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Research Article

The Investigation Protective Effect of *Tarantula Cubensis* Extract in Rats Induced Experimental Gentamicin Nephrotoxicity

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ABSTRACT

Gentamicin (GM), which is used in the treatment of infections caused by gram-negative bacteria, has limited clinical use due to its serious nephrotoxic side effects. *Tarantula cubensis* extract (TCE) is a homeopathic product that is widely used and proven to be effective in veterinary medicine to benefit from its regeneration, demarcation, antiphlogistic and resorptive effects. The aim of the study was to investigate the possible protective effects of TCE against these negative effects of Gentamicin, which is one of the drugs that trigger the formation of free radicals in the body, in terms of oxidative stress, apoptosis and antioxidant parameters. In this study, rats were divided into four equal groups. Groups; Control, GM, TCE, GM+TCE. Blood and kidney tissue samples were taken 24 hours after the last injection. Urea and creatinine analysis were performed in serum, MDA, SOD and TAS analysis were performed in kidney and serum samples. Bcl-2 and Bax analyzes and histopathological evaluations were performed in the kidney tissue. An increase in MDA, creatinine and urea levels, and a decrease in TAS and SOD levels were observed in the GM group compared to the control group. On the other hand, in the GM+TCE group, a decrease was observed in the parameters that increased compared to the GM group, and an increase in TAS and SOD levels was observed. In the histopathological and immunohistochemical examination of kidney tissue, it was determined that pathological disorders and increased apoptosis (decrease in Bcl-2, increase in Bax) decreased in the GM+TCE group. In conclusion, in the light of the data in this study, we believe that high-dose gentamicin causes side effects in the kidneys, while TCE may have antioxidant, antiapoptotic, protective and curative effects. However, additional studies are needed to confirm this assumption.

Keywords: *Antioxidant, apoptosis, gentamicin, nephrotoxicity, Tarantula cubensis extract.*

Deneysel Gentamisin Nefrotoksisitesi Oluşturulan Ratlarda *Tarantula Cubensis* Ekstraktının Koruyucu Etkisinin Araştırılması

ÖZET

Gram negatif bakterilerin neden olduğu enfeksiyonların tedavisinde kullanılan gentamisinin (GM), ciddi nefrotoksik yan etkileri nedeniyle klinik kullanımı sınırlıdır. *Tarantula cubensis* ekstraktı (TCE), rejenerasyon, demarkasyon, antiflojistik ve rezorptif etkilerinden yararlanmak için veteriner hekimlikte yaygın olarak kullanılan ve etkinliği kanıtlanmış homeopatik bir üründür. Bu çalışmanın amacı, vücutta serbest radikal oluşumunu tetikleyen ilaçlardan biri olan gentamisinin bu olumsuz etkilerine karşı TCE'nin olası koruyucu etkilerinin oksidatif stres, apoptoz ve antioksidan parametreler açısından araştırılmasıdır. Bu çalışmada sıçanlar dört eşit gruba ayrıldı. Gruplar; Kontrol, GM, TCE, GM+TCE'dir. Son enjeksiyondan 24 saat sonra kan ve böbrek doku örnekleri alındı. Serumda üre ve kreatinin, böbrek ve serum örneklerinde MDA, SOD ve TAS analizleri yapıldı. Böbrek dokusunda Bcl-2 ve Bax analizleri ile histopatolojik değerlendirmeler yapıldı. GM grubunda kontrol grubuna göre MDA, kreatinin ve üre düzeylerinde artış, TAS ve SOD düzeylerinde azalma gözlendi. GM+TCE grubunda ise GM grubuna göre artan parametrelerde azalma, TAS ve SOD düzeylerinde ise artış gözlendi. Böbrek dokusunun histopatolojik ve immunohistokimyasal incelemesinde, GM+TCE grubunda patolojik bozuklukların ve artan apoptozun (Bcl-2'de azalma, Bax'ta artış) azaldığı belirlendi. Sonuç olarak, bu çalışmadaki veriler ışığında, yüksek doz gentamisin böbreklerde yan etkilere neden olurken, TCE'nin antioksidan, antiapoptotik, koruyucu ve iyileştirici etkilerinin olabileceği kanısındayız. Ancak, bu varsayımı doğrulamak için ek çalışmalara ihtiyaç vardır.

Anahtar kelimeler: *Antioksidan, apoptoz, gentamisin, nefrotoksisite, Tarantula cubensis ekstraktı.*

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Introduction

Gentamicin (GM) is an aminoglycoside derivative broad-spectrum antibiotic used in the treatment of infections caused by gram-negative bacteria, and its clinical use is quite common due to its economic cost (Hodiamont et al., 2022). However, nephrotoxicity, which is a serious side effect of Gentamicin, limits its clinical use (Secilmis et al., 2005). It is known that 10-20% of cases with acute renal failure are caused by GM (Rizwana et al., 2022).

