What is the Benefit of Garlic Supplementation to Pb Intoxicated Rats?

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A B S T R A C T

The aim of this work is to investigate the protective effect of garlic Allium Sativum against Pb induced toxicity. Twenty one males rats were divided equally into three groups, in which the first group was taken as a control and the other were exposed to a diet containing (600 mg lead acetate/kg food) alone or combined with fresh garlic (30 g/kg food) during 6 weeks. Some renal, hepatic and testicular markers were evaluated and data of exposed groups were compared to the control. Results showed significant increase in the level of serum urea, creatinine, and also in the activities of Alanine Aminotransferase (ALAT) and Aspartate-Aminotransferase (ASAT) of the Pb group, with no remarkable change in the level of serum uric acid, cholesterol testosterone and alkaline phosphatase (ALP). Contrary, Pb-garlic group has shown only slight variations in the previous markers, except for urea and creatinine concentrations which were higher compared to the control. The hepatic, renal and testicular GSH content have been declined significantly in the Pb group. In the Pb-garlic group, the GSH content was only significantly different in the kidney tissue, whereas that of liver and testis was similar to the control. Concerning MDA content, it was higher in kidney, liver and testis when rats were exposed to Pb. The Pb-garlic group has elevated hepatic and renal MDA content, while that of testis was close to the control, which indicate the benefit use of garlic to protect testicular tissue from MDA formation when intoxicated by Pb. The histological profiles have showed hepatic capillary hemangioma, renal tubular necrosis in rats contaminated by Pb. However, in the Pb-garlic group, hepatic tissue was almost as that of the control, but an interstitial fibrosis still exists in the renal tissue. Concerning testis, seminiferous tubules were less filled with spermatozoa in the Pb group when compared to the control, whereas the garlic extract has not only protected spermatozoa, but it enhanced their number. To conclude, garlic has partially protected rats’ biomarkers from Pb contaminated diet.

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INTRODUCTION

Pollution by trace metals constitutes a serious interest in the world's population. These metals are used in many industries, and they have the ability to accumulate in living organisms posing a serious public health problem. Lead causes multiple biological alterations to different systems [1], where its accumulation provokes many alterations in liver cells [2]. Lead is also known as a risk factor for renal function [3], where it produces a proximal tubular alteration, which results in a leakage of low molecular weight proteins causing, moderate renal failure due to nephroclerosis and cortical atrophy [4]. Early chronic lead exposure alters glomerular development in pre-adolescent animals [5]. The action of Pb on reproduction resulted in spermatogenesis disruption, male sub fertility, impaired sperm production, decreased ejaculate volume, and alteration of testosterone level [6]. Pb was also reported to generate cell ROS production [7], the peroxidation of membrane lipids, and thus changes the balance between the generated free radicals and the antioxidant systems [8].

Garlic clove contains polysaccharides, amino acids, enzymes (alliinase, peroxidase, myrosinase), selenium and vitamins A, B1, B2 C [9]. In garlic, there are at least 100 bioactive volatile sulfur compounds and non-volatile with medicinal value that contribute to its pharmacological uses, some bioactive components of garlic include S-allylcysteine, saponins, ajoene, and flavonoid phenolic compounds [10]. The bioactive constituents of
garlic play therapeutic effects as they reduce the risk of cardiovascular disease (anti-hypercholesterolemia, anti-coagulant and anti-hypertensive) [11]. Garlic has antioxidant ability to protect cells against free radicals [12], antitumor activity [13] and it is traditionally used for its antimicrobial properties, for the treatment of certain bacterial infections and parasites [14].

Due to medicinal properties of garlic, this work was carried out to investigate the possible protective properties of *Allium sativum* extracts against Pb toxicity in male rats. Therefore, certain hepatic, renal and reproductive biomarkers were evaluated.

