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Therapeutic Effects of *Allium sativum* on Lead-induced Biochemical changes in Soft tissues of Swiss Albino Mice

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ABSTRACT

Allium sativum (Meaning pungent) belongs to the Alliaceae family and genus Allium, is generally known in the developing world for its characteristic flavor, a medicinal plant and a source of vegetable oil. Besides, the plant is reported to have various biological activities including hypocholesterolemic, antiatherosclerotic, anticoagulant, antibacterial, antifungal, anti-diabetic, anti-tumor agent; used for treating various disease such as inflammation, cardiovascular and liver diseases. The objective of this study is to investigate the therapeutic effects of *Allium sativum* on lead induced toxicity in mice. Chronic dose of lead (2 mg/Kg body weight, i.p.), showed significant decrease in antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and the nonenzymatic antioxidant as glutathione (GSH) and total protein content in the liver, kidney and brain. This decrease was accompanied with significant increase in lipid peroxidation and cholesterol level. Also, there were disturbances in the liver, kidney and brain functions manifested by significant changes in their functional markers. Efficacy of garlic to reduce tissue lead concentration was also evaluated. Mostly, all of the investigated parameters were restored nearly to the normal values after raw garlic extract treatment. In conclusion, garlic exerts its effects not only as an antioxidant but also as a sulfur donor. So, garlic has a promising role and it is worth to be considered as a natural chelating agent for lead intoxication.

KEYWORDS: Lead, *Allium sativum*, biochemical changes, liver, kidney, brain

INTRODUCTION

Lead is a heavy, low melting, bluish-gray metal that occurs naturally in the earth's crust. However, it is rarely found naturally as a metal. It is usually found combined with two or more other elements to form lead compounds (1). As it is one of the first discovered and most widely used metal in human history and is, therefore, one of the metal most commonly encountered in the environment. Its continued release into the environment as an exhaust emission product, as well as its widespread industrial use, has made lead a serious threat to human health (2). Most lead used by industry comes from mined ores "Primary" or from recycled scrap metal or batteries ("Secondary"). However, most lead today is "secondary" lead, obtained from lead-acid batteries. It is reported that 97% of these batteries are recycled (1).

Lead toxicity has affected human beings from antiquity through the industrial revolution and modern times. It has been written about and studied for more than two thousand years. Fortunately, over the last few decades, there has been a dramatic reduction in lead exposure among children (3), but many adults have problems with chronic lifetime exposures. Many organ systems are affected, in particular the kidney, leading to renal insufficiency and hypertension. Recent data have supported a link between exposure to low levels of lead and chronic kidney disease in patients with hypertension. Lead toxicity can

be an acute, a chronic, or acute-on-chronic nature. The symptoms are varied, depending on the level and timing of exposure and both history and physical examination are critical to making the diagnosis.

Although several hypotheses have been proposed, the exact mechanism of lead toxicity has not yet clearly defined. There are, however, studies which suggest that cellular damage mediated by reactive oxygen species (ROS) may be involved in the pathology associated with Lead intoxication (4). Lead causes oxidative stress by inducing the generation of ROS, reducing the antioxidant defense system of cells via depleting glutathione, interfering with some essential metal, inhibiting sulfhydryl dependent enzyme or antioxidant enzyme activities and increasing susceptibility of cells to oxidative attack by altering membrane integrity and fatty acids composition (5). The binding activity of lead compounds with oxidative stress factors and with the generation of reactive oxygen species, such as hydrogen peroxide and it's interaction with different metals are reported earlier (6, 7).

Considering that lead toxicity is currently one of the serious problem world wide, there is still no specific, reliable and safe treatment. Several chelating compounds have been used to manage lead toxicity in the event of exposure but none are suitable in reducing lead burden in chronic lead exposure (8). Moreover, these chelators in turn are potentially toxic (9) and often fail to remove Pb burden from all body tissues (10, 11).

Thus, there has been an increased interest in the therapeutic potential of plant products or medicinal plants having antioxidant properties in reducing free radical-induced tissue injury (12, 13).

