The protective effect of *Capparis ovata* in acute hepatotoxicity induced by paracetamol

Nurcan DOĞAN¹, Mustafa AKÇAM¹,², Tuğba KOCA¹, Duygu KUMBUL DOĞUÇ², Meltem ÖZGÖÇMEN²

¹Department of Pediatrics, Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey
²Department of Biochemistry, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey

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**Background/aim:** To investigate the efficiency of *Capparis ovata* as a protective agent against acute paracetamol toxicity of the liver.

**Materials and methods:** A total of 36 Wistar albino rats were divided into 4 groups: 1) paracetamol, 2) *Capparis ovata* + paracetamol, 3) *Capparis ovata*, and 4) control. Groups 2 and 3 were given *Capparis ovata* and Groups 1 and 4 distilled water for 8 days. On day 8, 3000 mg kg⁻¹ paracetamol was administered orally to Groups 1 and 2. Samples were taken on day 9. AST, ALT, total bilirubin, direct bilirubin, GGT, and ALP levels were assessed. Lipid peroxidation markers and thiobarbituric acid-reactive substance (TBARS) levels were measured in the blood and liver. Liver tissues were evaluated histologically.

**Results:** AST, ALT, and total bilirubin levels were lower in Group 2 than in Group 1 (P < 0.05). TBARS levels were lower in Groups 2 (P = 0.000), 3 (P = 0.001), and 4 (P = 0.001) than in Group 1. Degenerative findings were lower in the *Capparis ovata* + paracetamol group than in the paracetamol group (P < 0.05).

**Conclusion:** It can be concluded that *Capparis ovata* has a protective effect on the liver, both histopathologically and biochemically, against paracetamol-induced liver injury.

**Key words:** Antioxidant, *Capparis ovata*, hepatotoxicity, paracetamol

1. Introduction

Paracetamol is in widespread use as an effective analgesic and antipyretic. The liver is a vital organ for the detoxification of toxic substances present in the body, and hepatic injury is associated with excessive exposure to toxicants. An overdose of paracetamol has a toxic effect, which causes liver injury both in experimental animals and humans. Its toxicity has been shown to be initiated by cytochrome P450 metabolism to N-acetyl-p-benzoquinone imine (NAPQI) (1). The most common cause of acute liver failure worldwide is paracetamol, which is usually a nonprescribed drug. The current available treatment with N-acetylcysteine significantly reduces liver damage in the early stages; however, clinical studies have shown that there is no effective and safe way to prevent it completely (2). There have been many studies on this subject, but none have determined a treatment that is reliable, effective, and accessible. There is a need to develop new drugs or safer options from the currently available compounds in order to provide hepatoprotection.

*Capparis ovata* belongs to the family Capparidaceae and is a long-lasting shrubby plant found throughout the Mediterranean basin. *Capparis* occurs in various types (more than 350) and grows naturally in many different regions worldwide. For centuries it has been known as a traditional herbal medicine for its diuretic, antihypertensive, hypoglycemic, antihepatotoxic, analgesic, and hypolipidemic effects (3,4). Previous chemical studies have reported that alkaloids, lipids, polyphenols, flavonoids, and glucosinolates are present in the plant. Furthermore, *Capparis* extract is reported to be rich in antioxidants such as α-tocopherol, γ-tocopherol, and sitosterol, as well as in flavonoids such as kaempferol, rutinoside quercetin, and quercetin derivatives (5).

Thiobarbituric acid-reactive substances (TBARS) are formed as a byproduct of lipid peroxidation (i.e. as degradation products of fats), which can be detected by the TBARS assay using thiobarbituric acid as a reagent. An assay of TBARS measures malondialdehyde (MDA) present in the sample, as well as malondialdehyde generated from lipid hydroperoxides by the hydrolytic conditions of the reaction.

This study was undertaken to evaluate the hepatoprotective effect of *C. ovata* on paracetamol-
induced hepatotoxicity in rats through biochemical, antioxidant, and histological assessments, as this has not been previously investigated.

2. Materials and methods

2.1. Animals
A total of 36 female Wistar albino rats weighing 145–191 g (4–5 weeks old) were used in the study. The animals were kept in a 12-h light/dark cycle and allowed free access to food and water. All experiments were performed at a room temperature of 22–24 °C. The experimental protocol of the current study was approved by the Ethics Committee of the Medical Faculty of Süleyman Demirel University. The animals were kept and treated in accordance with the Animal Welfare Act and Guide for the Care and Use of Laboratory Animals prepared by Süleyman Demirel University.

