ORIGINAL ARTICLES

Garlic Powder Attenuates Acrylamide-Induced Oxidative Damage in Multiple Organs in Rat

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ABSTRACT

Background: Humans are exposed to dietary acrylamide (AA) during their lifetime. Accumulating evidence indicates that AA administration is associated with significant increase of oxidative stress; thus, dietary antioxidants may have a protective role against AA toxicity. It was reported that garlic, a natural antioxidant, is able to enhance the cellular antioxidant defence capacity, thereby protecting cells from oxidative stress. Aim: In this study, we evaluated the protective role of garlic on the oxidative damage induced by AA in rat tissues. Methods: Twenty eight Sprague Dawely male adult rats were divided into four groups (7 rats/ group). Group I, negative control group, was fed on basal diet for 22 days. Group II, positive control, fed on basal diet for 22 days and on the 11th day AA was added to the diet (0.34 g/Kg diet). The other two groups received the basal diet supplemented with either 2.5% or 5% garlic for 22 days and on the 11th day AA was added to the diet. Results: The administration of AA resulted in significant elevation in kidney, spleen, testes and brain malondialdehyde level (MDA) and significant reduction in the level of reduced glutathione (GSH) and the activity of copper-zinc superoxide dismutase (Cu/Zn SOD) in the same organs. Also serum urea and creatinine levels and lactate dehydrogenase (LDH) and alkaline phosphatase activities were significantly elevated whereas serum total proteins and albumin were significantly reduced in AA-treated rats as compared with negative control. Treatment with garlic prior to AA produced protective effects and attenuated these biochemical changes. The protective effects of garlic were more pronounced for the high dose. Conclusion: Garlic has been shown to possess antioxidant properties offering promising efficacy against oxidative stress induced by AA administration.

Key words: Garlic Powder, Acrylamide, Multiple Organs, Rat

Introduction

Acrylamide (AA) is an industrial chemical and has been known as an occupational hazard for decades (Bull et al., 2005). The concerns about public health risks from AA exposure have escalated by the finding that AA is formed in food during cooking (Rosen and Hellenas, 2002). Nowadays, one of the important sources of exposure of the general population to AA seems to be the consumption of high-acrylamide food (Klaunig, 2008). AA is metabolically converted to epoxide glycidamide (GA) in both humans and experimental animals. Glycidamide is considered to be more genotoxic and carcinogenic than acrylamide itself (Klaunig, 2008). In mice, the conversion of AA to glycidamide is mediated by cytochrome P450 2E1 (Ghanayem et al., 2005), whereas AA and glycidamide are conjugated to glutathione, which is the main pathway of AA metabolism in both rodents and humans (Bjellaas et al., 2007). Recent results suggest that acrylamide-induced oxidative stress and disturbances in the oxidative status (Teodor et al., 2011), which may be reduced by antioxidants. Thus, antioxidants may have a protective role against AA toxicity.

One effective approach against environmental agents-induced toxicity is prevention, so identification and development of effective chemopreventive agents especially from dietary constituents that block activation or enhance detoxification of environmental agents is an important aspect. Garlic (Allium sativum L.) is food widely used in the entire world like spices. Garlic has a variety of functions, including anticancer, antithrombotic, antiatherosclerotic, antidiabetic, renoprotective, antioxidant and immune modulation activities (Razo-Rodríguez et al., 2008 and Chihara et al., 2010). The major beneficial effects of alliums are attributable to the high content of organosulfur compounds produced when the garlic tissue is damaged, and the odorless precursors are converted by the alliinase enzyme (Vazquez-Prieto and Miatello, 2010).

In this study, we aimed to evaluate the antioxidant capacity of garlic powder in two concentrations against AA induced oxidative damage in some organs in rats.

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Materials and Methods

Materials:

Acrylamide (purity > 99.9%) was purchased from Merk-Schu-Chardt chemical company (Hohenbrunn, Germany). Commercial kits for biochemical analysis were purchased from Stanbio, Texas. Garlic was purchased from local markets and was sun-dried and powdered in a grinding mill and mixed with the basal diet at dose 5% according to Singh et al. (2006). Half of this dose was also tested. All other chemicals were of analytical grade.

