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## Bromelain diminishes renal consequences induced by gamma irradiation via suppressing ACE/Ang-II/aldosterone pathway in rats

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RADIATION causes renal damage by enhancing the renin-angiotensin-aldosterone system›s (RAAS) activation. This work aimed to evaluate the role of bromelain, an angiotensin-converting enzyme inhibitor (ACEI), in hindering the inflammatory and fibrotic effects of radiation by suppressing this pathway. Rats were subjected to a single dosage of 7.5 Gy then administered bromelain (30 mg/kg) orally for 14 days after one hour of irradiation. Gamma radiation caused a considerable increase in (RAAS) indices such as angiotensin II (AngII), aldosterone and angiotensin-converting enzyme (ACE) protein expression, in along with renal function: urea, creatinine and blood urea nitrogen with significant disturbance in oxidative stress markers: malondialdehyde (MDA), protein carbonyl (PCO), reduced glutathione (GSH), total thiol (tSH) and superoxide dismutase (SOD) in renal tissue homogenate. In addition, there was a notable increase in the renal inflammatory mediator's levels: tumor necrosis factor-α (TNF-α), nuclear factor kappa B (NF-κB) and interleukin-1β (IL-1β) alongside serum profibrotic markers: hyaluronic acid (HA), laminin (LN), type III procollagen (PCIII) and renal hydroxyproline (HYP) levels. Bromelain treatment dramatically improves the previously described parameters and modifies the radiation-induced renal histopathological damage in retaliation to the suppression of ACE, Ang II and aldosterone expressions.

Conclusions: Bromelain hinders radiation-induced inflammatory, fibrotic and oxidative stress response *via* remodelling the RAAS pathway in rats.

**Keywords**: Bromelain, ACE, radiation, Angiotensin II, aldosterone

## Introduction

Like many other cellular stressors, ionizing radiation can either activate or inhibit a wide range of signaling pathways, which can lead to increase cell growth or death. However, controlling the signaling mechanism varies according to the type of cell, radiation exposure, and culture circumstances (Munshi and Ramesh, 2013). Reactive oxygen species (ROS) are recognized as produced by radiation, leading to oxidative stress. Ionizing radiation's detrimental effects on biological systems could be exacerbated by water radiolysis, resulting in organ dysfunction and cellular damage (Mansour and Hafez, 2012; Cohen and Cohen, 2013). These ROS cause damage and dysfunction by attacking biological macromolecules like proteins, DNA, RNA, and cell membranes (Chittezhath and Kuttan, 2006). According to Hasan et al. (2020), radiation-induced kidney injury, which is typified by acute inflammation and fibrosis, is the most dangerous side effect of radiation therapy.

Damage to the kidney glomeruli, tubular epithelium, interstitium, and blood vessels has been found in the morphologic studies of radiation-induced nephropathy (Cohen and Robbins, 2003). The renin-angiotensin-aldosterone system (RAAS) is a multi-organ system comprising enzymes and the peptide substrates of those enzymes. RAAS is a crucial blood pressure and electrolyte regulator. The kidneys create renin that breaks down the liver's angiotensinogen

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into angiotensin I (AngI), which is subsequently changed into angiotensin II (AngII) by the lungs' angiotensin-converting enzyme (ACE). In renal disorders, particularly renal hypertension, intrarenal RAAS activation is a realistic event (Yang and Xu, 2017). RAAS-inhibition generally slows the progression of several renal disorders with numerous causes. It has a nephroprotective impact by lowering intraglomerular pressure, which lowers proteinuria and, in turn, tubulointerstitial damage (Meyer et al., 1987). Angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARBs) that stop RAAS damaging effects are strongly linked to halting cancer progression and overall survival, according to a recent meta-analysis (Sun et al., 2017). Hence, inhibition of AngII by bromelain helps to treat or prevent radiation-induced organ damage.

The pineapple-derived enzyme complex known as bromelain includes several thiol endopeptidases and others including various protease inhibitors, glucosidase, cellulase, phosphatase, peroxidase, and escharase (Agrawal et al., 2022). It has antithrombotic, fibrinolytic, anti-inflammatory, and antioxidant properties (Saptarini et al., 2019). Bromelain, an inexpensive by-product waste of pineapple extract, is rich in complex enzymes that play a vital role in various clinical applications and has a highly effective antioxidant effect in the treatment of nephrotoxicity and capable of ACE inhibition (Hanafi et al., 2018; Gürel and Kaya, 2022; Sulumer et al., 2024). The main active constituent of bromelain is cysteine protease fraction. This enzyme exerts anti-inflammatory, anti-apoptotic, and fibrinolytic actions that help attenuate and reducing kidney damage(Maurer, 2001).

Unlike pharmaceuticals and artificially made chemical components, bromelain is a natural, harmless substance that can cure several disorders. It is a powerful remedy for several illnesses, including osteoarthritis, cancer, inflammation, and infections (Chakraborty et al., 2021). In addition to exhibiting all of these actions, it can extensively retain its proteolytic action after being absorbed by the body without experiencing any serious adverse effects (Kwatra, 2019).

This study aimed to assess the novel hypothesis that bromelain, an ACE inhibitor, can effectively lessen the renal impacts of gamma irradiation by halting angiotensin II; thus, it is a very hopeful approach to treating renal damage.

