Menthol Prevents Liver Damage Induced by Paracetamol and CCl₄

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Abstract: Menthol, a terpenoid from Mentha piperita, was investigated for its possible protective effect against paracetamol and CCl₄-induced hepatic damage. Paracetamol produced 100% mortality at the dose of 1 g kg⁻¹ in mice while pre-treatment of animals with menthol (50 mg kg⁻¹) reduced the death rate to 40%. 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 transaminase (AST and ALT). Pre-treatment of rats with menthol 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 menthol was able to prevent the CCl₄-induced rise in serum enzymes and prolongation in pentobarbital sleeping time. In conclusion, menthol possesses hepatoprotective activity, demanding further research evaluations to validate its future role in hepato-biliary complications.

Key words: Menthol, essential oils, Mentha piperita, hepatoprotective, paracetamol, CCl₄

Introduction
Menthol is one of the monoterpeneoids present as essential oil in Mentha piperita and other related plants (McConkey et al., 2000). It exhibits multiple biological activities including carminative (Eccles, 1994), expectorant (Renselmann et al., 1997), antibacterial (Pattnaik et al., 1997), antifungal (Pattnaik et al., 1997), anti-inflammatory (Juregina et al., 1998), anti-parasite (Green and Endo, 1992) antiasthmatic (Wright et al., 1998), hypotensive (Futami, 1984b), cytotoxic (Russin et al., 1989), local anaesthetic (Gaetti et al., 2001), immunomodulator (Siddi et al., 1991), antiplatelets (Murayama and Kumauro, 1988), calcium channel blocking (Dierksee et al., 1997) and insecticidal (Loo et al., 2001) activities. Moreover, it is also used as a counter-irritant (Futami, 1984a) and as a pharmaceutical aid to increase skin permeation to facilitate the absorption of medications through intact skin (Kaplun-Fischoff and Tourtou, 1997). In present investigation, we describe its hepatoprotective activity against paracetamol and CCl₄-induced liver damage.

Materials and Methods
The study was conducted at Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi in 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 (47 x 34 x 18 cm²), mice (10/cage) and rats (5/cage), lined with sawdust renewed every 48 hours, in air-conditioned quarters and had free access to tap water and food.

Pharmacological materials: Paracetamol, CCl₄, menthol, ketamine hydrochloride and methyl cellulose were obtained from Sigma Chemicals Company, St Louis, MO USA and olive oil (P. Sasso e Figl, Neglia, 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 menthol against a lethal dose of paracetamol (1 g kg⁻¹). Animals were divided into two groups of ten animals each. One group was treated orally with menthol (50 mg kg⁻¹) followed after 1 h by oral administration of paracetamol. The second group served as control and received the same treatment except that normal saline (0.9% NaCl) was administered instead of menthol. 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) orally, whereas control animals received an equal volume of respective vehicle (1% methyl cellulose or olive oil), as described previously. Rats were divided into 3 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 similar to group 2, except that menthol (50 mg kg⁻¹, dissolved 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 anaesthetised 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 pentobarbitral sleeping time: The effect of menthol on CCl₄-induced prolongation in pentobarbitral sleeping time was studied in mice as described elsewhere (Montilla et al., 1990; Gilani et al., 1998) (Table 2). The animals were divided into three groups of ten animals each. Group 1 animals received four doses of normal saline (10 ml kg⁻¹) orally at 12-h interval and vehicle (olive oil) was administered as bolus dose (7.5 ml kg⁻¹; orally) 1 h after the last dose of saline followed after 24 h by pentobarbital (75 mg kg⁻¹, i.p.), while animals of group 2 were given the same treatment except vehicle was replaced by CCl₄ (1.5 ml kg⁻¹). Animals in group 3 were treated similar to group 2 except that menthol (50 mg kg⁻¹) was substituted for normal saline.

Statistical analysis: The results were expressed as mean ± SEM and all statistical comparisons were made by means of the student’s t-test and P < 0.05 was regarded as significant.

