Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org

Kafkas Univ Vet Fak Derg
27 (5): 559-566, 2021
DOI: 10.9775/kvfd.2021.25390

RESEARCH ARTICLE

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 Böbrek Hasarına *Tarantula cubensis* Ekstraktının Etkisi

Öz

Bu çalışmada ovariektomili ratlarda gentamisinle indüklenen akut böbrek hasarına *Tarantula cubensis* ekstraktının (TCE) etkisinin araştırılması amaçlandı. Çalışmada Wistar albino, dişi, yetişkin, 40 rat kullanıldı. Randomize şekilde her grupta 8 rat olacak şekilde 5 gruba ayrıldı. Gruplar; Kontrol grubu (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 tek doz TCE ile tedavi uygulandı. Değerlendirme için ötenazi sonrası kan, serum ve böbrek dokusu alındı. Total lökosit sayısı OT'de diğer gruplara oranla yüksek bulundu (P=0.002). Serum biyokimyasal parametrelerde, K ve O'ya kıyasla OT, OG ve OGT'de serum üre, kreatinin, aspartat aminotransferaz ve total protein seviyelerinde önemli artışlar belirlendi. OG'de serum ve böbrek dokusunda glutatyon seviyesi düşük, malondialdehit seviyesi diğer gruplara kıyasla yüksek bulundu (P<0.05). Ovariektomili ratlarda gentamisinle indüklenen akut böbrek hasarında TCE kullanımı sonucunda, OGT'de, OG'ye kıyasla serum kreatinin, üre ve malondialdehit seviyesinin düştüğü, glutatyon seviyesinin yükseldiği ve histopatolojik bulguların şiddetinin daha hafif düzeylere indiği görüldü. Sonuç olarak gentamisinle indüklenen akut böbrek hasarında TCE tek doz uygulanması böbrekteki hasarı kısmen azalttı.

Anahtar sözcükler: *Akut böbrek hasarı, 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

How to cite this article?

Akyüz E, Makav M, Kuru M, Bayram P: The effect of *Tarantula cubensis* extract on gentamicin-induced acute kidney injury in ovariectomized rats. *Kafkas Univ Vet Fak Derg,* 27 (5): 559-566, 2021.
DOI: 10.9775/kvfd.2021.25390

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Gram-negative bacteria. Although useful in many diseases, these drugs are also associated with some adverse side effects, including ototoxic, nephrotoxic, neurotoxic, and teratogenic effects. Even at normal treatment doses, they can cause kidney function losses when used for more than five days. Because of such side effects, antibiotics of the aminoglycoside group should not be the primary treatment choice. Especially in patients with renal dysfunction, they should be used with caution [1]. Medications such as aminoglycoside antibiotics, anti-inflammatory drugs, and those used for neoplastic conditions can have toxic effects on organs such as the kidneys [2-4]. These drugs can cause renal damage at high rates, even when used at standard treatment doses. Therapeutic doses of gentamicin may reportedly lead to acute renal failure in approximately 20% of patients [5-7]. The cause of kidney damage due to gentamicin has not been fully elucidated; however, some theories have been postulated. One such theory supported with experimental studies asserts that the negative effects of free oxygen radicals may play a role [8-10]. Different doses and application methods have been used to induce kidney damage in rats in experimental studies [9,10]. Gentamicin, which can cause loss of kidney function even with normal doses, is known to produce rapid effects at high doses. Nephrotoxicity has been induced by administering gentamicin intraperitoneally at a dose of 100 mg/kg/day for eight days [10].