Animals, diets and treatments:

Twenty eight adult male Sprague-Dawely albino rats (weighing 160-180 g) were purchased from the farm of the National Institute of Vaccination, Hellwan; Egypt. The animals were housed individually in mesh bottomed metallic cages and kept at a controlled temperature (23–25°C) and ambient humidity (50–60%). Lights were maintained on a 12-h light-dark cycle (lights on from 0600 to 1800 h). Basal diet was prepared as AIN-93 (Reeves et al., 1993). All animals were received basal diet and water ad-libitum for one week as an adaptation period.

Following one week of acclimatization, the rats were randomly divided into four groups (7 rats/group):

- **Group I**: Negative control, fed on basal diet for 22 days
- **Group II**: Positive control, fed on basal diet for 22 days, starting from the 11th day AA was added to the diet at a dose of 0.34 g/kg diet (Lehning et al., 2003).
- **Group III**: 2.5% garlic, fed on basal diet supplemented with 2.5% garlic powder for 22 days, starting from the 11th day AA was added to the diet at a dose of 0.34 g/kg diet.
- **Group IV**: 5% garlic, fed on basal diet supplemented with 5% garlic powder for 22 days, starting from the 11th day AA was added to the diet at a dose of 0.34 g/kg diet.

The consumed food and body weights of rats were recorded twice a week to monitor food intake and body weight change.

At the end of the experimental period, animals were over night fasted and allowed free access to water only. Then, rats were sacrificed under diethyl ether anesthesia. The blood samples were collected directly from portal vein into centrifuge tubes. Serum aliquots were separated by centrifugation at 3000 r.p.m for 15 minutes and were frozen at -20 °C for subsequent biochemical analysis. Immediately after sacrificing rats, kidneys, spleen, testes and brain were excised from the rats, washed in cold saline, plotted in filter paper, weighed and stored at -20 °C for biochemical assay.

Biochemical assays:

Lipid peroxidation was estimated in kidney, spleen, testes and brain homogenates by measuring the malondialdehyde (MDA) production formed in the thiobarbituric acid reaction (Mihara and Uchiyama, 1978). Glutathione concentration in the same organs was determined by the method of Beutler et al. (1963). Cu/Zn superoxide dismutase (Cu/Zn SOD) was determined as described by Winterbourne et al. (1975). Serum urea and creatinine were determined by the methods of Tabacco, (1979) and Hienegard and Diderstrom, (1973), respectively. Serum total proteins and albumin were estimated by standard spectrophotometric methods according to Henry et al. (1974) and Grant and Kachmar (1976), respectively. Serum ALP and LDH activities were assayed according Tietz et al. (1983) and Wacker et al. (1956) respectively.

Statistical analysis:

The data are expressed as mean ± standard error of mean (mean ± SEM). The significant differences among groups were determined by one-way analysis of variance using the SPSS package program, version 11. The results were considered significant if the value of p was <0.05, and Duncan's multiple range test was performed if differences were identified between groups (Bailey, 1994).

Results:

Results represented in table (1) showed that food intake and body weight gain of rats received AA decreased significantly (p<0.05) as compared with negative control. The absolute and relative weights of kidney and testes showed no significant changes, while the absolute weight of spleen was significantly (p<0.05) decreased and the relative weight of brain was significantly (p<0.05) increased in AA-treated rats as compared with negative control. Feeding garlic powder supplemented diets caused remarked improvement in body weights, food intake and organ weights.
Acrylamide treatment caused a significant \((p<0.05)\) elevation in MDA levels in kidney, spleen, testes and brain by 26.8\%, 22.8\%, 26\% and 27.3\%, respectively, as compared to negative control rats. Pre treatment with both doses of garlic significantly \((p<0.05)\) lowered these levels as compared to positive control. This effect was more pronounced for the high dose (Table 2).