Results
Effect of menthol on paracetamol-induced lethality: Paracetamol at the dose of 1 g kg⁻¹ killed all mice. In a group of animals pre-treated with menthol (50 mg kg⁻¹), the same dose of paracetamol killed only four out of ten resulting in 60% protection against the lethal effect of paracetamol (Table 1).
Table 1: Effect of Menthol on Paracetamol-induced Lethality in Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Menthol + Paracetamol (50 mg kg⁻¹ + 1 g kg⁻¹)</td>
<td>40</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + Paracetamol (10 ml kg⁻¹ + 1 g kg⁻¹)</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: Effect of menthol on CCl₄-induced prolongation in pentobarbital sleeping time in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>Sleeping time (minutes)</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>112 ± 17</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>211 ± 21*</td>
</tr>
<tr>
<td>3.</td>
<td>Menthol + CCl₄ + Pentobarbital (50 mg kg⁻¹ + 1.5 ml kg⁻¹ + 75 mg kg⁻¹)</td>
<td>127 ± 15*</td>
</tr>
</tbody>
</table>

Table 3: Effect of menthol on paracetamol-induced rise in serum enzyme levels in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>ALP</th>
<th>AST</th>
<th>ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (10 ml kg⁻¹ + 13 mg kg⁻¹)</td>
<td>211± 24</td>
<td>103± 18</td>
<td>44± 08</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + Paracetamol (10 ml kg⁻¹ + 640 mg kg⁻¹)</td>
<td>335± 31*</td>
<td>125± 298**</td>
<td>59± 147**</td>
</tr>
<tr>
<td>3.</td>
<td>Menthol + CCl₄ (50 mg kg⁻¹ + 640 mg kg⁻¹)</td>
<td>216± 20#</td>
<td>159± 31##</td>
<td>63± 13##</td>
</tr>
</tbody>
</table>

Group 3 animals received four doses of menthol (50 mg kg⁻¹) at 12-h interval before paracetamol (640 mg kg⁻¹) administration.

Table 4: Effect of menthol on CCl₄-induced rise in serum enzyme levels in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments</th>
<th>ALP</th>
<th>AST</th>
<th>ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Saline + Vehicle (10 ml kg⁻¹ + 7.5 ml kg⁻¹)</td>
<td>203± 20</td>
<td>98± 13</td>
<td>49± 14</td>
</tr>
<tr>
<td>2.</td>
<td>Saline + CCl₄ (10 ml kg⁻¹ + 1.5 ml kg⁻¹)</td>
<td>315± 27*</td>
<td>705± 186*</td>
<td>45± 121*</td>
</tr>
<tr>
<td>3.</td>
<td>Menthol + CCl₄ (50 mg kg⁻¹ + 1.5 ml kg⁻¹)</td>
<td>214± 19#</td>
<td>129± 27#</td>
<td>72± 23#</td>
</tr>
</tbody>
</table>

Values shown are mean ± SEM of 10 determinations expressed as IU. Group 3 animals received four doses of menthol (50 mg kg⁻¹) at 12-h interval before CCl₄ (1.5 ml kg⁻¹) administration. * P < 0.05; ** P < 0.01; Compared to group 1. # P < 0.05; ## P < 0.01; Compared to group 2.

