Protective role of garlic against cadmium toxicity in rats: Clinicopathological and histopathological studies

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SUMMARY
Cadmium (Cd) is an environmental and industrial pollutant of growing concern that adversely affects various organs in human and animal. This study was carried out to investigate the protective potentials of aqueous garlic extract (AGE) on Cd-induced toxicity in rats. Thirty male albino Wistar rats were randomly divided into five groups. The control group (1) received double distilled water; Cd group (2) received CdCl$_2$ (1.5 mg/100 g BW); AGE (0.5 ml/100 g BW) - treated group (3), and Cd plus AGE-treated group (4). The tested doses were orally given to rats once daily for 4 weeks, but the 5th group was pre-treated with CdCl$_2$ for 4 weeks, and then treated by AGE for additional 4 weeks.

CdCl$_2$ induced hepatorenal toxicity as indicated by a significant (P <0.001) elevations in the levels of serum alanine aminotransferase (ALT), aspartate aminotransferase, alkaline phosphatase (ALP), urea and creatinine with a significantly (P <0.001) decrease in the levels of creatinine-clearance in urine, serum proteins (P <0.01), and the activities of the hepatic and renal antioxidant-enzymes when compared with the control. A significant (P < 0.05) decrease in the sperm concentration and motility (%), with increased numbers of dead and abnormal sperm, when compared with the control. Furthermore, Cd induced multiple foci of hemorrhage, congestions, edema, coagulative necrosis and mononuclear-cell-infiltrations in the liver, kidneys and testes. These alterations that associated with Cd-toxicity were significantly alleviated by garlic administration. The protection level was better in rats of gp. (4) than gp. (5).

The present study provides evidence that the protective role of garlic against Cd toxicity could be due to enhanced antioxidant defense and metal chelating, therefore, garlic could be useful nutritional-supplement for alleviating the Cd-induced damage.

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INTRODUCTION

The contamination of food and feedstuff with heavy-metal ions represents problems for human and animal's health, thus attracting a world-wide attention (Satarug and Moore, 2004 and El-Kady et al., 2009). Cadmium (Cd) has been involved in historic poisoning episodes of human and animal populations as a result of increased anthropogenic activities including industrial uses (such as electroplating, plastic production, pigments, battery-manufactures and pesticides), besides lifestyles (Murugavel and Pari, 2007 and El-Kady et al., 2009). The risk of Cd toxicity is further increased because of its long biological half-life (17–30 years), resulting in accumulating and severe damaging effects, especially in the liver and kidneys (Shukla and Kumar, 2009). The pathobiochemical mechanism of Cd-induced-cytotoxicity or damage is mainly via induction of oxidative stress (Pathak and Khandelwal, 2006 and Suru, 2008). One mechanism by which Cd\(^{2+}\) ions can produce tissue-injury is the generation of reactive oxygen species (ROS) and lipid peroxidation, which in turn depress hepatic and renal functions (Shaikh et al., 1999 and Thevenod, 2003). If these ROS mediated stress events are not balanced by repair processes, the affected cells undergo apoptosis or necrosis (Thevenod, 2003). The epididymal-sperm-count is commonly used for measuring toxicological lesions of the male reproductive system (Strader et al., 1996). Cd-exposure is strongly associated with reproductive toxicity in both animal and human populations culminating in infertility and cancer of the reproductive tissues (El-Demerdash et al., 2004; Goyer et al., 2004 and Akinloye et al., 2006). The pathogenesis of testicular damage and sperm- abnormalities induced by Cd exposure is generally ascribed to oxidative damage (El-Demerdash et al., 2004 and Yang et al., 2006). Rats treated with Cd showed increased plasma-activities of ALT and AST, accompanied with a decrease in the hepatic activities of glutathione, superoxide-dismutase and catalase (Suru, 2008 and Obioha et al., 2009). Cd-intoxicated animals showed multiple foci of hemorrhage, cloudy swelling, necrosis and glomerular distension, besides individual coagulative necrosis, mononuclear cell infiltration, hepatic sinusoidal dilation, degenerative and necrotic changes in spermatocytes accompanied with decreased testicular sperm counts with a significant increase in sperm abnormality (Ahn et al., 1999; Pari et al., 2007; Jihen et al., 2008 and Manna et al., 2008).

