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Studies on the Protective Properties of Garlic oil against Acetaminophen-induced hepatotoxicity in the rat

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Pretreatment of rats with garlic oil for six days followed by a single Intraperitoneal dose of acetaminophen (125 mg/kg) protected against Acetaminophen-induced rise in liver alkaline phosphatase, glutamate oxaloacetate and glutamate pyruvate transaminase (GOT, GPT) activities. It also prevented the formation of thiobarbituric acid reactive substance (TBARS) and depletion of reduced glutathione (GSH) in the livers of acetaminophen treated rats.

ACETAMINOPHEN is the most commonly used analgesic and antipyretic drug. Higher doses of this drug have been shown to cause hepatic toxicity. The hepatotoxicity of acetaminophen is thought to be due to the cytochrome P450IIE1 catalysed formation of N-acetyl-p-benzoquinoneimine (NAPQI). The detoxication of this metabolite is brought about by conjugation with reduced glutathione, however, with excess dose of acetaminophen the hepatic GSH stores get depleted with consequent accumulation of free toxic metabolite which binds to tissue macromolecules. Moreover, reduced oxygen species responsible for membrane lipid peroxidation have also been implicated in acetaminophen-induced hepatotoxicity.

A synthetic compound, cysteamine in combination with N-acetylcysteine and deferoxamine, an

*For correspondence
Table 1
The activities of alkaline phosphatase, glutamic oxaloacetate and glutamic pyruvate transaminases in livers of control, acetaminophen and garlic oil plus acetaminophen treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Alkaline Phosphates(^c)</th>
<th>Glutamic(^d) Oxaloacetate Transaminase</th>
<th>Glutamic(^d) Pyruvate Transaminase</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Control</td>
<td>13.45 (± 0.86)</td>
<td>0.34 (± 0.03)</td>
<td>1.24 (± 0.06)</td>
</tr>
<tr>
<td>II. Acetaminophen(^a)</td>
<td>18.56 (± 0.91)(^e)</td>
<td>0.49 (± 0.04)(^e)</td>
<td>1.52 (± 0.08)(^e)</td>
</tr>
<tr>
<td>III. 5% Garlic Oil(^b) + Acetaminophen</td>
<td>16.55 (± 1.30)(^e)</td>
<td>0.49 (± 0.03)(^e)</td>
<td>1.50 (± 0.08)(^e)</td>
</tr>
<tr>
<td>IV. 10% Garlic Oil(^b) + Acetaminophen</td>
<td>15.96 (± 1.06)(^e)</td>
<td>0.40 (± 0.04)</td>
<td>1.47 (± 0.08)</td>
</tr>
</tbody>
</table>

\(a\) Male rats were given a single dose of 125 mg/kg bwt of acetaminophen intraperitoneally and sacrificed after 24 hrs.

\(b\) The rats were given 0.5 ml of appropriately diluted preparation of garlic oil in olive oil for 6 days prior to acetaminophen administration.

\(c\) μmole phenol liberated /min/mg protein

\(d\) μmole pyruvate formed /min/mg protein.

The values represent mean ± standard deviation.

\(e\) \(P < 0.05\) w.r.t. control

Table 2: Reduced glutathione (GSH) content of livers of control, acetaminophen and Garlic oil plus acetaminophen treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>glutathione (GSH) (µg/ml protein)</th>
<th>% Change</th>
<th>% Protection by garlic oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>5.21 (± 0.34)</td>
<td>-</td>
</tr>
<tr>
<td>II</td>
<td>Acetaminophen(^a)</td>
<td>4.13 (± 0.19)(^e)</td>
<td>20.73</td>
</tr>
<tr>
<td>III</td>
<td>5% Garlic Oil(^b) + Acetaminophen</td>
<td>4.13 (± 0.21)(^e)</td>
<td>20.73</td>
</tr>
<tr>
<td>IV</td>
<td>10% Garlic Oil(^b) + Acetaminophen</td>
<td>4.73 (± 0.35)</td>
<td>07.48</td>
</tr>
</tbody>
</table>

\(a, b\) Same as in Table - 1.

The values represent mean ± standard deviation.

\(e\) \(P < 0.05\) w.r.t. control.