Homeopathy originated as a form of treatment based on the principle that like things are treated with like, meaning that while something can cause disease symptoms when used in high doses, it can treat the same disease with minimal doses [11,12]. Supportive treatment is needed in some diseases in the field of veterinary medicine. One such application is *Tarantula cubensis* extract (TCE), a homeopathic product obtained from *T. cubensis* spiders used as supportive therapy [13]. Primarily used in veterinary medicine, it is prescribed to relieve edema, treat traumatic-necrotic disorders, infectious diseases, rapid epithelization, and some types of cancer [14,15]. Its effectiveness in different diseases in various animal breeds has been reported [13-17]. TCE is certified as a homeopathic product in veterinary medicine and available for use in some target species. Due to its antiphlogistic and regenerative properties, it is used in inflammatory diseases, ulcers and purulent lesions [13].

Glutathione in tissues occurs as a result of the peroxidation of fatty acids. It is intended to protect against oxidative damage, and the intracellular concentrations are very high. It was reported that the resistance of the proximal tubule to oxidative damage compared to the medullary parts is due to high glutathione levels [17,18].

Malondialdehyde is widely used in the measurement of oxidative stress. It can be detected in the blood as well as measured in the urine. It correlates well with the degree of lipid peroxidation since there is no specific indicator for the oxidation of fatty acids [17].

The kidneys are under the influence of sex hormones and factors. Estrogens have a positive role in the progression of some kidney diseases. Estrogen has more regenerative and immune system enhancing effects. In addition, estrogens have nephroprotective effects [19]. It has been reported that sex hormones have important effects on kidney damage [20,21]. During the cycle in rats, changes will occur in estrogen, progesterone and some hormone levels. Therefore, rats were ovariectomized to more accurately assess the effect of TCE on gentamicin-induced kidney damage and to minimize the effects of sex hormones.

This study aimed to examine the effects of TCE on gentamicin-induced kidney injury in ovariectomized rats by evaluating glutathione and malondialdehyde levels as well as hematological and histopatological parameters.

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 (22ºC), 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_2EDTA (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 -20ºC until analysis.

Biochemical and Hematological Analyses

Blood samples in K_2EDTA were assessed for total leukocyte count (WBC x10^3/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.[24] procedure and glutathione measurement according to the Beutler et al.[25] procedure. A spectrophotometric microplate reader device (Spectramax Plus®, Marshall Scientific, Product Code: MD-SMP, USA) was used to obtain the data.

Histopathological Procedure

Histopathological analyses were performed in the Histology and Embryology Laboratories, Faculty of Medicine, Kafkas University, Turkey. The kidney tissues were fixed with 10% buffered formalin for 72 h, washed under running water for 15-20 min to remove formalin, and then dehydrated by passing through graded alcohol (75%, 96%, 100%). After clearing the tissue with xylene and paraffin infiltration, paraffin blocks were prepared. Sections of 5-µm thickness were stained with hematoxylin-eosin (Sigma-Aldrich®, Merck, Germany). Micrographs were taken using a light microscope with a DP21 camera system (Olympus BX43®, Japan) to evaluate morphological alterations. Sections were graded according to histopathological findings as none (-), mild (+), moderate (++), or severe (+++).

Immunohistochemical Analysis

After deparaffinization with xylene and rehydration with graded alcohols (100%, 96%, 75%) for two min, sections were boiled with 10mM citrate buffer for ten min in a microwave for antigen retrieval. The sections were lined with a hydrophobic pen and washed with 0.1M phosphate buffer sodium (PBS). To block the endogenous peroxidase activity, they were incubated with 3% hydrogen peroxidase solution for five min at room temperature (RT), washed with PBS, and incubated with blocking solution for ten min at RT. After removing the blocking solution, the sections were incubated with anti-BAX (FNab000810, 1:100 dilution) and anti-Caspase9 (FNab01295, 1:100 dilution) primary antibody at +4ºC overnight. The kidney sections were washed with PBS and incubated with secondary antibodies for ten min at RT. Prior to horseradish peroxidase (HRP) polymer incubation for ten min at RT, blocks were washed with PBS. The sections were incubated with 3,3-diaminobenzidine (DAB) solution for two min, and the DAP reaction was stopped with distilled water. Harris hematoxylin was used as a counterstain. Sections were washed with distilled water and dehydrated with graded alcohols (75%, 96%, 100%) for two min and mounted with Entellan®-A Thermo Scientific™ UltraVision™ Quanto Detection System HRP DAB kit (Thermo Scientific™ TL-060-QHD) was used for hydrogen peroxide, blocking solution, secondary antibody, and HRP polymer.

