

*Araştırma Makalesi/Research Article***Protective Potential of Bromelain Against Chemotherapy-Induced Nephrotoxicity*****Bromelain'in Kemoterapi Kaynaklı Böbrek Toksisitesine Karşı Koruma Potansiyeli*****Alper YALÇIN¹, Sibel TÜREDİ²**

Abstract: Objective: Doxorubicin (DOX) is an antineoplastic drug, that has been extensively used for anticancer chemotherapy; however, it has toxic side effects, including nephrotoxicity. Bromelain (BRM); the main protease enzyme of pineapple has been used in several therapeutic treatments. In this experimental study, BRM was evaluated for its potential to suppress inflammation and apoptosis on DOX-induced nephrotoxicity. Methods: Twenty eight Wistar albino female rats were distributed into four groups: control (received normal saline via oral gavage (p.o.) for 10 days), BRM (40 mg/kg BRM was given via p.o. for 10 days), DOX (15 mg/kg DOX was given intraperitoneally on day 5), DOX+BRM. At the end of the study, the expression of caspase-3, tumor necrosis factor- α (TNF- α), and interleukin-2 (IL-2) in kidney tissues was determined using immunohistochemical staining, while a routine histopathological examination was performed using hematoxylin-eosin staining. Results: In microscopic evaluation, compared to the control group, severe histological damage, statistically increased immunoreactivities of caspase-3, TNF- α , and IL-2 were observed in the kidney tissues of DOX group. However, in the DOX + BRM group, BRM restored kidney histoarchitecture and inhibited the overexpression of DOX-induced apoptotic and inflammatory markers. Conclusions: BRM at dose 40 mg/kg has the capability to counteract the toxic effects of DOX on kidney. BRM might represent a novel approach in the therapy of DOX nephrotoxicity because of its anti-inflammatory and anti-apoptotic properties.

Keywords: Doxorubicin, Bromelain, Kidney, Caspase-3, TNF- α /IL-2 pathway

Öz: Amaç: Doksorubisin (DOX), kanser kemoterapisinde yaygın olarak kullanılan bir antineoplastik ilaçtır; ancak nefrotoksisite dahil olmak üzere toksik yan etkileri bulunmaktadır. Ananasın ana proteaz enzimi olan bromelain (BRM), çeşitli terapötik tedavilerde kullanılmaktadır. Bu deneysel çalışmada, BRM'nin DOX'un neden olduğu nefrotoksisite üzerinde inflamasyon ve apoptozu baskılama potansiyeli değerlendirilmiştir. Gereç ve yöntem: Yirmi sekiz dişi Wistar albino sıçan, dört gruba ayrıldı: kontrol grubu 10 gün boyunca oral gavaj yoluyla (p.o.) normal salin verildi, BRM (10 gün boyunca p.o. yoluyla 40 mg/kg BRM verildi), DOX (5. günde 15 mg/kg DOX intraperitoneal olarak verildi), DOX+BRM. Çalışmanın sonunda, böbrek dokularındaki kaspaz-3, tümör nekroz faktörü-alfa (TNF- α) ve interlökin-2 (IL-2) ekspresyonu immünohistokimyasal boyama yöntemiyle saptanırken, hematoksilin-eozin boyaması ile rutin histopatolojik incelemeye yapıldı. Bulgular: Mikroskopik değerlendirmede, kontrol grubuyla karşılaştırıldığında, DOX grubunun böbrek dokularında ciddi histolojik hasar, kaspaz-3, TNF- α ve IL-2 için istatistiksel olarak anlamlı düzeyde artmış immünoaktiviteler gözlemlendi. Bununla birlikte, DOX + BRM grubunda BRM, böbreğin histopatolojik yapısını iyileştirdi ve DOX'un neden olduğu inflamasyon ve apoptoz belirteçlerinin aşırı ekspresyonunu inhibe etti. Sonuç: 40 mg/kg dozundaki BRM, DOX'un böbrek üzerindeki toksik etkilerini önleme yeteneğine sahiptir. BRM, anti-inflamatuar ve anti-apoptotik özelliği nedeniyle, DOX nefrotoksisitesinin tedavisinde yeni bir yaklaşım sunabilir.

Anahtar Kelimeler: Doksorubisin, Bromelain, Böbrek, Kaspaz-3, TNF- α /IL-2 yolağı

¹Sorumlu yazar: Doç. Dr., Alper Yalçın, Kahramanmaraş Sütçü İmam Üniversitesi, Tıp Fakültesi, ORCID: 0000-0002-8975-1008, alperyalcin0171@hotmail.com
Assoc., Prof., Kahramanmaraş Sütçü İmam University, Faculty of Medicine, Kahramanmaraş/Türkiye.

²Dr. Öğretim Üyesi, Harran Üniversitesi, Tıp Fakültesi, ORCID: 0000-0001-2345-6789
Ass., Prof., Harran University, Şanlıurfa/Türkiye.