Gentamicin is eliminated from the body entirely by glomerular filtration from the kidneys and accumulates in lysosomes in the proximal tubules after being filtered by glomerular filtration (Li et al., 2008). The resulting tissue damage; basement membrane erosions, proximal tubule swelling, tubular atrophy or dilatation, interstitial inflammatory cell infiltration and reduction of basolateral membrane remnants (Volpini et al., 2006). The molecular mechanism of kidney failure due to GM has not been fully explained in the studies, but it has been suggested that it causes changes in different enzyme activities. It is thought that these mechanisms may cause nephrotoxicity by accumulation of superoxide anions, lysosomal enzyme changes and microsomal protein synthesis inhibition (Sharma, 2004; Secilmis et al., 2005; Leonard et al., 2023).

Homeopathy is a treatment system in which harmless and natural methods are used for diagnosis or treatment according to anamnesis. It is a treatment model that receives successful feedback in cases where chronic or modern medicine is insufficient in diagnosis and treatment. Mixtures used in homeopathic treatment are obtained from plants, metals, animal secretions or infected people (Basar, 2014). Homeopathic treatment is less known than modern medicine and it is an area open to development of new methods. It is an alternative system to modern medicine (Ilhan, 2018). Tarantula cubensis extract (TCE), which is used in homeopathic medicine, is obtained from the Tarantula cubensis spider. The extract from this spider was diluted in alcohol. It has been proven in different studies that this drug has regeneration, anti-inflammatory and antioxidant effects (Lotfollahzadeh et al., 2012; Karabacak et al., 2015).

Antioxidant defense mechanisms undertake the task of protecting the organism from the harmful effects of ROS. Most cells can tolerate low levels of oxidative stress. Repair systems find damaged molecules and remove them from the cell (Puppel et al., 2015; Akbari et al., 2022). The organism tries to counteract oxidative stress with enzymatic and/or non-enzymatic antioxidants. These are catalase, GSH and SOD, which are called natural antioxidants. The physiological function of SOD is to protect oxygen-using cells against the damage of superoxide radicals (Memisogullari, 2005).

Antioxidants are a combination of complex systems that protect tissues from the harmful effects of ROS and balance oxidative stress. The greatest contribution to TAS in the organism comes from the antioxidants in the plasma. In the antioxidant defense system, individual antioxidants can act together to protect organs against oxidative damage. For this reason, it is more accurate to measure the TAS level to evaluate the antioxidant defense system (Vural et al., 2007).

MDA is formed as a result of peroxidation of lipids and is widely used in the detection of oxidative stress levels. It can be analyzed in both urine and blood. Although there is no specific indicator for the peroxidation of lipids, it correlates well with the degree of peroxidation. For this reason, the detection of MDA is used as an indicator of lipid peroxide levels (Dimova et al., 2022).

In advanced healthy organisms, the increased number of cells by cell division is kept in balance by cell death. Cells that are not needed in the organism activate intracellular communication systems and initiate the process of death or suicide. This process is called “Apoptosis” or “Programmed Cell Death” (Mak, 2003; Aksit and Bildik, 2008). Apoptosis is a physiological requirement for the development of the organism, aging, as well as for the organism to maintain its balance. In addition, it acts as a protective mechanism in the organism against diseases or harmful agents (Vaux and Flavell, 2000; Elmore, 2007). Mitochondrial/Intrinsic pathway triggers mitochondrial degradation when sufficient levels of cytochrome-c are released (Bratton et al., 2000). This released cytochrome-c plays an important role in the activation of caspase-3 enzymes in apoptosis (Dirican et al., 2023). This pathway is regulated by Bcl-2 proteins. Antiapoptotic genes; c-myc, p-53 and bcl-2 family and the proteins they produce with the same name (Vervliet et al., 2023). Proapoptotic genes are Bax, Bad and Bid (Agena et al., 2023).

In this study, it was aimed to determine whether TCE has a protective effect on oxidative stress, apoptosis, antioxidant parameters and kidneys by experimentally induced renal toxicity with gentamicin, by biochemical, histopathological and immunochemical methods. In this study, it is aimed to fill an important literature deficiency in this field.

Materials and Methods

Drugs and chemicals

Gentamicin sulphate was purchased from Goldbio (Cas:1405-41-0). TCE (Theranekron®, alcoholic extract (1:100) of Tarentula cubensis in alcoholic solution 1 mg/ml) was obtained from Richter Pharma (Welss/Australia). TAS Assay kit was obtained from Rel Assay Diagnostics (Turkey). Urea (A2332) and creatinine (A2162) kits were obtained from Archem (İstanbul, Turkey). Antibodies against Bcl-2 (E1904), and Bax (G0104) were purchased from Santa Cruz Biotechnology (CA, USA). Seconder antibody (E0431) was obtained from Dako Cytomation (Glostrup, Denmark). All other chemicals of analytical grade were purchased from Merck (Darmstadt, Germany) or Sigma Chemical Co (St. Louis, MO, USA).

