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Original Article

Antioxidant Effects of Bromelain on Paracetamol-Induced Renal Injury in Rats

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ABSTRACT

Objective: Bromelain, a natural antioxidant, is the active ingredient of pineapple. Paracetamol is a nonsteroidal drug that is used worldwide as a pain reliever and causes kidney damage in high doses. This study was conducted to investigate the potential effects of bromelain on paracetamol-induced kidney damage.

Methods: 56 Sprague–Dawley rats were randomly divided into 7 groups, namely (1) control, (2) N-acetylcysteine (140 mg/kg), (3) bromelain (100 mg/kg), (4) paracetamol (2 g/kg), (5) paracetamol (2 g/kg) + N-acetylcysteine (140 mg/kg), (6) paracetamol (2 g/kg) + bromelain (50 mg/kg), (7) paracetamol (2 g/kg) + bromelain (100 mg/kg). At the end of the experiment, creatinine and urea levels from blood serum, malondialdehyde (MDA) and glutathione (GSH) levels from kidney tissue, and superoxide dismutase (SOD), glutathione peroxidase (GPx) activities were measured. Additionally, the kidney was evaluated histopathologically.

Results: It was determined that serum creatinine, urea levels, and kidney tissue MDA levels were significantly increased in rats in the paracetamol group compared to the control group, while SOD, GSH, and GPx activities were decreased ($P < .050$). N-acetylcysteine and bromelain applications were determined to decrease serum creatinine and urea levels and kidney tissue MDA levels caused by paracetamol and increased SOD, GSH, and GPx activities ($P < .050$). When the histopathological scores were examined, it was found that paracetamol-induced renal tissue damage was reduced by Bro50, Bro100, and N-acetylcysteine applications, and especially Bro100 application was more effective in reducing damage than N-acetylcysteine and Bro50 ($P < .050$).

Conclusion: It was determined that increased serum urea and creatine, tissue oxidative stress markers, and histopathological changes due to paracetamol have decreased thanks to the antioxidant property of bromelain. Additionally, it was determined that the Bro100 dose was more effective than the N-acetylcysteine treatment. It is thought that the obtained data will support different studies to be conducted on the usability of bromelain-supportive treatment in preventing paracetamol-induced kidney damage.

Keywords: Bromelain, renal injury, paracetamol, oxidative stress

INTRODUCTION

Paracetamol (Par), acetaminophen (4-hydroxyacetanilide), is used as an analgesic and antipyretic drug and generally has no side effects when used in therapeutic doses.[1] However, overdose and abuse are a growing concern worldwide. Clinicians often prescribe Par, and this drug is also available over-the-counter. Given its wide use and easy accessibility, toxicity from deliberate or unintentional drug overdose is a major concern.[2] Par is sold as an antipyretic and analgesic. However, it is partly included in the analgesic class by the World Health Organization and is prescribed for conditions such as low back pain and osteoarthritis.[3] The toxicity of Par is primarily due to its metabolism in the kidney and other tissues. The kidney is the second target organ of Par toxicity. Chronic use of Par can lead to dose-related renal failure.[1] Par is

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metabolized in the liver to N-acetyl-p-benzoquinonimine (NAPQI) by cytochrome P450.[4] NAPQI triggers oxidative stress by inducing the production of reactive oxygen species (ROS) in some tissues and causes damage by altering mitochondrial membrane permeability in the cell. It also causes cell death, DNA damage and sometimes acute kidney failure.[5] Oxidative stress occurs because of the disruption of the balance between the ROS and antioxidant defense systems.[6] As a result, oxidative stress occurs when the amount of oxidant is greater than the protective capacity of antioxidant enzymes and non-antioxidants and causes tissue damage. Among the antioxidant enzymes, Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and nonenzymatic antioxidant Glutathione (GSH) scavenge free radicals and superoxide ions in the cell.[7] One of the end products of lipid peroxidation (LPO) is malondialdehyde (MDA), a reactive species used as a redox marker, measured for high oxidative stress.[8]

The current clinical treatment for Par toxicity is N-acetylcysteine (Nac). Nac is metabolized to cysteine and increases intracellular GSH level.[4]

Research is needed to discover and develop agents with protective and therapeutic effects on Par-induced renal toxicity. Some natural products are important for treating renal toxicity caused by Par due to their safe, inexpensive, low side effects, and antioxidant properties. The function of antioxidants is to maintain the redox/oxidation balance in the body and to fight oxidative damage.[9]

Bromelain (Bro) is an enzyme complex derived from pineapple and contains protease inhibitors, peroxidase, and acid phosphatase. Bro gains importance due to its properties such as efficacy and safety. Bro agent acts as an antioxidant, anti-inflammatory, fibrinolytic, and antithrombotic.[10] Bro is considered an effective factor in tooth damage, cancer, inflammation, diseases such as osteoarthritis, and against various pathogens. Bro, a nontoxic and natural compound, can be used as an alternative to artificially produced chemical components and drugs for treating some diseases. Bro, a type of protease enzyme, breaks down other proteins by cutting amino acid chains.[11] While showing all these effects, it can be significantly absorbed in the body without losing its proteolytic activity and without having any significant side effects.[12] Bro has a half-life of 6–9 hours and a plasma concentration of 2.5–4 ng/mL after an oral dose of 8.6 g daily.[13]

This study was conducted to investigate the potential protective effects of Bro on renal injury caused by Par.

