6-Gingerol, an active ingredient of ginger, protects acetaminophen-induced hepatotoxicity in mice

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Objective: To investigate the hepatoprotective efficacy of 6-gingerol against acetaminophen-induced hepatotoxicity in mice.

Methods: Mice were injected with a single dose of acetaminophen (900 mg/kg) to induce hepatotoxicity, while 6-gingerol (30 mg/kg) or the standard drug silymarin (25 mg/kg) was given 30 min after the acetaminophen administration. The mice were sacrificed 4 h after acetaminophen injection to determine the activities of liver marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), total bilirubin in serum, and lipid peroxidation and antioxidant status (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione transferase and glutathione) in liver homogenate.

Results: The treatment of 6-gingerol and silymarin to acetaminophen-induced hepatotoxicity showed significant hepatoprotective effect by lowering the hepatic marker enzymes (AST, ALT, and ALP) and total bilirubin in serum ($P<0.05$). In addition, 6-gingerol and silymarin treatment prevented the elevation of hepatic malondialdehyde formation and the depletion of antioxidant status in the liver of acetaminophen-intoxicated mice ($P<0.05$).

Conclusion: The results evidently demonstrate that 6-gingerol has promising hepatoprotective effect which is comparable to the standard drug silymarin.

Keywords: 6-gingerol; plant extracts; hepatoprotective; antioxidants; lipid peroxidation; mice

Ginger (Zingiber officinale Roscoe, Zingiberaceae) is among the most healthy and frequently consumed spices throughout the world. It has been an important plant for the traditional Chinese and Indian pharmacopeia and widely used to relieve muscular aches, rheumatism, pains, coughs, sinusitis, sore throats, diarrhea, cramps, indigestion, loss of appetite, motion sickness, fever, flu, chills and other infectious diseases. 6-Gingerol ((S)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-decanone) is an aromatic polyphenol and the most pungent constituent of fresh ginger. It has varied pharmacological activities.

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including antioxidant, anti-inflammatory, anticancer, analgesic, and antiplatelet effects. It also has an inhibitory effect on xanthine oxidase responsible for generation of reactive oxygen species like superoxide anion. In addition, 6-gingerol has also been found to inhibit the expressions of cyclooxygenase-2, lipoxygenases and nuclear factor κB which play pivotal roles in progression of inflammation and cancer.

Acetaminophen is an over-the-counter drug widely used for its analgesic and antipyretic properties. Under therapeutic doses, it is biotransformed and eliminated as nontoxic glucuronide and sulfate conjugates. Only a small proportion of acetaminophen is converted to N-acetyl-p-benzoquinoneimine (NAPQI). However, overdose of acetaminophen causes depletion of the cellular glutathione level in liver, because NAPQI reacts rapidly with glutathione, which leads to oxidative stress, cell damage and death. Since liver disease is a widespread pathology and currently used hepatoprotective drugs are of doubtful worth and safety, thus, the development of new hepatoprotective drug of greater efficacy and protection is highly warranted. However, scientific literature behind the use of the 6-gingerol in acetaminophen-induced hepatotoxicity is not present. Therefore, the present study was aimed to investigate the potential of 6-gingerol against acetaminophen-induced hepatotoxicity in mice.

1 Materials and methods

1.1 Animals

Swiss albino mice of either sex weighing about 20 to 25 g were obtained from the School of Bio Sciences and Technology, Vellore Institute of Technology (VIT) University, Vellore, India. The mice were housed in large spacious cages. They were acclimatized for a week in a light and temperature-controlled room with a 12-hour dark-light cycle and fed with commercial pelleted feed obtained from Hindustan Lever Ltd. (Mumbai, India) and water ad libitum. The mice used in this study were treated and cared for well being in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Culture, Government of India, Chennai, India. The experimental protocol was approved by the VIT University animal ethical committee.

1.2 Drugs and chemicals

The commercially available 6-gingerol was obtained from the Natural Remedies Pvt. Ltd., Bangalore, India. Silymarin, a standard hepatoprotective drug and acetaminophen were obtained from the Micro Labs Ltd., Goa, India. All other reagents and chemicals used were of analytical grade.

1.3 Dose determination study

A preliminary study was carried out to determine the optimal dose of 6-gingerol by investigating serum hepatic marker enzyme activities in acetaminophen-intoxicated mice. 6-Gingerol suspended in normal saline was administered at different doses (namely, 10, 20 and 30 mg/kg body weight) to different groups of mice. Among the three doses, the 30 mg/kg body weight dose was more effective than the other two doses. Hence 30 mg/kg body weight was considered for this study. The dosage of acetaminophen (900 mg/kg body weight) and standard drug silymarin (25 mg/kg body weight) used in this study were selected based on our previous reports.