Several chelating agents and antagonists reduce the Cd-toxicity, some of these agents exhibited side
effects, especially the medicinal plants. Garlic (*Allium sativum*) is versatile vegetable which is often used in many dishes for flavor, aroma and taste enhancement (*Stajner* and *Varga, 2003*). It is a good source of dietary phytochemicals with proven antioxidant properties and ability to modulate the detoxification systems (*Nuutila et al., 2003; Stajner* and *Varga, 2003* and *El-Demerdlash et al., 2005*). These functional effects could be of great importance for their use in the prevention and treatment of several diseases (*Lau, 1998; Banerjee et al., 2001 and Ola-Mudathir et al., 2008*). The therapeutic and medicinal values of garlic are the subjects of many researches. It has anticarcinogenic, hepatoprotective, antidiabetic, anti-platelet aggregation, anti-biotic effects and may also act as antidote for heavy metal poisoning (*Agarwal, 1996; Lau, 1998* and *El-Demerdlash et al., 2005*).

This study was designed to evaluate the protective effects of garlic against the cadmium-induced biochemical changes, alterations in sperm characteristics histopathological alterations in liver, kidney and testes.

**MATERIAL AND METHODS**

**Preparation of extracts:**

Garlic bulbs were purchased from the local Market in Zagazig, Egypt. Its botanical identification was confirmed at the Field Crop Department, Faculty of Agriculture, Zagazig University, Egypt. Garlic extraction was performed according to *Flora et al. (2009)*. Briefly, garlic-bulbs were carefully undressed, then crushed with distilled water in a mixer (100 ml of distilled water per 100 g of garlic). The resultant slurry was squeezed through a double cheese cloth and the filtrate was quickly frozen (0-4°C) until used.

**Animals and experimental design:**

Thirty clinically healthy adult male albino Wistar rats weighing 150 -200 g were obtained from the breeding stock of Faculty of Veterinary Medicine, Zagazig University, Egypt. They were kept under standard laboratory conditions with ad libitum access to food and water in well-ventilated cages made of galvanized zinc plates. All the animal experiments were conducted in accordance with the Ethical Norms on Animal Care and Use approved by Faculty of veterinary Medicine, Zagazig University, Egypt. Rats were randomly divided into five equal groups and were treated accordingly (Table, 1).
Table 1. Experimental design:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Only vehicle (double distilled water) (0.5 ml/100 g BW, once daily for 4 weeks)</td>
</tr>
<tr>
<td>2</td>
<td>Cd* only (CdCl₂ in H₂O) (1.5 mg/100 g BW, once daily for 4 weeks)</td>
</tr>
<tr>
<td>3</td>
<td>Aqueous garlic extract, 0.5 ml/100 g BW, once daily for 4 weeks</td>
</tr>
<tr>
<td>4</td>
<td>Co-administration of Cd (CdCl₂ in H₂O) (1.5 mg/kg BW) and garlic extract (0.5 ml/100 g) once daily, for 4 weeks.</td>
</tr>
<tr>
<td>5</td>
<td>Cd (1.5 mg/kg BW once daily for 4 weeks), followed by garlic extract (0.5 ml/100 g BW, once daily, for additional 4 weeks)</td>
</tr>
</tbody>
</table>

*Cd Chloride (CdCl₂ in H₂O) 1000 mg Cd was obtained from Merck Ltd., Cairo, Egypt. The doses of garlic extract and CdCl₂ administered by gavage and were selected according to Ola-Mudathir et al. (2008).

Serum and tissue collection:

Blood samples were collected from the retro-orbital venous-plexus under diethyl ether anesthesia at the end of the experimental period and left to clot. The sera were separated by cooled centrifugation and stored at −20 °C until analysed. The liver and kidneys were immediately removed and washed with chilled saline-solution. Tissues were minced and homogenized in ice-cold 1.15% KCl (1g tissue/3 ml) in a Potter–Elvehjem type homogenizer. The homogenate was centrifuged at 5000 ×g for 20 min at 4 °C, and the resultant supernatant was used for antioxidant enzyme assay (Suru, 2008). Specimens were collected from the liver, kidneys and testes and immediately fixed in 10% buffered neutral formalin solution. Five-micron thick paraffin sections were prepared, stained by Hematoxyline and Eosin and examined microscopically (Bancroft and Stevens, 1996).

Biochemical analysis:

A- Measurement of hepatic parameters:

The activities of alanine transaminase (ALT) and aspartate transaminase (AST) were estimated according to Reitman and Frankel (1957). Serum alkaline phosphatase activity was measured according to Bowers and McComb (1972). The serum total
protein (TP) was determined according to Henry et al. (1974). Albumin concentration was determined by the method of Doumas et al. (1977). Globulin concentration was calculated as the difference between the total protein and albumin.