For immunohistochemical analysis, five different areas from each slide were photographed under 20× magnifications. H-SCORE rates were calculated with the H-SCORE=Σ Pi (i+1) formula [micrographs staining densities (i), pixel ratios (Pi)] [26].

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 graph 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

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

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

Parameters	Groups (Mean±SEM)					P Value
	C	O	OT	OG	OGT	
Total leukocytes count (x10³/µL)	5.52±0.35^{a}	4.87±0.71^{a}	10.27±1.16^{b}	5.87±0.74^{ab}	6.88±0.53^{ab}	0.002
Lymphocytes count (x10³/µL)	5.20±0.31	4.20±0.63	7.68±1.06	3.99±0.70	4.97±0.65	>0.05
Monocytes count (x10³/µL)	0.13±0.02^{a}	0.17±0.04^{a}	0.66±0.09^{b}	0.48±0.05^{b}	0.49±0.05^{b}	<0.001
Granulocytes count (x10³/µL)	0.19±0.04^{a}	0.51±0.13^{ac}	1.93±0.06^{b}	1.39±0.06^{bc}	1.42±0.22^{bc}	<0.001
Red blood cell count (x10⁶/µL)	7.11±0.18^{a}	7.29±0.24^{a}	12.58±2.34^{b}	7.17±0.11^{a}	7.52±0.13^{a}	0.038
Mean red cell volume (fL)	70.91±0.66^{a}	71.55±0.92^{a}	66.60±1.25^{ab}	64.21±0.55^{b}	62.60±1.21^{b}	<0.001
Hematocrit (%)	50.361.24^{a}	52.03±1.49^{a}	70.85±8.78^{b}	45.94±0.88^{a}	46.86±0.90^{a}	0.004
Mean erythrocyte hemoglobin (pg)	23.04±0.30^{a}	23.93±0.38^{ab}	19.38±2.03^{a}	24.69±0.18^{b}	23.41±0.20^{ab}	0.011
Mean hemoglobin volume (g/dL)	32.51±0.16^{a}	33.53±0.67^{a}	31.88±2.31^{a}	38.51±0.11^{b}	37.55±0.67^{b}	<0.001
Erythrocyte distribution width (fL)	10.45±0.44^{ab}	11.73±0.17^{a}	9.68±0.25^{b}	9.89±0.17^{b}	9.55±0.26^{b}	0.001
Hemoglobin (g/dL)	16.40±0.40^{a}	17.43±0.37^{a}	21.30±1.48^{b}	17.71±0.36^{a}	17.59±0.24^{a}	0.001
Platelet count (x10³/µL)	1049.88±27.29	2268.75±483.80	4477.00±1378.70	872.13±76.90	1097.38±114.47	>0.05
Mean platelet volume (fL)	5.16±0.04	5.23±0.13	5.30±0.06	5.21±0.05	5.15±0.04	>0.05
Platelets (%)	0.54±0.02^{ab}	1.14±0.23^{ab}	2.42±0.75^{a}	0.45±0.04^{b}	0.56±0.06^{ab}	0.017
Platelet distribution width (fL)	7.20±0.17^{a}	7.23±0.23^{a}	7.95±0.23^{ab}	8.55±0.23^{b}	8.21±0.09^{b}	0.007

C: *Control group,* ***O:*** *Ovariectomized group,* ***OT:*** *Tarantula cubensis group,* ***OG:*** *Gentamicin group,* ***OGT:*** *Gentamicin + Tarantula cubensis group,* ***SEM:*** *Standard error of mean*
$^{a\text{-}c}$ *The mean values with different letters in the same line represent the difference between groups. P<0.05: Expresses statistical significance*