**MATERIALS AND METHODS**

This study has been realized using 21 male wistar rats, weighting 180-315 g. Animals were reared in standard conditions of temperature, humidity and light and they fed standard diet *ad libitum*. Rats were divided into three groups; control, exposed to Pb (600 mg lead acetate/kg food) and exposed to fresh garlic (30 g/kg food) during 6 weeks. Both lead and garlic were prepared daily, mixed with the diet, and then given to animals. Food and water consumption were measured daily, whereas total body weight was weighed weekly. After 6 weeks experimental period, rats were sacrificed by decapitation; the blood was taken from jugular veins, in which it immediately received in polyethylene tubes containing EDTA. Blood was then centrifuged at 4000 rpm/15 minutes and plasma was used to measure urea, creatinine, uric acid, cholesterol, testosterone, Alanine-Aminotransferase (ALAT), Aspartate-Aminotransferase (ASAT) and alkaline phosphatase (ALP) by using commercial kits by means of automated apparatus (Urit 8030). Testosterone concentration was estimated by ELISA method using the commercial kit (Test ELISA RE 52151). Moreover, MDA and GSH were measured according to the method of [19], and [20], respectively.

Liver, kidney and testis were removed immediately and then preserved in the 1% formol in order to carry out the histological study.

Statistical analysis was realized using ANOVA, followed by Student *t*-test to compare each treated group with the control. Values were presented as mean ± SD. P≤ 0.05 level was considered statistically significant.

**Results:**

**Plasma biomarkers:**

Results of plasma biomarkers are presented in table 1. Compared to the control, lead has increased plasma urea and creatinine significantly, as did the combined treatment of Pb-garlic.

The ALAT and ASAT activities have been increased significantly in the Pb group, but their elevations have not marked any significant variation in the Pb-garlic group.

On the other hand, the activity of ALP, and the levels of uric acid, cholesterol and testosterone were not significantly varied in both treated groups compared to the control.

**Table 1:** Mean (±SD) Plasma biomarkers of rats exposed to Pb and Pb-garlic during 6 weeks.

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Control</th>
<th>Pb</th>
<th>Pb-garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/l)</td>
<td>38.36±0.95</td>
<td>50.81±2.72</td>
<td>40.8±6.26</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.68±0.01</td>
<td>0.82±0.13</td>
<td>0.7±0.09</td>
</tr>
<tr>
<td>Uric acid (g/l)</td>
<td>1.74±0.05</td>
<td>1.82±0.13</td>
<td>1.75±0.14</td>
</tr>
<tr>
<td>ASAT (U/L)</td>
<td>108.55±4.12</td>
<td>133.9±8.53</td>
<td>110.2±4.68</td>
</tr>
<tr>
<td>ALAT (U/L)</td>
<td>32.18±3.03</td>
<td>49.0±3.29</td>
<td>33.2±3.19</td>
</tr>
<tr>
<td>PAL (U/L)</td>
<td>188.41±7.81</td>
<td>220.7±6.76</td>
<td>193.7±7.68</td>
</tr>
<tr>
<td>Cholesterol (g/l)</td>
<td>0.55±0.04</td>
<td>0.45±0.06</td>
<td>0.51±0.05</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>12.23±0.24</td>
<td>9.06±1.48</td>
<td>12.3±0.38</td>
</tr>
</tbody>
</table>

S: Significantly different.

**Tissue GSH and MDA:**

Tissue biomarkers were presented in fig.1, 2 and 3. Mean hepatic, renal and testicular glutathion were significantly lower in the Pb group compared to the control. However, glutathion level has dropped in the kidney of Pb-garlic group, but it has not been affected in both liver and testis. Concerning MDA level, it is significantly elevated in the three organs by the Pb contaminated diet. Contrary, the concentration of MDA of the combined group was significantly decreased only in the testicular tissue, but it was remarkably higher in both liver and kidney.
Fig. 1: Mean (±SD) tissue content of glutathion (nmol/mg prot) and malondialdehyde (nmol/mg prot) in liver of rats exposed to Pb and Pb-garlic during 6 weeks.