**B-Measurement of renal parameters:**

The levels of creatinine and urea, in serum, were estimated spectrophotometrically, using commercial diagnostic kits (Sigma Diagnostics (I) Pvt Ltd, Cairo, Egypt). Creatinine clearance as an index of glomerular filtration rate was calculated from serum creatinine and a 24 h urine sample creatinine levels.

**C-Measurement of antioxidant enzymes:**

The protein content of the homogenized liver and kidneys was estimated by the method of Lowry et al. (1951), using the bovine serum albumin as a standard. The activities of the superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were measured according to the methods of Misra and Fridovich (1972), Sinha (1971) and Paglia and Valentine (1967), respectively. The enzyme levels were determined using commercial test kits according to the manufacturer’ instructions.

**Epididymal sperm characteristics:**

The epididymal spermatozoa were obtained by mincing the right and left epididymides with scissors and scalpels into small pieces and homogenized in a warmed Petri dish, containing 5 ml physiological saline and incubated at 37°C for 2 min (Linder et al., 1986). The suspension was stirred, one drop was placed on a warmed microscope slide, and a 22 x 22 mm coverslip was placed over the droplet. At least 10 microscopic fields were observed, at 400 x magnification, using a light microscope, and the percentage of motile sperm was recorded. Other slides were prepared and stained with 1% cosin B and 5% nigrosine in 3% sodium citrate dehydrate solution and examined at 400x magnification. A total of 400 sperm cells were counted for the assessment of the live, dead and abnormal spermatozoa. The same suspension was used for sperm counting, using a Neubauer hemocytometer. Three counts per sample were averaged (Strader et al., 1996).

**Statistical analysis:**

The data were expressed as means ±standard errors (SE). Differences between group means were estimated using a one-way analysis of variance (ANOVA) and the Duncan's Multiple Range Test was done for multiple comparisons using the SPSS 12.0 for Windows. Results
were considered statistically significant at $P < 0.05$.

RESULTS

Biochemical studies:

Cd - intoxicated rats, (gp. 2), showed severe deviations in the hepatic and renal parameters (Table, 2). A significant ($P < 0.001$) increase in the serum ALT, AST, and ALP activities, besides a significant ($p < 0.01$) decrease in the serum proteins (TP, albumin and globulins) were found, when compared with the control. The serum enzymes were reduced and protein-profile was increased in gp. (5). Gp. (4) showed that the elevated parameters were closed to the normal values. No significant change in any of the parameters was observed in gp. (3). A significantly ($P < 0.001$) increased level of serum urea and creatinine with a significantly ($P < 0.001$) decreased level of creatinine-clearance was observed in gp. (2). Co-administration of Cd and garlic extract gp. (4) did not affect the serum urea and creatinine levels and closely restored the level of creatinine-clearance to the control level. Gp. (5) showed a significant ($p < 0.05$) increase of serum urea, without any significant change in the serum creatinine and creatinine-clearance levels when compared with the control.

The activities of the hepatic SOD, CAT and GPx were significantly ($p < 0.001$) reduced in rats of gp. (2) as shown in table (3). The activities of the aforementioned enzymes were significantly ($P < 0.05$) enhanced in gp. (3). Challenging of garlic-treated rats with CdCl$_2$ gp. (4) was associated with activation of the antioxidant enzymes towards the control levels. However, treatments of CdCl$_2$-intoxicated rats with garlic extract gp. (5) significantly ($P < 0.05$) increased the hepatic CAT activity when compared with gp. (2), but did not return to the normal level of control.