Allium sativum Latin name (English: Garlic, Sanskrit/ Indian: Lasuna) belongs to the Alliaceae family. Garlic is believed by many people to be useful for disease prevention. Garlic has been cultivated for many centuries because of its characteristic flavor and medicinal properties (14, 15). In ancient Egypt and Rome, garlic was given to laborers and soldiers, possibly to mitigate fatigue or to prompt recovery from physical exhaustion (16). After that period, garlic was used to treat hypertension, cardiac diseases, inflammation, diabetes and cancer, according to folk medicines (16). Garlic has a wide range of beneficial effects (17). This is relevant in view of currently increasing public and scientific interest in herbal medicines. Garlic is known to stimulate the body's immune system and protect against heart disease and strokes by reducing cholesterol levels and hypertension (17, 18). Garlic has demonstrated anticancer effects owing to its potential to slow tumor growth, attributed to its chemical constituents mainly diallyl sulfide, diallyl disulfide (19). Garlic has also shown protection against damage from oxidation and free radicals (17, 20).

Researches have focused on the preventive and curative effects of garlic in lead and other heavy metal treatments (21–25) but still great emphasis is required for its use in reduction of lead from cell and body tissues. Keeping this perspective in mind and the aforementioned properties of garlic, the present investigation is planned to determine the effects of raw garlic extracts on lead induced tissue oxidative stress.

MATERIALS AND METHODS

Chemicals

All chemicals used in the study were of analytical reagent grade and were purchased from reliable firms (SRL (India), MERCK, RANBAXY, HIMEDIA and SUYOG).

Plant material

The experimental plant garlic (*Allium sativum*) was collected from medicinal plant garden, Banasthali University and was identified by a plant taxonomist of our Department as a local variety. Peeled garlic was minced and a homogenous suspension was prepared in distilled water a few minute before administration.

Animals and treatment

Male Swiss albino mice (*Mus musculus*) weighing 15–30 g was obtained from Haryana Agricultural University Hissar (India) for experimental purpose. The Institutional Animal Ethical Committee has approved the animal studies. Colony bred adult male albino mice were maintained in a well-ventilated animal house with 12 h light and 12 h dark schedule. Water is made available *ad libitum*. Essential cleanliness and sterile conditions were also maintained.

Experimental protocol

24 animals weighing 25–30 g were randomized into two groups of 6 and 18 mice each and were treated. The groups are as follows-

Group I- No treatment (Control); Group II- Lead nitrate treated (2 mg/Kg body weight, i.p).

After 10 days, lead exposed mice were divided into three groups of 6 mice each and given following treatment consecutively for 25 days-

Group II a) – Lead nitrate (2 mg/Kg body weight, i.p.); Group II b) - Lead nitrate (2 mg/Kg body weight, i.p.) + Raw garlic extract G-250 (250 mg/Kg body weight,

orally); Group II c) - Lead nitrate (2 mg/Kg body weight, i.p.) + Raw garlic extract G-500 (500 mg/Kg body weight, orally). The dose for lead was decided on the basis of LD 50 calculated in the laboratory and the plant doses were selected on the basis of experiments conducted in laboratory and on the basis of published reports, suggesting that *Allium sativum* is having prophylactic efficacy (26). After the administration of the last dose, the animals were provided rest overnight and were sacrificed. The organs liver, kidney and brain were collected and washed with phosphate buffer saline (pH-7.4) and immediately processed for biochemical assays and metal analysis.

Biochemical assays

Lipid peroxidation (LPO)

Lipid peroxidation was estimated colorimetrically by measuring malondialdehyde (MDA) as described earlier by Nwanjo (27). In brief, 0.1 ml of sample was treated with 2 ml of TBA-TCA-HCL reagent and placed in water bath for 15 min, cooled and centrifuged and then clear supernatant was measured at 535 nm against reference blank. The LPO was expressed as nmol of MDA formed $nmole^{-1}$ g^{-1}.

Superoxide dismutase (SOD)

The tissue SOD activity was assayed according to the method of Marklund (28). Procedure (for control): To 2.9 ml of tris buffer, 0.1 ml of pyrogallol solution was added and mixed; and reading was taken at 420 nm exactly after 1 min 30 s and 3 min 30 s. For sample: To 2.8 ml of tris buffer, 0.1 ml of sample was added and mixed; and the reaction was started by adding 0.1 ml of adjusted pyrogallol solution (as per control). Reading was taken at 420 nm exactly after 1 min 30 s and 3 min 30 s and the absorbance was recorded per 2 min. The enzyme activity is expressed as unit ml^{-1} and 1 unit of enzyme is defined as the enzyme activity that inhibits autooxidation of pyrogallol by 50%.