MAIN POINTS

- Paracetamol has cytotoxic effects and has a potential risk to human health.
- Bromelain ameliorates oxidative stress caused by paracetamol.
- Bromelain may serve as a potential method for paracetamol therapy.

METHODS

Animals

A total of 56 Sprague–Dawley female rats weight 250–300 g, obtained from the experimental animal laboratory of Kafkas University Experimental Research and Application Center, were used. The experimental animals were fed sufficient (ad libitum) water and pellet feed during the experiment. Rats were housed in separate groups at normal room temperature (22°C) and humidity (50-55%) conditions until the day of the experiment.

This study was approved by Kafkas University Animal Experiments Local Ethics Committee (Date: September 22, 2022, protocol number: 2022–8/144).

Experimental Groups

The animals were randomly divided into seven groups of eight rats in each group. The drug doses applied in the study were selected based on previous studies.[4,14,15]

Group 1: Control (Healthy)
Group 2: Nac140 mg/kg
Group 3: Bro100 mg/kg
Group 4: Par2 g/kg
Group 5: Par2 g/kg + Nac140 mg/kg
Group 6: Par2 g/kg + Bro50 mg/kg
Group 7: Par2 g/kg + Bro100 mg/kg

The Bro applied in this study was applied to the rats for 1 week. At the end of the 7th day, doses of Par and Nac were administered.

After oral administration of 2 g/kg Par, it was kept for 24 h and renal toxicity was created. At the end of the 8th day, the kidney tissues of the rats were taken under general anesthesia (50 mg/kg, thiopental sodium).

Biochemical Analysis

Preparation of Tissue Homogenates

Samples stored at –80°C until the day of analysis were first allowed to dissolve at –20°C and then at +4°C for a while. Kidney tissue was first ground in liquid nitrogen with Tissue Lyser II (Qiagen). The phosphate-buffered homogenate was then prepared. Then, the supernatant

obtained by centrifugation at 3000 rpm at +4°C for 30 min was used as a sample.

Kidney Function Tests

Blood serum from rats was collected for analysis. Commercial kits (Diasis Diagnostic Systems, İstanbul, Türkiye) were used to measure kidney function. Serum creatinine and urea were measured using a multiwell plate reader.

Lipid Peroxidation and Analysis of Antioxidants

MDA for lipid peroxidation level measurement, GSH level, SOD and GPx activities for antioxidant measurement were evaluated. LPO measurement was determined by analyzing the amount of MDA at 532 nm according to the method developed by Placer et al. The MDA level was expressed as nmol/g tissue.[16] The SOD activity was determined according to the method used by Sun et al. SOD activity was expressed as U/g protein.[17] GSH levels were determined based on the method developed by Sedlak and Lindsay. GSH results were expressed as nmol/g tissue.[18] The GPx activity was determined according to the method developed by Lawrence and Burk.[19] The results were expressed as U/g protein. Total protein analysis was performed using the Lowry et al. method.[20]

Histopathological Analysis

Kidney tissue samples taken for histopathological evaluation were kept in 10% formalin for fixation. After 24 hours of fixation, the tissues were washed for 4 hours, then the blocks were prepared by dehydration in increasing alcohol series, clearing in xylene, and infiltration in paraffin. Sections of 4-5 μm thickness were taken from the prepared paraffin blocks. Then, the hematoxylin–eosin staining procedure was applied. Histopathological analyses were photographed with a computer-assisted microscope and examined separately by 3 histologists. A kidney injury score test was also performed to show pathological changes in the kidney. Criteria used in damage: tubular atrophy, tubular dilatation, tubular epithelial cell shedding, degeneration, and vacuolization. This score was scored between 0 and 4 (0, normal structure; 1,<25%; 2, 25-50%; 3, 50-75; 4, >75%). Five nonoverlapping areas were randomly selected from each sample in kidney sections, and 200× magnification was evaluated.