Fig. 2: Mean (±SD) tissue content of glutathion (nmol/mg prot) and malondialdehyde (nmol/mg prot) in kidney of rats exposed to Pb and Pb-garlic during 6 weeks. S: Significantly different.

Fig. 3: Mean (±SD) tissue content of glutathion (nmol/mg prot) and malondialdehyde (nmol/mg prot) in testis of rats exposed to Pb and Pb-garlic during 6 weeks. S: Significantly different.
Histological study:

Kidney:

Fig. 4: Transitional histological section of kidney (H & E 200 x) from the control (A), Pb group (B) and Pb-garlic (C). NG: normal glomerulus, BC: Bowman’s capsule; NT: normal tubule, TN: tubular necrosis, FN: few nuclei, TM: tubular microcalcification.

Liver:

Fig. 5: Transitional histological section of liver (H & E 200 x) from the control (A), Pb group (B) and Pb-garlic (C). H: hepatocytes, S: sinusoids, T: tumor, B: bleeding, N: necrosis, CV: central vein, V: vessels.

Testis:

Fig. 6: Transitional histological section of liver (H & E 200x) from the control (A), Pb group (B) and Pb-garlic (C). L: lumen, SC: sertoli cell, ST: seminiferous tubule filled with spermatozoa.

Discussion:

The actual results showed remarkable elevation in urea and creatinine level of rats consumed Pb contaminate diet. It is known that kidney is considered as one of the principal target organ for metal toxicity [17]. Recently, [18] reported an increase in urea, creatinine and uric acid of rats injected with Pb acetate for one week. Such augmentations are explained on the basis that Pb might induce renal lesions, in which urea, creatinine and uric acid have increased respectively by 40%, 63% and 69% when compared to the control [3]. Moreover, it has been illustrated that the elevation in blood urea during Pb intoxication is likely to come from protein degradation [19], and/or from the disturbed renal filtration [20]. The reported positive correlation
between blood Pb and blood uric acid confirm that this metal reduce urinary excretion of uric acid [21]. Thus, the Pb chronic intoxication was associated with nephrosclerosis and cortical atrophy [4].

On the other hand, the diet containing fresh garlic has preserved urea and creatinine to their normal levels. Consequently, garlic sulfur compounds were able to neutralize the toxicity induced by sodium arsenite [22] and garlic flavonoids played important role in reducing metal bioavailability [23].

Concerning kidney histological profile of Pb exposed group, the distal tubules and corpuscles were denser, rounder and the capillary are surrounded by narrow gaps (fig. 4A). In addition, Pb exposure has provoked weak tubular necrosis and tubular microcalcification; it is the beginning of lithiasis, which supposed to come from metabolic disturbances. Pb also has decreased nuclei number significantly (fig. 4B). On the other hand, the presence of garlic has generated some protective effect on the injuries induced by Pb and showed an increased number in nuclei, with no amelioration in the kidney architecture, and the interstitial fibrosis remains unchanged (Fig.4C). Such results are in line with the finding of [3] who mentioned many histological alterations, including cell inflammations cytoplasmic vacuolization and dilation of certain tubules and proximal tubule necrosis during Pb toxicity. Likewise, 330 ppm of Pb was stated to induce renal swelling and glomerular hypertrophy [24]. Therefore, the histopathological profile observed in this study reflects the variations found in the serum biomarkers.