Catalase (CAT)

Catalase activity was estimated following the method of Aebi (29). In brief, 100 µl of tissue extract was placed in ice bath for 30 minutes and then for another 30 minutes at room temperature. Triton X-100 (10 µl) was added to each tube. In a cuvette containing 200 µl phosphate buffer and 50 µl of tissue extract was added to 250 µl of 0.066 M H_2O_2 (in phosphate buffer) and decrease in optical density was measured at 240 nm for 60 seconds. The molar extinction coefficient of 43.6 cm^{-1} was used to determine CAT activity. 1 unit of activity is equal to the moles of H_2O_2 degraded mg^{-1}.

Glutathione (GSH)

Reduced glutathione (GSH) was determined by the method of Ellman (30). In brief, 1 ml of supernatant (0.5 ml homogenate precipitated by 2 ml of TCA) was taken and 0.5 ml of Ellman's reagent (0.0198% DTNB in 1% sodium citrate) and 3 ml of phosphate buffer (pH 8.0) were added. The colour developed was read at 412 nm.

Protein estimation

The protein content was determined by using Bovine serum albumin as a standard by the method of Lowry *et al.* (31).

Cholesterol

The cholesterol estimation was determined by using cholesterol as a standard by the method of Zak's (32).

AST and ALT

Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed by the method of Reitman and Frankel (33). In brief 0.2 ml aliquot of tissue fraction and 1 ml of substrate (aspartate and 2-ketoglutarate for AST; alanine and 2-ketoglutarate for ALT) were incubated for 1 hr for AST and 30 minutes for ALT. To this 1ml aliquot of DNPH solution was added to arrest the reaction and kept for 20 min. at room temperature. After incubation, 1 ml of 0.4 N NaOH was added and the absorbance was read at 540 nm. Activities are expressed as IU L^{-1}.

Metal estimation

Lead concentration in liver, kidneys and brain were measured after HNO_3 digestion by an atomic absorption spectrophotometer.

Statistical analysis

Data are expressed as the Mean±S.E. Statistical analysis was done using analysis of variance (ANOVA). The multiple comparison test of Tukey was also used after the ANOVA test to compare the groups. Analysis was done by SPSS software program and the level of significance was set at $p < 0.05$.

RESULTS

Effects on hepatic oxidative stress and liver marker enzymes

Lead exposure produced significant adverse effects on the redox status of liver, which is evidenced by a significant increase in thiobarbituric acid reactive substances level

Table I. Lead induced changes in hepatic oxidative stress parameters and marker enzymes and their response to administration of raw garlic extract in mice.

Parameters	Normal (Group I)	$Pb(NO_3)_2$ induced (Group II a)	$Pb(NO_3)_2$+G-250 (Group II b)	$Pb(NO_3)_2$+G-500 (Group II c)
TBARS-	111.58±3.23	125.13±1.21*	116.96±1.23	114.01±1.90**
SOD-	1.13±0.04	0.95±0.03*	0.98±0.01*	1.09±0.01**
Catalase-	32.22±0.47	30.12±0.78	31.14±0.31	30.85±0.39
GSH-	7.62±0.23	5.49±0.13*	6.52±0.17*/**	6.41±0.20*/**
ALT-	32.08±0.24	42.39±0.22*	40.68±0.90*	39.73±0.72*/**
AST-	48.46± 0.34	58.04±0.42*	58.20±0.22*	53.32±1.58*/**
TP-	8.52±0.26	7.4±0.42	9.07±0.21**	10.65±0.39*/**
Cholesterol-	24.48±0.39	31.68±0.64*	25.96±0.19**	23.05±0.28**

Abbreviations- **TBARS**: Thiobarbituric acid reactive substances (MDA formed nmole^{-1} g^{-1}); **SOD**: Superoxide dismutase (unit ml^{-1}); **Catalase** (unit mg^{-1} protein); **GSH**: Reduced Glutathione (mg g^{-1} tissue); **ALT**: Alanine transaminase (IU L^{-1}); **AST**: Aspartate transaminase (IU L^{-1}); TP: Total Protein (g 100ml^{-1}); Cholesterol (m g^{-1}); Values are Mean±S.E; n=6.

