Aqueous Garlic Extract and Sodium Thiosulphate as Antidotes for Cyanide Intoxication in Albino Rats

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Abstract: This study was designed to elucidate the antidotal effect of aqueous garlic extract comparing to sodium thiosulphate as a classical antidote for cyanide poisoning. Subacute dose of potassium cyanide (9mg/kg bw), showed significant decrease in antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GSR), glutathione-S-transferase (GST) and in the nonenzymatic antioxidant as glutathione (GSH) content in the liver and kidney. This decrease was accompanied with high significant increase in lipid peroxidation in both organs. Also there were disturbances in the liver and renal functions manifested by significant changes in their functional markers. In addition, hypothyroidism was observed by significant decreases in triiodothyronine (T3) and tetraiodothyronine (T4) levels after cyanide intoxication. Mostly, all of the investigated parameters were restored nearly to the normal values after aqueous garlic extract and sodium thiosulphate treatment. In conclusion, garlic exerts its effects not only as an antioxidant but also as a sulfur donor. So, garlic has a promising role and it worth to be considered as a natural antidote for cyanide intoxication.

Key words: Cyanide intoxication, sodium thiosulphate, garlic extract, antidotes, thyroid hormones, antioxidant and detoxification, renal and kidney functions.

INTRODUCTION

Cyanide, in the environment, has been associated with many intoxication episodes in humans and animals resulting from the ingestion of foods, environmental pollution, chemical war, suicide, homicide, occupational factors and use in some drugs such as nitroprusside and laetrile[48]. In plants, cyanide can be found mainly as cyanogenic glycosides, as found in Manihot sp. (cassava), Linum sp., Lotus sp., Phaseolus lunatus, Sorghum sp.[11] and the content of this substance can be high as 100-800 mg/kg of the plant material[11,35]. Regardless the route of exposure, cyanide is rapidly absorbed into the blood stream and distributed throughout the body. Cyanide concentrates in erythrocytes through binding to methemoglobin[43,46]. Subacute oral administration of cyanide in rats produced changes in several biochemical indices and pathology in various organs[44]. In general, the propensity of cyanide to induce lipid peroxidation and impair antioxidant defense systems like, catalase and SOD can be considered as a cause for cyanide toxicity[44]. In the other study, after intake of cassava, mild damage to mitochondrial and/or cellular membranes of the liver indicated with high AST activity[23].

Cyanide toxicity was also found to cause hypothyroidism that leads to goiter[1,10,24]. This is because the detoxification product (thiocyanate) interferes with thyroid function by inhibiting iodine uptake by the thyroid gland[8] or blocking the entry of iodine and this leading to iodine deficiency goiter[1,24].

Various studies focused on the role of sodium thiosulphate in the cyanide detoxification. However, the principal detoxification pathway of cyanide to thiocyanate in the presence of sulfur donor like sodium thiosulphate is mainly catalyzed by a liver mitochondrial enzyme, rhodanese (Cyanide: thiosulphate sulphur transferase)[45]. Rhodanese activity level in catalyzing the transformation of thiosulphate to thiocyanate is limited by the availability of sulfur[7]. So, alternative antidotes against cyanide toxicity must be deduced. The antioxidant properties of allicin (dialkyl thiosulfinate), the main component in aqueous extract from raw garlic, may explain the possible role of garlic to protect against cyanide toxicity[34]. It has been found that allicin scavenges OH· and inhibits lipid peroxidation[36]. However garlic preparations and related organosulphur compounds have also been reported to protect against certain cytotoxicities[47] through promoting the induction of GST[25]. In addition organosulfurs enhance the synthesis of the cellular GSH content[49], which is catalyzed by antioxidant enzymes as γ-glutamyl transpeptidase[16]. So, this study aimed to benefit from such double actions of garlic in treating the cyanide intoxication.
MATERIALS AND METHODS

Preparation of Aqueous Garlic Extract: Peeled garlic (30 g) was crushed with distilled water in a mortar and further homogenization was occurred. The crushed material was carefully decanted by pressing and 60 ml of aqueous extract was extracted. One milliliters of aqueous extract contained 500 mg of garlic materials[40].