## Materials and methods

*Materials*

Bromelain was purchased from Sigma-Aldrich. Bromelain powder was stored at −20°C in tightly sealed, light-protected containers to prevent moisture uptake and degradation. Solutions were prepared immediately before administration. Every additional chemical that was involved in this investigation was analytical quality.

*Experimental animals*

The National Center for Radiation Research and Technology (NCRRT) offered male Wistar albino rats weighing 130-150 g. During the trial, rats were kept under standard conditions of humidity and temperature (22-24oC) and were fed on standard pellets of concentrated diet including all the necessary nutritive ingredients (23% protein, 4.68% lipids, and 2.6% fibers) and permitted full access to water. In compliance with worldwide ethical considerations, animal experiments were carried out with the guidelines for the appropriate care and use of laboratory animals (NIH publication No.85-23, amended 1985) and in compliance with regulations of the ethical committee for animal care at the NCRRT, Atomic Energy Authority, Cairo, Egypt. An effort was made to reduce the suffering of animals.

*Radiation facility*

Rats were given total body γ-irradiation at the NCRRT in Cairo, Egypt, using a Gamma cell-40 with a Caesium-137 irradiation source. Rats irradiated with 7.5 Gy as a single dose at a rate of 0.012 Gy/s (Mak et al., 2015).

*Experimental design*

Four groups of rats were randomly assigned (6 rats per group); the control group received saline; the irradiated group (IRR) was exposed to a single dose of 7.5 Gy; the (Bro) group received 30 mg/kg of bromelain dissolved in normal saline (0.9% NaCl) and administered orally for 14 days (Bennion et al., 2015a) and the (IRR+Bro) group received bromelain at a dose of (30 mg/kg) orally for 14 days post whole body γ-irradiation. Rats were anesthetized 24 hours after the last dose of bromelain and blood samples were taken by heart puncture. Serum was then separated by centrifugation, and kidney samples were taken right away. They were then washed with saline to remove any blood contamination, dried with filter paper, and then separated into two sections for different biochemical and histopathological analyses.

*Biochemical assessment*

*Evaluation of renal biochemical parameters*

The levels of serum urea, creatinine and blood urea nitrogen (BUN) were colorimetrically assayed using commercial kits in accordance with the manufacturer's instructions.

*Evaluation of oxidative stress in renal tissue*

Reduced glutathione (GSH) was estimated following the method of Ellman (1959). malondialdehyde (MDA) was measured according to Yoshioka et al. (1979). The protein carbonyl (PCO), total thiol (tSH) and superoxide dismutase (SOD) enzyme activity were estimated following the methods of (Sadeghi et al., 2019)**,** (Doustimotlagh et al., 2014) and Minami and Yoshikawa (1979) respectively in the kidney homogenates.

*Evaluation of renal inflammatory mediators*

Kidney homogenates were used for estimation of renal tumor necrosis factor-α (TNF-α), nuclear factor kappa B (NF-κB) and interleukin-6 (IL-1β) contents using ELISA kits (eBioscience, Inc., San Diego., USA) according to the manufacturer›s protocol.

*Evaluation of renal profibrotic biomarkers*

Serum levels of hyaluronic acid (HA), laminin (LN), and type III procollagen (PCIII) were evaluated using ELISA kits. In addition hepatic hydroxyproline (HYP) content was determined based on the method of (Woessner Jr, 1961).

*Western blot analysis*

Using the Western blot technique as outlined by Thomas et al. (2013), protein expression of angiotensin-converting enzyme (ACE), angiotensin II (Ang II), and aldosterone was assessed. The kidneys were, in short, homogenized in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1% Triton X-100, plus the entire protease inhibitor cocktail [Roche]), and then centrifuged at 20,000 g for 20 minutes at 4°C. The samples were moved to nitrocellulose membranes after being run on Invitrogen NuPAGE Bis-Tris gels. Membranes were imaged using a Bio-Rad Chemi Doc XRS+ imager after treatment with the corresponding antibodies. ACE (1:1000; Thermo Fisher Scientific, Cat. No.: 1636-MSM2-P0), Ang II (1:1000; Abbiotec, USA. Cat. No.: 251229), aldosterone (1:1000; Bio-Rad, Cat. No.: 0280-1379; USA), and β-actin (1:1000; Sigma) were the targets of the antibodies that were utilized. Following normalization for β-actin protein expression (as an internal control to ensure equal loading and transfer of protein), the results were ascertained.

*Histopathological examination*

Specimens of kidney tissue were collected from all animal groups and fixed in 10% neutral buffered formalin. They were trimmed, washed, and dehydrated in varying grades of alcohol, cleared in xylene, embedded in paraffin, sectioned at 4-6 μm thickness, and stained with hematoxylin and eosin in accordance with (Layton and Bancroft, 2013). Prepared slides sections were stained by hematoxylin and eosin and examined by light digital microscope (Olympus xc30. Tokyo. Japan).The frequency and severity of lesions in the kidney were assessed semi-quantitatively as previously reported by (Zhang et al., 2008) using a scale where, score 0, Normal histology score 1 Tubular epithelial cell degeneration, without significant necrosis or apoptosis score 2 Tubular epithelial cell necrosis and apoptosis <25% score 3Tubular epithelial cell necrosis and apoptosis <50% score 4Tubular epithelial cell necrosis and apoptosis <75% score 5 Tubular epithelial cell necrosis and apoptosis≥ 75% .