The activities of the kidney SOD, CAT and GPx were significantly ($P < 0.001$) reduced in rats of gp. (2) as shown in table (4). Treatment with garlic extract alone gp. (3) tended to significantly ($P < 0.05$) enhance the activities of SOD, CAT and GPx when compared with the control. Administration of garlic extract in combination with or following Cd significantly ($P < 0.01$) increased the level of these enzymes when compared with Cd-intoxicated rats.
Table 2. Effect of garlic extract on some hepatic and renal parameters of Cd-intoxicated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>1</th>
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<tbody>
<tr>
<td></td>
<td>ALT(IU/dl)</td>
<td></td>
<td>ALT(IU/dl)</td>
<td></td>
<td>ALT(IU/dl)</td>
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<tr>
<td></td>
<td>8.85 ± 1.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.80 ± 1.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.94 ± 1.40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.23 ± 1.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.92 ± 1.60&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>22.10 ± 2.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>27.50 ± 2.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.64 ± 3.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.50 ± 1.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.11 ± 0.45&lt;sup&gt;bc&lt;/sup&gt;</td>
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<td></td>
<td>19.54 ± 2.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>37.53 ± 3.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.24 ± 2.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.62 ± 2.50&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.00 ± 3.10&lt;sup&gt;bc&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>8.20 ± 1.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.10 ± 1.95&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.14 ± 1.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.11 ± 1.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.90 ± 1.60&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td></td>
<td>4.70 ± 1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.00 ± 0.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.51 ± 1.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.60 ± 1.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.30 ± 1.02&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>3.50 ± 0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.20 ± 0.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.63 ± 1.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.41 ± 1.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.20 ± 1.00&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>37.00 ± 4.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>62.23 ± 3.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.00 ± 3.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40.00 ± 2.50&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>45.4 ± 3.73&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.67 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.2 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.66 ± 0.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.69 ± 0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.74 ± 0.23&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.42 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.28 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.40 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37 ± 0.17&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.35 ± 0.23&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as means ±SE; n = 6 for each treatment group. Groups indicated with different superscript letters (a, b, c, d) in the same line are statistically significant (P < 0.05).
Table 3. Effect of Cd and garlic extract on the activities of the antioxidant enzymes and protein levels in the liver of rats. Values are expressed as means ±SE; n = 6 for each treatment group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (mg/ml homogenate)</td>
<td></td>
<td>27.50 ± 4.25</td>
<td>26.70 ± 3.77</td>
<td>28.12 ± 4.46</td>
<td>27.40 ± 3.60</td>
<td>27.11 ± 4.82</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td></td>
<td>3.61 ± 0.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.15 ± 0.16&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.93 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.40 ± 0.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.14 ± 0.47&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT (µg/mg tissue)</td>
<td></td>
<td>1.33 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.83 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.62 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.15 ± 0.55&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.00 ± 0.47&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPx (U/mg tissue)</td>
<td></td>
<td>0.90 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.42 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.99 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.86 ± 0.05&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.80 ± 0.04&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Groups indicated with different superscript letters (a, b, c, d) in the same line are statistically significant (P < 0.05).

Table 4. Effect of Cd and garlic extract on the activities of the antioxidant enzymes and protein levels in the kidney of rats. Values are expressed as means ±SE; n = 6 for each treatment group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (mg/ml homogenate)</td>
<td></td>
<td>26.50 ± 3.12</td>
<td>23.70 ± 5.00</td>
<td>25.00 ± 3.88</td>
<td>24.13 ± 4.16</td>
<td>23.11 ± 3.34</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td></td>
<td>1.68 ± 0.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.65 ± 0.27&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.80 ± 0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.53 ± 0.65&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.50 ± 0.53&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT (µg/mg tissue)</td>
<td></td>
<td>0.92 ± 0.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.44 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.99 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.87 ± 0.55&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.81 ± 0.47&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>GPx (U/mg tissue)</td>
<td></td>
<td>1.03 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.50 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.14 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.97 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

Groups indicated with different superscript letters (a, b, c, d) in the same line are statistically significant (P < 0.05).
**Sperm characteristics:**

Treatment of male rats with CdCl₂ gp. (2) caused a significant (p < 0.05) decrease in sperm concentration and motility (%). The dead and abnormal sperms were increased when compared to the control table (5). Treatment with garlic-extract alone, gp. (3) or in combination with CdCl₂ gp. (4) caused more significant (P < 0.05) increase in semen quality, and minimized the toxic effects of CdCl₂ than when Cd-intoxicated rats were treated with garlic extract gp. (5).

Table 5. Effects of garlic extract on epididymal sperm concentration (Sperm conc., 10⁶/ml), motility (%), dead (%) and abnormal sperm (%) of Cd-intoxicated male rats. Values are expressed as means ±SE; n = 6 for each treatment group.

<table>
<thead>
<tr>
<th>Groups Parameters</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm conc.</td>
<td>80.50 ± 6.44ᵃ</td>
<td>44.60 ± 4.33ᶜ</td>
<td>80.94 ± 7.54ᵃ</td>
<td>76.13 ± 6.13ᵇᵃ</td>
<td>63.55 ± 6.40ᵇ</td>
</tr>
<tr>
<td>Motility</td>
<td>75.46 ± 4.00ᵇ</td>
<td>35.00 ± 1.33ᵈ</td>
<td>81.0 ± 5.41ᵃ</td>
<td>75.40 ± 3.12ᵇ</td>
<td>70.0 ± 3.15ᵇᶜ</td>
</tr>
<tr>
<td>Dead</td>
<td>22.50 ± 1.10ᶜ</td>
<td>47.60 ± 2.50ᵃ</td>
<td>10.12 ± 1.10ᵈ</td>
<td>27.55 ± 2.50ᵇ</td>
<td>30.00 ± 3.10ᵇ</td>
</tr>
<tr>
<td>Abnormal</td>
<td>12.40 ± 1.30ᶜ</td>
<td>20.00 ± 1.70ᵃ</td>
<td>8.0 ± 0.49ᶜ</td>
<td>12.70 ± 1.33ᵇ</td>
<td>15.30 ± 1.60ᵇ</td>
</tr>
</tbody>
</table>

Groups indicated with different superscript letters (a, b, c, d) in the same line are statistically significant (P < 0.05).