1.4 Experimental protocol

In this experiment, mice were randomly allocated into five groups, each consisting of six mice. All mice were kept fasting for 24 h before the experiment. The first group which served as the control group received saline. The second group was treated with a single dose of acetaminophen (900 mg/kg body weight, intraperitoneally (i. p.)) on day 0. Acetaminophen was first dissolved in water at 70°C, and then cooled to 37°C before administration. The third group (6-gingerol plus acetaminophen) was given 6-gingerol (30 mg/kg body weight, i. p.) with a single dose of acetaminophen (900 mg/kg body weight, i. p.). The fourth group (silymarin plus acetaminophen) was given silymarin (25 mg/kg body weight, i. p.)
dissolved in saline) 30 min after the single injection of acetaminophen. The fifth group, 6-gingerol group, received 6-gingerol alone (30 mg/kg body weight, i. p., dissolved in saline). The dose selection for acetaminophen, silymarin, and 6-gingerol was based on our preliminary and previous experiments\textsuperscript{[11]}. The control and experimental mice were decapitated 4 h after the acetaminophen injection; the trunk blood was collected and the serum was separated and stored at $-70$ °C. Tissue samples from the livers were obtained for biochemical analysis.

1.5 Biochemical parameters The activities of alkaline phosphatase (ALP), alanine and aspartate aminotransferases (ALT and AST) and total bilirubin in serum were estimated by using commercial kits (Span Diagnostics, Chennai, India). In the hepatic tissue samples, lipid peroxidation was measured using thiobarbituric acid reactive substances (TBARS) according to the method of Ohkawa\textsuperscript{[11]}. The lipid peroxidation was expressed as micromoles of thiobarbituric acid (TBA) reactants per 100 g of tissue homogenate. Superoxide dismutase (SOD) was estimated in liver homogenate according to the method of Marklund\textsuperscript{[11]}. Catalase (CAT) was assayed by the method of Sinha\textsuperscript{[11]}. Glutathione peroxidase (GPx) was assayed by the method of Rotruck\textsuperscript{[11]}. Glutathione reductase (GR) that utilizes nicotinamide adenine dinucleotide phosphate (NADPH) to convert oxidized glutathione (GSSG) to the reduced form was assayed by the method of Bellomo\textsuperscript{[11]}. Glutathione transferase (GST) was assayed by the method of Habig\textsuperscript{[11]}. Total reduced glutathione (GSH) was determined by the method of Moron\textsuperscript{[11]}.

1.6 Statistical analysis Results were expressed as mean ± standard deviation and the statistical analysis was performed using analysis of variance, to determine the significant differences between the groups, followed by Student-Newman-Keuls test. $P<0.05$ implied significance.

2 Results

2.1 Effects of 6-gingerol on lipid peroxidation and antioxidant enzymes The levels of TBARS formation, SOD, CAT, GPx, GR, GST and total reduced GSH in liver of control and experimental mice were shown in Table 1. In acetaminophen-treated mice, SOD, CAT, GPx, GR, GST and total reduced GSH were found to be decreased, whereas TBARS level were increased compared with the control group $(P<0.05)$. However, treatment of 6-gingerol to acetaminophen-induced mice altered the above changes by regulating the TBARS level and antioxidant enzymes $(P<0.05)$.

2.2 Effects of 6-gingerol on liver marker enzymes and total bilirubin The activities of ALT, AST and ALP and content of total bilirubin in serum of control and experimental groups were shown in Table 2. The activities of ALT, AST and ALP and content of total bilirubin in serum were significantly increased in acetaminophen-induced mice compared with control group $(P<0.05)$. However, 6-gingerol treatment prevented the above changes observed in acetaminophen-induced mice $(P<0.05)$.

### Table 1 Effects of 6-gingerol on liver LPO levels and antioxidant enzyme activities in acetaminophen-intoxicated mice