Statistical Analysis

The Statistical Package for the Social Sciences (IBM SPSS Corp., Armonk, NY, USA) version 20.0 computer program was used for statistical analysis. One-way analysis of variance and Duncan multiple comparison test were used to analyze and compare the biochemical data of the groups. Statistical analysis results were expressed as mean ± standard deviation (S.D.). $P < .050$ was considered statistically significant.

RESULTS

Serum Biochemical Results

The creatinine and urea levels given in Figure 1A,B were significantly increased in the Par group compared with the control group ($P < .050$).

Both urea and creatinine levels of the groups given Bro and Nac with Par administration decreased compared with the Par group ($P < .050$). Looking at the results, it was

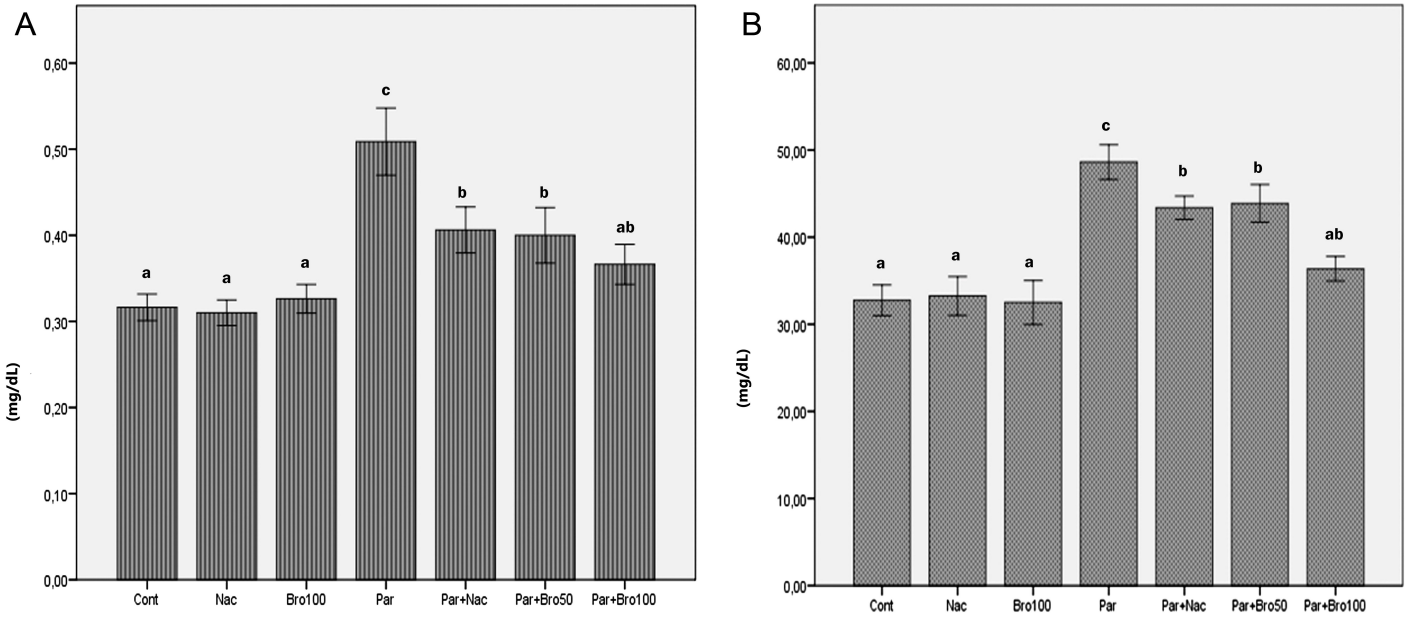

Figure 1. Comparison of creatinine (A) (mg/dL) and urea (B) (mg/dL) levels in all groups. Values are expressed as mean ± SD. Different letters (a,b,c) show the statistical differences between groups ($P < .050$). SD, standard deviation.

concluded that Bro treatment reduced creatine and urea. Additionally, it was observed that the Par + Bro100 group was more effective than the Nac group.

Kidney Biochemical Analysis Results

In this study, the MDA level was measured to determine lipid peroxidation. Figure 2A shows a significant increase in MDA level in the Par group compared to the control group ($P < .050$). MDA levels were found to be lower in the groups given Nac and Bro compared with Par, while high dose treatment was found to be more effective ($P < .050$).

As seen in Figure 2B, a significant increase was observed in all groups given SOD, Bro and Nac, one of the endogenous antioxidants, compared with the group administered Par ($P < .050$).

Likewise, as seen in Figure 2C,D, GSH and GPx levels were significantly increased in the groups given Nac and Bro compared with the group given Par ($P < .050$). Additionally, it was observed that the GSH level and GPx activity were higher and close to the control in the Par+Bro100 group. According to these results, it was concluded that the Bro factor had a protective effect on oxidative stress damage caused by Par.