Effect of menthol on CCl₄-induced prolongation in pentobarbital sleep: The effect of menthol on CCl₄-induced prolongation of pentobarbital sleeping time was studied in mice. Pentobarbital at a dose of 75 mg kg⁻¹, i.p., caused sleep in mice of control group for a period of 112 ± 17 min (mean ± SEM; n = 10). Whereas treatment of animals with CCl₄ prolonged the pentobarbital sleeping time to 211 ± 21 min, the value that is significantly higher (P < 0.01) than that of control (Table 2). However, prior treatment of animals with menthol (50 mg kg⁻¹) reduced this CCl₄-induced prolongation of pentobarbital sleeping time to 127 ± 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 menthol on paracetamol-induced hepatotoxicity: Control (saline + vehicle) serum values of ALP, AST and ALT in rats were found to be 211 ± 24, 103 ± 18 and 44 ± 08 IU respectively (Table 3), while toxic dose of paracetamol (640 mg kg⁻¹) raised significantly (P < 0.05) the respective serum enzyme values to 335 ± 31, 125 ± 298 and 59 ± 147 IU. Group 3 animals were pre-treated with menthol (50 mg kg⁻¹) to determine its effects on paracetamol-induced rise in serum enzymes. The serum enzymes in pre-treated group were found to be 216 ± 20 ALP, 159 ± 31 AST and 63 ± 13 ALT, which were significantly lower (P < 0.05) than the values of toxic control and similar to the control values (P > 0.05).

Effect of menthol on CCl₄-induced hepatotoxicity: The estimated values of serum alkaline phosphatase (ALP) and transaminase (AST and ALT) in control (saline + vehicle) group of rats were found to be 203 ± 20, 98 ± 13 and 49 ± 14 IU respectively (Table 4), which were raised significantly (P < 0.01) to the respective values of 315 ± 27, 705 ± 186 and 45 ± 121 IU after administration of a toxic dose of CCl₄ (1.5 ml kg⁻¹). However, pre-treatment of animals with menthol (50 mg kg⁻¹) reduced the serum ALP, AST and ALT values to 214 ± 19, 129 ± 27 and 72 ± 23 IU respectively, which were significantly lower (P < 0.05) 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 (Piba and Hewitt, 1982) and the extent of hepatic damage is assessed by the level of increased cytoplasmic enzymes (ALP, AST and ALT) in circulation (Sailie et al., 1991). Menthol 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 (Javatiakia et al., 1990). Whereas, pretreatment of animals with menthol prevented the CCl₄-induced prolongation in pentobarbital-sleeping time, suggesting a protective effect of menthol 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 isoforms of cytochrome P-450 (CYPs), i.e., CYP 2E1 (Tanaka et al., 2000), CYP 1A2 (Lehman, 2000), CYP 2A6 (Chen et al., 1996), CYP 3A4 (Dong et al., 2000) and CYP2D6 (Dong et al., 2000), whereas CCl₄ is activated to halogenated free radicals (HFR) by CYP 2E1 (Jeong, 1999). The massive production of reactive species may lead to depletion of protective physiological mitorides (glutathione and α-tocopherol, etc.), ensuing wide-spread propagation of the alkylation as well as peroxidation, causing damage to the macro molecules in vital biomembranes (Alldridge, 1991).

The inhibitors of CYPs are known to curtail the toxicity of paracetamol (Sunioka et al., 1998) as well as CCl₄ (Mizouka et al., 1999). Menthol 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 toxic exposure and the reported inhibitory effect of menthol on CYPs (De-Oliveira et al., 1995) might be the contributing factor towards the observed hepatoprotection. Moreover, menthol is reported to exhibit calcium channel blocking activity (Dierkes et al., 1997) and also tends to lower the intracellular Ca²⁺ by activation of Ca²⁺ sequestration mechanisms (Starling et al., 1994). Calcium content in liver cells are liable to be increased during the process of experimental liver damage (Tsuboe-Kuhn, 1989) and calcium channel blocking drugs, i.e., nifedipine and verapamil were found to inhibit the development of hepatic damage induced by different hepatotoxins including paracetamol and CCl₄ (Thibault et al., 1991). Similarly, hepatoprotective activity of menthol against paracetamol and CCl₄-induced damage reported in this study can be attributed to its calcium channel blocking activity.

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 (Greco and Endo, 1952) as well as inhibitory effect of menthol on lipooxygenase (Greco and Endo, 1952) and cyclooxygenase enzyme (Greco and Endo, 1952) might also be partly involved in the protective effect against paracetamol and CCL-induced hepatotoxicity observed in this study.

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

References