**Histopathological results:**

The light microscopic examination revealed a normal histological structure of all organs in the control gp. (1).

Animals treated with Cd gp. (2) showed enlarged hepatocytes, with severe hydropic degeneration and coagulative necrosis (Fig. 1). Fatty change was frequently observed accompanied with congestions and leukocytic infiltrations in the portal areas (Fig. 2). Congestions of the central veins and multiple hemorrhagic foci were observed in many cases. Sinusoidal
dilatation was also evident (Fig. 3). The kidneys showed congestion, multiple hemorrhagic foci, accompanied with focal interstitial mononuclear-cell-aggregations (Fig. 4). The renal tubular epithelium showed hyaline droplet formation, cloudy swelling and coagulative necrosis. These changes were accompanied with numerous swollen glomeruli with proliferation of the mesangial cells and presence of eosinophilic material in the lumen of some renal tubules (Figs. 5 & 6). Furthermore, numerous epithelial casts, and tubular dilatation were evident. The tests showed degeneration and necrosis of some seminiferous tubular epithelium, with defoliation of many spermatocytes into the lumen of seminiferous tubules. This was accompanied with congestion and oedema in the interstitial tissue (Fig.7). Moreover, multiple hemorrhagic foci were frequently noticed in the interstitial tissue. The tail of the epididymis showed marked depletion in the numbers of spermatozoa. In addition some tubules contained homogenous pink necrotic particles with absence of spermatozoa (Fig. 8).

There was no significant difference in the histology of the liver, kidneys and testes between gp. (3) and gp. (1). However, the hepatic tissues of the garlic-treated-group showed few eosinophilic cells infiltrations in the portal areas (Fig. 9).

The co-administration of garlic with Cd gp. (4) remarkably alleviated the lesions, induced by Cd, where the hepatic coagulative necrosis was absent and the congestions and sinusoidal widening was markedly reduced, but few slightly enlarged hepatocytes with light cytoplasm were observed (Fig. 10). The portal areas showed slight congestion and were infiltrated with few mononuclears. The kidneys showed few swollen glomeruli and minimal proliferation of the mesangial cells. The number of the mononuclears, in the interstitial tissue, became scanty, but the congestion was obvious (Fig. 11). Cloudy swelling and vacuolar degeneration were observed in the epithelium of some proximal and distal convoluted tubules. The testes of all animals in this group were structurally similar to the control, with only mild interstitial oedema and few degenerated spermatocytes (Fig. 12). The tails of the epididymides were filled with intact spermatozoa (Fig. 13).

Treatment of Cd-intoxicated rats with garlic extract gp. (5) resulted in a moderate improvement in the lesions induced by Cd, but it did not regain the control, as the liver which revealed numerous swollen hepatocytes with hydropic
degeneration accompanied with slightly congested central veins and mild sinusoidal dilatation. Moreover, coagulative necrosis was scanty and the portal areas were infiltrated with moderate numbers of leukocytes (Fig. 14). The kidneys showed few swollen glomeruli with proliferated mesangial cells together with congested capillaries and numerous minute hemorrhagic foci (Fig. 15). Some renal tubules showed cloudy swelling and hyaline droplet formation but coagulative necrosis was minimal. The interstitial tissue revealed a moderate number of mononuclear cells. The testes revealed mild congestions, few round cell infiltration and interstitial oedema, accompanied with a mild degeneration of the germinal epithelium (Fig. 16). The epididymis revealed mild congestion and few leukocytes in the interstitial tissue accompanied with mild depletion in the spermatozoa. The histopathological findings were graded in tables (6, 7 and 8).

Table (6): Histopathological findings in liver of rats of different groups.

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Groups</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congestion and sinusoidal dilatation</td>
<td></td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Vacuolar and hydropic degeneration</td>
<td></td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Fatty change</td>
<td></td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Apoptosis</td>
<td></td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mononuclear cell infiltrations</td>
<td></td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Hemorrhagic foci</td>
<td></td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Table (7): Histopathological findings in kidneys of rats of different groups.