Introduction

Doxorubicin (DOX) is a chemotherapeutic agent used in the treatment of various types of cancer (Wu et al., 2022). However, the toxicity of DOX on tissues limits its treatment (Kciuk et al., 2023). Kidney failure caused by DOX presents a major challenge to cancer application. Approximately 60% of cancer patients who must undergo chemotherapy experience kidney toxicity, which limits the effectiveness of the treatment (Fouad and Ahmed, 2022). Renal toxicity has an important risk of morbidity and death as a result of renal failure (Al-Naimi et al., 2019). Free radicals, causing oxidative injury and inflammation, is an important mechanism underlying DOX-induced nephrotoxicity (Ahmed et al., 2019; Arunachalam et al., 2022). DOX-mediated oxidative stress provokes mitochondrial permeability and apoptotic pathways (Vitale et al., 2024). There is currently no way that can protect organs from DOX without compromising its antitumor effect. Therefore, identifying an appropriate strategy is of great importance for cancer treatment practices (Chen et al., 2025).

Bromelain (BM) is the basic bioactive complex in pineapple has antioxidant, antibacterial and anti-inflammatory activities (Pereira et al., 2025; Djelti et al., 2026). It has health-promoting non-enzymatic components including glycoproteins, polysaccharides, and other compounds. In addition to β -carotene, vitamin C and flavonoids, BRM contains various antioxidants that contribute cell protection against free radical-provoked injury (Mótyán et al., 2013; Napper et al., 1994). BRM has therapeutic effects due to its immunomodulatory, and anticancer activities. BRM has been emphasized to be a significant candidate for the application of several inflammatory and oxidative stress-related diseases (Kansakar et al., 2024).

Evidence regarding the restorative potential of BRM in DOX-mediated renal toxicity is limited. The current study aimed to investigate the protective effects of BRM application against DOX-induced kidney injury using an experimental model that included immunohistochemical analyses of hepatic tumor necrosis factor-alpha (TNF- α), interleukin-2 (IL-2), caspase-3 expression along with routine histopathology.

Methods

Ethics approval of the current study was granted by the animal experiments local ethical committee of Harran University (Date;07.05.2026/Approval number;2026-004/01-04). A total of 28 Wistar Albino female rats (180-250 g) were used. Rats were kept at standard environmental conditions (12 hours light / dark, 23 ± 2 °C temperature), and fed by standard methods of food and water.

Rats were distributed into four groups as follows: Control group (n:6): Received normal saline via oral gavage (p.o.) for 10 days. BRM group (n:6): 40 mg/kg BRM was given via p.o. for 10 days (14). DOX group (n:8): 15 mg/kg DOX was given intraperitoneally (i.p.) on day 5 (days (15). DOX+BRM group (n:8): 15 mg/kg DOX was given intraperitoneally (i.p.) on day 5+ 40 mg/kg BRM was given via p.o. for 10 days.

Following the experimental period, kidney tissues of the experimental groups was removed and fixed in a 10% formalin solution for histological evaluation.

Histopathologic Analyses

For histopathological analyses, fixed kidney tissue samples from all groups were dehydrated in alcohol solutions, cleared with xylene, and embedded in paraffin. 5 µm sections were taken from the obtained paraffin blocks using a semi-automatic microtome (Thermo Shandon Finesse ME+ Microtome, Runcorn, UK) and stained with hematoxylin and eosin (H&E). Histopathological evaluation was performed under a light microscope by an impartial, experienced histologist. All findings and evaluations were photographed using a Zeiss Axioskop II microscope (Carl Zeiss Microscopy GmbH, Göttingen, Germany) equipped with a Zeiss Axiocam MRc camera (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) and saved to a computer. In each microscopic kidney tissue section, findings such as tubular epithelial damage, tubular dilatation, glomerular damage, Bowman's capsule/cavity damage, congestion, and edema were scored semiquantitatively, and the histopathological structure was examined. For the histopathological scoring system, each specimen was scored in ten different areas as absent (0), mild (+), moderate (++), or severe (+++) (Türedi et al., 2022)

Immunohistochemical Staining

To assess apoptosis and inflammation in kidney tissue samples, the immunohistochemical localization of caspase-3, TNF- α , and IL-2 was examined. Kidney sections (5 µm thickness) taken from the paraffin blocks were deparaffinized. Following the washing procedure, the sections were boiled in citrate buffer (pH: 6.0) after PBS buffer solution, and antigen retrieval was performed. The samples washed in PBS were treated to peroxidase blocking in a 3% H₂O₂ solution. Next, the tissue samples were incubated with 1:200 diluted caspase-3 (Santa Cruz Biotechnology, Inc., cat no. sc-56053), 1:200 diluted TNF- α (Santa Cruz Biotechnology, INC., cat no: sc-52746), and 1:100 diluted IL-2 (Santa Cruz Biotechnology, INC cat. no: sc-133118) primary antibodies at +4C. After incubation of the primary antibodies, samples were incubated with secondary antibody kit (Thermo Scientific, MA, USA, cat no: TP-060-HL) according to the manufacturer's instructions. The chromogen 3,3'-Diaminobenzidine (DAB) kit (Sigma-

Aldrich, St. Louis, USA, cat no: D3939) was poured on the sections. The samples were then counterstained with Mayer's hematoxylin, mounted with entellan, assessed under a light microscope, and photographed. For immunohistochemical analyses, TNF- α , IL-2, and caspase-3 positivity were observed as brown colour in the cell in each cross section and the staining intensity was scored by reporting according to the H-score analysis using; 0 for negative staining, 1+ for weak staining, 2+ for medium staining, 3+ for strong staining (Türedi et al., 2024).