*P<0.05 compared to normal animals.

**P<0.05 compared to Lead exposed animals.

Table II. Lead induced changes in renal oxidative stress parameters and marker enzymes and their response to administration of raw garlic extract in mice.

Parameters	Normal (Group I)	$Pb(NO_3)_2$ induced (Group II a)	$Pb(NO_3)_2$+G-250 (Group II b)	$Pb(NO_3)_2$+G-500 (Group II c)
TBARS-	99.80±2.66	130.73±2.21*	109.57±3.94**	107.39±2.38**
SOD-	1.08±0.08	0.93±0.01*	0.94±0.01*	0.99±0.02
Catalase-	30.43±0.97	28.45±1.08	29.01±0.27	31.18±0.40
GSH-	6.86± 0.15	5.84±0.14*	5.85±0.55**	5.86±0.07**
ALT-	17.72±0.30	26.16±0.12*	14.80±0.77*/**	18.00 ±0.71**
AST-	22.01±0.30	26.04±0.08*	25.03±0.55*	25.34±1.03*
TP-	5.75±0.26	4.95±0.31	5.17±0.23	4.69±0.40*
Cholesterol-	8.38±0.21	28.35±0.34*	24.98±0.17*/**	21.08±0.21*/**

Abbreviations- **TBARS**: Thiobarbituric acid reactive substances (MDA formed nmole^{-1} g^{-1}); **SOD**: Superoxide dismutase (unit ml^{-1}); **Catalase** (unit mg^{-1} protein); **GSH**: Reduced Glutathione (mg g^{-1} tissue); **ALT**: Alanine transaminase (IU L^{-1}); **AST**: Aspartate transaminase (IU L^{-1}); TP: Total Protein (g 100ml^{-1}); Cholesterol (mg g^{-1}); Values are Mean±S.E; n=6.

*P<0.05 compared to normal animals.

**P<0.05 compared to Lead exposed animals.

Table III. Lead induced changes in brain oxidative stress parameters and marker enzymes and their response to administration of raw garlic extract in mice.

Parameters	Normal (Group I)	$Pb(NO_3)_2$ induced (Group II a)	$Pb(NO_3)_2$+G-500 (Group II b)	$Pb(NO_3)_2$+G-500 (Group II c)
TBARS-	113.30±1.97	125.73±2.94*	120.78±1.55	114.51±2.44**
SOD-	1.11±0.07	0.99±0.05	1.11±0.04	1.14±0.04**
Catalase-	34.57±1.22	31.47±1.13	32.05±0.59	31.83±0.82
GSH-	2.47± 0.05	2.68±0.12	2.07±0.03*/**	2.44±0.04
ALT-	10.59±0.21	22.19±0.21*	7.69±0.37*/**	1.75±0.65**
AST-	14.64±0.59	29.03±0.18*	10.33±0.81*/**	18.83±0.75*/**
TP-	4.58±0.30	1.84±0.27*	2.08±0.10*	3.94±0.08*/**

Abbreviations- **TBARS**: Thiobarbituric acid reactive substances (MDA formed nmole^{-1} g^{-1}); **SOD**: Superoxide dismutase (unit ml^{-1}); **Catalase** (unit mg^{-1} protein); **GSH**: Reduced Glutathione (mg g^{-1} tissue); **ALT**: Alanine transaminase (IU L^{-1}); **AST**: Aspartate transaminase (IU L^{-1}); TP: Total Protein (g 100 ml^{-1}); Cholesterol (mg g^{-1}); Values are Mean±S.E; n=6.

*P<0.05 compared to normal animals.

**P<0.05 compared to Lead exposed animals.

Table IV. Tissue Lead concentrations (ppm)in liver, kidney and brain tissues of mice.