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Groups</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congestion</td>
<td></td>
<td>-</td>
<td>++++</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Cloudy swelling</td>
<td></td>
<td>-</td>
<td>++++</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Vacuolar and hydropic degeneration</td>
<td></td>
<td>-</td>
<td>++++</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Hyaline droplet formation</td>
<td></td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Hyaline and cellular casts</td>
<td></td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tubular necrosis</td>
<td></td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mononuclear cell infiltrations</td>
<td></td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Hemorrhagic foci</td>
<td></td>
<td>-</td>
<td>++++</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

Table (8): Histopathological findings in testes of rats of different groups.

<table>
<thead>
<tr>
<th>Lesions</th>
<th>Groups</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congestion</td>
<td></td>
<td>-</td>
<td>++++</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Tubular necrosis</td>
<td></td>
<td>-</td>
<td>++++</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Desquamations of spermatoocytes</td>
<td></td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Interstitial edema</td>
<td></td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Mononuclear cell infiltrations</td>
<td></td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hemorrhagic foci</td>
<td></td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

(-) absence of the change in all rats of a group, (++++) a change often found in all rats of a group, (++++) a change observed in almost all rats of a group, (++) a change not so often observed in all rats of a group and (+) a change which was rare within a group.
Figs. (1-4):
1- Liver of rat gp. (2) showing enlargement of the cell sizes, with severe hydropic degeneration (arrow) and individual coagulative necrosis (arrow heads), H&E., X.400.
2- Liver of rat gp. (2) showing fatty change (arrow head), with congestion (C), and leukocytic infiltrations (arrows), in the portal areas, H&E., X.400.
3- Liver of rat gp. (2) showing sinusoidal dilatation (arrows), H&E., X.400.
4- Kidney of rat gp. (2) showing congestions (C), hemorrhages (H), and focal interstitial mononuclear inflammatory cell aggregations (arrow), H&E., X.400.
Figs. (5-8):
5- Kidney of rat gp. (2) showing cloudy swelling (arrow), and coagulative necrosis (arrow heads), of the tubular epithelium, H&E., X.400.
6- Kidney of rat gp.(2) showing hyaline droplet formation (arrow head), swollen glomeruli with proliferated mesangial cells (arrow), and presence of eosinophilic material in the lumen of some renal tubules (E), H&E., X.400.
7- Testis of rat gp.(2) showing congestion (C), interstitial oedema (E), and defoliation of many spermatocytes into the lumen of seminiferous tubules (arrow ), H&E., X.400.
8- Tail of epididymis of rat gp. (2) showing marked depletion in the numbers of spermatozoa (arrow), with presence of homogenous pink necrotic particles with absence spermatozoa in some tubules (arrow heads), H&E., X.50.
Figs. (9-12):
9- Liver of rat gp. (3) showing showed few eosinophilic cells infiltrations (arrow), in the portal areas, H&E., X.400.
10- Liver of rat gp. (4) showing slightly enlarged hepatocytes with light cytoplasm (arrow), and marked reduction in the congestions (C), and sinusoidal dilatation (arrow head), H&E., X.400.
11- Kidney of rat gp. (4) showing scanty numbers of mononuclear cells in interstitial tissue (arrow head), with marked congestion (C), of the renal blood, H&E., X.50.
12- Testis of rat gp. (4) showing mild interstitial oedema (arrow), and few degenerated spermatocytes (arrow head), H&E., X.400.
Figs. (13-16):
13- Tail of piddymis of rat gp. (4) showing epididymal tubules filled with spermatozoa (arrows), H&E., X.100.
14- Liver of rat gp. (5) showing numerous swollen hepatocytes with hydropic degeneration (arrow), scanty individual coagulative necrosis (arrow heads), slightly congested central veins (C), and moderate portal leukocytic infiltrations (L.), H&E., X.100.
15- Kidney of rat gp. (5) showing few swollen glomeruli with proliferated mesangial cells (arrow heads), together with congested capillaries (C), and numerous minute hemorrhagic foci (H), H&E., X.400.
16- Testis of rat gp. (5) showing mild congestion (C), and edema (arrow) in the interstitial tissue accompanied with mild degeneration of the germinal epithelium (arrow head), H&E., X.400.
DISCUSSION

Cadmium is a poisonous metal that is widely used in different industries. It promotes an early oxidative stress and afterward contributes to the development of serious biochemical, spermiotoxic and pathological conditions because of its long retention in some tissues. The treatment strategies for Cd-toxicity include chelation and antioxidant therapies (Obioha et al., 2009). The antagonism of the garlic to Cd-toxicity was evaluated on the basis of biochemical alterations, alterations in sperm characteristics and histopathological findings in liver, kidneys and testes.