Histopathological Results

When histological images of the control group kidneys were examined, glomeruli, Bowman's space, and tubules showed normal morphological structure (Figure 3A).

In the Nac and Bro100 groups, the kidney architecture was close to the intact control (Figure 3B,C).

In the Par group, it was remarkable that the glomeruli were atrophied, especially the dilatation of the bowman space.

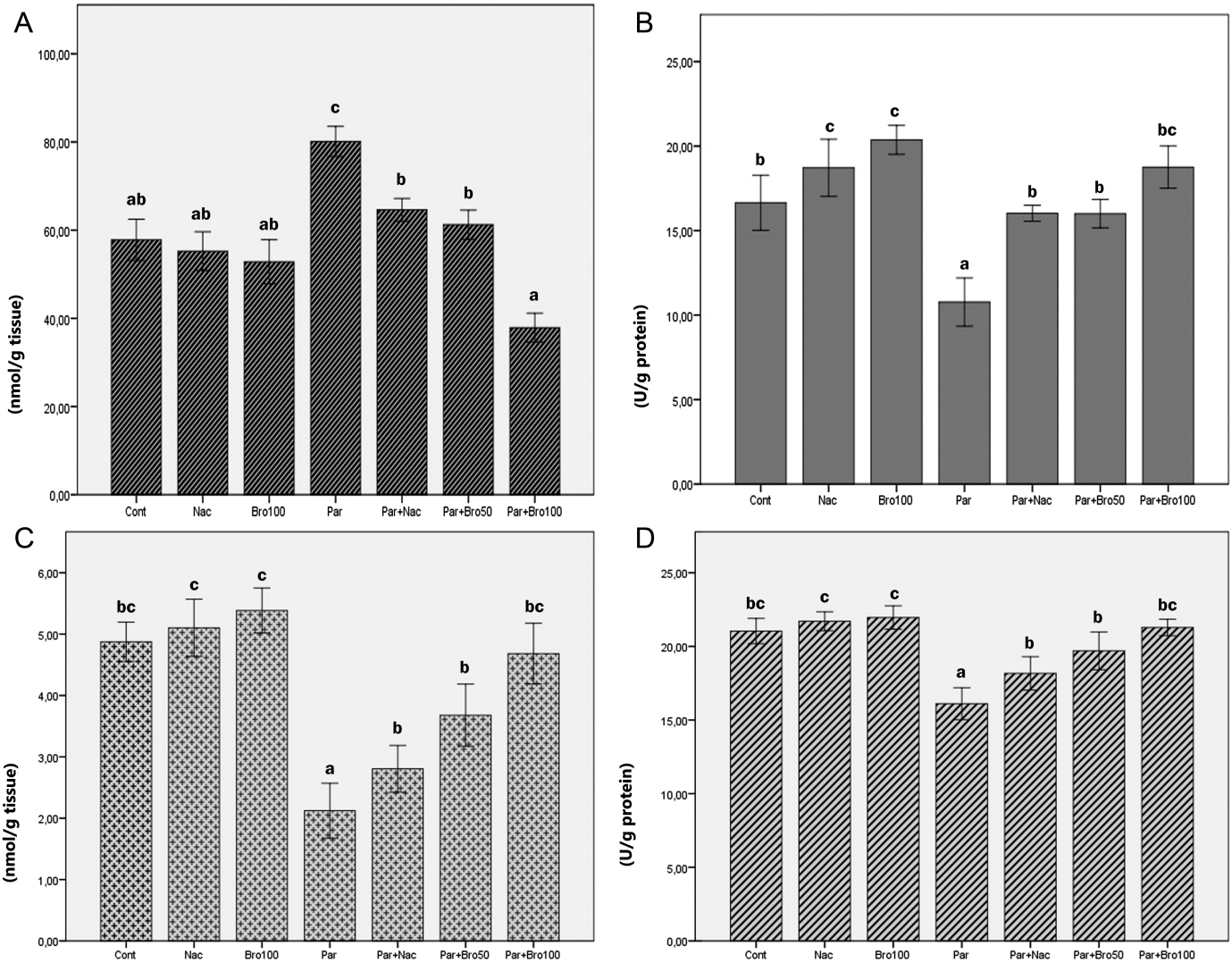

Figure 2. Comparison of MDA (A) (nmol/g tissue), SOD (B) (U/g protein), GSH (C) (nmol/g tissue), GPx (D) (U/g protein) activity in all groups. Values are expressed as mean ± SD. Different letters (a,b,c) in the groups show the statistical differences between ($P < .050$). GPx, glutathione peroxidase; GSH, glutathione; MDA, malondialdehyde; SD, standard deviation; SOD, superoxide dismutase