Statistical Analyses

SPSS software, version 25.0 (IBM Corp., Chicago, IL, USA), was used for statistical analyses. Since the assumption of normal distribution was not met in between-group comparisons, the nonparametric Kruskal-Wallis H test was used. In cases where significant differences were detected, the post-hoc Mann-Whitney U test was applied to determine the source of the difference. To reduce the risk of Type I error in multiple comparisons, Bonferroni correction was applied, and the adjusted significance threshold (alpha) was set at $p < 0.0083$ (or $p < 0.0833^*$) according to the number of comparisons performed. Descriptive statistics are presented as Median (Min–Max).

Power Analyses

Power analysis was performed using G*Power version 3.1.9.7 for one-way ANOVA (fixed effects, omnibus test). Assuming a large effect size (Cohen's $f=0.60$), a significance level of 0.05, and a statistical power of 86%, the minimum required sample size was calculated as 24 animals. Therefore, a total of 28 rats were included in the study. Animals were randomly allocated to the experimental groups using a randomization procedure. Owing to the anticipated treatment-related mortality and greater biological variability following DOX administration, the DOX-treated groups each included eight animals, whereas the Control and BRM groups each included six animals. Animals were randomly allocated to the experimental groups using a randomization procedure.

Results

Histopathologic Findings

The results of the histopathological scoring using H&E staining for all experimental groups were represented in Table 1. It was observed that the control and BRM groups had a normal morphological structure; the boundaries between the cortex and medulla had a normal histological appearance, the Bowman's membrane was intact; the Bowman's space was of normal width; the glomerular structure had a normal histological appearance; and the proximal

and distal tubular epithelium had a regular structure (Figure 1 A, B). In the kidney tissue of the DOX group, histopathological findings such as tubular dilatation, the loss of glomerular architecture, degeneration resulting from dilation of the Bowman's space and detachment of the Bowman's membrane, tubular epithelial cell detachment and loss were observed (Figure 1C, D). In the DOX+BRM group, however, the tubular epithelial structure was preserved, glomerular integrity was partially maintained, and improvement in the signs of damage was observed. (Figure 1E, F). In histopathological scoring, when the DOX group was compared to the control group, statistically significant increases were observed in tubular epithelial damage, glomerular degeneration, Bowman's space/capsule damage, vascular congestion, and edema compared to the control group ($P < 0.0083$). On the other hand, in the DOX+BRM group, tubular epithelial damage, glomerular degeneration, and Bowman's space/capsule damage were statistically significantly reduced compared to the DOX group ($P < 0.0083$), while no difference was observed in terms of vascular congestion and oedema ($P > 0.0083$) (Table 1).

Table 1. Renal Histopathological Scoring

Groups	Tubular epithelium damage	Tubular dilatation	Glomerular degeneration	Bowman space/capsula damage	Vascular congestion and oedema
Control	0.30 (0.20 - 0.30)	0.20 (0.10-0.30)	0.20 (0.10-0.40)	0.10 (0.00-0.20)	0.05 (0.00-0.10)
BRM	0.35 (0.20 - 0.50)	0.25 (0.10-0.40)	0.30 (0.20 - 0.40)	0.20 (0.10-0.30)	0.10 (0.00-0.10)
DOX	2.30 (2.00 -3.00) ^a	1.60 (1.40-2.00) ^a	2.00 (1.80 - 2.60) ^a	2.10 (1.80-2.60) ^a	0.40 (0.30-0.60) ^a
DOX+BRM	1.00 (0.80 - 1.20) ^{a,b}	0.60 (0.40 - 0.80) ^{a,b}	0.80 (0.60 - 1.00) ^{a,b}	0.80 (0.60-1.00) ^{a,b}	0.40 (0.20-0.60)
^o P value	<0.001	<0.001	<0.001	<0.001	<0.001

^o: Kruskal–Wallis H test was used. Mann-Whitney U test with Bonferroni correction was used ($p < 0.0083$).
 Values are expressed as median (min-max).
 a: $P < 0.0083$ compared with the control group
 b: $P < 0.0083$ compared with the DOX group

Immunohistochemical Findings for Caspase-3

To evaluate the effects of BRM on DOX-induced apoptosis in kidney tissue, caspase-3 was used as a marker. Caspase-3 expression was similar in the control and BRM groups ($P > 0.0083$) (Figure 2 A-B). However, a significant increase in caspase-3 positivity was observed in the DOX group compared with the control group ($P < 0.0083$) (Figure 2 C). In contrast to the DOX-treated group, caspase-3 positivity was significantly decreased in the DOX + BRM group ($P < 0.0083$) (Figure 2 D), (Table 2).

