The Effect of Tarantula cubensis Extract on Gentamicin-Induced Acute Kidney Injury in Ovariectomized Rats

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Article ID: KVFD-2021-25390    Received: 06.06.2021    Accepted: 18.09.2021   Published Online: 18.09.2021

Abstract
This study examined the effect of Tarantula cubensis extract (TCE) on gentamicin-induced acute kidney injury in ovariectomized rats. A total of 40 female Wistar albino rats were randomly divided equally into five groups: Control (C), ovariectomy (O), Tarantula cubensis (OT), gentamicin (OG), and gentamicin + Tarantula cubensis (OGT). All rats except those in the C were ovariectomized. Kidney damage was created with gentamicin for OG and OGT. The OT and OGT were treated with a single dose of TCE. Blood, sera, and kidney tissue were taken at necropsy for evaluation. Total leukocyte count was higher in the OT compared to the others (P=0.002). Significant increases were also determined in serum urea, creatinine, aspartate aminotransferase, and total protein levels in the OT, OG, and OGT compared to the C and O. The glutathione level was low in the serum and kidney tissue of the OG, and the malondialdehyde level was high compared to the others (P<0.05). As a result of the use of TCE in gentamicin-induced acute kidney injury in ovariectomized rats, serum creatinine, urea, and malondialdehyde levels decreased in the OGT compared to the OG, the glutathione level increased, and the severity of histopathological findings decreased to milder levels. As a result; single dose of TCE partially reduced kidney damage in rats with gentamicin-induced acute kidney injury.

Keywords: Acute kidney injury, Gentamicin, Rat, Tarantula cubensis

Ovariektomili Ratlarda Gentamisin İle İndüklenen Akut Börek Hasarına Tarantula cubensis Ekstraktinin Etkisi

Öz
Bu çalışmada ovariektomili ratlarda gentamisinle indicteden akut böbrek hasarına Tarantula cubensis ekstraktinin (TCE) etkininin araştırılması amaçlandı. Çalışmada Wistar albino, dişi, yetişkin, 40 rat kullanıldı. Randomize şekilde her grupta 8 rat olacak şekilde 5 grubu ayrıldı. Gruplar: Kontrollü (K), ovariektomi grubu (O), Tarantula cubensis grubu (OT), gentamisin grubu (OG), gentamisin + Tarantula cubensis grubu (OGT) olarak belirlendi. K dışındaki tüm ratlara overektomi operasyonu yapıldı. OG ve OGT'de gentamisin ile böbrek hasarı oluşturuldu. OT ve OGT'de teko TCE ile tedavi uygulandı. Degerlendirme için ötenazi sayıdı, serum ve böbrek dokusu alındı. Total lökosit sayısının OT ve OGT'de diğer gruplara oranla yüksek bulunması (P=0.002). Serum biyokimyasal parametrelerinde, OG ve OGT'de serum üre, kreatinin, aspartat aminotransferaz ve total protein seviyelerinde önemli artışlar belirlendi. OG'de serum ve böbrek dokusunda glutatyon seviyesi düşüktü, malondaldehit seviyesi diğer gruplara kıyasla yüksek bulundu (P<0.05). Ovariektomili ratlarda gentamisinle indüklenen akut böbrek hasarına TCE kullanıldığı sonucunda, OG'de, OGT'ye kıyasla serum kreatinin, üre ve malondaldehit seviyelerinin düştüğü, glutatyon seviyesinin yükseltiği ve histopatolojik bulguların şiddetinin daha hafif düzeylerde indiği görüldü. Sonuç olarak gentamisinle indüklenen akut böbrek hasarında TCE tek doz uygulaması böbrek etkisi hisseden azalttı.

Anahtar sözcükler: Akut böbrek hasar, Gentamisin, Rat, Tarantula cubensis

INTRODUCTION
Antibiotics are classified as killing bacteria or preventing their growth. Aminoglycosides act by killing bacteria. Antibiotics exert their negative effects on bacteria in different ways. Aminoglycosides show their effect on bacteria by inhibiting protein synthesis. This narrow-spectrum antibiotic group is especially effective against...
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Material and Methods

Ethical Statement

Upon the approval of the Local Ethics Committee of Animal Experiments of Kafkas University (KAU-HADYEK/2020-100), this study was conducted in the Department of Internal Medicine of the Faculty of Veterinary Medicine, Kars, Turkey.