Animals

40 male Sprague Dawley rats were used. Rats were obtained from Balikesir University Experimental Animals Production, Care, Application and Research Center, Balikesir, Turkey. Standard commercial pellet food and water were provided ad libitum. All tests and procedures were performed according to the European Economic Community Guidelines for the care and use of laboratory animals and were proved by the Local Ethics Committee of Balikesir University (Ethics Committee Approval Decision No: BAU-HADYEK 2019/2-3).

Experimental procedure

Rats were acclimatized for one week prior to the study. 40 male Sprague Dawley rats were divided into four equal groups randomly. Groups; Control (0.5 ml isotonic saline, ip for 8 days), GM (100 mg/kg ip in isotonic saline for 8 days), TCE (200 µl/kg/day, sc for 14 days), GM (100 mg/kg ip for 8 days) + TCE (200 µl/kg/day sc, a total of 14 days, 3 days before and 3 days after GM application). GM dose was chosen based on the previous studies (Kalkan et al., 2012; Khaskari et al., 2021). TCE dose was chosen based on the study by Karabacak et al. 2015. 24 hours after the last application, blood samples were taken, serum was separated and stored at -80 °C. Kidney tissues were immediately dissected after sacrificed under isoflurane anesthesia, rinsed from blood in isotonic saline and then half of them was stored at -80 °C until analysis, the other half of the tissues were fixated in 10% phosphate buffered formalin for histopathological and immunohistochemical examinations.

Measurement of biochemical parameters

The serum creatinine and urea levels were measured using the biochemical autoanalyzer (Sinnowa D280, Nanjing, China) and commercially available diagnostic kits (Archem, İstanbul, Türkiye).

Kidney tissues were weighed and homogenized in ice-cold 1.15% Potassium chloride to prepare a 10% (w/v) tissue homogenate at 1300 rpm for 3 min with homogenizator (Stuart SHM 1, UK). The half of homogenate was used for the determination of MDA described by Yoshioka et al. 1979. The other half of homogenate was centrifuged at 5000 g for 60 min at 4°C and supernatant samples were separated for the determination of SOD and TAS analysis. Total protein level of the tissues homogenate and supernatant was analyzed using the Lowry method (Lowry et al., 1951). The levels of SOD in tissue supernatant were assessed following the methods of Sun et al. 1988. The levels of TAS were measured using commercially available kit according to procedure in supernatants. Serum MDA, SOD and TAS analyzes were also performed according to the same procedures.

Histopathological examination

Kidney tissues samples in formalin fixation for 72 hours were blocked by passage through alcohol, xylol and were embedded in paraffin block. 3 µm sections were taken in the microtome (Leica 2245, Nussloch, Germany) and then stained with hematoxlin and eosin (H&E). The preparations were closed with entellan. All slides of each group were examined under a light microscope. Histopathological evaluation scored as follows in kidney tissues: Tubulointerstitial inflammatory infiltrates, (tubulointerstitial inflammatory infiltrates: none = 0, leukocytes confined within the interstitium = +1, and leukocytes infiltrating the interstitium and tubular epithelial cells = +2). Pathologic Proteinous Casts, (no damage (0), mild (1, unicellular, patchy isolated damage), moderate (2, damage less than 50%), severe (3, damage more than 50%), The degree of medullary congestion no congestion (0), mild (1, vascular congestion with identification of erythrocytes by ×400 magnification), moderate (2, vascular congestion with identification of erythrocytes by ×200 magnification), severe (3, vascular magnification with identification of erythrocytes by ×100 magnification). Tubular necrosis (0 = normal cortex, 1 = small and one or two areas of tubule damage, 2 = tubular damage up to 50% of the cortex, 3 = tubular damage covering more than 50% of the cortex) (Said, 2011; Kader et al., 2017).

Immunohistochemical examination

Following the follow up and blocking procedures 3 µm sections were taken in polylysine coated slides. Immunohistochemistry was performed on sections using avidin biotin peroxidase complex (ABC, Daco Cytomation, Denmark) method according to manufacturer recommendation.