*Data analysis*

One-way analysis of variance (ANOVA) and Tukey-Kramer's Multiple Range test were used in the statistical package of social science (SPSS) version 21.0 for Windows. P values < 0.05 were regarded as significant, and the data gathered were shown as means ± standard error of mean (SEM).

## Results

*Modulatory action of bromelain versus γ-radiation-induced kidney structural damage.*

To examine the impact of γ-radiation on renal damage, the protein expression of ACE, Ang-II and aldosterone in renal tissue with serum kidney function (Urea, Creatinine and BUN) was unveiled. The bromelain group's results revealed an insignificant change in these markers compared with the control group (Fig. 1 and 2). In contrast, the results of these indicators in the IRR group showed a substantial increase (p £ 0.05) in the expression of ACE, Ang-II and aldosterone and kidney function parameters in comparison to the control group. The data observation of IRR+Bro group indicated a noteworthy change (p £ 0.05) in these metrics following bromelain treatment in contrast to the irradiated group (Fig. 1 and 2).

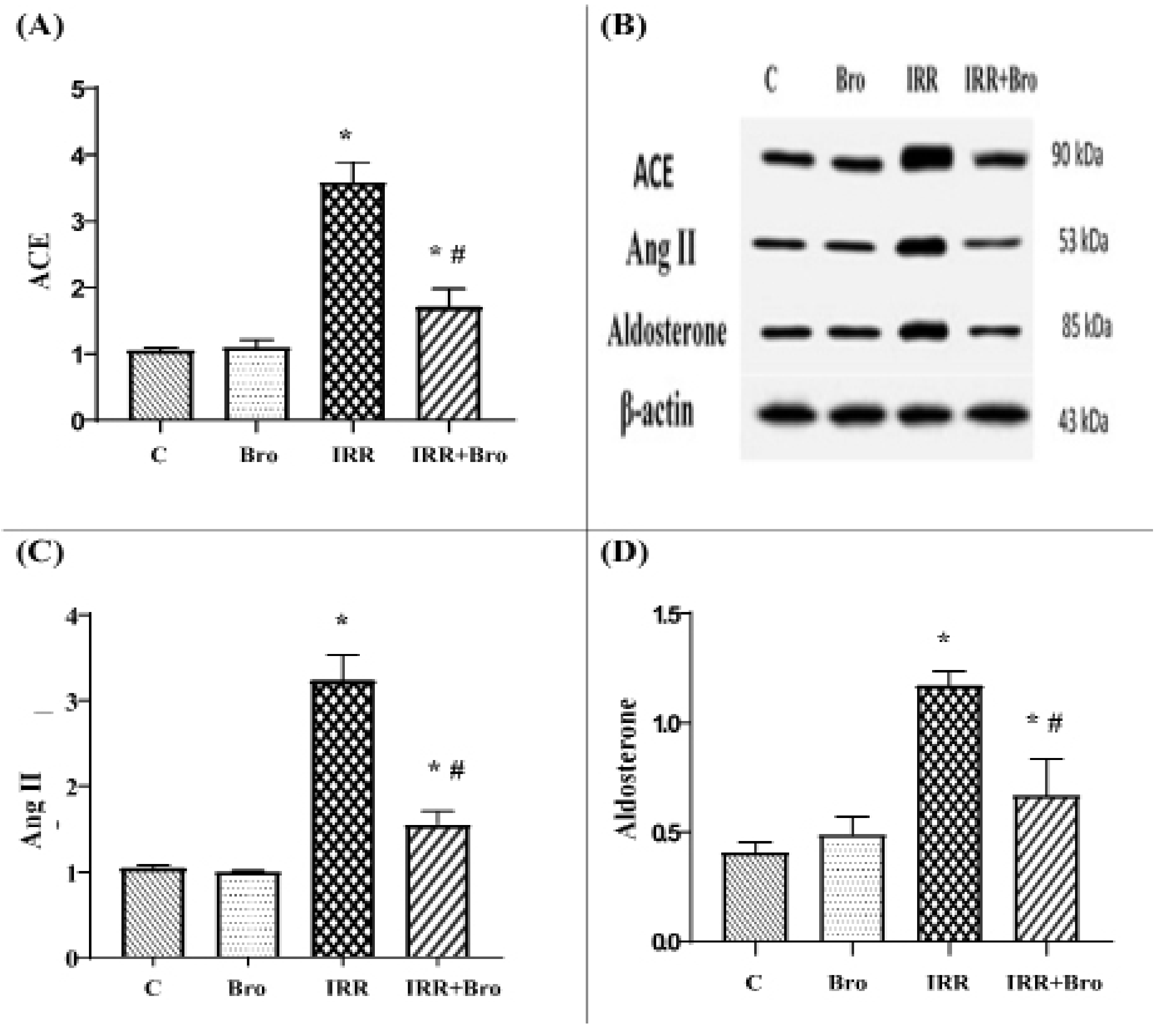


**Fig. 1. Effect of bromelain against renal consequences induced by γ-radiation *via* suppressing ACE/Ang-II/ aldosterone pathway.**

Relative protein expression was quantified by densitometry and corrected by reference to b-actin. Each value represents Mean± SEM (n=6). Columns denoted with (*) significant from control, (#) significant from irradiated group at (p £ 0.05) using One-way ANOVA followed by Tukey–Kramer as a post-ANOVA test. C: control rats, Bro: rats treated with bromelain, IRR: rats exposed to 7.5 Gy, IRR+Bro: rats exposed to 7.5 Gy and treated with bromelain. AngII: Angiotensin II, ACE: Angiotensin Converting Enzyme.

