Protective Antioxidant Efficiency of Garlic against Lead-Induced Renal and Testicular Toxicity in Adult Male Rats

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Abstract

Rational: Leads is one of the common heavy metal pollutants which have toxicological effects on various organs in humans and animals. Lead (Pb) exposure leads to the production of Reactive Oxygen Species (ROS) which affected kidney and testes functions.

Objectives: The current study was performed to evaluate the effect of lead on the alterations of biochemical markers related to oxidative stress in kidney and testes of adult male rats and to highlight the protective effect of garlic administration on these biomarkers.

Results: The results revealed that lead exposure caused significant elevations in creatinine, urea and uric acid as indicators to renal function, cytokines and inflammatory mediators, concentration of lead in kidney and testis tissues, oxidative stress with significant reduction in weights of kidney and testes, sexual hormones and antioxidants in kidney and testis tissues. Lead intoxicated rats showed marked increase in the percentage of dead sperms and abnormal sperm rate, while significant decrease in sperm concentration and sperm motility.

Conclusion: Pre-treatment with garlic prevent degenerative changes induced by lead, reduced oxidative stress, cytokines and inflammatory mediators, and restored the biochemical changes occurring in the kidney and testis tissues to approximately close to normal group.

Keywords: Lead; Kidney; Testis functions; Garlic; Oxidative stress; Antioxidants; Cytokines

Introduction

Lead is a toxic heavy metal that causes adverse health effects in humans and animals. Lead exposure mainly occurs through the respiratory and gastrointestinal systems, alters biomarkers of kidney function, and increases lipid per-oxidation and decreases antioxidants function [1]. Lead induces overproduction of Reactive Oxygen Species (ROS), alters many biological activities at the molecular, cellular and intracellular levels, which may result in morphological alterations [2]. Lead induces kidney damage [1], evokes prominent testicular toxicity, deterioration of sperm characters and disruption of serum gonad/pituitary hormone levels Luteinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and testosterone, markedly raises testicular oxidative stress (lipid peroxides). While reduces the activity of Superoxide Dismutase (SOD), reduced glutathione (GSH) and catalase [3].

Garlic has many pharmacological properties and medical application. It contains more than 200 chemical compounds, including volatile oil with sulphur containing allicin, allin, ajone, allinase, peroxidase and myrosinase [4]. The biological effects attributed to garlic includes induction of endogenous antioxidant in rat tissue organs, stimulation of immune function, enhancement detoxification of foreign compounds, antimicrobial and antioxidant effects [5], inhibition of lipid peroxidation, attenuation of urea and creatinine levels and promotion of the activities of antioxidant enzymes [6].

The present study was performed to evaluate the effect of lead on the biochemical biomarkers related to oxidative stress in kidney and testes of adult male rats and to clarify the protective effect of garlic on these biomarkers.
Materials and Methods

Experimental animals

Forty healthy adult male albino rats of similar age and weight (200 ± 20 g) were used in this study. The animals were obtained from the National Institute of Vaccination, Hellwan, Egypt. They were kept under standard laboratory conditions (25 ± 2°C and 12 h light-dark cycle). Basal diet and clean water were provided ad libitum for two weeks as an adaptation period. All animals’ procedures are in accordance with the guidelines of Ethical Committee of the National Institutes of Health Guide for care and use of laboratory animals (Publication No. 85-23 revised 1996).

Chemicals

Lead acetate ([Pb(CH3COO)2], 99% pure) was purchased from El-Nsar pharmaceutical chemicals company (Zagazig, Egypt). Lead acetate was dissolved in saline solution (0.9% NaCl) and injected intraperitoneally at a dose of 8 mg/kg b.w. daily. Garlic (dry garlic powder 300 mg in each tablet, 50 tablets ATOS Pharma, Cairo, Egypt) was grounded and suspended in distilled water and administered by gavage at a dose of 200 mg/kg b.w. daily. All other chemicals and reagents were of analytical grade and obtained from standard commercial suppliers.

Experimental design

The animals were randomly divided into four groups of ten rats each:

Group 1: Rats received a daily oral administration of distilled water and injected saline (ip) for ten weeks and served as normal control.

Group 2: Rats received garlic (200 mg/kg b.w. daily for ten weeks by gavage).

Group 3: Rats received lead acetate (8 mg/kg b.w. daily for ten weeks ip).