The sections were deparaffinized, rehydrated and blocked with 3% hydrogen peroxide. And then incubated with Bax (dilution: 1:100, Santa Cruz, CA, USA) or Bcl-2 (dilution: 1:100, Santa Cruz, CA, USA) antibodies. Sections were incubated with biotinylated goat anti-rabbit secondary antibody (Dako Corporation, Carpinteria, USA) and streptavidin peroxidase complex (ABC; Dako Corporation, Carpinteria, USA). The slides were treated with 3,3-diaminobenzidine (DAB) in Phosphate buffered saline (PBS) (0.5 mg DAB/ml) containing hydrogen peroxide 30% v/v. The Bax and Bcl-2 positive cells were examined semi-quantitatively under a light microscope and then photographed.

Statistical analysis

All data were expressed as mean and standard error (as mean ± SE) in each group. Statistical analysis of differences between groups was performed using ANOVA. Post hoc multiple comparisons were performed using Duncan's test. If the obtained data were not normally distributed (Histopathological examination), non-parametric Kruskal–Wallis data analysis was applied for the comparative test. All analyses were performed using the SPSS (Version 17.0, Chicago, IL, USA) software program. The difference between the groups in terms of the parameters examined was considered significant at the $P<0.05$ level.

Results

Biochemical results

The results of this study showed that serum urea, creatinine, and tissue and serum MDA levels increased with GM administration, while SOD and TAS levels were significantly decreased compared to the control group. Serum urea and creatinine, tissue and serum MDA levels decreased, TAS and SOD levels were increased in GM+TCE group compared to GM group (Table 1 and Table 2). The levels of serum and tissue kidney parameters of rats in all groups are presented in Table 1, 2.

Histopathological findings and immunohistochemical evaluation

Bax and Bcl-2 were assessed by immunohistochemically. Histopathological alterations in kidney tissues were examined (HxE). Immunohistochemical examination showed that apoptosis induced (up regulation of Bax and down regulation of Bcl-2) in kidney tissue in GM group. Bcl-2 activity was increased, Bax positive intensity of kidney tissue was decreased in GM+TCE group compared to GM group. The immunohistochemical findings showed that slight apoptotic activity in control and TCE groups when compared with GM and GM+TCE (Figure 1 and 2).

Histopathological alteration in kidney tissues of all study groups are shown in Table 3, Figure 3. Microscopical

Table 1. Serum biochemical parameters in study groups (Mean±SE)

Parameters	Control (n=10)	TCE (n=10)	GM (n=10)	GM+TCE (n=10)
MDA (µmol/L)	20.40±0.77^{c}	21.59±0.50^{c}	36.95±1.45^{a}	27.55±0.35^{b}
SOD (U/L)	6.94±0.07^{a}	6.90±0.05^{a}	3.43±0.08^{c}	4.91±0.09^{b}
TAS (mmol trolox equiv./L)	2.35±0.07^{a}	2.31±0.06^{a}	1.51±0.03^{c}	1.83±0.03^{b}
Urea (mg/dl)	35.40±0.85^{c}	35.00±1.23^{c}	72.70±2.17^{a}	58.70±1.02^{b}
Creatinine (mg/dl)	0.56±0.01^{c}	0.49±0.02^{c}	1.39±0.05^{a}	0.88±0.03^{b}

$^{a, b, c}$: Between groups with different letters in the same row mean difference is significant (P<0.05). TCE: *Tarantula cubensis* extract, GM: Gentamicin.

Table 2. Biochemical parameters of kidney tissue samples in study groups (Mean±SE)

Parameters	Control (n=10)	TCE (n=10)	GM (n=10)	GM+TCE (n=10)
MDA (µmol/mg protein)	9.26±0.21^{c}	10.01±0.30^{c}	17.61±0.34^{a}	13.58±0.32^{b}
SOD (U/mg protein)	5.70±0.17^{a}	5.37±0.19^{a}	2.95±0.15^{c}	4.27±0.11^{b}
TAS (mmol trolox equiv./mg protein)	2.39±0.04^{a}	2.46±0.05^{a}	1.58±0.05^{c}	2.03±0.02^{b}
Urea (mg/dl)	35.40±0.85^{c}	35.00±1.23^{c}	72.70±2.17^{a}	58.70±1.02^{b}
Creatinine (mg/dl)	0.56±0.01^{c}	0.49±0.02^{c}	1.39±0.05^{a}	0.88±0.03^{b}

$^{a, b, c}$: Between groups with different letters in the same row mean difference is significant (P<0.05). TCE: *Tarantula cubensis* extract, GM: Gentamicin.

