygen species in kidney [29]. 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 [30].

Cervello et al [31] 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 [32]. 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 [33] and GST contain sulfhydryl groups at their active site hence become inactive due to direct binding of lead to sulfhydryl group [34]. Glutathione reductase, the enzyme responsible for recycling of glutathione from the oxidized form (glutathione disulfide; GSSG) to the reduced form (reduced glutathione; GSH) was also deactivated by lead. Catalase is an efficient decomposer of H_2O_2 and known to be susceptible to lead toxicity [35]. Inhibition of heme synthesis by lead is well reported and since CAT is a heme-containing enzyme, its activity decreases [36]. A significant decrease in the activity of tissue superoxide dismutase (SOD), a free radical scavenger and metalloenzyme (zinc/copper) on lead exposure have been reported [37].

Group V (lead+ginger-I), group-VI (lead+ginger-II) showed recovered levels of GPx, GR, GST, CAT and SOD when compared to lead controlled rats. Several chelating agents have been used to reduce the burden of the toxic effect of lead, but these have also produced a toxic potential themselves. This has necessitated researches into the therapeutic potential of various medicinal plants and herbs [38]. Siddaraju and Dharmesh [39] elucidated that ginger-free phenolic and ginger hydrolysed phenolic fractions of ginger exhibited free radical scavenging, inhibited lipid peroxidation, DNA protection and reduced power abilities indicating strong antioxidant properties. Ansari et al [40] reported that the ethanolic *Z. 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 group-VII(std.drug silymarin treated group) showed significantly recovered levels of SOD, CAT, GPx, GR, and GST 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. In support of our work Karimi et al [41] reported that silymarin has antinephrotoxic activity against cisplatin induced nephrotoxicity in albino rats.

4. CONCLUSION

In conclusion, the results generated from this study is suggestive of the fact that lead acetate has adverse effects on the antioxidants of kidney tissue of rats which could lead to initiation of dysfunction of proximal renal tubules 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. Recovered levels of antioxidant enzymes in kidney 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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