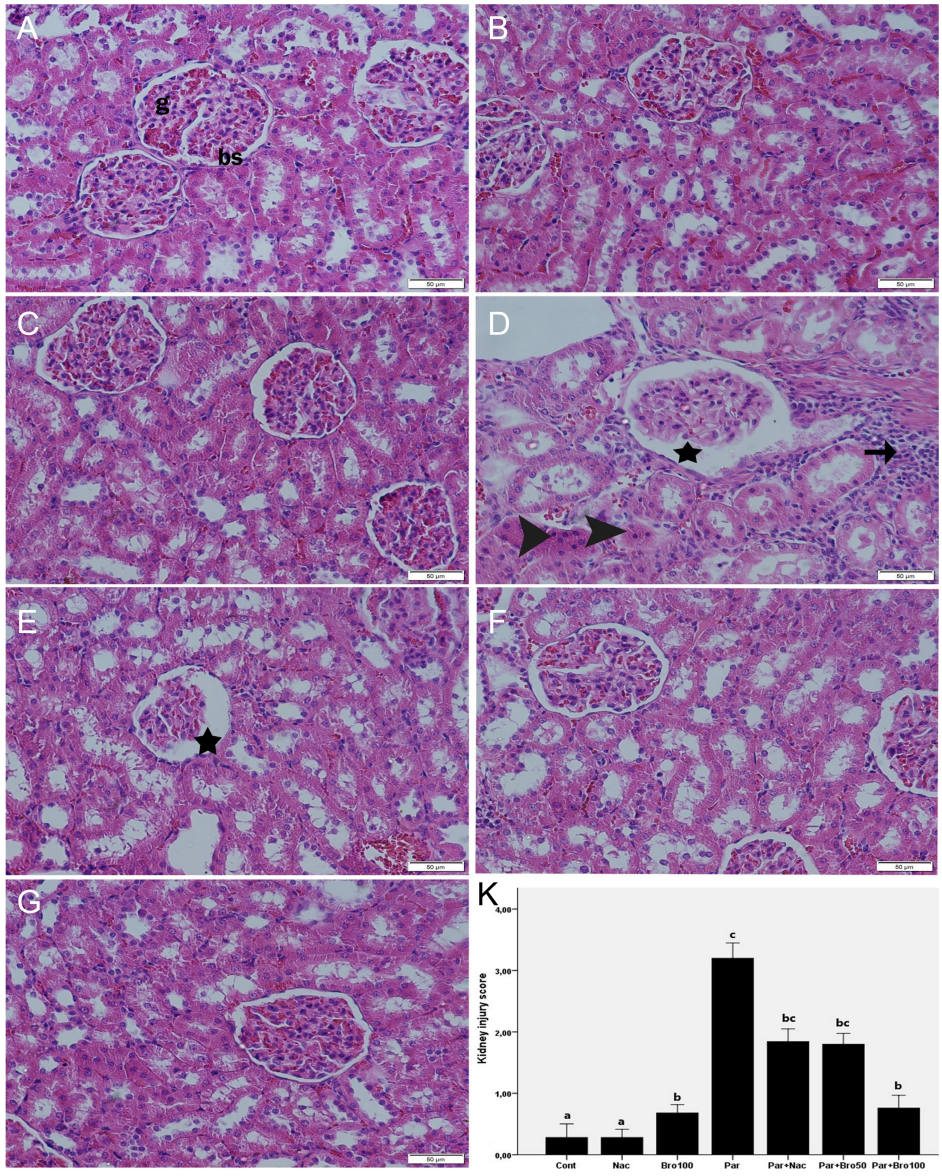

Figure 3. Light micrographs of kidney tissue. (A) Control group; g, glomerulus; bs, Bowman's space, (B) Nac group; (C) Bro100 group; (D) Par group; star, Bowman's space dilation; arrow, inflammatory cell infiltration; arrowhead, eosinophilic cell, (E) Par+Nac group; (F) Par+Bro50 group, (G) Par+Bro100 group. K, kidney tubular injury scores. Values are expressed as mean ± SD. Different letters (a,b,c) the groups show the statistical differences between ($P < .050$). H&E staining; original magnifications: 200×. Bro100, bromelain 100 mg/kg; Bro50, bromelain 50 mg/kg; H&E, hematoxylin and eosin; NAC, N-acetylcysteine; Par, paracetamol; SD, standard deviation

In addition, a decrease in the cells of the tubules, intense pycnotic staining, and necrotic changes in the nuclei were observed. Also, inflammatory cell infiltration in the interstitial area was remarkable (Figure 3D).

A moderate architectural improvement was observed in the kidney tissue of the groups treated with Nac and Bro50 after Par (Figure 3E, F).