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

Parameters	Groups (Mean±SEM)					P Value
	C	O	OT	OG	OGT	
Alanine aminotransferase (U/L)	51.11±4.02	47.13±3.33	50.51±5.53	62.03±5.80	54.74±2.40	>0.05
Aspartate aminotransferase (U/L)	134.62±8.54^{a}	137.03±6.81^{a}	141.49±5.37^{a}	177.84±15.64^{b}	195.51±6.73^{b}	<0.001
Urea (mg/dL)	56.57±5.98^{a}	57.36±7.06^{a}	68.74±3.23^{ab}	94.38±16.02^{b}	79.56±7.62^{ab}	0.017
Creatinine (mg/dL)	0.66±0.11^{a}	0.84±0.09^{a}	0.93±0.06^{a}	2.53±0.08^{b}	2.25±0.23^{b}	<0.001
Total protein (g/dL)	7.06±0.19^{a}	7.15±0.13^{ab}	7.70±0.15^{bc}	8.08±0.19^{c}	7.73±0.11^{bc}	<0.001
Glutathione (umol/mL)	1.42±0.11^{ab}	1.31±0.09^{ab}	1.52±0.12^{b}	1.09±0.05^{a}	1.12±0.06^{a}	0.007
Malondialdehyde (nmol/mL)	1.43±0.06^{a}	1.46±0.05^{a}	1.51±0.14^{a}	2.15±0.11^{b}	2.02±0.12^{b}	<0.001
Kidney tissue glutathione (umol/g)	7.90±0.12^{a}	8.15±0.26^{a}	9.33±0.50^{a}	7.62±0.25^{a}	11.89±0.81^{b}	<0.001
Kidney tissue malondialdehyde (nmol/g)	9.51±0.34^{a}	9.69±0.49^{a}	10.58±0.40^{ab}	13.51±0.56^{c}	12.25±0.45^{bc}	<0.001

C: *Control group,* ***O:*** *Ovariectomize group,* ***OT:*** *Tarantula cubensis group,* ***OG:*** *Gentamicin group,* ***OGT:*** *Gentamicin + Tarantula cubensis group.* ***SEM:*** *Standard error of mean.* $^{a\text{-}c}$ *The mean values with different letters in the same line represent the difference between groups. P<0.05: Expresses statistical significance*