Immunohistochemical Findings for TNF- α

TNF- α expression was similar in both the control and BRM groups ($P > 0.0083$) (Figure 2 A-B). On the other hand, a marked increase in TNF- α positivity was observed in the group

compared with the control group ($P < 0.0083$) (Figure 2 C). In contrast to the DOX-treated group, TNF- α positivity was significantly decreased in the DOX + BRM group ($P < 0.0083$) (Figure 2 D), (Table 2).

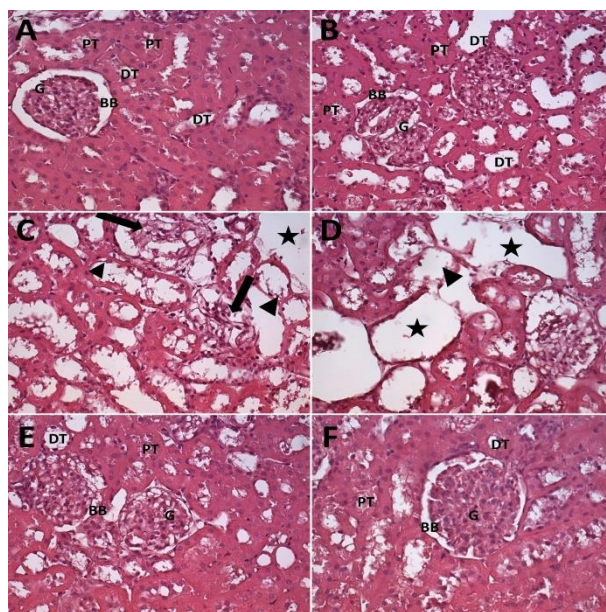


Figure 1. Light Microscopic Micrographs of Kidney Tissue from the Experimental Groups. Control group (A), BRM group (B), DOX group (C, D), DOX+BRM group (E, F). Tubular dilation (asterisk), tubular epithelial degeneration (arrowhead), tubular dilation (brackets), glomerular and bowman's capsule damage (arrow). DT, Distal Tubule; PT, Proximal Tubule; G, Glomerulus; BB, Bowman's Capsule; (H&E, 40X).

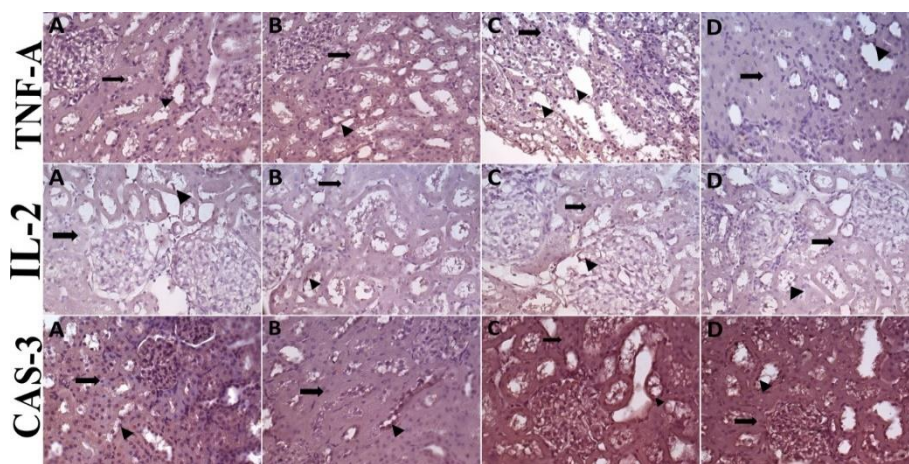


Figure 2. Immunohistochemical micrographs of sections stained with TNF- α , IL-2, and caspase-3 from the experimental groups. Control group (A), BRM group (B), DOX group (C), DOX+BRM group (D). TNF- α , IL-2, and caspase-3 positive staining (arrowhead), negative staining (arrow), (40X).

Immunohistochemical Findings for IL-2

The control and BRM groups showed similar IL-2 expression ($P > 0.0083$) (Figure 2 A-B). A significant increase in IL-2 immunoreactivity was observed in the DOX group ($P <$

0.0083) (Figure 2C). IL-2 immunostaining significantly decreased in the DOX + BRM group compared with the DOX group ($P < 0.0083$) (Figure 2D), (Table 2).