Animals

Wistar albino rats were obtained from Kafkas University Experimental Animals Application and Research Center, Kars, Turkey. Daily rat pellet feed and water consumption of rats kept in standard cages were tracked. During the study, all rats were provided with a relative humidity of 40%-60%, optimal room temperature (22°C), and 12 h of light and 12 h of darkness. A total of 40 female rats weighing 255-300 g were utilized for the study. Following a 15-day adaptation period, the rats were randomly divided into five groups: Control (C), ovariectomy (O), Tarantula cubensis (OT), gentamicin (OG), and gentamicin + Tarantula cubensis (OGT), each having eight rats.

Methods

Ovariectomy procedures were performed on all rats, except for those in the control group (C). The rats were anesthetized with a combination of 5-10 mg/kg/IP xylazine HCl (Rompun®2%-Bayer) and 35-50 mg/kg/IP ketamine HCl (Ketalar®-Pfizer) for the operation. The median line area was shaved and cleaned, and the skin, muscle layers, and peritoneum were incised to reach the ovaries. The right and left ovaries and suspensory ligaments and vessels were ligated with 3-0 polyglactin 910 (Vicryl® Ethicon) and removed. The peritoneum and muscles were closed with simple continuous sutures and the skin with simple interrupted sutures. Enrofloxacin (Baytril 10%®, Bayer, Germany) was injected intramuscularly at a dose of 10 mg/kg for four days postoperatively. The incision areas of the rats were checked daily for peritonitis and inflammation [22].
Once the rats were determined to be healthy 15 days after the operation, kidney damage (acute tubular necrosis) was created with gentamicin, and TCE (Theranekron D6®, Richter Pharma, Austria) was applied to the experimental groups.

Group (C) did not undergo an ovariectomy or any other application. All rats in the other four groups were ovariectomized, and group (O) did not receive any additional application. Group (OT) received a single dose (0.3 mg/kg/SC) of TCE. Group (OG) was administered gentamicin (80 mg/kg/IP) once a day for one week to induce acute kidney injury [23]. Group (OGT) was administered gentamicin (80 mg/kg/IP) for one week and a single dose of TCE (0.3 mg/kg/SC).

**Blood and Tissue Samples Taken**

All rats were euthanized (cervical dislocation) under the general anesthesia at the end of the study, and blood samples were taken from the heart into serum tubes with gel (BD Vacutainer®, BD, UK) and tubes with K$_2$EDTA (BD Vacutainer®, BD, UK). Blood samples taken for serum were kept at room temperature for about one hour and centrifuged at 3000 rpm for ten min (Hettich Rotina 380R®, Hettich, Germany). Kidney tissue samples taken for biochemical analysis were homogenized in phosphate buffer, and homogenates were removed. All samples were stored at -20°C until analysis.

**Biochemical and Hematological Analyses**

Blood samples in K$_2$EDTA were assessed for total leukocyte count (WBC x 10$^9$/µL) and other hematological parameters using a complete blood count device (VG-MS4e®, Melet Schloesing, France). Serum urea, creatinine, total protein (TP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured with a fully automatic biochemistry device (Mindray BS120®, Mindray Medical Technology Istanbul, Turkey). Measurement of malondialdehyde from serum and tissue homogenates was carried out according to the Yoshioka et al. procedure [24].

**Statistical Analyses**

Data were given as mean ± standard error of mean (SEM). The groups were not in accordance with the normal distribution according to the histogram, Q-Q method and Shapiro-Wilk test. The Kruskal-Wallis H test was used for multiple comparisons of the groups, and the Mann-Whitney U test was used for pairwise comparisons. Adjusted P values were taken into account by applying Bonferroni correction to the P value obtained after the Mann-Whitney U test. SPSS (SPSS Version 23.0®, Chicago, IL, USA) program was used for all statistical analyses. The differences between the groups in terms of the parameters examined were considered significant at the P<0.05 level.

**RESULTS**

**Hematological and Biochemical Evaluation**

The total leukocyte count was higher in the OT group compared to the other groups (P=0.002). Regarding other
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Hematological parameters, statistical significance was found between the groups in terms of lymphocytes, granulocytes, monocytes, erythrocytes, hematocrit percentage, hemoglobin, and platelet counts (P<0.05) (Table 1). Regarding serum biochemical parameters, significant increases were determined in serum urea, creatinine, aspartate aminotransferase, and TP levels in the OT, OG, and OGT groups compared to the C and O groups (P<0.05) (Table 2). The OG group had the lowest glutathione level in serum and kidney tissue and the highest malondialdehyde level (P<0.05) (Table 2).