We observed that the pathological effects were reversed in the Bro100 group. Glomeruli and tubules were found to be in normal morphology. Additionally, a decrease in inflammatory cell infiltration was observed (Figure 3G).

When the kidney damage score was evaluated, the damage increased significantly in the Par group compared to the control group. The damage score was significantly lower in the Bro and Nac groups compared to the Par group ($P < .050$). It was also observed that the Par+Bro100 group was more effective on damage than the other treatment groups (Figure 3K).

DISCUSSION

Par is an analgesic and antipyretic drug and is widely used around the world. However, there is evidence that long-term use and overdose of this drug cause nephrotoxicity.

Toxicity is caused by covalent binding to lipids, proteins, and DNA due to increased production of the reactive metabolite NAPQI. Because of this binding, cell death occurs due to the increase in ROS.[21] Despite many studies on the mechanism of action of Par, a clear conclusion has not been reached yet.

New active substances are required to avoid long-term use of Nac for Par treatment and to avoid drug dependence. Among these agents, much emphasis is placed on natural products.

Bro is an active ingredient with anticancer, anti-inflammatory, and antioxidant properties.[10] The mechanism of this factor is not clear. As far as our knowledge, this study is the first to show the effect of Bro on Par. This study investigated Bro's effect on renal functions, oxidative damage, and histological changes on Par-induced nephrotoxicity.

The kidney, which is a vital organ in humans, plays an active role in blood pressure, acid-base electrolytes, and cell fluid balance. Especially some drugs, toxic substances, pesticides, and chemotherapy factors are taken into the body to change this order and impair kidney functions. Kandemir et al. found that Par disrupts kidney function and increased creatinine and urea levels.[22] When we look at our results, we found that the overdose of Par increased serum urea and creatinine, which indicates kidney functions, in accordance with the experimental and clinical results in the literature.[22,23] In our study, in addition to the healing effect of Nac, it was observed that the urea and creatine levels in the groups given Par+Bro50 and Par+Bro100 had a significant decrease compared with the Par group. Therefore, it was concluded that the administration of Bro has a similar effect to Nac and that this treatment regulates kidney functions.

One of the important effects of Par toxicity is the damage caused by free radicals. MDA, a lipid peroxidation product, is an indicator of tissue damage. MDA results in the study were increased in the Par group, similar to the previous literature findings.[24] MDA levels were upregulated in the groups given Bro after Par. It was observed that a 100 mg/kg dose of Bro agent was more therapeutic than the Nac drug on kidney damage caused by Par. A recent toxicity study showed evidence that Bro reduces MDA levels.[25] Looking at these results, we concluded that Bro may be protective against membrane-induced damage.

The increase in ROS due to the increase in NAPQI may disrupt the antioxidant balance in the body. Additionally, increasing MDA level increases oxidative stress by detoxifying the SOD, GSH, and GPx antioxidant defense system. Antioxidants actively role in scavenging free radicals from the body by enzymatic and nonenzymatic means.[22] SOD, a cellular antioxidant, plays a role in reducing the level of superoxide. Consistent with the literature, there was a significant decrease in SOD activity in the Par group in our study.[26] Similar to the effect of Bro in the toxicity study by Chaudhary et al., Bro decreased the SOD activity in our study.[27] This result is thought to be due to the ability of the Bro agent to scavenge free radicals directly. Additionally, it was determined that Bro indirectly suppressed oxidative stress by regulating antioxidant enzyme activities.

GSH, which is abundant in the cell, is a nonenzymatic antioxidant. This antioxidant acts as a substrate for enzymes such as GR, GPx, and GST. The decreased amount of GSH causes excessive accumulation of reactive species. The increase in NAPQI production due to the administration of Par causes glutathione depletion. Recently, glutathione precursor Nac has been preferred to increase GSH levels.[22,28] Nac administration is the only drug used in the treatment of Par. However, the Bro we use is also effective in raising the GSH level. Our study results show that the decrease in GSH level in the Par group was remarkable. Furthermore, we observed that the high dose of Bro was more effective than the low-dose treatment of Bro, which had similar results with the Nac application. Our results aligned with the results of Alves et al.'s study.[29] As a result, it was determined that high-dose Bro administration with Par increased GSH level to close to the control group. GPx, one of the antioxidant enzymes, protects cell membrane lipids from oxidative damage and catalyzes the reaction of hydroperoxide radicals with GSH to form disulfide glutathione (GSSG).[30] In the current study, it was determined that GPx activity decreased due to Par administration, and GPx activity increased similarly in Nac and low-dose Bro treatment. However, it was observed that the Par+Bro100 group was more effective than the other treatments and brought the GPx activity to levels close to control. This result was in line with the results of the Bro study by El-Demerdash et al.[30] Therefore, Bro protects cells by activating antioxidant enzymes such as GPx, which detoxifies the ROS produced by Par.