Animals and experimental design: Twenty-five adult male rats (100-120g) were used in this study. The animals were divided into five groups.

- Control group: received tap water daily for the experimental period of 30 days.
- Cyanide group: received KCN solution at concentration 9.0 mg/kg in the drinking tap water (0.09g/L); every day the amount of KCN administered in the drinking water was adjusted to the body weight and the water consumption and a fresh solution of cyanide provided[42]. The stability of KCN in the drinking water is stable for at least 4 days after preparation[29].
- Cyanide and garlic group: received KCN (9.0 mg/kg) and intraperitoneal injection of garlic extract at 500 mg/kg[40].
- Cyanide and Na-thiosulphate received KCN (9.0 mg/kg) and intraperitoneal sodium thiosulphate at 1000 mg/kg[29].
- Cyanide and Garlic & Na-thiosulphate: received KCN (9.0 mg/kg) and intraperitoneal injection of both garlic extract at 500mg/kg and sodium thiosulphate at 1000 mg/kg.

On day 30, the rats were sacrificed then blood collected, centrifuged and sera stored at -20 °C. Liver and kidneys were obtained and homogenized and then tissue homogenates were collected and stored at -20 °C for the biochemical studies.

Lipid peroxidation was measured in liver and kidney homogenates according to the modification of the method of Ohkawa et al.[31]. GST enzyme activity in rat liver and kidneys was determined according to the method of Habig and Jakoby.[37]. CAT activity was measured according to the method of[35]. Total SOD activity was assessed according to the method of[30]. GSR enzyme activity was estimated according to[6]. GSH level of the liver and kidney homogenates were measured by the method of[37]. Serum ALT and AST were determined by[41]. Serum bilirubin and albumin levels were estimated by the method of[13,22] respectively. Urea and creatinine levels in sera and urine were estimated according to the methods of[19,33] respectively. Serum uric acid was estimated by the method of[14]. Serum T3 and T4 were measured according to the method of[12,20] by Immulite system DPC (Diagnostic Product Corporation), USA.

In kidney and liver homogenates lipid peroxidation was measured according to the modification of Ohkawa et al.[31] method. GST[37], CAT[35], total SOD[30] and GSR[40] and GSH[37] was measured in liver and kidney homogenates. Serum ALT and AST[41], serum bilirubin and albumin levels[13,22], urea and creatinine levels[33,19] and serum uric acid[41] and serum T3 and T4 were measured according to the method of Hollandez and Shenkanin[20] were determined in sera samples.

Statistics: The findings were expressed as the mean±SD. Statistical and correlation analyses were undertaken using the One-way ANOVA followed by post-hoc LSD (Least Significant Difference) test. A P value < 0.05 was accepted statistically significant. Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA).

RESULTS AND DISCUSSIONS

Table 1 showed a very high significant decrease (p<0.001) in the activities of liver and kidney catalase and SOD enzymes compared to the control, but there were a very high significant increase (p<0.001) in malondialdehyde level in the investigated tissues.

In Table 2 cyanide administered rats showed a very high significant decrease (p<0.001) in glutathione level, activities of glutathione-s-transferase and glutathione reductase in both liver and kidneys.

Table 3 showed very high increases (p<0.001) of AST and ALT enzyme activities and in serum globulin level. But there was a high significant decrease in serum albumin level in cyanide-administered group.

Table 4 showed significant increases in creatinine and urea levels both in serum and urine in rats drinking water contaminated with cyanide. Also there was a high significant increase in serum uric acid level.

Table 5 showed very high significant decrease in both T3 and T4 levels after cyanide administration.

All the tested parameters restored nearly to the normal control values after intraperitoneal injection of aqueous garlic extract and sodium thiosulphate, but garlic was more efficient than sodium thiosulphate when each injected alone and also it was more efficient than the mixture of both garlic extract and sodium thiosulphate.