In the present study, Pb contaminated diet has increased the ALAT and the ASAT activities. This result is in line with that reported by [18] and also by [8] and [25], in which Pb has conducted to an important elevation in the activities of ASAT, ALAT and ALP because of cell lysis and the inhibition of K and Ca channels. Moreover, Pb fed female rats has even diminished liver weight [26]. In the actual study, PAL activity has not been affected significantly by Pb contaminated food. Thus, Pb is known to enter in competition with zinc found in the structure of PAL, and may inhibit the enzyme active site, which might cause a reduction in PAL activity [27, 28]. Pb is reported to bind the thiol groups situated in the hepatocyte membranes inducing leakage of cytosolic enzymes principally ASAT and ALAT [2]. Moreover, the oxidative stress generated by Pb acetate exposed rats has led to an enormous increase in the activity of serum transaminase [29].

Concerning Pb-garlic group, transaminases and PAL activities were not significantly different that of the control. The results of [6] are in line with this finding, where the aqueous extract of garlic has reduced the augmentation of ASAT, ALAT and PAL induced by Pb nitrate. However, this suggests that garlic organosulfur compounds could preserve the tissue structural integrity [30]. Garlic contains cystine, S-allyle, mercaptocysteine and alliin [31]. Such compounds were already used against Pb toxicity [32], where they increased rat Pb excretion in urine and feces [33].

The liver histological profiles of the Pb exposed group is characterized by a big nuclei, rare binuclei and the cells bordering the sinusoids can be distinguished easily from the hepatocytes by their flattened nuclei, condensed and less colored cytoplasm (Fig. 5A). Pb also provoked vascular hemangioma and the inflammatory cells were increased around blood vessels and blood sinusoids causing bleeding (Fig. 5B). In parallel, rats treated with lead acetate showed massive dilated hepatic cell aggregation with increased mononuclear inflammatory cells surrounding the blood vessels and sinusoids and the nuclei were fragmented [25].

However, the garlic supplementation has generally reduced the negative effect of Pb to some extent; where the general architecture is much better than that of Pb group, and it relatively similar to that of the control (Fig. 5C).

Plasma cholesterol concentration was not varied significantly in both treated groups. This result is similar to those of [34] where they found non-change of rat plasma cholesterol level intoxicated with 400 mg Pb/kg during two weeks. Contrary, serum cholesterol level was significantly higher after exposure of rats to Pb nitrate and that by activating cholesterol biosynthetic enzymes and inhibiting cholesterol catabolic enzymes [35, 6]. On the other hand, the supplementation of wistar rats by 8% *Allium sativum* has reduced tissue total cholesterol level remarkably [36]. The mechanism of action was proposed by [37], who indicated that garlic is capable to reduce lipid concentrations by inhibiting cholesterol and fatty acids’ enzymes. It was suggested also that garlic allyl-disulphure group is probably responsible on cholesterol diminution by inhibiting oxidase 4α-methyl sterols [38].

Plasma testosterone concentration has recorded slight reduction in the Pb exposed group. This hormone was decreased significantly in battery production workers after many years of exposure [39]. In animals, Pb acetate was suggested to affect the steroidogenesis enzymes responsible on testosterone synthesis [40, 41].

In this work, Pb-garlic group has normal concentration of testosterone as that of the control. It has been indicated that testicules are sensible to oxidative stress because they contain large amount of polyunsaturated fatty acids [42, 6]. In addition, the garlic organosulfur compounds modulate the activity of glutathione S-transferases and cytochrome P450, which play important roles in xenobiotic detoxifications [43]. However, oral administration of garlic juice added to Pb nitrate has significantly reduced the concentration of Pb in blood and tissues, indicating that garlic can be a tool of protection and has therapeutic potential against Pb toxicity [44].

The testicular histological profile of the control group shows seminiferous tubules filled with sperm, lined by epithelium consisting of two cell types; the cells in different stages of spermatogenesis and spermiogenesis,
which are called spermatogenic cells and Sertoli cells (Fig. 6A). Pb seems to be able to inhibit the different stages of spermatogenesis, where seminiferous tubules are less filled with sperm (Fig. 6B). Though, garlic extract protects the effect provoked by Pb and even enhance the number of spermatozoa than that of the control (Fig. 6C). That is to say garlic does not only offer protection against Pb intoxication, but it offer also nutritive elements to boost spermatogenesis. The immune-histochemistry of animals heavily intoxicated has shown that the negative effects of Pb on the production of progesterone and testosterone were due mainly to the decrease in the expression of cytochrome P450c17 that catalyzes the hydroxylation of progesterone, and then its transformation into androstenedione, the immediate precursor of testosterone [38].

