Hepatoprotective Effect of Thymol on Chemical-induced Hepatotoxicity in Rodents

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Abstract: The hepatoprotective activity of thymol, a terpenoid from essential oils of plant origin was investigated against paracetamol and CCl₄-induced hepatic damage. The results showed that paracetamol produced 100% mortality at the dose of 1 g kg⁻¹ in mice while pre-treatment of animals with thymol (150 mg kg⁻¹) reduced the death rate to 30%. Oral administration of paracetamol (640 mg kg⁻¹) produced liver damage in rats as manifested by the rise in serum enzyme levels of alkaline phosphatase (ALP) and transaminases (AST and ALT). Pre-treatment of rats with thymol (150 mg kg⁻¹) prevented the paracetamol-induced rise in serum enzymes. The hepatotoxic dose of CCl₄ (1.5 ml kg⁻¹; orally) also raised the serum ALP, AST and ALT levels. The same dose of thymol (150 mg kg⁻¹) was able to prevent the CCl₄-induced rise in serum enzymes. The results indicated that thymol also prevented the CCl₄-induced prolongation in pentobarbital sleeping time confirming hepatoprotective. It was concluded that thymol possesses anti-hepatotoxic activity.

Key words: Thymol, essential oils, anti-hepatotoxic, paracetamol, CCl₄

Introduction
Thymol is one of the terpenoids present as essential oil in plants (Ali-Shayeh et al., 1997). The compound exhibited multiple biological activities including anti-bacterial (Didry et al., 1994), anti-fungal (Mahmoud, 1994), anti-inflammatory (Azuma et al., 1986), hypo-cholesteremic (Case et al., 1995), cytotoxic (He et al., 1997), immunomodulating (Suzuki and Furuta, 1988) and also molluscidal (Singh et al., 1999) activities. The toxin-induced hepatic damages are known to be mediated through involvement of oxidized reactive intermediates (Aldridge, 1981) and compounds exhibiting anti-oxidant (Attri et al., 2000), free radical scavenging (Sadanobu et al., 1999) and anti-lipid per-oxidation (Liu et al., 2000) properties are reported to exhibit anti-hepatotoxic activities. Thymol possesses anti-oxidant (Aeschbach et al., 1994), free radical scavenging (Fujiisawa and Kadorn, 1992) and anti-lipid per-oxidation (Beach and Giroux, 1992) properties and is likely to curtail the sequence of events leading towards hepatocellular damage. The present study was undertaken in an attempt to assess the possible anti-hepatotoxic potential of thymol against paracetamol and CCl₄-induced hepatotoxicity.

Materials and Methods
This study was conducted at Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi during July-August, 2000.

Animals: Swiss male mice (20-25 g) and male albino wistar rats (200-250 g) were obtained from the Animal House of The Aga Khan University. The animals were housed in plastic cages (47x34x8 cm³), mice (10 cage⁻¹) and rats (5 cage⁻¹), lined with sawdust renewed every 48 h, in air-conditioned quarters and had free access to tap water and food.

Pharmacological materials: Paracetamol, CCl₄, thymol, ketamine hydrochloride and methyl cellulose were obtained from Sigma Chemicals Company, St Louis, MO USA and olive oil (P. Sasso e Figili, Ongelia, Italy) was purchased from local market. Paracetamol and CCl₄ were suspended in 1% methyl cellulose (50 mg ml⁻¹) and olive oil (20% v/v), respectively.

Lethality study in mice: Preliminary experiments were performed on mice to estimate the protective effect of thymol against a lethal dose of paracetamol (1g kg⁻¹). Animals were divided into two groups of 10 animals each. One group was treated orally with thymol (150 mg kg⁻¹) followed after 1 h by oral administration of paracetamol. The 2nd group served as control and received the same treatment except that normal saline (0.9% NaCl) was administered instead of thymol. The mortality was observed for 24 h post-administration of paracetamol.

Hepatoprotective study: Hepatic injury in rats was induced separately by paracetamol (640 mg kg⁻¹) as well as CCl₄ (1.5 ml kg⁻¹), administered orally, whereas control animals
received an equal volume of respective vehicle (1% methyl cellulose or olive oil) as described previously (Janbaz and Gilani, 1995).

Rats were divided into three groups of 10 animals each. Group 1 served as vehicle control and received normal saline (10 ml kg⁻¹) and vehicle (1% methyl cellulose; 13 ml kg⁻¹, orally). Group 2 was given 4 doses of normal saline at 12 h intervals and paracetamol was administered orally 1 h post-treatment of the last dose. Group 3 was treated similarly to group 2, except that thymol (150 mg kg⁻¹, suspended in 10 ml saline) was administered instead of saline.