Table 2. Histoscore of Caspase 3, TNF- α , and IL-2 Immunoreactivities in Kidney Tissue

Variables	Control group	BRM group	DOX group	DOX+BRM group	θ P value	ϕ Post-hoc (MWU)
Caspase-3	0.60 (0.40 – 0.80)	0.60 (0.60 – 0.80)	1.60 (1.20 – 1.80)	0.80 (0.60– 1.00)	<0.001	1-2: p=0.03865 1-3: p=0.0040 1-4: p=0.275 2-3: p=0.0039 2-4: p=0.0693 3-4: p=0.0043
TNF- α	0.50 (0.40 – 0.60)	0.60 (0.40 – 0.60)	1.80 (1.60 – 2.00)	0.60 (0.60– 0.80)	<0.001	1-2: p=0.6404 1-3: p=0.0043 1-4: p=0.0379 2-3: p=0.0041 2-4: p=0.0689 3-4: p=0.0041
IL-2	0.20 (0.10 – 0.40)	0.40 (0.30 – 0.60)	01.20 (1.00 – 1.40)	0.70(0.60– 0.80)	<0.001	1-2: p=0.5946 1-3: p=0.0043 1-4: p=0.0080 2-3: p=0.0040 2-4: p=0.0074 3-4: p=0.0041
θ : Kruskal–Wallis H test was used ϕ : Mann-Whitney U test with Bonferroni correction was used ($p < 0.0083$). Values are expressed as median (min-max).						

Discussion

DOX has been reported therapeutic potential on several cancer types (Yalcin et al., 2024). However, it has toxic side effects on glomeruli and renal tubules (Wu et al., 2021). The current study proposed to investigate the restorative ability of BRM on DOX-provoked nephrotoxicity with respect to routine histopathology, apoptosis and inflammation. The findings showed the protective potential of BRM against DOX-mediated kidney damage. Its ability to restore kidney histoarchitecture, to inactivate the TNF- α /IL-2 pathway, as well as its capacity to reduce renal apoptosis, seems to be the possible mechanisms underlying the action of BRM.

DOX was emphasized to cause atrophy of the glomeruli and raise the permeability of glomerular capillaries in rat kidneys (Saad et al., 2001). Following DOX accumulation, significant glomerular injury was produced as the result of oxidative stress (Khodeer et al., 2021). DOX toxicity has been reported to show remarkable histopathological alterations, such as tubular dilatation, and congestion, glomerular atrophy with Bowman's capsular gap expansion, as well as necrosis, and leukocytic infiltration (Gad et al., 2025). Rasheed et al. (2025) reported that histopathological alterations such as widened and irregular Bowman's space, degenerated tubular epithelial cells, and distorted glomeruli in DOX treated kidney. In line with these previous studies, the present study determined that DOX led histopathological alterations in kidney, such as tubular dilatation, glomerular disorganization, dilatation of the Bowman's space and detachment of the Bowman's membrane, tubular epithelial cell detachment and loss ($P < 0.0083$) compared with the control group. The renal damage observed in kidney tissue may due to the toxic effect of DOX on tissues (Kciuk et al., 2023). Restorative potential of BRM on renal histopathology has been reported in previous reports (Ali et al.,

2024). Similarly, kidney sections from DOX+BRM group showed an important remarkable improvement of the histopathological changes mediated by DOX. These BRM protective effects were verified by statistically significant amelioration of tubular epithelial damage, glomerular degeneration, and Bowman's space/capsule injury.

Oxidative stress provokes tissue injury and a pro-inflammatory production (Liao et al., 2018). Inflammation has been reported to participate in renal pathology (Wang et al., 2018). Abd El-Aziz et al. (2012) reported significant release of pro-inflammatory cytokines following post-DOX administration. Our results showed that DOX administration caused to significant expression in renal inflammatory markers; TNF- α and IL-2 in line with the previous studies (Tu et al., 2013). Elevated production of kidney TNF- α and IL-2 may be related ROS and oxidative stress (Abdelmeguid et al., 2010; Carvalho et al., 2009). However, the release of both cytokines were significantly reduced in the kidneys of DOX-induced rats pre-treated with 40 mg/kg BRM in the DOX+BRM group. The obtained findings of the current study may contribute to literature reporting BRM as an important strategy for treating inflammation in kidney tissue (Pansare et al., 2025).

DOX significantly induces apoptosis by increasing free radical production and lipid and protein oxidation, ultimately leading to a decrease in tubular function and glomerular number (Nagai et al., 2018). DOX triggers apoptosis by provoking the caspase-3 mitochondrial pathway (Su et al., 2015). In the present work, DOX led an important increase in the expression of caspase-3 in the cytoplasm of renal cells in line with the previous reports (Kaymak et al., 2022; Gad et al., 2025). Apoptosis in tubular cells was attributed to oxidative stress caused by reactive oxygen species as well as inflammation (Mohammed et al., 2022). These findings are consistent with those of Rashid et al. 2013, which explain the activation of apoptotic DOX leading to DNA damage and mitochondrial DNA damage. BRM exerts cytoprotective effects by regulating apoptotic pathways. It modulates caspase activity and protects mitochondrial function, preventing tubular cell apoptosis and maintaining renal tissue architecture (Pavan et al., 2012). The current study also showed the suppression of caspase-3 by BRM in kidneys exposed to DOX. This anti-apoptotic effect of BRM on kidney tissue was in concomitant with the previous studies (Sulumer et al., 2024).