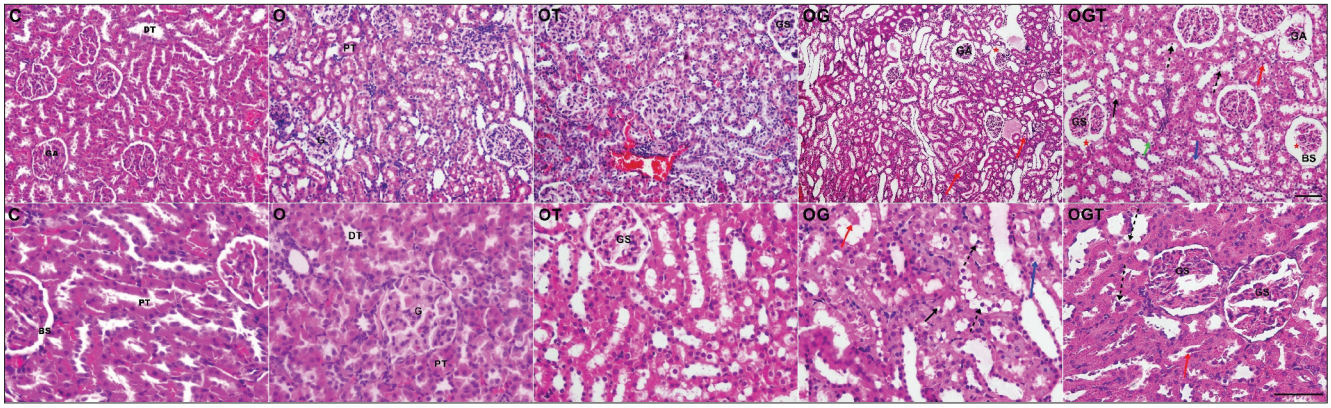


Fig 1. Photomicrographs showing the histopathological changes of hematoxylin-eosin stained rat kidney (H&E 20X and 40X). Kidney tissues of group C and group O had a normal appearance and the OT group had mild glomerular segmentation (GS). In the OG and OGT groups, it was observed cytoplasmic vacuoles *(black arrow),* glomerular atrophy (GA), the dilatation of Bowman's space *(star),* hyaline cylinders *(red arrow)* in tubule lumen, cytoplasmic degeneration *(blue arrow),* cell in proximal tubule lumen *(black dashed arrow)* and enlarged cells *(green arrow);* although there was variation in their density. G: Glomeruli, DT: Distal tubule, PT: Proximal tubule, C: Control group, O: Ovariectomized group, OT: *Tarantula cubensis* group, OG: Gentamicin group, OGT: Gentamicin + *Tarantula cubensis* group. Bar (top row): H&E X20, Bar (bottom row): H&E X40

Table 3. *Score of histological damage in the kidney tissue*

Parameters	**C**	**O**	**OG**	**OT**	**OGT**
Dilatation in Bowman space	-	-	+++	-	++
Glomerular segmenting	-	-	+++	+	+++
Cells in tubules lumen	-	-	++	-	++
Cytoplasmic vacuole in tubules cells	-	-	+++	+	++
Hyaline cylinders in tubules lumen	-	-	++	-	+

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*

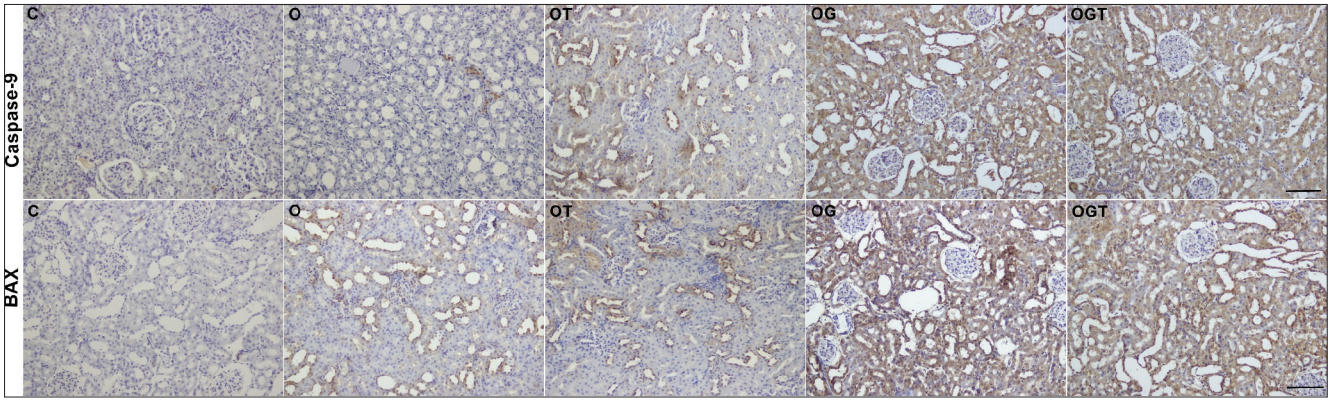


Fig 2. Photomicrographs showing immunohistochemical labeling with Bax and Caspase9 primary antibody in rat kidney (20X magnification). Brown staining indicates positive immune-reactivity. Caspase-9 and Bax proteins are secreted when cells are prone to apoptosis. Positive immune-reactivity areas indicate the susceptibility of cells to apoptosis. Compared to the other groups, the OG and OGT groups have more apoptotic cells rates because of positive immune-reactivity. C: Control group, O: Ovariectomized group, OT: Tarantula cubensis group, OG: Gentamicin group, OGT: Gentamicin + Tarantula cubensis group