In a parallel study on 3 similar groups of rats (n = 10), normal saline (10 ml kg⁻¹) and vehicle (olive oil; 7.5 ml kg⁻¹) were administered orally to vehicle control group, whereas the remaining 2 groups were treated similarly to the study mentioned above except that paracetamol was replaced by CCl₄.

Animals were anesthetized with ketamine (100 mg kg⁻¹, i.m.) 24 h after the last treatment and blood (3 ml) was collected by cardiac puncture using sterile disposable syringes. Serum was separated by centrifugation (3000 rpm for 15 min) and serum alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) were estimated on the same day spectrophotometrically using Merck diagnostic kits.

**Modification of CCl₄-induced prolongation in pentobarbital sleeping time:** The effect of thymol on CCl₄-induced prolongation in pentobarbital sleeping time was studied in mice (Montilla et al., 1990; Gilani et al., 1998) (Table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Sleeping time (min)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saline + Vehicle + Pentobarbital (10 ml kg⁻¹ + 7.5 ml kg⁻¹ + 75 mg kg⁻¹)</td>
<td>124±11</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Saline + CCl₄ + Pentobarbital (10 ml kg⁻¹ + 1.5 ml kg⁻¹ + 75 mg kg⁻¹)</td>
<td>219±27**</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Thymol + CCl₄ + Pentobarbital (150 mg kg⁻¹ + 1.5 ml kg⁻¹ + 75 mg kg⁻¹)</td>
<td>132±15**</td>
<td>20</td>
</tr>
</tbody>
</table>

Values represent the mean ± SEM of 10 determinations. Saline/Thymol/CCl₄ was given orally, while pentobarbital was given intraperitoneally. *P<0.01; Compared to group 1 (control); **P<0.05; Compared to group 2.

**Effect of thymol on paracetamol-induced lethality:** Paracetamol at the dose of 1 g kg⁻¹ killed all mice. In a group of animals pre-treated with thymol (150 mg kg⁻¹), the same dose of paracetamol killed only two out of ten resulting in 80% protection against the lethal effect of paracetamol (Table 1).

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<tr>
<td>1</td>
<td>Thymol + Paracetamol (150 mg kg⁻¹ + 1 g kg⁻¹)</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>Saline + Paracetamol (10 ml kg⁻¹ + 1 g kg⁻¹)</td>
<td>100</td>
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**Effect of thymol on CCl₄-induced prolongation in pentobarbital sleep:** Pentobarbital at a dose of 75 mg kg⁻¹, i.p., caused sleep in mice of control group for a period of 124±11 min (mean ± SEM; n = 10). Whereas treatment of

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Values shown are mean ± SEM of 10 determinations expressed as IU. Group 3 animals received four doses of thymol (150 mg kg⁻¹) at 12 h interval before paracetamol (640 mg kg⁻¹) administration. *P<0.01; Compared to group 1. **P<0.01; Compared to group 2.

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<th>AST</th>
<th>ALT</th>
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<tbody>
<tr>
<td>1</td>
<td>Saline + Vehicle (10 ml kg⁻¹ + 13 ml kg⁻¹)</td>
<td>207±15</td>
<td>97.0±13</td>
<td>48.0±69</td>
</tr>
<tr>
<td>2</td>
<td>Saline + Paracetamol (10 ml kg⁻¹ + 640 mg kg⁻¹)</td>
<td>208±19²</td>
<td>94.3±181*</td>
<td>438.0±196*</td>
</tr>
<tr>
<td>3</td>
<td>Thymol + Paracetamol (150 mg kg⁻¹ + 640 mg kg⁻¹)</td>
<td>211.0±21²</td>
<td>119.0±209*</td>
<td>75.0±19**</td>
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Values shown are mean ± SEM of 10 determinations expressed as IU. Group 3 animals received four doses of thymol (150 mg kg⁻¹) at 12 h interval before CCl₄ (1.5 ml kg⁻¹) administration. *P<0.01; Compared to group 1 (control). **P<0.05; ***P<0.01; Compared to group 2.

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<tr>
<td>1</td>
<td>Saline + Vehicle (10 ml kg⁻¹ + 7.5 ml kg⁻¹)</td>
<td>205.0±17</td>
<td>92.0±11</td>
<td>51.0±12</td>
</tr>
<tr>
<td>2</td>
<td>Saline + CCl₄ (10 ml kg⁻¹ + 1.5 ml kg⁻¹)</td>
<td>318.0±32*</td>
<td>781.0±169*</td>
<td>449.0±114*</td>
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ALP, alkaline phosphatase; AST, aspartate transaminase; ALT, alanine transaminase
animals with CCL₄ prolonged the pentobarbital sleeping time to 219 ± 27 min, the value that is significantly higher (P < 0.01) than that of control (Table 2). However, prior treatment of animals with thymol (150 mg kg⁻¹) returned this CCL₄-induced prolongation of pentobarbital sleeping time to 132 ± 15 min, which is significantly lower than group 2 animals (P < 0.05) and close to the control sleeping time (P > 0.05).