Cyanide is predominantly transformed by the liver into thiocyanate mainly catalyzed by a liver mitochondrial enzyme, rhodanese (Cyanide: thiosulphate sulphur transferase) which is readily eliminated as thiocyanate by the kidneys[45]. However, cyanide accumulates in various body cells through binding to metalloproteins or enzymes such as catalase and cytochrome C oxidase[46]. So, it is a...
potent cytotoxic agent that kills the cell by inhibiting cytochrome oxidase of the mitochondrial electron transport chain[9,20]. Cyanide toxicity also caused by increased generation of superoxide anion and lipid peroxidation[22] with inhibition of antioxidants enzymes[24]. In the present study, cyanide intoxication showed increased MDA level (a marker of lipid peroxidation) with decreased catalase and SOD activities in liver and kidney, indicating hepatic and renal toxicity. Fortunately, aqueous extract of raw garlic scavenges hydroxyl radical[27] and superoxide anion[27] and modulating lipid peroxidation[29]. This may

### Table 1: Liver and kidney malondialdehyde (MDA) (nmole/g) level, superoxide dismutase (SOD) and catalase (CAT) (u/min/g) activities in different rat groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>control</th>
<th>cyanide</th>
<th>Cyanide&amp; garlic</th>
<th>Cyanide&amp; thiosulphate</th>
<th>Cyanide&amp; mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA(nmole/g)</td>
<td>Liver</td>
<td>76.1±13.4</td>
<td>378.4±19.8*</td>
<td>92.9±20.7</td>
<td>109.9±18.0*</td>
<td>112.9±6.9*</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>34.9±2.4</td>
<td>181.7±16.6*</td>
<td>41.2±5.2</td>
<td>94.1±5.8*</td>
<td>58.8±9.2*</td>
</tr>
<tr>
<td>SOD(u/min/g)</td>
<td>Liver</td>
<td>107.5±8.1</td>
<td>46.5±2.9*</td>
<td>105.5±22.4</td>
<td>62.3±11.3*</td>
<td>81.7±6.9*</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>83.2±13.6</td>
<td>36.2±6.7*</td>
<td>73.6±5.9</td>
<td>45.3±4.4*</td>
<td>74.1±8.6</td>
</tr>
<tr>
<td>CAT(u/min/g)</td>
<td>Liver</td>
<td>0.81±0.03</td>
<td>0.65±0.02*</td>
<td>0.76±0.01*</td>
<td>0.77±0.02*</td>
<td>0.78±0.01*</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>0.43±0.03</td>
<td>0.35±0.03</td>
<td>0.39±0.02</td>
<td>0.39±0.02</td>
<td>0.40±0.02</td>
</tr>
</tbody>
</table>

Data are expressed as means±SD, *p<0.001, †p<0.01, ‡p<0.05

### Table 2: Liver and kidney glutathione (GSH) (mg/g) content and glutathione-s-transferase (GST) and glutathione reductase (GSR) (u/min/g) activities in different rat groups.

<table>
<thead>
<tr>
<th>Parameters (mg/g)</th>
<th>Groups</th>
<th>control</th>
<th>cyanide</th>
<th>Cyanide&amp; garlic</th>
<th>Cyanide&amp; thiosulphate</th>
<th>Cyanide&amp; mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH</td>
<td>Liver</td>
<td>6.1±0.27</td>
<td>4.9±0.34*</td>
<td>5.7±0.13</td>
<td>5.3±0.13*</td>
<td>5.8±0.36</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>4.5±0.29</td>
<td>3.9±0.15*</td>
<td>4.2±0.10</td>
<td>4.00.19†</td>
<td>4.1±0.24†</td>
</tr>
<tr>
<td>GST (u/min/g)</td>
<td>Liver</td>
<td>4.7±0.72</td>
<td>2.8±0.48*</td>
<td>3.9±0.53</td>
<td>5.2±0.91</td>
<td>4.3±0.91</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>4.4±0.74</td>
<td>1.6±0.86*</td>
<td>2.5±0.38</td>
<td>2.8±0.53</td>
<td>3.2±0.57</td>
</tr>
<tr>
<td>GSR (u/min/g)</td>
<td>Liver</td>
<td>61.0±1.37</td>
<td>55.6±2.26*</td>
<td>59.4±0.83</td>
<td>58.7±1.14†</td>
<td>59.3±2.3</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>54.5±1.69</td>
<td>41.7±1.45*</td>
<td>48.1±1.69</td>
<td>46.0±1.47†</td>
<td>47.1±1.43†</td>
</tr>
</tbody>
</table>