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

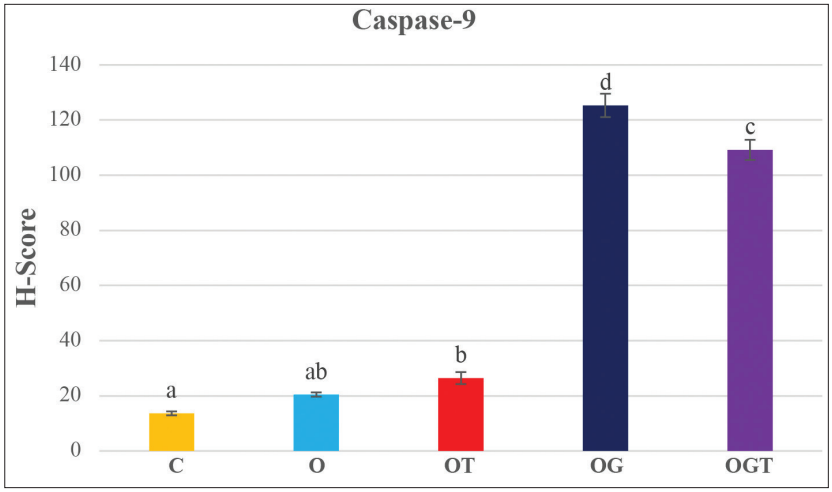
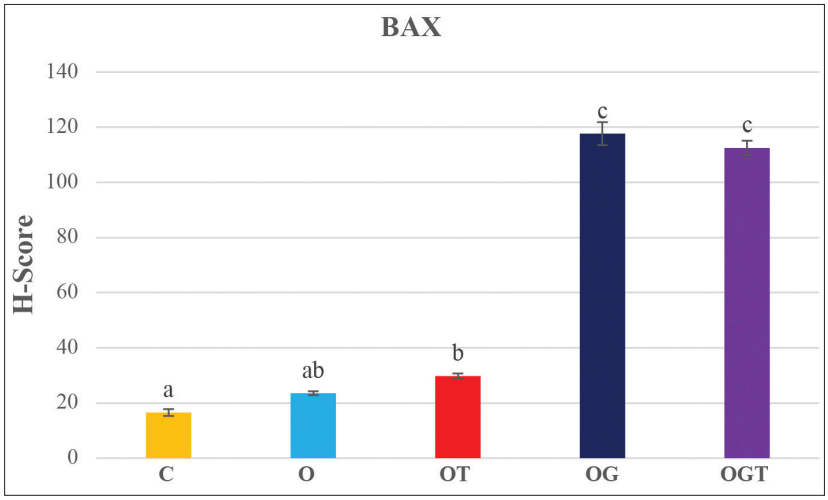


Fig 3. H-score of Caspase-9 by groups. **C:** Control group, **O:** Ovariectomized group, **OT:** *Tarantula cubensis* group, **OG:** Gentamicin group, **OGT:** Gentamicin + *Tarantula cubensis* group. [a-c] Different letters represent the difference between groups. P<0.05: Expresses statistical significance

Fig 4. H-score of BAX by groups. **C:** Control group, **O:** Ovariectomized group, **OT:** *Tarantula cubensis* group, **OG:** Gentamicin group, **OGT:** Gentamicin + *Tarantula cubensis* group. [a-c] Different letters represent the difference between groups. P<0.05: Expresses statistical significance



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

measure oxidative stress [17,18,35]. Our results suggested that an increased oxidative stress load led to low glutathione and high malondialdehyde levels in the OG group, which is also in line with the literature. Histopathological changes in the kidney tissues of rats with gentamicin-induced nephrotoxicity were also in line with the results of previous studies [34,36]. Similar to the literature, 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 OGT 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.

Availability of 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.

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