Group 4: Rats received garlic (200 mg/kg b.w. followed after two h by lead acetate (8 mg/kg b.w. daily for ten weeks).

Blood collection

At the end of the experiment, all rats were fasted overnight, euthanized and blood samples were drawn from the retro-orbital venous plexus in centrifuge tubes for serum separation, centrifuged at 3000 rpm for 15 min (4°C) and stored at -20°C as aliquots for further determination of creatinine, urea, uric acid, cytokines and inflammatory mediator and sexual hormones.

Tissue samples

All animals were rapidly sacrificed after blood collection and kidney and testes were immediately excised, washed several times from blood by ice-cold isotonic saline, weighed and blotted dry. Testes, epididymis, seminal vesicles and ventral prostate were dissected from any adhering connective tissue weighed and shock-freeze in liquid nitrogen (-170°C) and stored at -20°C. The specimens of kidney and testes were homogenized individually with tissue homogenizer to make 10% of homogenate to assay the tissue oxidants and antioxidants. The homogenates were prepared for analysis by centrifugation at 18000 rpm (4°C) for 30 min and the supernatant was kept at -2°C for analysis of oxidative stress markers i.e. superoxide anion and Lipid Peroxidation (LP), antioxidant enzymes, i.e., Superoxide Dismutase (SOD), catalase (CAT) and the content of reduced glutathione (GSH).

Biochemical analysis

Serum analysis: Serum creatinine, urea and uric acid as indicators to kidney function were measured spectrophotometrically using standardized commercially diamond diagnostic kits (Egypt) following the instruction of the manufactures. Cytokines interleukin -1β (IL-1β) and tumor necrosis factor alpha (TNF-α) as well as inflammatory mediator monocyte chemoattractant protein-1 (MCP-1) were determined by Enzyme-Linked-Immunno-Sorbent-Assay (ELISA) using rat IL-1β, TNF-α and MCP-1 kits (Biosource International USA) and microtiter plate reader, Fisher Biotech (Germany) according to the methods of Chen et al., [7]; Ono et al., [8] and Zajkowska et al., [9] with the same respect. Serum sexual hormones including LH, follicle-stimulating hormone (FSH) (Cusabio ELISA Kit), PRC and total testosterone (DRG ELISA Kit, Germany) were estimated using enzyme linked immuno sorbent assay kits following the manufacturer recommendations.

Tissue homogenate analysis: Tissue superoxide anion was determined according to the modified method of Hassoun and Stohs [10]. The amount of malondialdehyde (MDA) in tissue homogenate of kidney and testes as an index of LP was determined by Enzyme-Linked-Immunno-Sorbent-Assay (ELISA) using rat IL-1β, TNF-α and MCP-1 kits (Biosource International USA) and microtiter plate reader, Fisher Biotech (Germany) according to the methods of Chen et al., [7]; Ono et al., [8] and Zajkowska et al., [9] with the same respect. Serum sexual hormones including LH, follicle-stimulating hormone (FSH) (Cusabio ELISA Kit), PRC and total testosterone (DRG ELISA Kit, Germany) were estimated using enzyme linked immuno sorbent assay kits following the manufacturer recommendations.

Sperm characteristics: Sperm quantity was counted according to the modified method of Yokoi et al., [16] using a hemo-cytometer and light microscope at 200x magnification. Sperm motility was determined as described by Sonmez et at., [17] on the basis of visual estimation under light microscope at 400x magnification. Sperms were assigned as either motile or non-motile. The percentage of abnormal spermatozoa was estimated according to Turk et al., [18]. Sperms were assigned as either motile or non-motile. The percentage of abnormal spermatozoa was estimated according to Turk et al., [18]. Slides were stained with India ink and visually estimated under light microscope at 400x magnification and any head, tail and entire sperms abnormalities were expressed as a percentage.
Statistical analysis

The obtained data were expressed as mean ± SD. Homogeneity of variance for each variable was analysed using the Levine test. The data were statistically analysed by means of one-way analysis of variance (ANOVA) using SPSS software (SPSS Inc, Chicago, IL). Intergroup comparison was performed by Duncan’s multiple rank tests using the mSTAT-c computer program. A difference was considered significant at p<0.05.