Data are expressed as means±SD, *p<0.001, †p<0.01, ‡p<0.05

### Table 3: Serum aspartate transaminase (AST), alanine aminotransferase (ALT) (IU/L), and bilirubin and albumin (mg/dl) levels in different rat groups.

<table>
<thead>
<tr>
<th>Parameters (IU/L)</th>
<th>Groups</th>
<th>control</th>
<th>cyanide</th>
<th>Cyanide&amp; garlic</th>
<th>Cyanide&amp; thiosulphate</th>
<th>Cyanide&amp; mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>Liver</td>
<td>38.4±2.01</td>
<td>80.8±6.1*</td>
<td>45.2±3.7*</td>
<td>61.2±6.01*</td>
<td>43.6±1.5</td>
</tr>
<tr>
<td></td>
<td>ALT (IU/L)</td>
<td>16.2±0.84</td>
<td>26.0±1.0*</td>
<td>18.2±2.7*</td>
<td>23.0±1.0*</td>
<td>17.6±0.89</td>
</tr>
<tr>
<td></td>
<td>Billirubin (mg/dl)</td>
<td>0.58±0.08</td>
<td>1.5±0.11*</td>
<td>0.76±0.21</td>
<td>0.62±0.16</td>
<td>0.74±0.15</td>
</tr>
<tr>
<td></td>
<td>Albumin (mg/dl)</td>
<td>3.1±0.21</td>
<td>2.3±0.21*</td>
<td>3.3±0.37</td>
<td>2.8±0.11</td>
<td>2.9±0.24</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD, *p<0.001, †p<0.01, ‡p<0.05

### Table 4: Serum and urine creatinine, urea, and uric acid (mg/dl) levels in different rat groups.

<table>
<thead>
<tr>
<th>Parameters (mg/dl)</th>
<th>Groups</th>
<th>control</th>
<th>cyanide</th>
<th>Cyanide&amp; garlic</th>
<th>Cyanide&amp; thiosulphate</th>
<th>Cyanide&amp; mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>serum</td>
<td>0.47±0.1</td>
<td>2.1±0.44*</td>
<td>0.68±0.06</td>
<td>0.82±0.08†</td>
<td>1.04±0.13†</td>
</tr>
<tr>
<td></td>
<td>urine</td>
<td>3.2±0.13</td>
<td>15.1±0.49*</td>
<td>3.0±0.2</td>
<td>3.9±0.23†</td>
<td>7.3±0.25†</td>
</tr>
<tr>
<td>Urea</td>
<td>serum</td>
<td>19.9±2.1</td>
<td>57.8±1.1*</td>
<td>46.5±0.76*</td>
<td>40.4±1.5*</td>
<td>30.1±0.89*</td>
</tr>
<tr>
<td></td>
<td>urine</td>
<td>114.2±3.3</td>
<td>128.4±1.6*</td>
<td>94.2±4.0*</td>
<td>124.9±1.0*</td>
<td>120.4±2.7†</td>
</tr>
<tr>
<td>Uric acid</td>
<td>serum</td>
<td>1.5±0.25</td>
<td>4.2±0.54*</td>
<td>2.7±0.22*</td>
<td>3.4±0.21*</td>
<td>2.3±0.13*</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD, *p<0.001, †p<0.01, ‡p<0.05