Limitations of The Study

In the current study BRM was tested at a single dose protocol whose efficacy had previously been demonstrated in the literature; however, the potential protective effects of different BRM doses were not evaluated.

As the experimental period was only 10 days, this duration may not be sufficient to evaluate the long-term effects of DOX and BRM.

While the most basic apoptosis (caspase-3) and inflammation markers (TNF- α , and IL-2) were analyzed, further contributions to the literature would be supported by more detailed molecular and mechanistic-level investigations.

In future studies, we plan to evaluate different dose ranges, application durations, and additional biochemical/molecular parameters which may contribute to a more comprehensive understanding of BRM's nephroprotective abilities.

Conclusion and Recommendations

In conclusion, our results show that BRM has nephroprotective effects against DOX-induced nephrotoxicity. The beneficial potential of BRM may be partly related to the inhibition of TNF and IL-2 expression, resulting in a reduction in apoptosis in kidney tissue of rats. BRM might be a therapeutic option for the treatment of inflammatory renal diseases through its anti-apoptotic and anti-inflammatory abilities. Therefore, we recommend that BRM be used in future experimental and clinical studies to develop functional foods with kidney-protective effects against renal complications caused by DOX.

Acknowledgments: The authors thank Prof. Fatih Uckardes (Adıyaman University Faculty of Medicine, Department of Biostatistics and Medical Informatics, Türkiye) for his support in statistical analysis.

Conflicts of Interest: The authors declare no conflict of interest.

Funding: This research received no external funding.

Ethical Statement: Ethics approval of the current study was granted by the animal experiments local ethical committee of Harran University (Date;07.05.2026/Approval number;2026-004/01-04).

Author Contributions: Concept/Idea: AY; Design: AY, ST; Supervision/Consulting: AY, ST; Sources: ST; Materials: AY, ST; Data Collection and/or Processing: AY, ST; Analysis and/or Interpretation: AY, ST; Literature Review: AY, ST; Writing: AY; Critical Review: AY, ST. All authors reviewed the results and approved the final version of the article.

Peer Review: External independent.

References

Abd El-Aziz, T. A., Mohamed, R. H., Pasha, H. F., & Abdel-Aziz, H. R. (2012). Catechin protects against oxidative stress and inflammatory-mediated cardiotoxicity in adriamycin-treated rats. *Clinical and experimental medicine*, 12(4), 233-240. <https://doi.org/10.1007/s10238-011-0165-2>