Table 3. Histopathological alteration in kidney tissue of study groups (H&E) (Mean ± SE)

Parameters	Control (n=10)	TCE (n=10)	GM (n=10)	GM+TCE (n=10)	P
Pathologic Proteinous Casts	0.50±0.22^{b}	0.70±0.21^{b}	1.80±0.33^{a}	1.30±0.30^{a,b}	0.014
Tubulointerstitial inflammatory infiltrates	0.20±0.13^{b,c}	0.10±0.10^{c}	0.80±0.29^{a,b}	1.20±0.29^{a}	0.009
Tubular necrosis	0.40±0.16^{b,c}	0.10±0.10^{c}	1.20±0.29^{a}	0.90±0.23^{a,b}	0.005
The degree of medullary congestion	0.80±0.25^{b}	1.10±0.31^{a,b}	1.80±0.20^{a}	1.40±0.16^{a,b}	0.040

$^{a, b, c}$: Between groups with different letters in the same row mean difference is significant. P value is from Asymp. Sig. Kruskal-Wallis test. TCE: *Tarantula cubensis* extract, GM: Gentamicin.

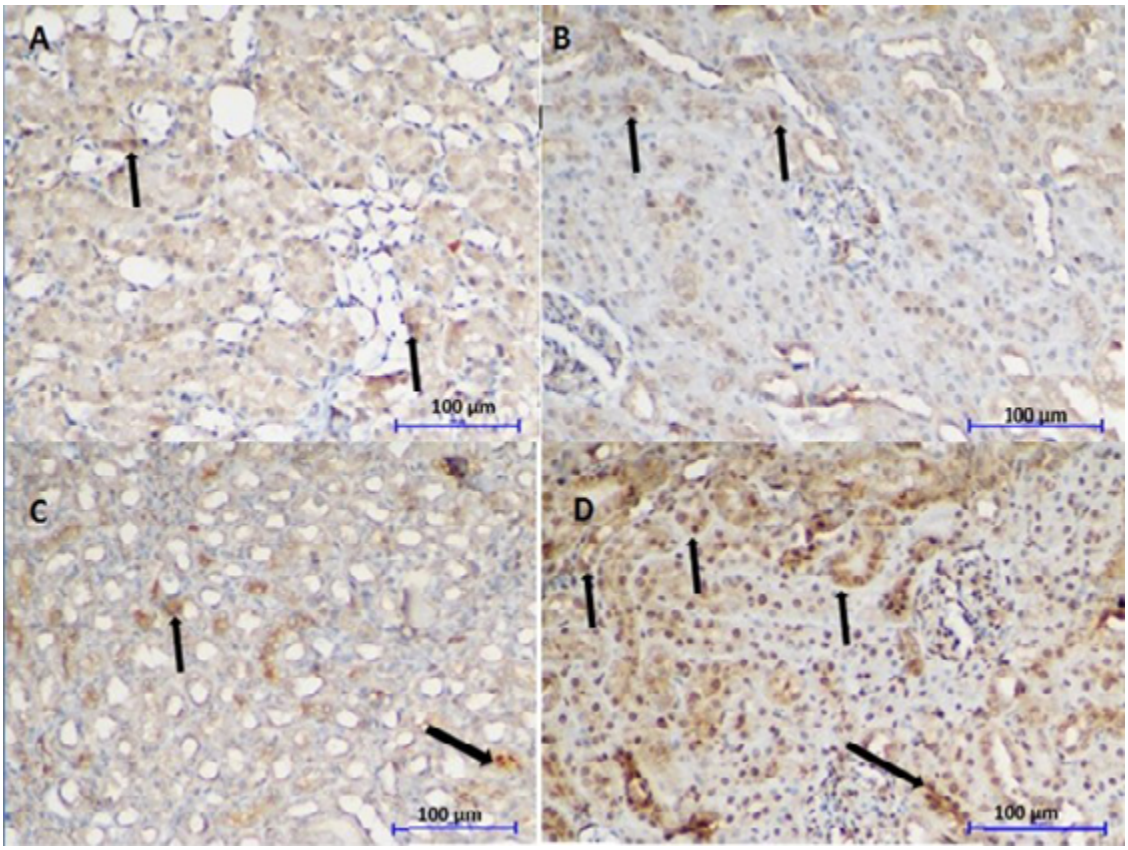

Figure 1. Immunohistochemical photomicrographs of Bcl 2 in rat kidney tissue. Control group (A), TCE group (B), GM group (C), GM+TCE group (D), arrow marks Bcl-2 positive cells, (200x).