Results

Organs weight

The data given in Table 1 show that garlic administration enhanced the weights of both kidney and testes. However, Pb-intoxicated rats showed significant decrease in right and left kidney, right and left testis, right and left epididymis, seminal vesicles and ventral prostate. Pre-treatment Pb-intoxicated rats with garlic resulted in considerable increase in the kidney and testes weights as compared to the Pb-treatment alone. Thus, pre-treatment Pb-intoxicated rats with garlic brought back the weights of kidney and testes too approximately close to control group.

Table 1: Effect of garlic administration on kidney and testes weights of lead (Pb) intoxicated male rats. Values are expressed as means ± SE. Means followed by the same alphabetical letter are not significantly different at p<0.05.

|                | Kidney (g) | Testes (g) | Epididymis (mg) | Seminal Vesicles (g) | Prostate (g) |
|----------------|------------|------------|-----------------|----------------------|--------------|
|                | Right      | Left       | Right           | Left                 | Right       | Left       | Right       | Left       | Right       | Left       |
| Control        | 2.1 ± 0.4ab| 2.0 ± 0.4ab| 1.9 ± 0.18a     | 1.8 ± 0.16b         | 0.52 ± 0.04ab  | 0.50 ± 0.04ab| 0.60 ± 0.07ab | 0.38 ± 0.04ab | 0.4 ± 0.03ab |
| Garlic         | 2.4 ± 0.5a | 2.2 ± 0.3a | 2.0 ± 0.20a     | 1.9 ± 0.17ab        | 0.54 ± 0.05a  | 0.53 ± 0.06a | 0.81 ± 0.08a  | 0.39 ± 0.12a  | 0.4 ± 0.03 |
| Lead           | 1.6 ± 0.2c | 1.9 ± 0.2c | 1.5 ± 0.14c     | 1.4 ± 0.12c         | 0.42 ± 0.03c  | 0.40 ± 0.03c | 0.62 ± 0.05c  | 0.26 ± 0.03c  | 0.5 ± 0.04 |
| Garlic+Lead    | 2.0 ± 0.3bc | 2.1 ± 0.3ab| 2.1 ± 0.23a     | 2.0 ± 0.20a         | 0.49 ± 0.05b  | 0.48 ± 0.04b | 0.75 ± 0.07b  | 0.36 ± 0.04b  | 0.6 ± 0.03 |

Tissue analysis

It is evident from the data presented in Table 3 that garlic administration tended to decrease insignificantly the accumulation of Pb and oxidative stress (O-2 and MDA) in both kidney and testes tissues, and insignificantly increased the antioxidants (SOD, CAT and GSH) in both tissues. However, Pb-intoxicated rats showed significant elevations in the accumulation of Pb (183% and 222%), superoxide anion (85% and 111%) and MDA (71% and 40%) in both kidney and testes tissues, respectively as compared to the control group. Otherwise, the antioxidants were significantly decreased by 55% and 62% for SOD activity, 46% and 46% for CAT activity and 61% and 49% for GSH level in both kidney and testes tissue, with the same respect.

Pre-supplementation Pb-intoxicated rats with garlic revealed significant reduction in the accumulation of Pb, oxidative stress (O-2 and MDA), while significantly increased antioxidants (SOD, CAT and GSH) in kidney and testes tissues as compared to the Pb treatment alone. Pre-treatment Pb-intoxicated rats with garlic brought back the concentration of Pb, the levels of O-2, MDA and GSH as well as the activities of SOD and CAT to near the normal control group. It is interest to mention that there was insignificant difference between pre-treatment Pb-intoxicated rats with garlic and the control group concerning the levels of Pb, O-2, MDA, GSH and the activities...
of SOD and CAT in kidney and testes tissues reflecting the therapeutic value of garlic.