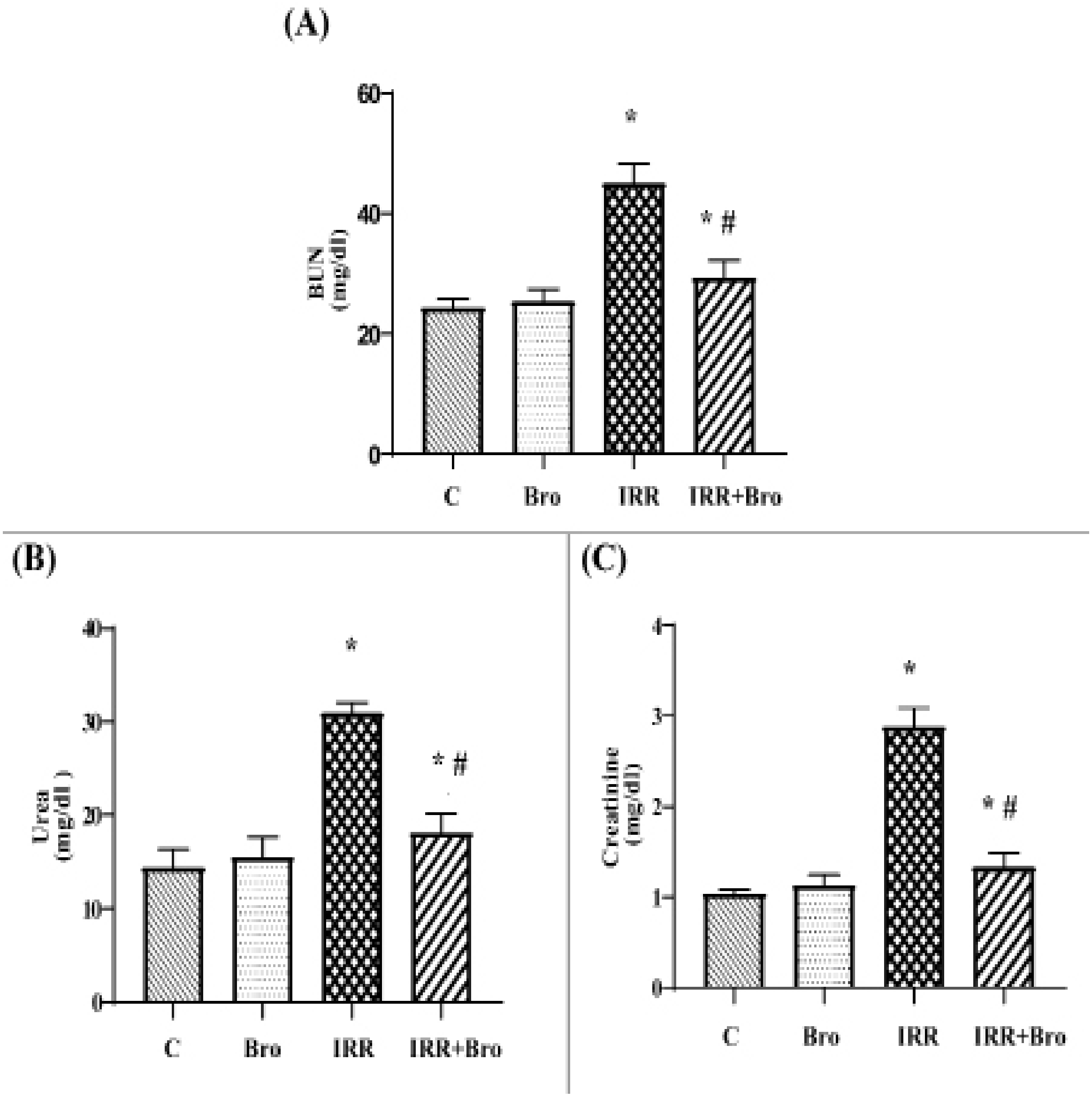


**Fig. 2. Effect of bromelain against renal function disturbance induced by γ-radiation.**
Each value of serum Urea, Creatinine and blood urea nitrogen (BUN) represents Mean± SEM (n=6). Columns denoted with (*****) significant from control, **(#)** significant from irradiated group at (p £ 0.05) using One-way ANOVA followed by Tukey–Kramer as a post-ANOVA test. **C**: control rats, **Bro**: rats treated with bromelain, **IRR**: rats exposed to 7.5 Gy, **IRR+Bro**: rats exposed to 7.5 Gy and treated with bromelain.

*Impact of bromelain on γ-radiation-caused renal oxidative stress.*

In the kidney, the bromelain group's data demonstrated an insignificant change in all indicators when compared with the control, as demonstrated in Fig. 3. Rats exposed to radiation experienced oxidative stress, as evidenced by a notable rise (p £ 0.05) in PCO and MDA levels accompanied by a remarkable decline (p £ 0.05) in tSH, SOD and GSH contents in IRR and IRR+Bro groups, respectively, compared with the control group (Fig. 3). Concurrent bromelain considerably enhanced this impact, as seen by a considerable elevation (p £ 0.05) in tSH, SOD and GSH contents alongside a marked reduction (p £ 0.05) in PCO and MDA levels compared with the irradiated group (Fig. 3).