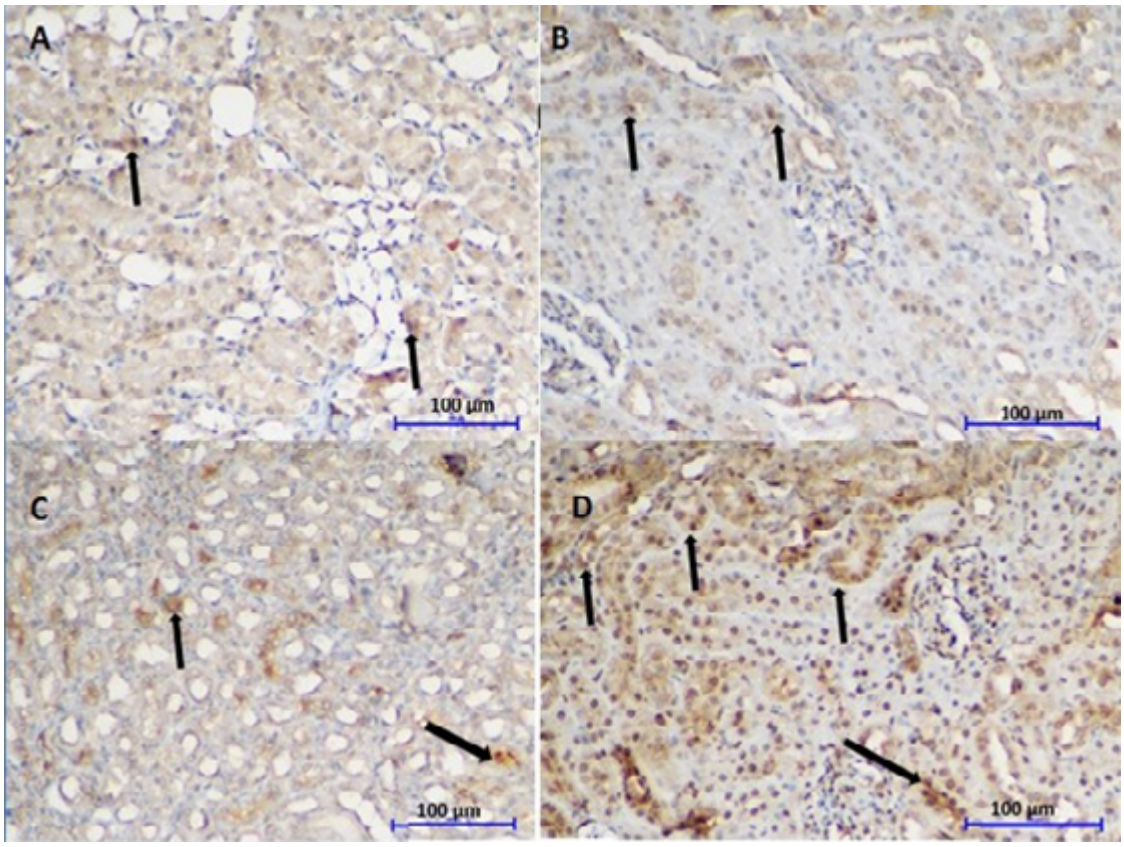

Figure 2. Immunohistochemical photomicrographs of Bax in rat kidney tissue. Control group (A), TCE group (B), GM group (C), GM+TCE group (D), arrow marks Bax positive cells, (200x).