Compatible with our biochemical results, Par administration was also reflected in the histological results. Similar to the studies in the literature, it is obvious that Par application causes atrophic glomerular structure, dilatation in Bowman's space, and lymphocyte infiltration.[31] Bro showed wound healing ability, reduced lipid peroxidation, and increased antioxidant levels. Our histopathological data confirmed this evidence. It was observed that Bro, in particular, not only improved the architectural structure of the deteriorated kidney but also reduced inflammatory cells at high doses.[30] We observed that our results agreed with studies looking at the therapeutic effect of Bro on

some toxic effects. This healing effect is thought to be due to the antioxidant property of Bro.

As a result, it was revealed that Bro suppressed Par-induced lipid peroxidation, increased the antioxidant level, and reduced tissue damage in this way. Compared with Nac, 50 mg/kg Bro administration had similar effects. However, 100 mg/kg Bro was more effective than the Nac treatment. These results show that Bro is a potential adjuvant for Par-induced renal injury. The data obtained are important in terms of contributing to different and more advanced studies on the subject.

Ethics Committee Approval: Ethics committee approval was received for this study from the Kafkas University Animal Experiments Local Ethics Committee (Date: September 22, 2022, protocol number: 2022–8/144).

Informed Consent: Since rats were used in the study, patient consent was not obtained.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – N.A.; Design – N.A., E.T.; Supervision – N.A., E.T.; Resources – N.A., E.T.; Materials – N.A., E.T., N.A.C., N.Y., H.İ.Ö.; Data Collection and/or Processing – N.A., E.T., N.A.C., N.Y., H.İ.Ö.; Analysis and/or Interpretation – N.A., E.T., N.A.C., N.Y., H.İ.Ö.; Literature Search – N.A., H.Ş.; Writing Manuscript – N.A., H.Ş.; Critical Review – N.A., E.T., H.Ş.; Other – N.A.

Declaration of Interests: The authors declare that they have no competing interest.

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REFERENCES

1. Hilal MA, Elsayed RM, Mahmoud SF, Abdallah AA, Kasem SE. Ameliorative role of alpha lipoic acid on paracetamol induced renal toxicity and oxidative stress in rats. *Egypt J Forensic Sci Appli Toxicol*. 2019;19(1):87-106.
2. Chatterjee S, Bhattacharya S, Choudhury PR, et al. Drynaria quercifolia suppresses paracetamolinduced hepatotoxicity in mice by inducing Nrf-2. *Bratisl Lek Listy*. 2022;123(2):110-119. [CrossRef]
3. McCrae JC, Morrison EE, MacIntyre IM, Dear JW, Webb DJ. Long-term adverse effects of paracetamol - a review. *Br J Clin Pharmacol*. 2018;84(10):2218-2230. [CrossRef]
4. Canayakin D, Bayir Y, Kilic Baygutalp N, et al. Paracetamol-induced nephrotoxicity and oxidative stress in rats: the protective role of Nigella sativa. *Pharm Biol*. 2016;54(10): 2082-2091. [CrossRef]
5. Fadda L, Ali HM, Aldrees GA, Alquraishi NM. Nano ubiquinone: promising candidate for treatment of renal toxicity induced by over dose of paracetamol. *Toxicol Rep*. 2019; 6:712-717. [CrossRef]
6. Aparicio-Trejo OE, Aranda-Rivera AK, Osorio-Alonso H, et al. Extracellular vesicles in redox signaling and metabolic regulation in chronic kidney disease. *Antioxidants (Basel)*. 2022;11(2):356. [CrossRef]
7. Kandemir FM, Caglayan C, Darendelioğlu E, Küçükler S, İzol E, Kandemir Ö. Modulatory effects of carvacrol against cadmium-induced hepatotoxicity and nephrotoxicity by molecular targeting regulation. *Life Sci*. 2021;277:119610. [CrossRef]
8. Çomaklı S, Kandemir FM, Küçükler S, Özdemir S. Morin mitigates ifosfamide induced nephrotoxicity by regulation of NF-kappaB/p53 and Bcl-2 expression. *Biotech Histochem*. 2022;97(6):423-432. [CrossRef]
9. Ferah Okkay I, Okkay U, Cicek B, et al. Neuroprotective effect of bromelain in 6-hydroxydopamine induced in vitro model of Parkinson's disease. *Mol Biol Rep*. 2021;48(12):7711-7717. [CrossRef]
10. Saptarini NM, Rahayu D, Herawati IE. Antioxidant activity of crude bromelain of pineapple (ananas comosus (l.) merr) crown from subang district, Indonesia. *J Pharm Bioallied Sci*. 2019;11(4)(suppl 4):S551-S555. [CrossRef]
11. Chakraborty AJ, Mitra S, Tallei TE, et al. Bromelain a potential bioactive compound: a comprehensive overview from a pharmacological perspective. *Life (Basel)*. 2021;11(4):317. [CrossRef]
12. Kwatra B. A review on potential properties and therapeutic applications of bromelain. *World J Pharm Pharm Sci*. 2019;8(11):488-500.
13. Tallei TE, Fatimawali Yelnetty A, Idroes R, et al. An analysis based on molecular docking and molecular dynamics simulation study of bromelain as anti-SARS-CoV-2 variants. *Front Pharmacol*. 2021;12:717757.
14. Karcioglu SS, Palabiyik SS, Bayir Y, et al. The role of RAAS inhibition by aliskiren on paracetamol-induced hepatotoxicity model in rats. *J Cell Biochem*. 2016;117(3):638-646. [CrossRef]
15. Şehirli AÖ, Sayiner S, Savtekin G, Velioğlu-Öğünç A. Protective effect of bromelain on corrosive burn in rats. *Burns*. 2021;47(6):1352-1358. [CrossRef]
16. Placer ZA, Cushmanni LL, Johnson BC. Estimation of products of lipid peroxidation (as malondialdehyde) in biochemical systems. *Anal Biochem*. 1966;16:359-364.
17. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem*. 1988;34(3):497-500. [CrossRef]
18. Sedlak J, Lindsay RH. Estimation of total protein bound and nonprotein sulfhydryl groups in tissue with Ellmann's reagent. *Anal Biochem*. 1968;25(1):192-205. [CrossRef]
19. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun*. 1976;71(4):952-958. [CrossRef]
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem*. 1951;193(1):265-275. [CrossRef]
21. El Menyiy N, Al-Waili N, El Ghouizi A, Al-Waili W, Lyoussi B. Evaluation of antiproteinuric and hepato-renal protective activities of propolis in paracetamol toxicity in rats. *Nutr Res Pract*. 2018;12(6):535-540. [CrossRef]
22. Kandemir FM, Kucukler S, Eldutar E, Caglayan C, Gülçin İ. Chrysin protects rat kidney from paracetamol-ınduced oxidative stress, ınflammation, apoptosis, and autophagy: A Multi-Biomarker Approach. *Sci Pharm*. 2017;85(1):4. [CrossRef]
23. Naguib YM, Azmy RM, Samaka RM, Salem MF. Pleurotus ostreatus opposes mitochondrial dysfunction and oxidative