*Defensive impact of bromelain against γ-radiation-induced inflammation.*

The renal inflammatory status of the bromelain group showed an insignificant change in renal levels of TNF-α, NF-κB, and IL-β1 compared with the control group. The IRR and IRR+Bro groups› data showed a substantial increase (p£ 0.05) in all inflammatory markers, respectively, compared with the control group. IRR+Bro group revealed a substantial drop (p£ 0.05) in aforementioned parameters respectively, in comparison to the irradiation group (Fig. 4).

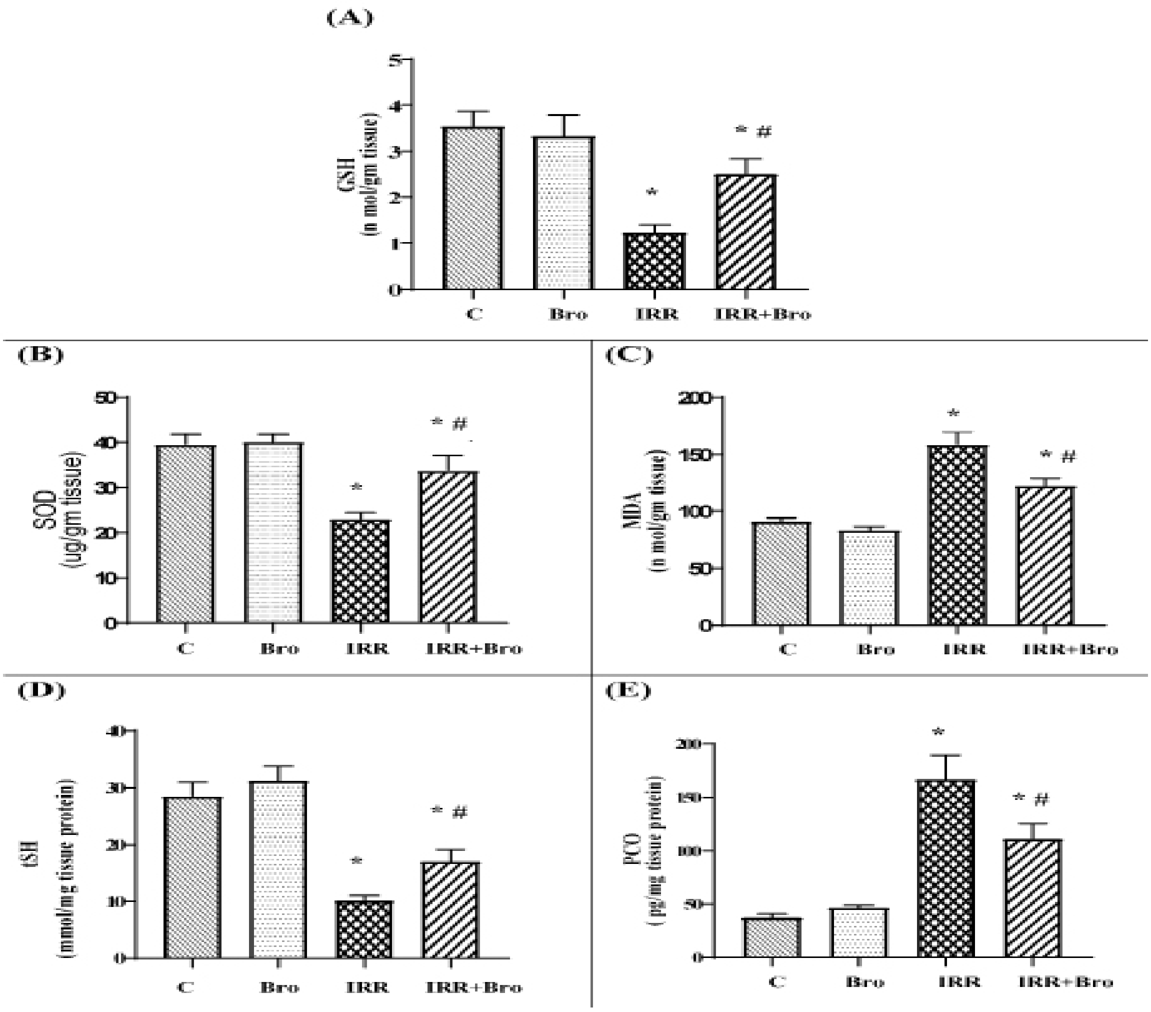


**Fig. 3. Effect of bromelain against renal oxidative stress induced by γ-radiation.**

Each value of reduced glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA), total thiol (tSH) and protein carboryl (PCO) content in rat kidney tissues represents Mean± SEM (n=6). Columns denoted with (*****) significant from control, **(#)** significant from irradiated group at (p £ 0.05) using One-way ANOVA followed by Tukey–Kramer as a post-ANOVA test. **C**: control rats, **Bro**: rats treated with bromelain, **IRR**: rats exposed to 7.5 Gy, **IRR+Bro**: rats exposed to 7.5 Gy and treated with bromelain.