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>LPO (μmol of TBA reactants per 100 g tissue)</th>
<th>SOD (U per mg protein per min)</th>
<th>CAT (μmol of H$_2$O$_2$ consumed per mg protein per min)</th>
<th>GPx (μg of GSH utilized per mg protein per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>0.62±0.10</td>
<td>180.83±7.19</td>
<td>160.67±7.26</td>
<td>85.16±3.97</td>
</tr>
<tr>
<td>Acetaminophen (900 mg/kg body weight, i. p.)</td>
<td>6</td>
<td>1.59±0.32</td>
<td>105.33±6.05</td>
<td>79.28±6.73</td>
<td>45.50±3.73</td>
</tr>
<tr>
<td>Acetaminophen plus 6-gingerol (30 mg/kg body weight, i. p.)</td>
<td>6</td>
<td>0.94±0.14</td>
<td>161.50±9.40</td>
<td>147.17±9.28</td>
<td>74.00±3.35</td>
</tr>
<tr>
<td>Acetaminophen plus silymarin (25 mg/kg body weight, i. p.)</td>
<td>6</td>
<td>0.97±0.02</td>
<td>156.67±9.83</td>
<td>131.00±7.38</td>
<td>68.33±6.02</td>
</tr>
<tr>
<td>6-gingerol (20 mg/kg body weight, i. p.)</td>
<td>6</td>
<td>0.66±0.11</td>
<td>174.50±12.20</td>
<td>157.43±9.81</td>
<td>79.33±10.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>GR (nmol of NADPH oxidized per mg protein per min)</th>
<th>GST (nmol of CDNB-GSH conjugate formed per mg protein per min)</th>
<th>Total reduced GSH (nmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>45.14±6.47</td>
<td>83.16±6.18</td>
<td>36.83±5.56</td>
</tr>
<tr>
<td>Acetaminophen (900 mg/kg body weight, i. p.)</td>
<td>6</td>
<td>19.71±3.90</td>
<td>47.50±10.4</td>
<td>19.00±3.58</td>
</tr>
<tr>
<td>Acetaminophen plus 6-gingerol (30 mg/kg body weight, i. p.)</td>
<td>6</td>
<td>32.71±7.83</td>
<td>64.00±5.10</td>
<td>24.50±3.27</td>
</tr>
<tr>
<td>Acetaminophen plus silymarin (25 mg/kg body weight, i. p.)</td>
<td>6</td>
<td>34.00±5.45</td>
<td>58.83±5.46</td>
<td>29.33±4.13</td>
</tr>
<tr>
<td>6-gingerol (30 mg/kg body weight, i. p.)</td>
<td>6</td>
<td>42.57±4.89</td>
<td>76.83±5.56</td>
<td>38.16±5.12</td>
</tr>
</tbody>
</table>

$^*$ $P<0.05$, vs control group; $^\Delta$ $P<0.05$, vs acetaminophen group. LPO: lipid peroxidation; TBA: thiobarbituric acid; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; GR: glutathione reductase; NADPH: nicotinamide dinucleotide phosphate; GST: glutathione transferase; CDNB: 1-chloro-2,4-dinitrobenzene; GSH: glutathione; i. p.: intraperitoneally.
### Table 2 Effects of 6-gingerol on liver functional markers and total bilirubin in acetaminophen-intoxicated mice in serum (Mean±standard deviation)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
<th>Total bilirubin (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>896.0±65.6</td>
<td>423.3±51.3</td>
<td>173.3±11.4</td>
<td>0.41±1.73</td>
</tr>
<tr>
<td>Acetaminophen (900 mg/kg body weight, i.p.)</td>
<td>6</td>
<td>3 408.3±169.0*</td>
<td>2 211.7±173.0*</td>
<td>464.3±34.7*</td>
<td>0.83±1.89*</td>
</tr>
<tr>
<td>Acetaminophen plus 6-gingerol (30 mg/kg body weight, i.p.)</td>
<td>6</td>
<td>1 661.7±82.6&lt;sup&gt;Δ&lt;/sup&gt;</td>
<td>591.6±57.4&lt;sup&gt;Δ&lt;/sup&gt;</td>
<td>239.8±17.1&lt;sup&gt;Δ&lt;/sup&gt;</td>
<td>0.49±2.43&lt;sup&gt;Δ&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetaminophen plus silymarin (25 mg/kg body weight, i.p.)</td>
<td>6</td>
<td>1 281.7±51.2&lt;sup&gt;Δ&lt;/sup&gt;</td>
<td>725.7±52.9&lt;sup&gt;Δ&lt;/sup&gt;</td>
<td>242.4±18.6&lt;sup&gt;Δ&lt;/sup&gt;</td>
<td>0.43±1.19&lt;sup&gt;Δ&lt;/sup&gt;</td>
</tr>
<tr>
<td>6-gingerol (30 mg/kg body weight, i.p.)</td>
<td>6</td>
<td>1 003.3±55.4</td>
<td>538.6±53.5</td>
<td>201.3±12.5</td>
<td>0.43±1.07</td>
</tr>
</tbody>
</table>

* P<0.05, vs control group; △ P<0.05, vs acetaminophen group. ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; i.p.: intraperitoneally.