Our results indicated that Cd-intoxication caused a significant increase in the serum ALT, AST, ALP, urea, creatinine and a decrease in serum TP, albumin and globulins. This was accompanied with a decrease creatinine-clearance levels in urine and a decline in the levels of SOD, CAT and GPx in the hepatic and renal tissues. Similar results were reported (El-Demerdash et al., 2004; Pari and Murugavel, 2005; Chanta et al., 2007 and Yadav and Khandelwal, 2009). A key finding of the present study is the significant increase in the morphologically abnormal sperms and dead sperms coupled with substantial decrease in sperm count and motility in rats intoxicated with Cd. The same results were previously reported (WHO, 1992; Yang et al., 2006 and Ola-Mudathir et al., 2008).

The disturbances in the activities of the enzymes (ALT, AST, ALP) with serum urea and creatinine, as well as creatinine-clearance levels in urine represented biomarkers for hepatic and renal damage (Chater et al., 2009; EL-Kady et al., 2009; Shukla and Kumar, 2009). Furthermore, the administration of Cd (3 mg/kg BW/day) in rats for 3 weeks induced renal damage, which was evident by significantly increased levels of serum urea and creatinine with a significant decrease in creatinine-clearance in urine (Pari and Murugavel, 2005; Pari et al., 2007). The decrease in serum proteins may be due to the increased excretion of high molecular weight protein (proteinurea) (El-Demerdash et al., 2004 and El-Kady et al., 2009). Furthermore, the increased serum creatinine and lowered creatinine-clearance points out to renal damage (Jarup, 2002).

The decreased activities of the antioxidants (SOD, CAT and GPx), in the hepatic and renal tissues, indicated a failure of the antioxidant-defense-system to overcome the influx of ROS on Cd. Our results agree with Pari et al. (2007). The significant decrease in the activities of SOD and CAT in the Cd group
may be attributed to a direct inhibitory effect on SOD and CAT activities via Cd–enzyme interaction (Patra et al., 1999 and Casalino et al., 2002). The GPx depletion may be due to its enhanced utilization to conjugate Cd, counteract ROS and lipid peroxidative products (Singhal et al., 1987). Similarly, Jurczuk et al. (2004) reported that the exposure to 50 mg Cd/l drinking water led to a decrease in the activities of SOD in the liver and CAT in the liver and kidney, and an increase in the kidney activity of SOD.

The gonadotoxic and spermiotoxic effects of Cd may be due to a direct effect on the testes and epididymides or by altering the post-testicular events such as sperm-progress, motility and/or viability, which may culminate in hypogonadism and infertility (WHO, 1992 and Akinloye et al., 2006). The observed spermatogenic damage is consistent with the report of El-Demerdash et al. (2004) in rats and Acharya et al. (2008) in mice. Generation of reactive oxygen species (ROS) by Cd-toxicity and consequent oxidative damage may increase the meiotic errors and sperm deformation (Acharya et al., 2008). The decreased sperm counts may be a direct outcome of increased and consistent lipid peroxidation (LPO) and altered membrane properties that led to germ cell death at different stages of development (Hew et al., 1993). Increased sperm membrane LPO has been shown to impede sperm progress motility, and increase percent of the total sperm abnormalities and cause a dramatic loss in the fertilizing potential of sperm (Sharma and Argawal, 1996 and El-Demerdash et al., 2004). Cd disrupts the tight-junctions between the Sertoli cells and alters the Sertoli-germ cell-adhesion, with consequent exfoliation of immature cells into the lumen of seminiferous tubules and, in turn, leads to a reduction of viable sperm count in the epididymides (Hew et al., 1993).

Also, Cd intoxication resulted in several structural changes in the liver, kidneys and testes, where the hepatic cells suffered vacuolar and hydropic degenerations, fatty change and coagulative necrosis. The renal tubular epithelium showed hyaline droplet formation, cloudy swelling and coagulative necrosis. The seminiferous tubules revealed severe degeneration and necrosis with partial loss of spermatogenic cells and the epididymides showed marked depletion in the numbers of spermatozoa. Moreover, leukocytic infiltration and multiple hemorrhagic foci were frequently observed in the liver, kidneys and testes. Many investigators have noted similar or more pronounced changes in the hepatic, renal and testicular tissues.
under Cd effect (Ahn et al., 1999, Koyu et al., 2006, Jihen et al., 2008, Pari et al., 2007 and Manna et al., 2008). These lesions are mainly due to oxidative stress where the cytosolic Cd interacted with mitochondria, peroxisomes, and microsomes, resulting in excessive generation of ROS capable of depleting endogenous antioxidant status and causing oxidative damage to biomolecules such as membrane lipids, DNA and a variety of transport proteins, including Na+/K+-ATPase, which results in cell-death and organ-dysfunction (Shaikh et al., 1999; Thevenod and Friedmann, 1999; Tang and Shaikh, 2001 and Wang et al., 2004).