GSH levels were significantly \((p<0.05)\) depleted in kidney, spleen, testes and brain by 26.8\%, 22.8\%, 26\% and 27.3\%, respectively, as compared with negative control rats. This depletion in GSH levels was attenuated by pre-feeding of garlic powder, as there was a significant \((p<0.05)\) increase in GSH levels by 28\%, 18.3\%, 12.1\% and 13.4\% in kidney, spleen, testes and brain, respectively, in rats fed on 2.5\% garlic diet and by 28.9\%, 19.68\%, 16\% and 23.13\%, respectively in rats fed on 5\% garlic diet as compared with positive control (Table 2).

Moreover, SOD activity in tissue homogenates of AA-treated rats was significantly \((p<0.05)\) reduced in all organs as compared with the negative control. This effect was improved by pretreatment with garlic powder as compared with positive control (table 2).

Serum urea and creatinine levels and ALP and LDH activities were significantly increased while serum Proteins and albumin were significantly decreased \((p<0.05)\) in AA-treated rats as compared with negative control. Pretreatment with garlic powder significantly \((p<0.05)\) attenuated these effects (Table 3).

### Table 1: Effects of dietary supplementation with garlic powder on food intake, body weight change, absolute and relative weights of organs in acrylamide treated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative Control</td>
</tr>
<tr>
<td></td>
<td>Food Intake (g/day/Rat)</td>
</tr>
<tr>
<td></td>
<td>Body weight Change (g/1/day)</td>
</tr>
<tr>
<td>Brain</td>
<td>Absolute weight (g)</td>
</tr>
<tr>
<td></td>
<td>Relative weight (g%)</td>
</tr>
<tr>
<td>Kidneys*</td>
<td>Absolute weight (g)</td>
</tr>
<tr>
<td></td>
<td>Relative weight (g%)</td>
</tr>
<tr>
<td>Spleen</td>
<td>Absolute weight (g)</td>
</tr>
<tr>
<td></td>
<td>Relative weight (g%)</td>
</tr>
<tr>
<td>Testes*</td>
<td>Absolute weight (g)</td>
</tr>
<tr>
<td></td>
<td>Relative weight (g%)</td>
</tr>
</tbody>
</table>

* Means of two kidneys or testes.

The values are expressed as mean ± SEM \((n=7\) rats/group). The same letters means that there is no significant difference between groups. The different letters means that there is a significant difference between groups at \(p<0.05\).

### Table 2: Effects of garlic powder on levels of GSH and MDA and activity of SOD in some organs of acrylamide treated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental groups</th>
<th>Negative Control</th>
<th>Positive Control</th>
<th>2.5% Garlic</th>
<th>5% Garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney GSH (mg/g tissue)</td>
<td>22.05±7.12</td>
<td>16.14±1.89</td>
<td>20.67±0.58</td>
<td>20.81±0.79</td>
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</tr>
<tr>
<td>MDA (n mole/g tissue)</td>
<td>175.28±7.3</td>
<td>34.28±23.38</td>
<td>238.14±11.07</td>
<td>206.85±18.56</td>
<td></td>
</tr>
<tr>
<td>Spleen GSH (mg/g tissue)</td>
<td>32.61±2.62</td>
<td>25.15±1.19</td>
<td>29.7±1.377</td>
<td>30.1±1.43</td>
<td></td>
</tr>
<tr>
<td>MDA (n mole/g tissue)</td>
<td>73.92±3.88</td>
<td>203±10.3</td>
<td>155.7±15.89</td>
<td>132.7±11.66</td>
<td></td>
</tr>
<tr>
<td>Testes GSH (mg/g tissue)</td>
<td>565.28±85.68</td>
<td>206.28±20.18</td>
<td>424.7±22.55</td>
<td>500.42±27.68</td>
<td></td>
</tr>
<tr>
<td>Brain GSH (mg/g tissue)</td>
<td>39.44±1.5</td>
<td>29.15±1.88</td>
<td>32.68±1.31</td>
<td>35.82±1.46</td>
<td></td>
</tr>
<tr>
<td>MDA (n mole/g tissue)</td>
<td>229.28±23.96</td>
<td>493.7±24.04</td>
<td>269.5±24.79</td>
<td>211±30.3</td>
<td></td>
</tr>
<tr>
<td>SOD (U/g tissue)</td>
<td>505.85±49.9</td>
<td>211±30.3</td>
<td>380±22.94</td>
<td>395±19.7</td>
<td></td>
</tr>
<tr>
<td>MDA (n mole/g tissue)</td>
<td>239.7±19.33</td>
<td>385.7±26.5</td>
<td>172.5±8.7</td>
<td>169.67±9.97</td>
<td></td>
</tr>
<tr>
<td>SOD (U/g tissue)</td>
<td>235±32.9</td>
<td>148.85±28.7</td>
<td>357.58±10.6</td>
<td>302.81±20.92</td>
<td></td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SEM \((n=7\) rats/group). The same letters means that there is no significant difference between groups. The different letters means that there is a significant difference between groups at \(p<0.05\).