Tissues	Normal (Group I)	$Pb(NO_3)_2$ induced (Group II a)	$Pb(NO_3)_2$+G-250 (Group II b)	$Pb(NO_3)_2$+G-500 (Group II c)
Liver-	0.044±0.006	3.90±0.11*	2.68±0.12*/**	1.24±0.02*/**
Kidney-	0.94±0.011	8.98±0.12*	8.21±0.4*	4.94±0.17*/**
Brain-	0.13±0.31	2.46±0.27*	2.04±0.2*	1.49±0.15*/**

Values are Mean±S.E; n=6.

*P<0.05 compared to normal animals.

**P<0.05 compared to Lead exposed animals.

and a decrease in the levels of biochemical variables indicative of oxidative stress. Decline in catalase activity suggests oxidative injury following lead exposure. Other enzymatic and non-enzymatic antioxidants such as SOD and GSH decreased significantly following lead exposure. Administration of crude extract of *Allium sativum* post lead exposure produced significant recovery in the above mentioned biochemical variables (Table I).

Level of marker enzymes such as ALT and AST increased significantly in lead treated groups, ($p<0.05$), as compared to normal control. The increase in cholesterol level and decrease in total protein was observed in lead treated mice (Table I). Raw garlic extract treatment caused significant decrease in ALT and AST and cholesterol and enhanced the level of T.P. (Table I).

Effects on renal oxidative stress and kidney marker enzymes

The involvement of free radicals in the pathogenesis of kidney injury has been investigated for many years by using chronic lead poisoning. Table II reports the changes in some kidney biochemical variables, indicative of renal oxidative stress following lead exposure and post exposure treatment with raw garlic extract. A significant decrease in GSH level and SOD accompanied by a significant increase in TBARS level suggest kidney oxidative stress. Table II shows that lead administration increased ALT and AST significantly which indicated severe kidney damage. The mean values of total protein decreased while cholesterol level significantly increased by lead intake when compared with normal group. Administration of *Allium sativum* showed significant protection in almost all the above biochemical variables.

Effects on brain oxidative stress and brain marker enzymes

Table III reports the changes in some brain biochemical variables indicative of oxidative stress following lead exposure and post exposure treatment with *Allium sativum*. A significant elevation of LPO and non significant depletion of SOD and GSH was noted. Table III showed significant increase of AST and ALT enzymes and cholesterol level and a significant decrease in total protein level after lead administration. Administration of *Allium sativum* extract produced significant recovery in almost all the above mentioned biochemical variables (Table III).

Effects on Lead concentration

Table IV shows tissue lead accumulation pattern in the experimental mice groups. Administration of raw garlic extract reduced tissue lead accumulation.

DISCUSSION

In general, the results obtained indicate that dietary supplementation with crude garlic extract significantly reduced the toxic effects of lead nitrate. In the present study, there was a concomitant decrease in protein levels. Lead binds to plasmatic proteins, where it causes alterations in a high number of enzymes. It can also perturb protein synthesis in hepatocytes (34). The observed decrease in protein content of plasma of rats treated with Pb may be due to decreased hepatic DNA and RNA (35). Hassanin *et al.* (36) and El-Zayat *et al.* (37) reported a decrease in hepatic total protein content in response to lead intoxication.

Several studies demonstrated that lead intake is associated with significant increase in plasma cholesterol (38). It was found that administration of Pb to rats (39) elevates plasma LDL (low density lipoprotein) and reduces plasma HDL (high density lipoprotein). There is evidence that linking increased of serum cholesterol and LDL levels to a higher risk for developing coronary heart diseases (40). In the present study lead intake increased the mean values of cholesterol significantly while after treatment with garlic, these values significantly decreased. These results were in agreement with Mukherjee *et al.* (41). The mechanism of action was suggested by Sodimu *et al.* (42) who indicated that garlic oil prevented an increase of cholesterol, triglyceride and total lipids by inactivation of thiol group enzymes such as HMG-CoA reductase and CoASH, the rate limiting enzyme for cholesterol biosynthesis and the multi-enzyme complex for fatty acid biosynthesis.