Table 2: Effect of garlic on some renal function, cytokines & inflammatory mediator and sexual hormones in serum of lead (Pb) intoxicated male rats. Values are expressed as means ± SE. Means followed by the same alphabetical letter are not significantly different at p<0.05.

| Renal function | Cytokines and inflammatory mediator | Sexual hormones |
|----------------|-----------------------------------|-----------------|
| Creatinine (mg/dl) | Urea (mg/dl) | Uric acid (mg/dl) | IL-1β (pg/ml) | TNF-α (pg/ml) | MCP-1 (pg/ml) | LH (ng/ml) | FSH (ng/ml) | Testosterone (ng/ml) |
| Control | 0.90 ± 0.05 | 22.2 ± 2.3d | 1.92 ± 0.16bc | 15.2 ± 1.5d | 18.6 ± 2.6d | 80.6 ± 7.8cd | 2.2 ± 0.21bc | 9.3 ± 0.81a | 7.2 ± 0.62ab |
| Garlic | 0.85 ± 0.04d | 21.8 ± 1.9d | 1.85 ± 0.17b | 13.7 ± 1.4d | 16.8 ± 2.4d | 78.5 ± 7.2d | 2.4 ± 0.92a | 9.8 ± 0.92a | 7.6 ± 0.70a |
| Lead | 1.9 ± 0.14a | 34.6 ± 3.5a | 3.24 ± 0.28a | 32.5 ± 2.8a | 36.4 ± 4.8a | 142.3 ± 9.5a | 0.82 ± 0.07c | 5.2 ± 0.43c | 3.5 ± 0.40c |
| Garlic + Lead | 1.1 ± 0.09b | 24.5 ± 2.6bc | 2.12 ± 0.19b | 17.3 ± 1.8bc | 20.2 ± 2.8bc | 82.4 ± 7.6bc | 1.9 ± 0.16b | 7.8 ± 0.61b | 7.1 ± 0.66b |

Table 3: Effect of garlic administration on the concentration of lead, oxidative and antioxidant levels in kidney and testes tissues of lead intoxicated male rats. Values are expressed as means ± SE. Means followed by the same alphabetical letter are not significantly different at p<0.05.

| Kidney tissue | Testes tissue |
|---------------|---------------|
| Pb conc µg/g tissue | O-2 (nmol/g tissue) | MDA (nmol/g Tissue) | SOD u/g tissue | CAT u/g tissue | GSH nmol/g tissue | Pb conc µg/g tissue | O-2 (nmol/g tissue) | MDA (nmol/g Tissue) | SOD u/g tissue | CAT u/g tissue | GSH nmol/g tissue |
| Control | 1.2 ± 0.15a | 14.5 ± 2.6d | 42.6 ± 3.8d | 10.4 ± 1.8c | 9.9 ± 0.9a | 2.2 ± 0.2b | 0.9 ± 0.02c | 11.6 ± 1.9b | 29.6 ± 2.8cd | 2.1 ± 0.3b | 8.5 ± 0.7ab | 1.8 ± 0.3ab |
| Garlic | 1.0 ± 0.12c | 13.2 ± 1.8d | 39.5 ± 3.2d | 11.6 ± 1.2c | 10.2 ± 1.1a | 2.4 ± 0.3a | 0.8 ± 0.01c | 10.5 ± 1.8c | 27.8 ± 2.6d | 2.8 ± 0.4a | 9.2 ± 0.8a | 2.1 ± 0.5a |
| Lead | 3.4 ± 0.41ab | 26.8 ± 3.6a | 72.8 ± 6.8a | 4.7 ± 0.9a | 5.3 ± 0.6bc | 0.86 ± 0.09c | 2.9 ± 0.24ab | 24.5 ± 3.2a | 41.3a ± 3.8a | 0.8 ± 0.07c | 4.6 ± 0.5c | 0.92 ± 0.08c |
| Garlic+Lead | 1.6 ± 0.14bc | 17.3 ± 2.9bc | 44.2 ± 4.1bc | 11.2 ± 1.3b | 8.9 ± 0.9b | 2.1 ± 0.3b | 1.0 ± 0.09bc | 14.6 ± 2.6b | 30.5 ± 2.9bc | 1.9 ± 0.12b | 8.2 ± 0.8b | 1.7 ± 0.2b |

Sperm characteristic

Administration of garlic insignificantly increased sperm quantity, motility and insignificantly decreased the percentages of dead and abnormal sperms as compared to the control group (Table 4). Otherwise, daily IP injection of rats with lead acetate (8 mg/kg b.w.) for ten weeks produced marked reduction in sperm concentration and sperm motility amounting to -37% and -42%, respectively as compared to the control group. Whereas, the percentages of dead and abnormal sperms were significantly increased by 162% and 133% respectively as compared to the control group. On the other hand, pre-treatment Pb-intoxicated rats with garlic brought back sperm characteristics to approximately near the control group, reflecting the preventive effect of garlic against the adverse effect of Pb on sperm characteristics.