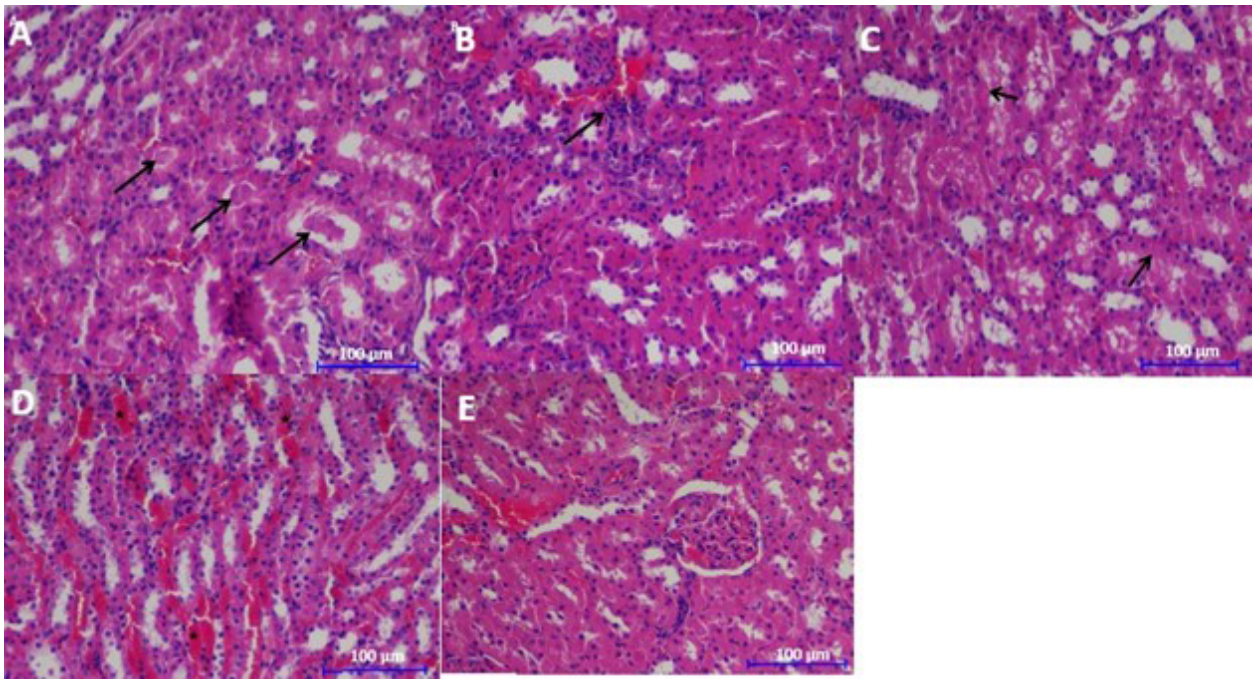

Figure 3. Renal histopathological disorders and normal kidney tissue determined in the study groups; Pathologic Proteinous Casts, arrow marks (A), Tubulointerstitial inflammatory infiltrates, arrow marks (B), Tubular necrosis, arrow marks (C) Medullary congestion, star marks (D), Normal kidney tissue of control group (E) (H&E), (200x)kidney tissue of control group (E) (H&E), (200x).

evaluation of kidney tissue was found severe pathologic proteinous casts, tubulointerstitial inflammatory infiltrates, medullary congestion and tubular necrosis in the GM group compare to control and TCE groups. Control and alone TCE treated groups showed normal tissue morphology. Significant reduction in some histopathological scores was found in GM group administrated with TCE (Table 3).

Discussion

Nephrotoxicity is among the most common side effects of GM, which is frequently used in clinics due to its wide spectrum of action and rapid effects (Rizwana et al., 2022). Although the nephrotoxic mechanism of action due to the use of GM has not yet been determined, many factors are thought to play a role, especially the accumulation of oxygen radicals (Sakr and Kamel, 2023). It has been reported that chemical agents generally used in the treatment of diseases cause complications and oxidative stress (Li et al., 2023). ROS affects intracellular proteins, nucleic acids and membrane lipids, causing changes in their structures and functions (Wang et al., 2023).

TCE, which is a homeopathic agent, has been observed in veterinary medicine to provide very rapid healing in foot diseases, ulcers, abscesses, all kinds of inflammatory and necrotic cases, even with a single dose. It is stated that TCE also has regeneration, demarcation, resorption and antiphlogistic effects (Kacar et al., 2007).

In many studies in experimental animals, GM is used to cause acute kidney injury and the effects of different substances are investigated in order to reduce the severity of the nephrotoxic effect (Abdelzaher et al., 2023; Elkhoely, 2023). Most agents thought to have antioxidant properties, especially extracts from different plants and animals, have been used in experimental GM nephrotoxicity (Aldahmash et al., 2016; Yarjani et al., 2016; Yılmaz et al., 2018; Ozsayın, 2019; Samy et al., 2023).

Some researchers reported that GM cause reactive oxygen metabolites. Increased ROS production leads to lipid peroxidation and oxidative damage. Under normal conditions the antioxidant-oxidant system is in balance, while there is an increase in MDA and a decrease in SOD and TAS during oxidative stress. SOD enzyme is a major antioxidant defense component (Yaman and Balikci, 2010). ROS plays a vital role as an inducer of apoptosis. Apoptosis is a form of programmed cell dead resulting in interconnected intracellular caspase proteins (Tanyeli et al., 2019).

The increase in MDA levels in GM-induced nephrotoxicity has been shown in many studies (Ulutas et al., 2006 ; Wijayanti et al., 2023). In different studies, it has been determined that gentamicin impairs redox reactions by reducing antioxidant enzyme activities in the organism, such as SOD. It has been reported in studies that ROS cause peroxidation of lipids (Yadav et al., 2017; Leonard et al., 2023). In terms of these parameters, this study is compatible with the literature.

Ozsayin (2019) reported that nephrotoxicity was created with gentamicin in rats, oil, water and ethanol extracts of the Cyclotrichium niveum plant were applied and it was determined that there was a significant decrease in MDA, serum urea and creatinine levels in all extracts. In addition, in the histopathological examination, it

was determined that the disorders in the tissues were restored. In another study by Karabulut (2016), it was determined that the increased MDA and oxidative stress levels in GM nephrotoxicity induced in rats decreased in the GM+L-arginine combination, while the decreased SOD level increased. As a result of the findings obtained in this study, it was determined that the increase in MDA, urea, creatinine levels and histopathological damage obtained as a result of GM nephrotoxicity, and the decrease in SOD level changed in the opposite direction when TCE was used, and the findings are consistent with the literature.