In the present work, lead intake significantly increased tissue marker enzymes (ALT and AST). These results were in agreement with Al-Wabel *et al.* (43). It has been evidenced that excess generation of reactive oxygen species in liver leads to hepatic injury as manifested by an increase in the activities of AST and ALT accompanied by tendency of apoptosis (44, 45). In the current study; these enzymes decreased significantly by garlic treatment. These results were in support with Eidi *et al.* (46) and Eman *et al.* (47), who showed that oral administration of the garlic extract significantly decreased serum glucose, total cholesterol, triglycerides, AST and ALT levels in normal rats ($p<0.05$). The observed decrease in these enzymes showed that raw garlic extract preserve the structural integrity of the organs from the toxic effects of lead. Decrease in GSH, SOD and CAT levels were also observed in the present study. Administration of garlic restored almost all the values of these antioxidant related parameters.

Lead have a high affinity for sulfhydryl (SH) groups; mercaptides are formed with the SH group of cysteine and less stable complexes with other amino acid side chains. Lead exerts its toxicity through reaction with sulfhydryls that exist in the cell.

GSH is a tripeptide containing cysteine that has a reactive SH group with reductive potency. Lead binds exclusively to the SH group (48, 49), which decreases the GSH levels (50) and interfere the antioxidant activity of GSH. Garlic components may provide the sulfhur source required for the synthesis of GSH (51, 52). So, it restores glutathione level and increases the activities of glutathione reductase and glutathione-s-transferase. On the other hand, GPx, catalase, and SOD are metalloproteins and accomplish their antioxidant functions by enzymatically detoxifying peroxides, H_2O_2 and free oxygen radical, respectively. Since these antioxidant enzymes depend on various essential trace elements for proper molecular structure and enzymatic activity, they are potential targets for lead toxicity (53). Catalase is another major antioxidant enzyme having heme as the prosthetic group. Lead is known to reduce the absorption of iron in the gastrointestinal tract and to inhibit the heme biosynthesis (54). Decreased catalase activity observed in lead-exposed animals was attributed to the interference of lead by both processes (55, 56). SOD plays an important role in protecting the cells against the toxic effects of oxygen radical by catalyzing its dismutation reactions. Several studies pointed to decreased RBC SOD activity in lead-exposed rats (53, 57).

Lead intake also increased LPO, which results from direct interaction of lead to biological membranes. Several studies have focused on the possible toxic effects of lead on membrane components and identified a correlation between these effects and lead-induced oxidative damage. Yiin and Lin (58) demonstrated a marked enhancement in MDA concentrations following incubation of linoic, linolenic, and arachidonic acid with lead (58).

Mice administered with *Allium sativum* significantly restored the altered levels of antioxidant molecules suggesting that the active ingredients in the garlic possess antioxidant properties and protects tissues against lead induced oxidative stress. Garlic contains sulfur-containing amino acids, like S-allyl cystine, S-allyl mercaptocystein and alliin (59). Sulfur-containing amino acids like cystein have already been reported for their chemoprophylactic use in lead toxicosis (60–63). The efficiency of garlic was perhaps due to the presence of these sulfur-containing amino acids and compounds having free carboxyl (-C = 0) and amino ($-NH_2$) groups in their structures. These biologically active compounds might have chelated lead and enhanced its excretion from the body resulting in reduced lead accumulation in tissues and blood. Further published reports also showed that garlic extracts increased the lead concentration in the urine as well as in the feces of rats (23) lending credence to this hypothesis. Besides chelation, other components of garlic (S-allyl cystein, S-allyl mercaptocystein and some micronutrients) also prevent absorption of lead from the gastro-intestinal tract (64). Therefore it can be suggested that the ameliorative potential of garlic was probably due to the combined effects both on metal absorption and on excretion from the body. In addition, garlic compounds have been found to inhibit lipid peroxidation, which is considered one of the main features of aging in liver cells (65, 66).

Lead concentration was reduced in liver, kidney and brain tissues of mice given both lead and garlic simultaneously or garlic as a supplement after lead treatment. Our study also confirms earlier reports suggesting that garlic powder has the ability to reduce lead in soft tissues (67).

CONCLUSION

On the basis of above results, it could be concluded that raw garlic extract not only has a potent antioxidant activity but also has chelating property against lead toxicity. Chelating property of *Allium sativum* is because of its sulfur containing amino acids. Thus, from the present study we recommended that a suitable selection and manipulation of our diet might be useful to a major extend in reducing toxicity risk in population exposed to lead.

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