### Table 3: Effects of dietary supplementation with garlic powder on some biochemical parameters in acrylamide treated rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental groups</th>
<th>Negative control</th>
<th>Positive control</th>
<th>2.5% Garlic</th>
<th>5% Garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea mg/dl</td>
<td>25.14±1.72</td>
<td>34.97±2.48</td>
<td>26.51±1.75</td>
<td>42.84±1.4</td>
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</tr>
<tr>
<td>Creatinine mg/dl</td>
<td>0.967±0.0513</td>
<td>1.71±0.14</td>
<td>1.25±0.039</td>
<td>1.26±0.18</td>
<td></td>
</tr>
<tr>
<td>Total Protein g/dl</td>
<td>6.0±0.42</td>
<td>5.7±0.58</td>
<td>6.04±0.11</td>
<td>6.01±0.28</td>
<td></td>
</tr>
<tr>
<td>Albumine g/dl</td>
<td>3.2±0.075</td>
<td>2.9±0.11</td>
<td>2.98±0.085</td>
<td>3.05±0.11</td>
<td></td>
</tr>
<tr>
<td>Alkaline Phosphatase U/L</td>
<td>74.7±7.3</td>
<td>80.84±13.11</td>
<td>76.48±7.62</td>
<td>79.9±9.36</td>
<td></td>
</tr>
<tr>
<td>Lactate dehydrogenase U/L</td>
<td>140.5±10.22</td>
<td>246.6±30.6</td>
<td>163.67±7.3</td>
<td>165.5±20.1</td>
<td></td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SEM \((n=7\) rats/group). The same letters means that there is no significant difference between groups. The different letters means that there is a significant difference between groups at \(p<0.05\).
Discussion:

AA is well known to generate free radicals, disturbing the antioxidant status and ultimately leading to oxidative stress and carcinogenesis (Gey, 1993). Lipid peroxidation leads to the formation of several toxic products, such as MDA and 4-hydroxynonenal. These products can attack cellular targets including DNA, thereby inducing carcinogenicity (Banakar et al., 2004). The increase in lipid peroxidation was reported during AA-induced hepatocarcinogenesis (Jeyabal et al., 2005). In line with this finding, the data of the present study revealed a significant increase in the level of lipid peroxidation in the kidney, spleen, testes and brain of rats treated with AA.

The present data showed that AA treatment caused depletion of GSH, which may be partially responsible for the increased lipid peroxidation (Pradeep et al., 2007). A major pathway of AA metabolism is GSH conjugation (Miller et al., 1982). AA like other α and β unsaturated electrophiles reacts with nucleophiles possessing –SH, -NH or -OH reactive groups. Therefore it reacts with GSH in a similar manner and formed GSH-S-conjugates (Awad et al., 1998). Depletion of GSH caused by AA-GSH metabolites has been reported in vivo (Martensson et al., 1990) and in vitro (Park et al., 2002). AA also binds to cysteine residues of proteins; this may in turn depleted GSH as cysteine is a known limiting substance in GSH biosynthesis (Reed and Fariss, 1984). Moreover, in vitro co-treatment of N-acetyl-L-cysteine, a sulphhydryl group donor, with AA prevented AA-induced reduction of GSH, while DL-buthiomone-sulfoximin, an inhibitor of GSH synthesis exacerbated the toxicity of AA (Srivastava et al., 1983).