2.2. Extraction of Capparis ovata extracts
The C. ovata samples were collected from the area around Isparta, in the Mediterranean region of Turkey. Distilled water extracts of the flowers were prepared at the Forestry Industrial Engineering Department of Süleyman Demirel University, Isparta, Turkey. The total phenols were measured as 292 µg L⁻¹ and flavonoids as 170 µg L⁻¹ with a T80 + UV/VIS spectrometer (Germany). One gram of Capparis flowers was put into 50 mL of distilled water, boiled for 10 min, cooled for 15 min, and then brought to a 2% solution (2 mg/100 mL) (6), and 1.5 mL was administered orally (7).

2.3. Experimental design
Thirty-six animals were randomly divided into 4 groups as follows:

Group 1: Paracetamol group (PS, n = 10): 1.5 mL of distilled water was given orally via gastric gavage for 8 days. On day 8, 3000 mg/kg paracetamol was administered orally (7).

Group 2: C. ovata + paracetamol (CAP + PS, n = 10): 1.5 mL of C. ovata was administered orally to the animals for 8 days. On day 8, 3000 mg/kg paracetamol was administered orally.

Group 3: C. ovata (CAP, n = 8): 1.5 mL of C. ovata was given orally for 8 days.

Group 4: Control (C, n = 8): 1.5 mL of distilled water was given orally for 8 days.

2.4. Anesthesia, blood collection, and preparation of blood samples
Twenty-four hours after paracetamol administration, on day 9, the rats were anesthetized with a cocktail of ketamine hydrochloride (80 mg kg⁻¹) and xylazine (10 mg kg⁻¹) administered i.p. before the sacrifice of each rat and the withdrawal of blood samples. Blood (4–6 mL) was taken from the aorta using a sterile injector. Blood samples were centrifuged at 5000 × g for 5 min and serum was recovered. At the end of the study, the abdomen of the rats was opened under anesthesia through a midline incision, and the right lobes of the liver were removed for histologic examination.

2.5. Biochemical parameters
A Beckman Coulter Inc. (Japan) autoanalyzer was used to determine the serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transferase (GGT), total bilirubin (T. Bil.), direct bilirubin (D. Bil.), and alkaline phosphatase (ALP).

2.6. Lipid peroxidation (TBARS) determinations
TBARS was determined by the double heating method on serum and liver tissues. The method consisted of a spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBA) with MDA. The concentration of TBARS was calculated by the absorbance coefficient of MDA–TBA complex as expressed in the serum (µM/mL) and liver (µM/g tissue).

2.7. Histopathological examinations
For light microscopic evaluation, sections of each liver were fixed in 10% neutral phosphate-buffered formalin solution. Following dehydration in an ascending series of ethanol solutions (70%, 80%, 96%, 100%), tissue samples were cleaned in xylene and embedded in paraffin. The paraffin-embedded specimens were cut into 5-µm-thick sections, stained with hematoxylin and eosin (H&E), and examined using a light microscope (Olympus BX50). The liver tissues were examined by a histologist (MÖ), who was blinded to the protocol. The liver tissues were examined for connective tissue growth, hemorrhage, mononuclear cell infiltration, necrotic cells, pyknotic-core cells, apoptosis, granular degeneration, bile duct proliferation, and hygroscopic-core cells, as described by Abdel-Wahhab et al. (8). All findings were scored as (– = 0), (+ = 1), (++) = 2), or (+++ = 3).

2.8. Statistical analysis
The Kruskal–Wallis test was used to compare the biochemical data and histopathologic scores of the multiple groups. For two-group comparisons, the Mann–Whitney U test was performed and a significance level of P < 0.05 was accepted. The results were expressed as mean ± standard deviation (SD). Statistical analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Biochemical results
The effects of C. ovata on the paracetamol-induced modification on the serum AST, ALT, GGT, ALP, T. Bil, and D. Bil. levels are shown in Table 1. AST, ALT, and T. Bil. levels were lower in Group 2 (CAP + PS) than in Group 1 (PS) (P < 0.05).
3.2. Results of lipid peroxidation (TBARS)
The effects of *C. ovata* on the paracetamol-induced modification in the serum and liver tissue TBARS levels are shown in Figure 1. Liver tissues and blood TBARS levels were lower in Group 2 (CAP + PS) (*P* < 0.0001 for both), Group 3 (CAP) (*P* < 0.0001 and *P* = 0.001, respectively), and Group 4 (control) (*P* < 0.0001 and *P* = 0.001, respectively) than in Group 1 (PS).