### Table 5: Serum triiodothyronine (T3) (ng/ml) and tetraiodothyronine (T4) (µg) levels in different rat groups.

<table>
<thead>
<tr>
<th>Parameters (ng/ml)</th>
<th>Groups</th>
<th>control</th>
<th>cyanide</th>
<th>Cyanide&amp; garlic</th>
<th>Cyanide&amp; thiosulphate</th>
<th>Cyanide&amp; mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3</td>
<td>Liver</td>
<td>155.6±4.3</td>
<td>89.8±3.82*</td>
<td>131.0±5.66*</td>
<td>92.2±3.85*</td>
<td>128.6±3.05*</td>
</tr>
<tr>
<td></td>
<td>T4 (µg/ml)</td>
<td>5.1±0.44</td>
<td>2.9±0.19*</td>
<td>4.6±0.26*</td>
<td>3.9±0.48*</td>
<td>3.7±0.19*</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD, *p<0.001, †p<0.01, ‡p<0.05
explain the alleviation of MDA level, catalase and SOD activities in the investigated tissues after injection of garlic extract.

In addition, the cyanide-induced oxidative stress leads to a decrease in GSH content, GSR and GST activities. However, GSH, GSR and GST were restored nearly the control levels after garlic treatment, because it has antioxidant activity[5]. It exerts its effect by enhancing the nonenzymatic antioxidant (GSH) and the detoxifying enzyme (GST)[30]. Also, garlic components (as diallyl disulfide and diallylsulfide) may provide the sulfur source required for the synthesis of GSH[36,49]. So it restores glutathione level and increases the activities of glutathione reductase and glutathione-S-transferase[39]. Previous studies have showed that the organosulfur components occurring in garlic oil promote the induction of GST[25]. In addition organosulfurs enhance the synthesis of the cellular GSH content in red blood cells[49], which is catalyzed by antioxidant enzymes as -glutamyl transpeptidase[16]. Thus, garlic increase the antioxidant activities by enhancing homeostatic regulation of cellular GSH contents as well as by promoting detoxification of metabolic intermediates through induction of phase II metabolizing enzymes such as GST[32]. GST is a detoxification enzyme which catalyzes the conjugation of many electrophile agents with GSH[18], so it may be bind to CN and this explain their decreases in both liver and kidneys. In the present study, cyanide induced hepatotoxicity was reflected by the observed increases in serum ALT and AST activities, as well as in bilirubin level with a decreased albumin content. Organosulfur components (as diallyl sulfide) present in garlic oil exhibit hepatoprotective effects against toxicants[29]. As the liver is the main target of many toxicants, so it always needs additional helpers like garlic and other related preparations.

On the other hand, the recorded renal toxicity was also detected by the elevation in serum uric acid as well as in serum and urine creatinine and urea levels. However, the protective effect of garlic on kidney indices could be attributed to its antioxidant properties because it has been found that ROS may be involved in the impairment of glomerular filtration rate[21]. Garlic acts also (like sodium thiosulphate) as a sulfur donor, so it may help rhodanese to detoxify cyanide in the liver into thiocyanate which is then excreted by kidneys[45]. However, in the present study the protective effect of garlic was more pronounced if compared to that of sodium thiosulphate when injected separately.

As in the present data, cyanide also found to cause thyroid disorder, as in agreement with[26,3]. This indicated by the decreased thyroid hormones (T3 and T4). Cyanide intoxication is characterized by high plasma level of thiocyanate, the main metabolite of cyanide. The decrease in T3 and T4, in table (5), might explain as thiocyanate acts as a competitive inhibitor of iodide accumulation in the thyroid, it could produce goiter and/or hypothyroidism[24]. Garlic extract restore the thyroid hormones level nearly to the control values, because it can induce the formation of GSH[15] that is important in detoxifying the CN, by reducing the free thiocyanate level in the plasma, hence iodine will be available for thyroid function.

**Conclusion:** Aqueous garlic extract exerts its effects not only by modulating lipid peroxidation and enhancing the antioxidant and detoxifying enzyme systems, but it also acts as a sulfur donor like sodium thiosulphate (a classical antidote against cyanide). So, I.P injection of aqueous garlic extract (500mg/kg bw/daily for 30 days) has a promising role and it worth to be considered as an antidote against cyanide toxicity.

**REFERENCES**