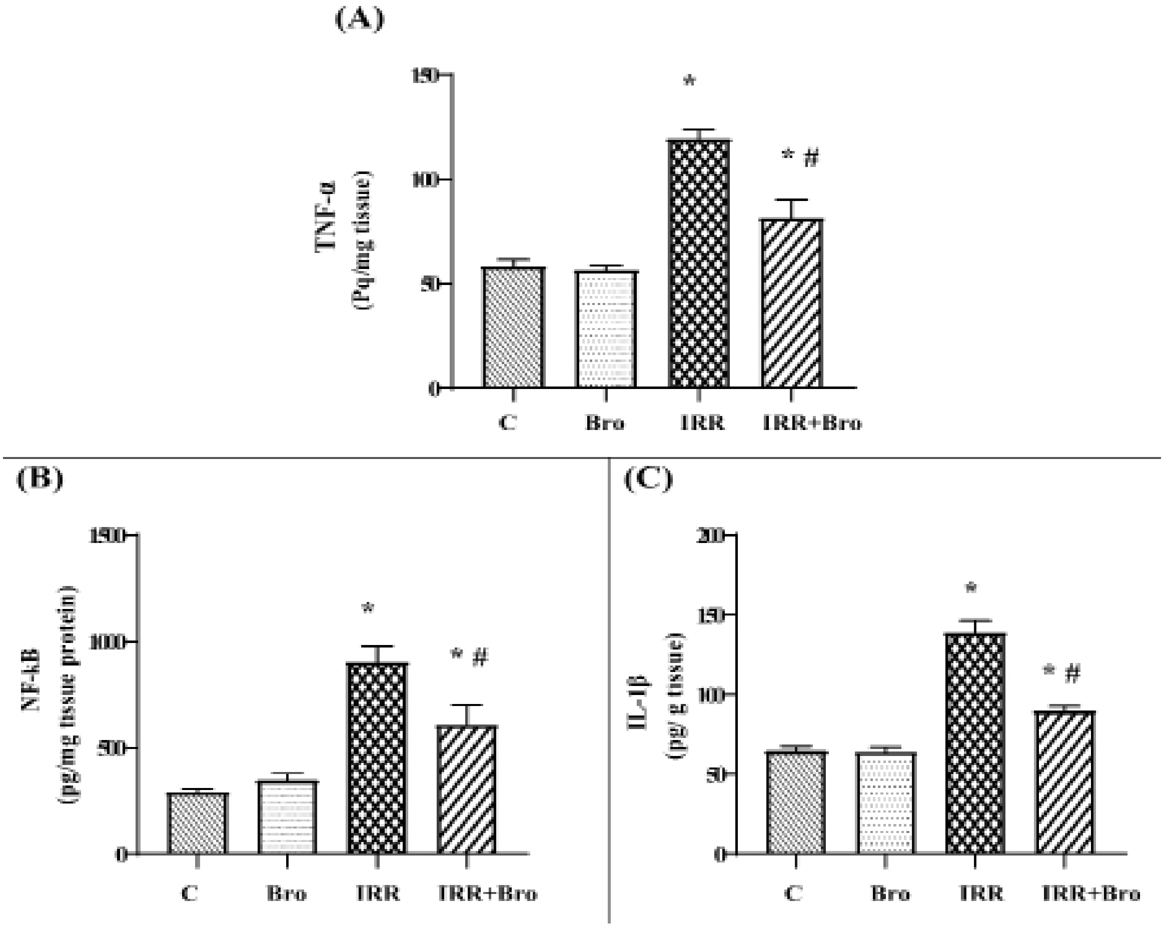


**Fig. 4. Effect of bromelain against renal inflammatory biomarkers induced by γ-radiation.**
Each value of tumor necrosis factor-α (TNF-α), nuclear factor kappa (NF-κB) and Interleukin-β1 (IL-β1) content in rat kidney tissues represents Mean± SEM (n=6). Columns denoted with (*****) significant from control, **(#)** significant from irradiated group at (p £ 0.05) using One-way ANOVA followed by Tukey–Kramer as a post-ANOVA test. **C**: control rats, **Bro**: rats treated with bromelain, **IRR**: rats exposed to 7.5 Gy, **IRR+Bro**: rats exposed to 7.5 Gy and treated with bromelain.

*Impact of bromelain on renal profibrotic index.*

According to Fig. 5, the systemic fibrosis of the bromelain group showed no discernible change in HA, LN, PCIII and HYP levels as renal profibrotic biomarkers in comparison to the control group. While rats subjected to γ-radiation in the IRR and IRR+Bro groups revealed a substantial elevation ($p<0.05$) in the profibrotic index in comparison to the control group. Whereas, in the IRR+Bro group, manifested a noticeable drop (p£ 0.05) in profibrotic biomarkers concentrations, compared with the irradiated group.

*Histopathological examination of kidney*

The histopathological examination of the kidney tissues of different animal groups was showed in Fig. 6. The C-group showing circumscribes glomeruli with normal structure of capillary tufts and Bowman's capsule. The renal tubules of both proximal and distal convoluted tubules showed intact epithelial lining and regular arrangement score (0) Fig. 6 (a-b). Kidney tissue section of irradiated group revealed hypercellularity of glomerular tufts and narrowing of Bowman's space. Renal tubules showed swelling of tubular epithelial lining accompanied with narrowing and occlusion of tubular lumen by albuminus and cellular casts. Tubular epithelial cell necrosis and apoptosis <50% score (3) were seen Fig. 6 (**c**). On the other hand, IRR+Bro group showed mild histological changes of renal tubular epithelial lining appeared in form of mild swelling of tubular epithelial cell lining without significant necrosis or apoptosis was seen. Intra-tubular albuminus droplets were seen score (1) Fig. 6 (d).