Histopathological Evaluation

Hematoxylin-eosin staining was performed to evaluate the morphological changes in kidney tissues caused by TCE in rats with nephrotoxicity induced by gentamicin (Fig. 1). Kidney tissues of group C had a normal appearance, and group O was similar to group C. The glomerulus and Bowman’s space were normal in the OT group; however, different from group O, mild glomerular segmentation was observed. Swelling and cytoplasmic vacuoles were observed in the proximal tubule cells in the OG group, with

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**Table 1. Hematological data in the study according to groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups (Mean±SEM)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>O</td>
<td>OT</td>
</tr>
<tr>
<td>Total leukocytes count (x10^3/µL)</td>
<td>5.52±0.35a</td>
<td>4.87±0.71a</td>
</tr>
<tr>
<td>Lymphocytes count (x10^3/µL)</td>
<td>5.20±0.31</td>
<td>4.20±0.63</td>
</tr>
<tr>
<td>Monocytes count (x10^3/µL)</td>
<td>0.13±0.02a</td>
<td>0.17±0.04a</td>
</tr>
<tr>
<td>Granulocytes count (x10^3/µL)</td>
<td>0.19±0.04a</td>
<td>0.51±0.13ac</td>
</tr>
<tr>
<td>Red blood cell count (x10^12/µL)</td>
<td>7.11±0.18a</td>
<td>7.29±0.24a</td>
</tr>
<tr>
<td>Mean red cell volume (µL)</td>
<td>70.91±0.66a</td>
<td>71.55±0.92a</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>50.36±1.24a</td>
<td>52.03±1.49a</td>
</tr>
<tr>
<td>Mean platelet volume (fL)</td>
<td>16.40±0.40a</td>
<td>17.43±0.37a</td>
</tr>
<tr>
<td>Platelet count (x10^3/µL)</td>
<td>1049.88±27.29</td>
<td>2268.75±483.80</td>
</tr>
<tr>
<td>Platelets (%)</td>
<td>5.16±0.04</td>
<td>5.23±0.13</td>
</tr>
<tr>
<td>Platelet distribution width (fL)</td>
<td>7.20±0.17a</td>
<td>7.23±0.23a</td>
</tr>
</tbody>
</table>

C: Control group, O: Ovariectomized group, OT: Tarantula cubensis group, OG: Gentamicin group, OGT: Gentamicin + Tarantula cubensis group, SEM: Standard error of mean

*a-c* The mean values with different letters in the same line represent the difference between groups. P<0.05: Expresses statistical significance

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**Table 2. Biochemical parameters in serum and kidney tissue according to groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups (Mean±SEM)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>O</td>
<td>OT</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/L)</td>
<td>51.11±4.02</td>
<td>47.13±3.33</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/L)</td>
<td>134.62±8.54a</td>
<td>137.03±6.81a</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>56.57±5.98ab</td>
<td>57.36±7.06a</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.66±0.11a</td>
<td>0.84±0.09a</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>7.06±0.19a</td>
<td>7.15±0.13ab</td>
</tr>
<tr>
<td>Glutathione (µmol/mL)</td>
<td>1.42±0.11a</td>
<td>1.31±0.09ab</td>
</tr>
<tr>
<td>Malondialdehyde (nmol/mL)</td>
<td>1.43±0.06a</td>
<td>1.46±0.05a</td>
</tr>
<tr>
<td>Kidney tissue glutathione (umol/g)</td>
<td>7.90±0.12a</td>
<td>8.15±0.26a</td>
</tr>
<tr>
<td>Kidney tissue malondialdehyde (nmol/g)</td>
<td>9.51±0.34a</td>
<td>9.69±0.49a</td>
</tr>
</tbody>
</table>

C: Control group, O: Ovariectomized group, OT: Tarantula cubensis group, OG: Gentamicin group, OGT: Gentamicin + Tarantula cubensis group. SEM: Standard error of mean. *a-c* The mean values with different letters in the same line represent the difference between groups. P<0.05: Expresses statistical significance
some areas showing cytoplasm degeneration. Compared to groups C and O, dilatation in Bowman’s space, atrophy of glomeruli, and segmentation stood out. The OG group exhibited many cells in the tubule lumen, indicative of acute tubular necrosis. In addition, the hyaline cylinder increase in the proximal tubule lumen was remarkable. Comparison of the OGT group and OG group showed dilatation in Bowman’s space, cells in the tubule lumen indicative of acute tubular necrosis, and the proportion of hyaline cylinders in the proximal tubule lumen were decreased. Glomerular segmentation was at the same level. Score of histological damage in the kidney tissue give in Table 3. It was observed that apoptosis was induced as a result of nephrotoxicity caused by gentamicin in the OG