Iron chelator, have protective effects against liver injury caused by acetaminophen\(^7\). Popov et al\(^8\) have shown potent antioxidant effect of garlic extract and Umalakshmi and Devaki\(^9\) have demonstrated
Table - 3: Thiobarbituric acid reactive substances (TBARS) in livers of control, acetaminophen and acetaminophen plus garlic oil treated rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (n moles MDA formed /min/mg protein)</th>
<th>% Change</th>
<th>% Protection by garlic oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Control</td>
<td>0.27 (± 0.02)</td>
<td>--</td>
</tr>
<tr>
<td>II.</td>
<td>Acetaminophen(^a)</td>
<td>0.33 (± 0.02)(^b)</td>
<td>23.02</td>
</tr>
<tr>
<td>III.</td>
<td>5% Garlic Oil(^b)</td>
<td>0.32 (± 0.02)(^b)</td>
<td>20.38</td>
</tr>
<tr>
<td></td>
<td>+ Acetaminophen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV.</td>
<td>10% Garlic Oil(^b)</td>
<td>0.29 (± 0.02)</td>
<td>08.68</td>
</tr>
<tr>
<td></td>
<td>+ Acetaminophen</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) and \(^b\) Same as in Table - 1.

The values represent mean ± standard deviation

\(^e\) P <0.05 w.r.t. control.

The inhibition of ethanol-induced mitochondrial lipid peroxidation by garlic oil. In view of such observations we have carried out the present study on the effects of garlic oil against acetaminophen-induced hepatoxicity in rats.

Male albino rats of porton strain weighing 100-125 g were used in the present study. The animals were maintained on Hind. Liver Pellet diet and had free access to water. The garlic oil (Ranbaxy Lab., India) was appropriately diluted with olive oil. The solutions were prepared 1 hour before use.

The rats were divided into four groups of six animals each. Rats of group I were not given any treatment of serve as the control. Rats of group II were treated (ip) with acetaminophen (125 mg/kg). The rats of group III and group IV were administered 5 per cent and 10 percent garlic oil in olive oil respectively for six days followed by a single intraperitoneal dose of acetaminophen (125 mg/kg.)

The animals were euthanized after anesthetizing with ether. The livers were perfused with cold normal saline, removed and homogenized in four volumes of 0.15 M potassium chloride and 100 mM potassium phosphate buffer, pH 7.5.

Standard methods were used for measuring TBARS, GSH, GOT, GPT, alkaline phosphatase and protein in liver samples. Unpaired students t-test was employed for the significance of the results.

An intraperitoneal dose of acetaminophen (125 mg/kg) was found to show hepatotoxic changes in rat liver. The activities of serum alkaline phosphatase, GOT and GPT were significantly increased following acetaminophen treatment (data not shown) with concomitant increased in their activities in the livers of acetaminophen treated animals (Table - 1). Also the depletion in reduced GSH levels (Table-2) and an increased in the formation of TBARS (Table-3) showing enhanced lipid peroxidation due to acetaminophen treatment was noted.

In rats pretreated with 5 and 10 percent garlic oil for six days prior to acetaminophen administration, the liver alkaline phosphatase, GOT and GPT activities were found to be closer to those of the normal animals (Table 1). Also garlic oil pretreatment of rats at 10 percent level was found to completely
prevent depletion of reduced GSH (Table-2) as well as the formation of TBARS (Table-3) due to acetaminophen treatment. It has been reported that the reactive metabolite NAPQI is detoxified by conjugation with reduced GSH which when present in excess can cause depletion of reduced glutathione\textsuperscript{10}. Recent report using cultured rodent hepatocytes suggest that partially reduced oxygen species cause enhanced lipid peroxidation in acetaminophen toxicity\textsuperscript{11}.

The prevention of depletion of reduced glutathione might be explained due to the presence of sulfur compounds like diallylsulfide (DAS) in garlic oil which can significantly inhibit the cytochrome P450IE1 in rat liver\textsuperscript{12}. This isozyme of cytochrome P450 has been shown to be responsible for the metabolism of acetaminophen to NAPQI\textsuperscript{13}.

The observation regarding the prevention of acetaminophen-induced TBARS formation of garlic oil might also be linked to the prevention of GSH depletion. This inference is supported by the findings of Wendel et al\textsuperscript{14} who have shown that it acetaminophen toxicity, the lack of substrate availability for glutathione peroxidase is responsible for cytochrome P450 mediated increase in generation of reduced oxygen species leading to enhanced lipid peroxidation.

The results presented in this paper, show that garlic oil is endowed with protective properties against acetaminophen-induced hepatotoxicity due to its ability to check GSH depletion and prevent lipid peroxidation-mediated harmful effects of acetaminophen.

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