- Abdelmeguid, N. E., Chmaisse, H. N., & Abou Zeinab, N. S. (2010). Protective effect of silymarin on cisplatin-induced nephrotoxicity in rats. *Pakistan journal of nutrition*, 9(7), 624-636. <https://doi.org/10.3923/pjn.2010.624.636>
- Ahmed, Z. A., Abtar, A. N., Othman, H. H., & Aziz, T. A. (2019). Effects of quercetin, sitagliptin alone or in combination in testicular toxicity induced by doxorubicin in rats. *Drug Design, Development and Therapy*, 3321-3329. <https://doi.org/10.2147/DDDT.S222127>
- Ali, H. I., Mutlak, B. H., Alshareef, S. A., & Alhuwaymil, Z. (2024). Effect of CCl₄ on Renal Tissues of Male Mice and Prevented by Pretreatment with Bromelain Loaded with Gold Nanoparticles: Histopathological and Caspase-9 Expression Alterations. *Trends in Sciences*, 21(12), 8358-8358. <https://doi.org/10.48048/tis.2024.8358>
- Al-Naimi, M. S., Rasheed, H. A., Hussien, N. R., Al-Kuraishy, H. M., & Al-Gareeb, A. I. (2019). Nephrotoxicity: Role and significance of renal biomarkers in the early detection of acute renal injury. *Journal of advanced pharmaceutical technology & research*, 10(3), 95-99. DOI: 10.4103/japtr.JAPTR_336_18
- Arunachalam, S., Nagoor Meeran, M. F., Azimullah, S., Jha, N. K., Saraswathiamma, D., Subramanya, S., ... Ojha, S. (2022). α -Bisabolol attenuates doxorubicin induced renal toxicity by modulating NF- κ B/MAPK signaling and caspase-dependent apoptosis in rats. *International journal of molecular sciences*, 23(18), 10528. <https://doi.org/10.3390/ijms231810528>
- Carvalho, C., Santos, R. X., Cardoso, S., Correia, S., ... Moreira, P. I. (2009). Doxorubicin: the good, the bad and the ugly effect. *Current medicinal chemistry*, 16(25), 3267-3285. <https://doi.org/10.2174/092986709788803312>
- Chen, M., He, K., Wang, K., Cai, Y., ... Jiang, J. (2025). Low-dose radiation ameliorates doxorubicin-induced renal injury via reducing oxidative stress and protecting mitochondrial function. *Plos one*, 20(2), e0313649. <https://doi.org/10.1371/journal.pone.0313649>
- Djelti, F., Hani, M., Belaadi, A., Chetbani, Y., ... Ghernaout, D. (2026). In Silico Characterization of the Bromelain–NLRP3 inflammasome Interaction: Insights from Homology Modeling, molecular docking, and MD simulation. *Chemical Engineering Journal Advances*, 101187. <https://doi.org/10.1016/j.ceja.2026.101187>
- Fouad, G. I., & Ahmed, K. A. (2021). The protective impact of berberine against doxorubicin-induced nephrotoxicity in rats. *Tissue and Cell*, 73, 101612. [10.1016/j.tice.2021.101612](https://doi.org/10.1016/j.tice.2021.101612)
- Gad, N. S., Shabana, S. M., Amer, M. E., Othman, A. I., & El-Missiry, M. A. (2025). Naringin mitigated doxorubicin-induced kidney injury by the reduction of oxidative stress and inflammation with a synergistic anticancer effect. *BMC Pharmacology and Toxicology*, 26(1), 121. <https://doi.org/10.1186/s40360-025-00947-7>
- Kansakar, U., Trimarco, V., Manzi, M. V., Cervi, E., ... Santulli, G. (2024). Exploring the therapeutic potential of bromelain: applications, benefits, and mechanisms. *Nutrients*, 16(13), 2060. <https://doi.org/10.3390/nu16132060>
- Kaymak, E., Öztürk, E., Akin, A. T., Karabulut, D., & Yakan, B. (2022). Thymoquinone alleviates doxorubicin induced acute kidney injury by decreasing endoplasmic reticulum stress, inflammation and apoptosis. *Biotechnic & Histochemistry*, 97(8), 622-634. <https://doi.org/10.1080/10520295.2022.2111465>
- Kciuk, M., Gielecińska, A., Mujwar, S., Kołat, D., Kałuzińska-Kołat, Ż., Celik, I., & Kontek, R. (2023). Doxorubicin—an agent with multiple mechanisms of anticancer activity. *Cells*, 12(4), 659. <https://doi.org/10.3390/cells12040659>
- Khodeer, D.M., Mehanna, E.T., Abushouk, A.I., Abdel-Daim, M.M. Protective Effects of Evening Primrose Oil against Cyclophosphamide-Induced Biochemical, Histopathological, and Genotoxic Alterations in Mice. *Pathogens* 2020, 9, 98. <https://doi.org/10.3390/pathogens9020098>
- Liao, D., Xiang, D., Dang, R., Xu, P., ... Jiang, P. (2018). Neuroprotective effects of dl-3-n-butylphthalide against doxorubicin-induced neuroinflammation, oxidative stress, endoplasmic reticulum stress, and behavioral changes. *Oxidative Medicine and Cellular Longevity*, 2018(1), 9125601. <https://doi.org/10.1155/2018/9125601>
- Majid FA, Chobotova K. Cytoprotective effects of bromelain in oxidative stress models. *Mol Cell Biochem*. 2008; 308(1–2): 131–137. doi:10.1007/s11010-007-9646-4.
- Mohamed, H. K., Mobasher, M. A., Ebiya, R. A., Hassen, M. T., ... Awad, N. S. (2022). Anti-inflammatory, anti-apoptotic, and antioxidant roles of honey, royal jelly, and propolis in suppressing nephrotoxicity induced by doxorubicin in male albino rats. *Antioxidants*, 11(5), 1029. <https://doi.org/10.3390/antiox11051029>
- Mótyán, J. A., Tóth, F., & Tőzsér, J. (2013). Research applications of proteolytic enzymes in molecular biology. *Biomolecules*, 3(4), 923-942. <https://doi.org/10.3390/biom3040923>
- Nagai, K., Fukuno, S., Otani, K., Nagamine, Y., ... Konishi, H. (2018). Prevention of doxorubicin-induced renal toxicity by theanine in rats. *Pharmacology*, 101(3-4), 219-224. <https://doi.org/10.1159/000486625>
- Napper, A. D., Bennett, S. P., Borowski, M., Holdridge, M. B., Leonard, M. J., Rogers, E. E., ... & Shames, S. L. (1994). Purification and characterization of multiple forms of the pineapple-stem-derived cysteine