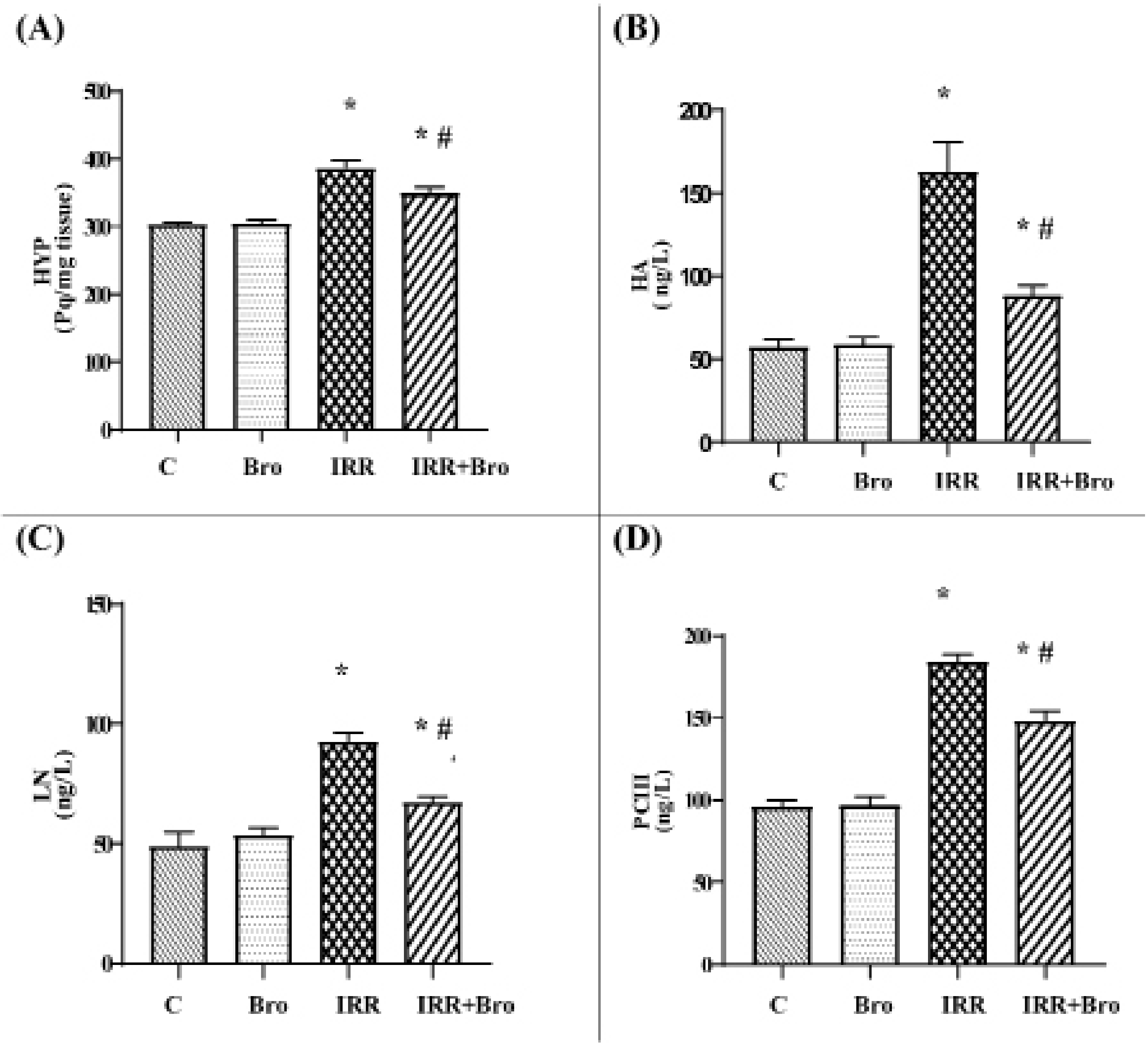


**Fig. 5. Effect of bromelain against renal profibrotic biomarkers induced by γ-radiation.**
Each value of Hydroxyproline (HYP), Hayluronic acid (HA), Laminin (LN) and III Procollogen (PCIII) content in rat kidney tissues represents Mean± SEM (n=6). Columns denoted with (*****) significant from control, **(#)** significant from irradiated group at (p £ 0.05) using One-way ANOVA followed by Tukey–Kramer as a post-ANOVA test. **C**: control rats, **Bro**: rats treated with bromelain, **IRR**: rats exposed to 7.5 Gy, **IRR+Bro**: rats exposed to 7.5 Gy and treated with bromelain.