Free radical scavenging enzymes such as superoxide dismutase (SOD) protect the biological systems from oxidative stress. The current study showed a significant decrease in SOD activity in rat tissues treated with AA. The inhibition of SOD activity in AA administered rats may be related to the impairment of GSH metabolism and increased oxidative damage to proteins and DNA (Pradeep et al., 2007). Another possible explanation might be that with less GSH available to conjugate H₂O₂ the later becomes elevated to the point that it provides negative feedback on superoxide dismutase (Pigeolet et al., 1990).

In this study, AA treatment caused reduction in the levels of serum total proteins. The rate of protein synthesis was reported to be decreased in response to AA exposure (Chatterjea and Shinde, 2002), which may be related to its adverse effects on the liver (Doerge et al., 2005), which is the main organ for protein synthesis.

Current data showed that ALP and LDH activities were significantly elevated in AA treated rats. ALP has been reported to be the marker enzyme for plasma membrane and is required in certain amounts for proper functioning of organs (Matosoka et al., 1996). Increase in the ALP and LDH activities indicated the increased permeability, damage, and/or necrosis of cells (Awad et al., 1998).

On the other hand, groups treated with garlic displayed a significant reduction in lipid peroxidation and a significant increase in GSH content SOD activities in rat tissues when compared to animals treated with AA alone, and these effects were more pronounced for the high dose. It has been reported that administration of garlic significantly decreased lipid peroxidation and increased endogenous antioxidants, such as SOD, CAT, and GSH (Kiruthiga et al., 2007).

The observed reduction in the level of lipid peroxidation in garlic treated animals was presumably due to its ability to scavenge the hydroxyl and peroxy radicals (Horie et al., 1999). It has been shown that garlic extract protects vascular endothelial cells from H₂O₂-induced oxidative damage by inhibiting lipid peroxidation (Yamasaki and Lau, 1997). Also, Arivazhaga et al. (2000) reported that rats treated with N-methyl-N-nitro-N-nitrosoguanidine as garlic extract increased GSH level. Since administration of aqueous garlic extract prevented the hepatic GSH depletion, it appears that the protective effect of garlic extract involves the maintenance of antioxidant capacity in protecting the hepatic tissue against oxidative stress. Furthermore, Razo-Rodriguez et al. (2008) reported that garlic powder ameliorated cisplatin-induced renal injury and this was associated with its antioxidant properties.

Garlic contains different biologically active components and sulfur-containing amino acids. The primary sulfur-containing compound of intact garlic bulb is γ-glutamyl cysteine which can be hydrolyzed and oxidized to form alliin. Alliin is converted to odoriferous thiosulfinate allicin by alliinase after processing such as crushing, cutting, chewing or dehydration which contributes heavily to the health benefits of garlic (Amagase, 2006). In addition to alliin, other garlic organosulfurs, such as, allyl cysteine, allyl disulfide, and diallyl disulfide, possess antioxidant properties and can neutralize several types of ROS. The consequence of synergism between various compounds is responsible for the antioxidant activity of garlic (Chung, 2006). Moreover, it was reported that the total polyphenol content of garlic is 3.67 mg/g (Jang et al., 2008). Therefore, the polyphenol compounds could be responsible for strong antioxidant activity of garlic.

The inhibition of the cytochrome system is effective for reducing the toxicity of a wide variety of toxic agents ( Hosono-Fukao et al., 2009). Cytochrome P450 2E1 mediated the conversion of AA to glycidamide in mice (Ghanayem et al., 2005). Diallyl trisulfide, which is found in garlic oil was reported to down regulate cytochrome P450 2E1 and, as a result, suppressed oxidative stress (Hosono-Fukao et al., 2009).
Conclusions:

From these observations it can be concluded that garlic may suppress the formation of AA induced oxidative damage in rats by alleviating lipid peroxidation through scavenging of free radicals, or by enhancing the activity of antioxidants. These results highlight the importance of the consumption of natural vegetables and also contribute to the understanding of the beneficial effects of functional foods in the prevention of oxidative damage induced by acrylamide.

References