3.3. Results of histopathological changes
The histological findings of degeneration scores are shown in Table 2. The histological score of Group 2 (CAP + PS) was lower than that of Group 1 (PS) (*P* < 0.05). The histopathological appearance of the liver tissue samples is shown in Figures 2–4.

4. Discussion
To the best of our knowledge, this is the first study to investigate *C. ovata* as protection against paracetamol-induced hepatotoxicity.

Analgesic toxicity is the leading cause of toxicity in Turkey, similarly to most developed countries (9,10). The most frequent agent is reported to be paracetamol (11). Previous studies have shown that *Capparis* species have antioxidant activity and liver protection effects. In one study, the antioxidant activity of methanolic acid extract was shown to depend on the phenol it contained (12). Germano et al. (3) reported that p-methoxybenzoic acid in *Capparis spinosa* extract prepared with methanol has a protective effect on hepatotoxicity when prepared in vivo.

### Table 1. Serum AST, ALT, GGT, ALP, and bilirubin levels.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (PS) n = 10</th>
<th>Group 2 (PS + CAP) n = 10</th>
<th>Group 3 (CAP) n = 8</th>
<th>Group 4 (C) n = 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>189.50 ± 39.70</td>
<td>108.10 ± 4.37</td>
<td>113.75 ± 5.44</td>
<td>109.00 ± 7.30</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>112.12 ± 20.09</td>
<td>64.90 ± 2.86</td>
<td>52.00 ± 2.99</td>
<td>48.12 ± 2.15</td>
</tr>
<tr>
<td>T. Bil. (mg/dL)</td>
<td>0.15 ± 0.015</td>
<td>0.10 ± 0.013</td>
<td>0.10 ± 0.009</td>
<td>0.09 ± 0.004</td>
</tr>
<tr>
<td>D. Bil. (mg/dL)</td>
<td>0.03 ± 0.005</td>
<td>0.02 ± 0.003</td>
<td>0.02 ± 0.002</td>
<td>0.03 ± 0.002</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>1.25 ± 0.16</td>
<td>1.60 ± 0.26</td>
<td>1.75 ± 0.31</td>
<td>1.62 ± 0.26</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>252.75 ± 46.99</td>
<td>226.50 ± 22.29</td>
<td>224.50 ± 22.12</td>
<td>213.37 ± 27.35</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SEM. PS: paracetamol, CAP: *Capparis ovata*, C: control.

* *P* = 0.027 versus control group; †*P* = 0.003 versus control group; ‡*P* = 0.011 versus control group; §*P* = 0.033 versus PS group; ¶*P* = 0.029 versus PS group; ‖*P* = 0.025 versus PS group; ‡‡*P* = 0.045 versus CAP group; ‡§*P* = 0.006 versus CAP group; ‡‖*P* = 0.031 versus CAP group.
with carbon tetrachloride (CCL4) and paracetamol, and in vitro with thioacetamide and galactosemia. Siracusa et al. (13) demonstrated that phenolic acid in Capparis spinosa has an antioxidant function. In a study by Bonina et al., antioxidant activity was reported due to flavonoids and hydroxycinnamic contained in Capparis (14). In addition, Baijal et al. (15) reported that Liv. 52 DS tablets containing Capparis spinosa have a liver protection effect when used against acute hepatitis. In a study by Goyal et al., Capparis decidua extracts were used and a decrease was determined in plasma triglycerides, total lipid, and phospholipid concentrations (16).

In a study on rats by Gadgoli et al. (12), the effect of Capparis spinosa was researched against liver damage induced with carbon tetrachloride (CCL4) and paracetamol. There was a statistically significant decrease in AST, ALT, ALP, and T. Bil. levels in the group that received CCL4 and Capparis compared to the group that only received CCL4. In another study on rats by Madhavan et al., the effect of Capparis spinosa was investigated against acute hepatotoxicity induced with acetaminophen. There was a statistically significant decrease in AST, ALT, ALP, T. Bil., and D. Bil. levels in the group administered acetaminophen and Capparis compared to the group that only received acetaminophen (17).