Rats received garlic alone gp. (3) did not show any biochemical or structural lesions with the exceptions of few leukocytic infiltrations in the portal areas. Previously, several reports indicated that treatment with garlic did not show any toxicological or physiological effects in rats (Ola-Mudathir et al., 2008) and mice (Flora et al., 2009). However, garlic administration significantly enhanced the activities of SOD CAT and GPx in the hepatic and renal tissues, and improved the semen quality. These benefits are mainly attributed to the antioxidative activities of garlic (Zeng et al., 2008) and suggested that garlic influences the host-detoxification processes (Das and Saha, 2009).

The results showed that the hepatic, renal and testicular protection by garlic against Cd-induced toxicity was evident, particularly when administered in combination with Cd. The alterations in the serum levels of ALT, AST, ALP, proteins, urea and creatinine, besides the creatinine- clearance levels in urine induced by Cd were close to the normal values by the garlic extract administration, especially in gp. (4). The lowered trend in liver and renal functional parameters implicated that garlic alleviated the hepatic and renal damage. Similar results were reported by Nakagawat et al. (2006) and Flora et al. (2009).

The levels of antioxidant enzymes (SOD, CAT and GPx), in the hepatic and renal tissues, were increased by garlic treatment, however, the hepatic CAT activity didn’t restore to the control level, especially in gp. (5). Similar results were reported by (Nuutila et al., 2003 and El-Beshbishy, 2008). The increase in these enzymes reduced the mitochondrial-generated-reactive radicals from causing oxidative stress and cellular damage (Flora et al., 2009). The antioxidative activities of garlic could be related to its contents of cysteine-containing bioactive compounds, diallyl sulfur compounds, seleno-
compounds and flavonoids (quercetin, allixin, anthocyanins, and kaempferol), which are known to exert antioxidant effects (Banerjee and Maulik, 2002). Moreover, it is possible that garlic extracts could have spared the consumption of GPx, SOD, and CAT occasioned by Cd-induced oxidative stress, and enhance the endogenous antioxidant status of the liver and kidneys (catalase and SOD) (Banerjee et al., 2001 and Massadeh et al., 2007). The testicular protection could be due to the antioxidant properties of garlic (Nuutila et al., 2003; Murugavel and Pari, 2007). Also, the protective effects of garlic may be related to their ability to chelate/sequester Cd via formation of Cd–flavonoid complexes (Massadeh et al., 2007). Moreover, Garlic is well documented for the attenuation of oxidant-mediated renal, hepatic and testicular tissue damage induced by various agents (Kabasakal et al., 2005; Pari et al., 2007; suru 2008 and Obioha et al 2009). Also garlic significantly protected the testes, epididymides and spermatozoa against Cd-toxicity by reversing these altered parameters and increasing the semen quality. Rats exposed to Cd for 4 weeks, garlic was effective in ameliorating Cd induced biochemical and morphological changes in the liver, kidneys and epididymal sperm (Yadav and Khandelwal, 2008). The histopathological results were coincided with the above findings, where the hepatic coagulative necrosis was absent and the congestions, sinusoidal widening and leukocytic infiltrations were markedly reduced. The kidneys showed few swollen glomeruli and proliferation of the mesangial cells with minimal mononuclear cells in the interstitial tissue. Moreover, the testes of group (4) were structurally similar to the control with the exception of only mild interstitial edema and few degenerated spermatocytes. The epididymal tubules were filled with spermatozoa. These results are in agreement with Pari et al. (2007), Jihen et al. (2008), Manna et al. (2008) and Ola-Mudathir et al. (2008)

It could be concluded that the intoxication by Cd resulted in severe toxic effects on the liver, kidneys and testes. Garlic supplement counteracted these toxic effects, thus it is recommended to supplement the diet with garlic, particularly in areas where Cd-contamination is expected.

REFERENCES


Jihen, E.H.; Imed, M.; Fatima, H. and Abdelhamid, K.
(2008): "Protective effects of selenium (Se) and zinc (Zn) on cadmium (Cd) toxicity in the liver and kidney of the rat: Histology and Cd accumulation." Food and Chemical Toxicology, 46 (11): 3522–3527.


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