In this study, rats given a Pb contaminated diet has remarkably reduced the GSH level in kidney, liver and testis, while Pb has upraised the concentration of MDA in these organs. Previously, Pb was found to affect the glutathione negatively, accompanied with an elevation in free radicals [45, 46]. Another experiment has showed that Pb intoxication was responsible on the induction of lipid membrane peroxidation, conducting to the formation of MDA, modifying then the balance between free radicals and the antioxidant system [47,48]. The actual results have confirmed previous findings of MDA renal and hepatic augmentation in rats exposed to Pb nitrate and Pb acetate [25, 18]. Furthermore, frog exposed to Pb nitrate for 10 days had decreased levels of glutathione in kidneys, liver, heart, brain and muscles [49]. Thus, metallic ion interactions with thiol groups of proteins and glutathione are one of the main mechanisms of Pb toxicity towards cell components [50]. Consequently, cell damage occurs when antioxidants and metallothionein levels are very low [51], that make the possibility of Pb to bind cell components, disturbing, however their functions. In this study, treatment of rats by the combination of Pb-garlic causes a significant improvement, where the rates of hepatic and testicular glutathione are almost normal, except for kidney, but the MDA concentration was higher in kidney and liver alike. Therefore, garlic extract was able to ameliorate the level of hepatic and testicular GSH and the MDA testicular concentration. On the other hand, the MDA concentration was still higher in both kidney and liver even in the presence of garlic, probably because these organs receive high amount of Pb and they considered as target for Pb accumulation and toxicity. Furthermore, testicular MDA level was similar to the control when garlic was added, which could be explained on the basis that this organ does not accumulate too much Pb, especially the GSH was also in its normal level and may not been exhausted by ROS.

Accordingly, treatment of rats with a combination of Pb nitrate and garlic aqueous extract produced significant increase of renal GSH content and a decrease in lipid peroxidation [6]. Moreover, the supplementation of onion and/or garlic extract to rats poisoned by cadmium respectively reduced and improved testicular MDA and GSH level [52]. Such onion and garlic components are able to prevent ROS elevation and subsequent lipid peroxidation [53]. Furthermore, the observed reduction in the level of total antioxidant capacity of humans exposed to Pb was returned to normal when they have been supplemented with vitamin E and C [54].

Due to wide variety of antioxidant compounds, such as flavonoids, vitamins, phenolic acids and sulfur compounds, garlic has been involved in the cleaning of the hydroxyl radicals and superoxide anions [55]. That to say organosulfurs (allicine, alliin, allyle-cysteine, allyle-disulfur) are important garlic constituents, in which their antioxidant properties might neutralize many ROS types [56,57]. Hence, glutathion is very abundant in cytosol, nuclei and mitochondria and its antioxidant capability comes from sulfur atom which is easily gaining the lost electron generated by Pb toxicity [58].

**Conclusion:**

Male rats exposed to Pb contaminated diet for 6 consecutive weeks have increased plasma urea, creatinine levels and aminotransferase activities. However, the Pb-garlic group has kept these enzymes within normal ranges. Renal, hepatic and testicular glutathion and MDA concentrations were respectively lower and higher in the Pb group, but in the presence of garlic, the levels of hepatic glutathion, in addition to testicular glutathion and MDA have resisted Pb intoxication. The histological profiles of kidney, liver and testis are generally in line with the plasma biomarkers. Thus garlic had beneficial action on testis better than liver and kidney. Such results indicate the partial protective role of garlic against Pb induced toxicity.

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