### 3 Discussion

In this study, we investigated the effect of 6-gingerol against acetaminophen-induced liver damage in mice. Noticeable indication of liver injury is the release of cellular enzymes into the plasma due to the interruption caused by chemicals in the transport functions of the hepatocytes<sup>[19]</sup>. The evaluation of enzymes in the serum is marker for liver damage. In the present study, acetaminophen administration caused a significant increase in liver marker enzymes AST, ALT and ALP, total bilirubin and lipid peroxidation compared with the normal mice. The turnaround of increased serum enzymes, total bilirubin and lipid peroxidation observed in 6-gingerol-treated acetaminophen-intoxicated mice was supported by the limited degree of histological changes. The reversal of transaminases level in serum with the healing of hepatic parenchyma and the regeneration of hepatocytes is commonly accepted agreement<sup>[20]</sup>. This hepatoprotective effect of 6-gingerol may be due to the prevention of the intracellular enzyme release by its membrane stabilizing and antioxidant activity.

Large dose of acetaminophen causes hepatic glutathione depletion because excess NAPOI reacts rapidly with GSH which exacerbates oxidative stress in conjunction with mitochondrial dysfunction. The NAPOI-induced depletion of cytosolic and mitochondrial GSH trigger the loss of cellular homeostasis leading to liver injury<sup>[21]</sup>. The actions of SOD, CAT, and GPx are insightful indexes in hepatorenal damage as they scavenge the reactive oxygen species leading to diminishing of the toxic effects. In consistence with our previous report<sup>[9]</sup>, in this present study, acetaminophen induction (900 mg/kg body weight) significantly increased the lipid peroxidation levels, and caused the depletion of antioxidant status (SOD, CAT, GPX, GR, GST and GSH). However, treatment with 6-gingerol and silymarin abrogated the acetaminophen-induced decreases in antioxidant enzymes and GSH in mice. The restored hepatic GSH level observed in acetaminophen-induced mice indicates an involvement of 6-gingerol in facilitating the rapid and efficient consumption of reactive oxygen species generated by acetaminophen P450 bioactivation. Therefore, the observed antioxidant protection from 6-gingerol against acetaminophen-induced hepatotoxicity might be partially due to the increased GSH. A recent study has shown that ginger is gifted with strong antioxidant effects against acetaminophen-induced hepatotoxicity<sup>[22]</sup>. The antioxidant action observed by the 6-gingerol in our study is in accordance with the other previous reports suggesting that, it is well documented against reactive oxygen species-mediated damages<sup>[23, 24]</sup>. Recently, it has been reported that 6-gingerol protected against β-amyloid-induced cytotoxicity and apoptosis by inhibition of intracellular accumulation of reactive oxygen species and subsequent oxidative and/or nitrosative damages<sup>[25]</sup>.

Several investigations have shown that silymarin improved liver function related to hepatocellular necrosis and increased membrane permeability through its antioxidant capacity<sup>[26]</sup>. The protective effect of silymarin observed in our study was attributed to its antioxidant and free radicals-scavenging properties, which have been already well established<sup>[27]</sup>.

In conclusion, the results of this study reveal that 6-gingerol has a strong hepatoprotective effect on acetaminophen-induced liver damage in mice due to its antioxidant effect. However, further pharmacological evidence at molecular level is required to establish the actual mechanism of the action of the active compound which is underway.

### 4 Competing interests

The authors declare that they have no competing interests.

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生姜提取物6-姜酚对对乙酰氨基酚致小鼠肝脏毒性的保护作用

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目的：研究生姜提取物6-姜酚对对乙酰氨基酚致小鼠肝脏毒性的保护作用。
方法：实验组小鼠在腹腔注射对乙酰氨基酚（900 mg/kg）30 min后，分别给予6-姜酚（30 mg/kg）或标准对照药水杨酸（25 mg/kg）。注射对乙酰氨基酚4 h后处死小鼠，检测血清中天冬氨酸氨基转移酶、丙氨酸氨基转移酶、碱性磷酸酶活性，总胆红素的含量及肝匀浆中脂质过氧化及其氧化情况，如超氧化物歧化酶、过氧化氢酶、谷胱甘肽过氧化物酶、谷胱甘肽还原酶、谷胱甘肽转移酶活性及还原型谷胱甘肽含量。
结果：与对照组相比，6-姜酚及水杨酸均能显著降低对乙酰氨基酚引起的小鼠血清中天冬氨酸氨基转移酶、丙氨酸氨基转移酶、碱性磷酸酶活性及总胆红素含量的升高（P＜0.05）。此外，6-姜酚及水杨酸有有效控制了肝脏内丙二醛的形成及各种抗氧化酶的降低（P＜0.05）。
结论：本研究的结果证实了6-姜酚具有与水杨酸相当的保肝作用。
关键词：6-姜酚；植物提取物；保肝药；抗氧化剂；脂质过氧化作用；小鼠