Karabacak et al. (2015) investigated the effects of TCE on the toxic effects of aflatoxin in kidney, liver and other organs in a study conducted in rats. They observed that MDA levels increased in the kidney tissue of rats given aflatoxin, and a decrease in MDA levels with TCE administration. In a study conducted by Ozbek (2019), it was determined that TCE provided histopathological improvement in renal ischemic reperfusion. However, they found that TCE reduced the effects of ischemic reperfusion-induced oxidative stress and inflammation. In this study, it was determined that the MDA level increased in kidney toxicity caused by GM and decreased with TCE administration, which is consistent with the literature.

Bai et al. (2023) reported that blood urea and creatinine levels increased significantly when they administered 100 mg/kg ip GM to experimental animals for 10 days. Renal tubules showed necrosis and vacuolation with occasional desquamation appeared on epithelial cells of the proximal convoluted tubules. Mild inflammatory cell infiltration, edema and extravasated red blood corpuscle were determined histopathologically in the interstitium. The Bax protein expression decreased and the Bcl-2 protein expression increased with the applications of antioxidant substance *C. deserticola* in GM-induced nephrotoxicity in rats significantly. When the current study is evaluated in terms of these parameters, it is compatible with the literature.

Geshnigani et al. (2023) determined that concentrations of BUN, creatinine and malondialdehyde were significantly increased. But, the level of glutathione and activities of glutathione peroxidase, and superoxide dismutase decreased during treatment with gentamicin. On the other hand, the concentrations of creatinine, BUN, nitric oxide, malondialdehyde, were significantly reduced, and the glutathione level, activities of glutathione peroxidase were significantly increased via co-administration with antioxidant activities of diosmin. Veljkovic et al. (2016) reported that GM disrupts the kidney morphology, thus causing necrosis in the kidney tubules, degenerations in the cytoplasm and causing interstitial inflammation. Yilmaz et al. (2018) observed atrophy in the histopathological examination of the renal tissues of experimental animals administered GM. They stated that there are degenerative changes in tubule epithelial cells and capsule. In this study, in the histopathological examination of renal tissues, it was observed that pathological proteinaceous casts were formed in the GM group, increases in tubulointerstitial inflammatory infiltrates and medullary congestion were formed. In the group in which the GM+TCE combination was used, these pathological findings were observed to be alleviated. But, there is no significant difference between the GM and the GM+TCE groups in some pathological disorders.

Nadeem et al. (2023) reported GM provoked an upsurge in the relative kidney weight and serum level of urea and creatinine. The MDA level was markedly boosted, with a decline in the level of TAS, SOD, and Nrf2 expression in the renal tissue. Additionally, caspase-3 and Bax expression were elevated, whereas the Bcl-2 level was reduced. Furthermore, histological examination revealed inflammation, degradation, and necrosis. GM-provoked pathological abnormalities were reversed by antioxidant treatment, which restored normal kidney architecture. When this study was evaluated in terms of these parameters, similar results were obtained.

In this study, in the nephrotoxicity model induced by GM, an increase was observed in the MDA level due to oxidative stress in the GM group, however, it was determined that the MDA level decreased in the GM+TCE group and decreased the oxidative stress. A significant decrease was detected in the SOD level, which is another evaluation criterion of this study, in the group given GM, however, in the group given the GM+TCE combination, the SOD level increased and the ROS in the cells were removed. However, while increases in serum creatinine and urea levels, which are markers of renal impairment, were observed in the GM group, a significant decrease was observed in serum urea and creatinine levels in the GM+TCE combination group. It was determined that in GM-induced nephrotoxicity, there was a decrease in antiapoptotic Bcl-2 gene expression in the GM group, and an increase in apoptosis-inducing Bax. An increase in Bcl-2 gene expression and a significant decrease in Bax were observed in the GM+TCE group. Differences were determined in the cells in the cortex and medulla layer of the kidney tissue. In the cortex layer, it was determined histopathologically that tubule necrosis was high in areas with tubule damage, and low apoptosis was determined immunohistochemically. On the contrary, it was observed that the number of apoptotic cells was higher in the medulla.

Conclusion

In this study, GM was used to induce nephrotoxicity. The findings support that GM administration at the administered dose causes renal damage and that oxidative stress contributes to this damage. Although different agents thought to be antioxidant effective were used in GM-induced nephrotoxicity studies, TCE application was tried for the first time. In the study, the protective effect of TCE against kidney damage induced by GM was

investigated biochemically, immunohistochemically and histopathologically. As a result, it was concluded that GM-induced kidney damage can be prevented by the antioxidant and anti-apoptotic effects of TCE, and these findings should be supported by further studies. This is the first study to examine the protective effect of TCE on GM-induced nephrotoxicity and will be a reference for future studies.

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Conflict of Interest

No conflict of interests was declared by the authors.

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