Discussion

Lead has proved to be extremely toxic metal and is considered as a serious occupational hazard throughout the world [19]. It induced over production of ROS, increased oxidative stress in various rat organs including kidney and testis tissues [2]. The current study aimed to evaluate the possible protective effect of garlic against lead induced renal and testicular toxicity and oxidative stress in rats. The results of the present study revealed that administration of garlic insignificantly enhanced the weights of kidney and testis as compared to the control group.

On the other hand, Pb-intoxicated rats showed significant reduction in the weights of both kidney and testis as compared to the control group. These results are in the same
line with the finding of Aprioku and Obianime [20] who reported that Pb-treated rats significantly decreased the weights of kidney (-33%) and testis (-56%), this reduction may be due to anemia, lymphocytosis and neuroprotineia induced by administration of Pb.

Table 4: Effect of garlic administration on sperm characteristics of lead (Pb) intoxicated male rats. Values are expressed as means ± SE. Means followed by the same alphabetical letter are not significantly different at p<0.05.

|               | Sperm concentration million/g | Sperm motility % | Dead sperms % | Abnormal sperms rate% |
|---------------|-------------------------------|------------------|---------------|-----------------------|
|               | Head                          | Tail             | Entire        |                       |
| Control       | 295 ± 16.2<sup>ab</sup>       | 78.4 ± 5.8<sup>b</sup> | 14.9 ± 1.9<sup>c</sup> | 2.6 ± 0.12<sup>cd</sup> | 4.1 ± 0.35<sup>c</sup> | 6.7 ± 0.45<sup>c</sup> |
| Garlic        | 310 ± 18.5<sup>a</sup>        | 82.2 ± 6.4<sup>a</sup> | 12.1 ± 1.4<sup>c</sup> | 2.1 ± 0.14<sup>d</sup> | 3.6 ± 0.38<sup)b</sup> | 5.7 ± 0.42<sup>c</sup> |
| Lead          | 185 ± 12.1<sup>c</sup>        | 45.3 ± 3.8<sup>c</sup> | 39.1 ± 2.5<sup>a</sup> | 7.4 ± 0.82<sup>a</sup> | 8.2 ± 0.86<sup>a</sup> | 15.6 ± 1.2<sup>a</sup> |
| Garlic+Lead   | 286 ± 14.5<sup>b</sup>        | 70.4 ± 5.7<sup>b</sup> | 21.1 ± 2.2<sup>b</sup> | 3.2 ± 0.24<sup>bc</sup> | 5.3 ± 0.42<sup>b</sup> | 8.5 ± 1.1<sup>b</sup> |

Co-administration of garlic with Pb significantly enhanced the weight of both kidney and testes as compared to lead group. Similar finding was reported by Cha [21]. The biological effects attributed to garlic include induction of endogenous antioxidant in rat tissue organs, stimulate immune function and enhanced detoxification of foreign compounds [5].

Pb-intoxicated rats showed significant elevations in serum creatinine, urea, uric acid, IL-1β, TNF-α and MCP-1 and significantly decreased the levels of sexual hormones as compared to the control group. These results are generally in agreement with other studies on Pb nephrotoxicity [1,2] and testicular toxicity [3,22]. Creatinine and urea are waste products of protein metabolism that needs to be excreted by the kidney; therefore the marked increase of these parameters is an indication of functional damage to the kidney. The increase of creatinine in Pb-intoxicated rats might be due to impaired kidney function in this case. This view could be supported by Kluwe [23] who indicated that an elevation of creatinine level in the blood is indicative of impaired kidney function.

The increase of uric acid, as a result of Pb intoxication, stimulates the release of pro-inflammatory mediator MCP-1 from vascular cells [24] and cause renal vasoconstriction and chronic renal disease [25]. Hyperuricemic rats showed significantly greater tubular injury and proliferation with significantly greater macrophage infiltration and increased expression of MCP-1. Uric acid may exacerbate renal injury in a model of acute renal failure. The mechanism may relate to a pro-inflammatory pathway involving chemokine expression with leukocyte infiltration [26].

In addition, the increases of TNF-α, obtained in the current study from Pb intoxicated rats, enhances the expression of other inflammatory cytokines and chemokine such as IL-1β and MCP-1 which cause nephrotoxicity in rats [27].