stress in acetaminophen-induced hepato-renal injury. *BMC Complement Altern Med*. 2014;14:494. [CrossRef]
24. Yu Y, Wu Y, Yan HZ, et al. Rosmarinic acid ameliorates acetaminophen-induced acute liver injury in mice via RACK1/TNF-α mediated antioxidant effect. *Pharm Biol*. 2021;59(1):1286-1293. [CrossRef]
25. Al-Otaibi WR, Virk P, Elobeid M. Ameliorative potential of stem bromelain on lead-induced toxicity in Wistar rats. *Acta Biol Hung*. 2015;66(2):149-160. [CrossRef]
26. Akgun E, Boyacioglu M, Kum S. The potential protective role of folic acid against acetaminophen-induced hepatotoxicity and nephrotoxicity in rats. *Exp Anim*. 2021;70(1):54-62. [CrossRef]
27. Chaudhary B, Bist R. Protective manifestation of bacoside A and bromelain in terms of cholinesterases, gamma-amino butyric acid, serotonin level and stress proteins in the brain of dichlorvos-intoxicated mice. *Cell Stress Chaperones*. 2017;22(3):371-376. [CrossRef]
28. BinMowyna MN, AlFaris NA. Kaempferol suppresses acetaminophen-induced liver damage by upregulation/activation of SIRT1. *Pharm Biol*. 2021;59(1):146-156. [CrossRef]
29. Alves EHP, Carvalho ADS, Silva FRP, et al. Bromelain reduces the non-alcoholic fatty liver disease and periodontal damages caused by ligature-induced periodontitis. *Oral Dis*. 2020;26(8):1793-1802. [CrossRef]
30. El-Demerdash FM, Baghdadi HH, Ghanem NF, Mhanna ABA. Nephroprotective role of bromelain against oxidative injury induced by aluminium in rats. *Environ Toxicol Pharmacol*. 2020;80:103509. [CrossRef]
31. Ahmad S, Zeb A. Nephroprotective property of Trifolium repens leaf extract against paracetamol-induced kidney damage in mice. *3 Biotech*. 2020;10(12):541. [CrossRef]