### Table 2. The histopathologic scores of groups.

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (PS)*</th>
<th>Group 2 (PS + CAP)</th>
<th>Group 3 (CAP)</th>
<th>Group 4 (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connective tissue growth</td>
<td>2.00 ± 0.53*</td>
<td>1.12 ± 0.64</td>
<td>0.37 ± 0.51</td>
<td>0</td>
</tr>
<tr>
<td>Granular degenerations</td>
<td>2.50 ± 0.75*</td>
<td>1.00 ± 0.53</td>
<td>0.37 ± 0.51</td>
<td>0</td>
</tr>
<tr>
<td>Mononuclear cell infiltration</td>
<td>2.50 ± 0.53*</td>
<td>1.12 ± 0.35</td>
<td>0.12 ± 0.35</td>
<td>0</td>
</tr>
<tr>
<td>Necrotic cells</td>
<td>2.00 ± 0.53*</td>
<td>1.00 ± 0.53</td>
<td>0.25 ± 0.46</td>
<td>0</td>
</tr>
<tr>
<td>Vascular congestion</td>
<td>2.62 ± 0.51*</td>
<td>1.00 ± 0.53</td>
<td>0.25 ± 0.46</td>
<td>0</td>
</tr>
</tbody>
</table>

PS: paracetamol, CAP: Capparis ovata, C: control. *Group 1 is compared with all groups: P < 0.05.
*P = 0.002 versus PS + CAP group; *P < 0.0001 versus PS + CAP group; *P < 0.0001 versus PS + CAP group; *P = 0.002 versus PS + CAP group; *versus PS + CAP group P = 0.000.

**Figure 2.** Histologic findings of Group 1 (PS). Connective tissue growth (thin arrow), infiltration (thick arrow), bile duct proliferation (star) (H&E, 10×).

**Figure 3.** Histologic findings of Group 2 (PS + CAP). Infiltration (thin arrow), granular degeneration (thick arrow) (H&E, 10×).
In the present study, there was a statistically significant decrease in AST, ALT, ALP, and T. Bil. levels in the group that received *C. ovata* and paracetamol compared to the group that only received paracetamol, just as in other studies conducted with other *Capparis* species.

In a study on rats by Anju Anıyathi et al., there was a significant decrease in MDA levels, which was produced by peroxidation and was controlled in the liver tissue in the group that received *Capparis brevispina* against hepatotoxicity created by paracetamol (18). In a study performed by Kumar Mishra et al., the antioxidant activity of an extract prepared from *Capparis zeylanica* and ethanol was investigated (19). Daily extracts of *Capparis zeylanica* for rats were administered as doses of 100 mg kg\(^{-1}\), 200 mg kg\(^{-1}\), and 400 mg kg\(^{-1}\) with the gastric lavage method. There was a significant decrease in serum TBARS levels, especially in the 400 mg kg\(^{-1}\) dose of extract, when compared to the control group.

In the current study, the oxidative situation to create experimental toxicity by paracetamol in the groups was evaluated by the TBARS method, both in the serum and liver tissue. The TBARS levels in the paracetamol group were high in the liver tissue and in the blood. The TBARS levels in the group that received *Capparis* and paracetamol were significantly lower compared to the paracetamol group. However, there was no statistically significant difference between the control and *Capparis* groups. The results of this study have shown that *C. ovata* has a protective effect against oxidative stress.

In a study on rats by Aghel et al., the protective effect of *Capparis spinosa* on liver tissue was researched against hepatotoxicity created by CCl\(_4\). There was less necrosis, lymphoid infiltration, and lipid change in the group that received CCl\(_4\) and *Capparis spinosa* compared to the group that only received CCl\(_4\) (20).

In a study conducted with *Capparis brevispina* extract prepared with ethanol against paracetamol-induced hepatotoxicity in rats, cellular necrosis and mononuclear infiltration were observed to be significantly less in the group that received paracetamol and *Capparis* compared to the paracetamol only group (18).

In the present study, there was a statistically significant decrease in connective tissue growth, granular degeneration, cell infiltration, and necrotic cell and vascular conventions in the paracetamol and *Capparis* group compared to the paracetamol-only group, similar to the histopathological evaluation.

In conclusion, the results of this study have demonstrated that *C. ovata* has antioxidant and hepatoprotective activities, which is consistent with other studies conducted with other species of *Capparis*. However, this study can be considered to be an important contribution to the literature, as it is the first experimental study on weaned rats in particular, which shows the activity of *C. ovata* against paracetamol toxicity for the pediatric age group.

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References