Concerning the sexual hormones, it has been shown that LH and FSH activity depends on both the quantity of these hormones and the number of specific receptors in the testis. Leydig cells of the testis are responsible for the biosynthesis and secretion of androgens and reproductive function in male rats. Testosterone production is directed by LH. However, FSH affects sterol’s cells, in that it triggers the formation of spermatogonial cells testosterone binding protein. Lead exposure recorded the number of sertoli cells and diminished the development of leydig cells [28].

Pre-supplementation Pb intoxicated rats with garlic brought back the levels of creatinine, urea, uric acid, cytokines and inflammatory mediator as well as sexual hormones to approximately near to the control group. These results could be explained on the basis that garlic contains more than 200 chemical compounds including volatile oil with sulphur containing allicin, allin, ajone, allinase, peroxidase and myrosinase [4]. These biologically active compounds might have chelated Pb by the formation of ionic bonds between sulphur containing compound and enhanced its excretion from the body. Garlic also enhanced detoxification of foreign compounds and has antioxidant effects [5].

In the present study, Pb-intoxicated rats revealed significant elevation in the accumulation of Pb, superoxide anion, MDA and significant decrease in SOD, CAT and GSH in both kidney and testes tissues as compared to the control group. The previous findings [29,30] supported the obtained results. The accumulation of lead in kidney and testes tissues produces damaging effects in renal and reproductive system. It caused severe damage to mitochondria and the tissues became unable to perform normal functions [31,32]. Pre-supplementation Pb-intoxicated rats with garlic showed significant reduction in the concentration of Pb, superoxide anion and MDA, while significant increase in SOD, and CAT activities as well as GSH level in both kidney and testes tissues as compared to the Pb-intoxicated animals. The values of the obovemention parameters as a result of garlic administration were approximately near or close to the values of the control animals.

These results could be discussed on the basis that garlic organo-sulfur compounds could preserve the tissue structural integrity [33]. Garlic contains cysteine, s-allyl, mercaptocysteine and allin, ajone, allinase, peroxidase [4]. Such compounds were already used against Pb toxicity [34], where they increased rat Pb excretion in urine and feces. In addition, garlic enhanced detoxification of foreign compounds and has antioxidant effects [5].
Superoxide anion (O-2) directly affects the activity of catalase and peroxidase by affecting intracellular enzymes [35]. MDA is an important indicator of LP which causes impaired membrane structure and functions. SOD plays an important role in protecting the cells against the toxic effects of (O-2) by catalyzing its dismutation reactions. In addition CAT is a heme protein, which catalyzes the reduction of hydrogen peroxides and protects tissues from highly reactive hydroxyl radicals. Meantime GSH is one of the most important compounds which helps in the detoxification and excretion of heavy metals [29].

Concerning sperm characteristics, it is evident that Pb intoxicated rats showed significant decrease in sperm concentration, sperm motility and significant increase in the percentages of dead sperm and the rate of abnormal sperms as compared to the control group (Table 4). However, pre-supplementation Pb-intoxicated animals with garlic revealed significant increase in sperm concentration, sperm motility and significant decrease in the percentage of dead sperms and the rate of abnormal sperms as compared to the Pb-intoxicated rats. Thus the values of sperm characteristics due to garlic administration were insignificantly different when compared to the control group. The negative effects of Pb on rat sperm characteristics may attributed with the accumulation of Pb in testicular tissues, the reduction of testis weight, sexual hormones GSH level, SOD and CAT activities in testicular tissues and elevations of superoxide anion and MDA. On the other hand, the positive effect of garlic administration may be attributed with the biological effects of garlic including volatile oil with sulfur containing alllicin, allin, ajone, allinase, peroxidase and mycoron [4], induction of endogenous antioxidant in rat testicular tissues, stimulate immune function, enhanced detoxification of heavy metal [5], inhibit LP and enhance the activities of antioxidant enzymes [6].

Conclusion

The results of the currents study reinforce the significant role of ROS in the pathogenesis of Pb-induced oxidative damage in the rat kidney and testes and also it is assumed that garlic had beneficial effect for prophylaxis of Pb-induced renal and testicular damage for its potent cyto-protective effect against the deleterious toxic effects caused by Pb.

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