- proteinases ananain and comosain. *Biochemical Journal*, 301(3), 727-735. <https://doi.org/10.1042/bj3010727>
- Pansare, K., Ahire, Y., & Bairagi, V. (2025). Nephroprotective Effect of Naringenin and Bromelain Against Cisplatin-Induced Renal Toxicity in Rats. *Trends in Sciences*, 22(11), 10681-10681. <https://doi.org/10.48048/tis.2025.10681>
- Pavan R, Shraddha, Jain S, Kumar A. Mechanisms of bromelain-induced cytoprotection. *Biotechnol Res Int*. 2012; 2012: 976203. doi:10.1155/2012/976203.
- Pereira, I. C., Pedrosa-Santos, Á. M. C., Martins, J. A., de Sousa, A. A., ... Torres-Leal, F. L. (2025). Bromelain and liver health: A comprehensive systematic review of preclinical studies. *Clinical nutrition ESPEN*, 66, 437-445. <https://doi.org/10.1016/j.clnesp.2025.02.001>
- Rasheed, R. A., Sadek, A. S., Khattab, R. T., Saad, D. Z., ... Abdelfattah, H. A. (2025). Could hesperetin ameliorate doxorubicin-induced nephrotoxicity in rats via its antioxidant, antiapoptotic, and anti-inflammatory properties?. *Tissue and Cell*, 96, 102951. <https://doi.org/10.1016/j.tice.2025.102951>
- Rashid, S., Ali, N., Nafees, S., Ahmad, S. T., ... Sultana, S. (2013). Alleviation of doxorubicin-induced nephrotoxicity and hepatotoxicity by chrysin in Wistar rats. *Toxicology mechanisms and methods*, 23(5), 337-345. <https://doi.org/10.3109/15376516.2012.759306>
- Saad, S. Y., Najjar, T. A., Al-Rýkaby, A. C. The preventive role of deferoxamine against acute doxorubicin-induced cardiac, renal and hepatic toxicity in rats. *Pharmacol. Res.* 2001, 43, 211–218. <https://doi.org/10.1006/phrs.2000.0769>
- Su, Z., Ye, J., Qin, Z., & Ding, X. (2015). Protective effects of madecassoside against Doxorubicin induced nephrotoxicity in vivo and in vitro. *Scientific reports*, 5(1), 18314. doi: 10.1038/srep18314 (2015).
- Sulumer, A. N., Palabıyık, E., Avcı, B., Uğuz, H., & Aşkın, H. (2024). Histopathological and Antioxidant Effects of Bromelain on Kidney Tissue of Tyloxapol-Induced Hyperlipidemic Rats. *Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 29(2), 413-422. <https://doi.org/10.53433/yyufbed.1381717>
- Tu, Y., Sun, W., Wan, Y. G., Che, X. Y., ... Shi, X. M. (2013). RETRACTED: Huangkui capsule, an extract from *Abelmoschus manihot* (L.) medic, ameliorates adriamycin-induced renal inflammation and glomerular injury via inhibiting p38MAPK signaling pathway activity in rats. <https://doi.org/10.1016/j.jep.2013.03.006>
- Türedi, S., Çelik, H., Dağlı, ŞN., Taşkın, S., ... Deniz, M. An Examination of the Effects of Propolis and Quercetin in a Rat Model of Streptozotocin-Induced Diabetic Peripheral Neuropathy. *Curr Issues Mol Biol*. 2024 Mar 2;46(3):1955-1974. doi: 10.3390/cimb46030128. <https://doi.org/10.3390/cimb46030128>
- Türedi, S., Hancı, H., Odacı, E., Çelik, H. Prenatal dönemleri boyunca günde bir saat kesintisiz 900 MHz elektromanyetik alan etkisine maruz kalan 75 günlük Sprague Dawley dişi sıçanların böbrek ve mesane dokularının histopatolojik olarak incelenmesi. *ADYÜ Sağlık Bilimleri Derg.* 2022;8(2):142-150. doi:10.30569/adiyamansaglik.1023728. <https://doi.org/10.30569/adiyamansaglik.1023728>
- Vitale, R., Marzocco, S., & Popolo, A. (2024). Role of oxidative stress and inflammation in doxorubicin-induced cardiotoxicity: a brief account. *International Journal of Molecular Sciences*, 25(13), 7477. <https://doi.org/10.3390/ijms25137477>
- Wang, Z., Huang, W., Li, H., Tang, L., ... Zhang, L. (2018). Synergistic action of inflammation and lipid dysmetabolism on kidney damage in rats. *Renal failure*, 40(1), 175-182. <https://doi.org/10.1080/0886022X.2018.1450763>
- Wu, B. B., Leung, K. T., & Poon, E. N. Y. (2022). Mitochondrial-targeted therapy for doxorubicin-induced cardiotoxicity. *International journal of molecular sciences*, 23(3), 1912. <https://doi.org/10.3390/ijms23031912>
- Wu, Q., Li, W., Zhao, J., Sun, W., ... Gao, K. (2021). Apigenin ameliorates doxorubicin-induced renal injury via inhibition of oxidative stress and inflammation. *Biomedicine & Pharmacotherapy*, 137, 111308. <https://doi.org/10.1016/j.biopha.2021.111308>
- Yalçın, T., Kaya, S., & Kuloğlu, T. (2024). Resveratrol may dose-dependently modulate nephrin and OTULIN levels in a doxorubicin-induced nephrotoxicity model. *Toxicology mechanisms and methods*, 34(1), 98-108. <https://doi.org/10.1080/15376516.2023.2268717>