Table 3. Score of histological damage in the kidney tissue

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>O</th>
<th>OG</th>
<th>OT</th>
<th>OGT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilatation in Bowman space</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Glomerular segmenting</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Cells in tubules lumen</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cytoplasmic vacuole in tubules cells</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hyaline cylinders in tubules lumen</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Sections were evaluated according to histopathological findings as none (-), mild (+), moderate (++) and severe (+++). C: Control group, O: Ovariectomized group, OT: Tarantula cubensis group, OG: Gentamicin group, OGT: Gentamicin + Tarantula cubensis group.
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and OGT groups (Fig. 2). The H-score of BAX and caspase-9 according to the groups was statistically significant (P<0.05) (Fig. 3 and Fig. 4).

**DISCUSSION**

Changes in hematological and serum biochemical parameters provide physicians important information related to the severity of diseases, the effectiveness of treatment, metabolic events, and organ functions [27,28]. A study conducted on rats showed similar results in terms of hematological parameters with our OG group, especially in WBC. We believe that the higher WBC level in the OT group compared to the other groups is due to the regenerative properties of TCE on the tissue.

Gentamicin administered at high doses to induce nephrotoxicity enters the cytoplasm by disrupting the cell membrane. Gentamicin in the cytosol activates the intrinsic apoptosis pathway by affecting mitochondria [29], thus increasing Bax [29,30] expression. Bax, in turn, increases cytochrome-c production, which increases apoptosis. Some previous studies showed that cytochrome-c activates the caspase pathway [31]. The current study showed that gentamicin triggered apoptosis as a result of nephrotoxicity, which is consistent with the literature.

One of the most important side effects of gentamicin is nephrotoxicity. Certain increases in serum creatinine indicate nephrotoxicity, which in general, is directly proportional to the dose and duration of administration [32,33]. A study conducted on rats found increased serum urea and creatinine levels in a gentamicin group compared to a control group [34]. Similar results in the OG and OGT groups in the current study show that nephrotoxicity occurred. Another study determined that TCE had a protective effect on nephrotoxicity and decreased urea and creatinine concentrations [17]. Consistent with the literature, the current study determined that serum creatinine and urea concentrations were lower in the OGT and OG groups, which showed that TCE partially reduced acute kidney injury. We reason this result is due to the antiphlogistic and regenerative properties of TCE as well as its curative effect on necrotic tissues. A study conducted on rats reported that the malondialdehyde level in a gentamicin group was higher than in control and TCE groups [17]. Glutathione in tissues is intended to protect against oxidative damage, and malondialdehyde is widely used to...
and therapeutic effect of TCE will be enhanced when and therapeutic effect. We believe that the protective dose of TCE was injected.

Histopathological comparison of the OGT tubule lumen decreased. However, cell desquamation and cytoplasm, and the proportion of hyaline cylinders in the OG group. With the addition of TCE, it was observed that oxidative stress [17,18,35]. Our results suggested that measure oxidative stress [17,18,35]. Our results suggested that cell desquamation and and glomerular atrophy, hyaline cylinders in tubule lumens [37], tubular vacuolization, cell desquamation in tubule lumens [38], and glomerular segmentation [39] were observed in the OG group. With the addition of TCE, it was observed that Bowman’s space dilatation, vacuoles in the tubular cell cytoplasm, and the proportion of hyaline cylinders in the tubule lumen decreased. However, cell desquamation and the rate of glomerular segmentation in the tubule lumen did not change. Histopathological comparison of the OG and OG groups showed only slightly different results, which is thought to be due to the fact that only a single dose of TCE was injected.

Based on our results, we determined that the single-dose administration of TCE provided a partial protective and therapeutic effect. We believe that the protective and therapeutic effect of TCE will be enhanced when administered repeatedly. Additional studies are needed to confirm this assumption.

Available Data and Materials
The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Funding Support
There is no funding source.

Conflict of Interest
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

Author Contributions
EA, MM and MK conceived and supervised the study, EA and MK collected and analyzed data. EA and MM made laboratory measurements. PB applied the histopathological examination of the study. All authors contributed to the critical revision of the manuscript and have read and approved the final version.

References