**Effect of thymol on paracetamol-induced hepatotoxicity:**
Control (saline + vehicle) serum values of ALP, AST and ALT in rats were found to be 207 ± 15, 97 ± 13 and 48 ± 09 IU, respectively (Table 3), while toxic dose of paracetamol (640 mg kg⁻¹) raised significantly (P < 0.01) the respective serum enzyme values to 309 ± 28, 943 ± 181 and 438 ± 119. Group 3 animals were pre-treated with thymol (150 mg kg⁻¹) to determine its effect on paracetamol-induced rise in serum enzymes. The serum values of enzymes in pre-treated group were found to be 208 ± 19 (ALP), 133 ± 37 (AST) and 69 ± 21 (ALT), which were significantly lower (P < 0.01) than the values of toxic control and similar to the control values (P > 0.05).

**Effect of thymol on CCL₄-induced hepatotoxicity:**
The estimated values of serum alkaline phosphatase (ALP) and transaminases (AST and ALT) in control (saline + vehicle) group of rats were found to be 205 ± 17, 92 ± 11 and 51 ± 12 IU, respectively (Table 4), which were raised significantly (P < 0.01) to the respective values of 318 ± 32, 781 ± 169 and 449 ± 114 after administration of a toxic dose of CCL₄ (1.5 ml kg⁻¹). However, pretreatment of animals with thymol (150 mg kg⁻¹) returned the serum ALP, AST and ALT values to 211 ± 21, 119 ± 29 and 75 ± 19 IU, respectively, which are significantly lower (P < 0.05; 0.01; 0.01) than values of toxic control and were close to normal values (P > 0.05).

**Discussion**
Paracetamol and CCL₄-induced hepatic injuries are commonly used models for hepatoprotective drug screening (Plaa and Hewitt, 1982) and the extent of hepatic damage is assessed by the level of increased cytoplasmic enzymes (ALP, AST and ALT) in circulation (Sallie et al., 1991). Thymol when administered prophylactically exhibited protection against paracetamol-induced lethality in mice suggesting hepatoprotective actions. The treatment of mice with CCL₄ caused a damage to microsomal drug metabolizing enzymes in hepatocytes leading to a substantial decrease in hepatic drug metabolizing capacity, being reflected in prolongation of pentobarbital-induced sleeping time (Javatilaca et al., 1990). Whereas, pretreatment of animals with thymol prevented the CCL₄-induced prolongation in pentobarbital-sleeping time, suggesting a protective effect of thymol against CCL₄-induced damage to hepatocytes.

Paracetamol is converted to a toxic reactive intermediate called N-acetyl-p-benzoquinone imine (NAPQI) following metabolism by a number of isozymes of cytochrome P-450 (CYPs), i.e., CYP 2E1 (Tanaka et al., 2000), CYP 1A2 (Venkatakrishnan et al., 1998), CYP 2A6 (Chen et al., 1998), CYP 3A4 and CYP2D6 (Dong et al., 2000), whereas CCL₄ is activated to halogenated free radicals (HFR) by CYP 2E1 (Jeong and Park, 1998). The massive production of reactive species may lead to depletion of protective physiological molecules (glutathione and *-tocopherol, etc.), ensuing widespread propagation of the alkylolation as well as peroxidation, causing damage to the macromolecules in vital biomembranes (Aldridge, 1981). The reactive species mediated hepatotoxicity can be effectively managed upon administration of such agents possessing anti-oxidants (Attri et al., 2000), free radical scavengers (Sadanobu et al., 1999) and anti-lipid peroxidation (Lim et al., 2000) activities. Thymol treatment was able to ameliorate the paracetamol and CCL₄-induced hepatocellular damage as evidenced by prevention of any increase in serum enzymes (ALP, AST and ALT) levels subsequent to toxin exposure and the reported anti-oxidant (Aeschbach et al., 1994), free radical scavenging (Fujisawa and Kadoma, 1992) and anti-lipid peroxidation (Beach and Giroux, 1992) properties might be the contributed factor towards the observed hepatoprotection.

Inflammation plays a central role during drug-induced acute hepatitis and products of arachidonic acid metabolism have been extensively involved in inflammatory processes (Perez-Alvarez et al., 1993). Similarly, the reported anti-inflammatory (Azuma et al., 1986) and cyclooxygenase inhibitory (Anamura et al., 1988) activities of thymol may also be partly involved in the protective effect against paracetamol and CCL₄-induced hepatotoxicity observed in this study.

In conclusion thymol exhibited protection against paracetamol and CCL₄-induced liver injuries as manifested by the reduction in toxins-mediated rise in serum enzymes in rats, protection against lethal dose of paracetamol in mice and prevention of CCL₄-induce increase in pentobarbital sleeping time possibly through multiple pathways.

**References**