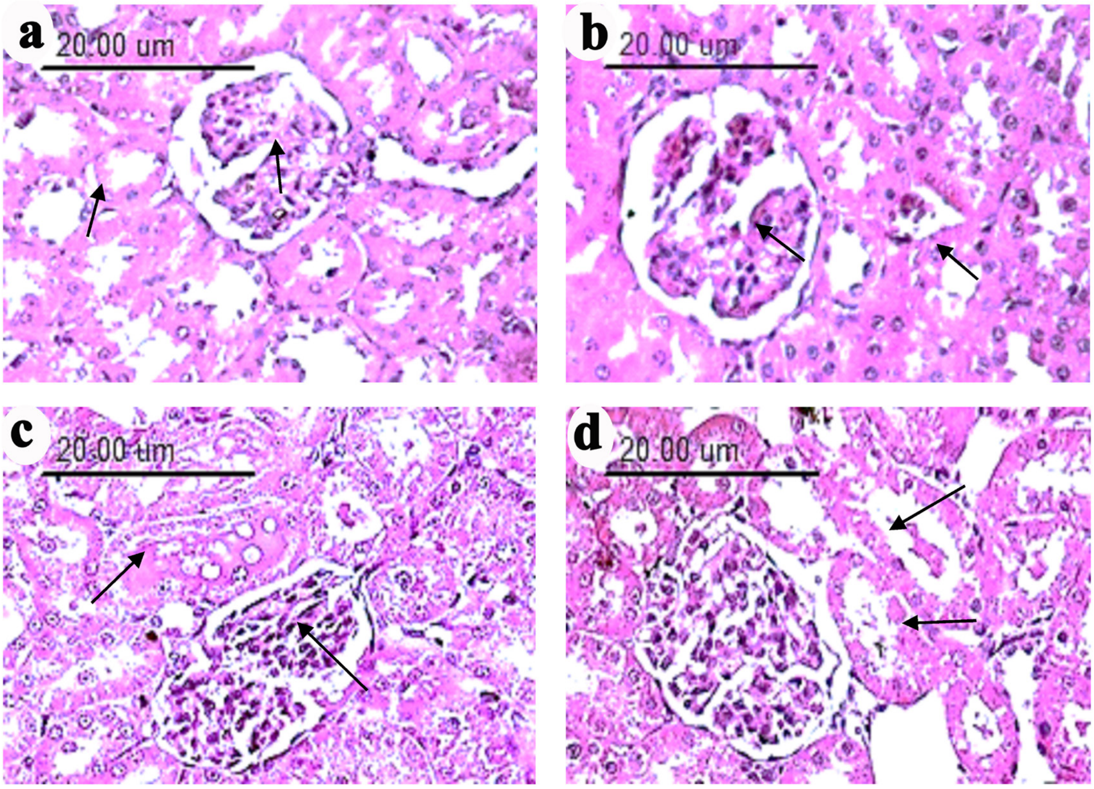


**Fig. 6. Histopathological examination of kidney in different animal groups.**
The tissues sections stained with hematoxylin and eosin, magnification ×400 (kidney; H&E x 400).
a: normal rats, b: rats treated with bromelain, c: rats exposed to 7.5 Gy, d: rats exposed to 7.5 Gy, and treated with bromelain.
(a&b) normal histological structure of glomerulus and renal tubules **arrow** (c) hypercellularity of glomerular tufts and necrosis of tubular epithelial lining **arrow** (d) mild swelling of tubular epithelial lining with intralumenal cast **arrow** (Scale bar =20).

## Discussion

The potential molecular and cellular signaling pathways of radiation renal impacts begin with DNA damage and its repair mechanisms in the kidney. Radiation renal impacts may involve cell death, oxidative stress, vascular dysfunction, cellular senescence, inflammation, the release of profibrotic substances, and the activation of the Renin-angiotensin-aldosterone system (RAAS) (Klaus et al., 2021). Further research in this field has discovered that ACE activity in rats was elevated following irradiation. It was found that there is a direct relation between the dosage of irradiation and ACE activity (Korystova et al., 2018). Through activating the angiotensin II receptor type 1 (AT1R), the ACE product angiotensin II (Ang II) promotes vasoconstriction, salt and water reabsorption, inflammation and oxidative stress (Bader and Ganten, 2008).

Cao and Wu (2012) found that mice exposed to 20 Gy of radiation had higher levels of AngII. Renin activation eventually results in the production of AngII, which acts as a signal for the adrenal cortex to release aldosterone (Morris et al., 2008). Aldosterone induces oxidative stress, inflammation, and endothelial dysfunction, which results in vascular damage in the kidneys, heart and brain through activation of the mineralocorticoid receptor (Joffe and Adler, 2005).

The current study assumed that bromelain, as an ACE inhibitor, can downregulate and degrade the AngII and reduce renal damage caused by radiation via suppressing the ACE/Ang-II/aldosterone pathway. In the precise same vein, our data indicated that radiation-upregulated ACE/Ang-II/aldosterone levels exert a pathological condition in the kidney.

Comprehending the various effects of AngII might be required to stop renal disease from getting worse. AngII, a powerful vasoconstrictor, plays a significant role in the genesis and progression of radiation nephritis (Hasan et al., 2020)Q□6F. Additionally, these results match previous research which indicated that the lessening of AngII production accounted a treatment for radiation nephropathy (Hasan et al., 2020)84’8484#848484848484848484ÿÿÿ.

Mahdy (1991) revealed that radiation increased the activity of glutamate dehydrogenase, which forms urea by interacting with carbonyl phosphate synthetase. Rats’ levels of urea and creatinine elevated three, seven, and ten days after receiving doses of three, six, and seven Gy of radiation (Ramadan et al., 2002; Abdel-azeem and Mohamed, 2005). Consistent with earlier results, irradiated rats showed noticeably elevated levels ($P < 0.05$) of creatinine, BUN, and urea, suggesting an impairment in kidney function. However, when bromelain was administered to irradiated rats, kidney functions were recovered.

According to earlier research, AngII is capable of double the amount of vascular ROS produced (Rajagopalan et al., 1996). AngII is a powerful stimulant of NADPH oxidase, which is the main cause and origin of ROS production in a variety of tissues (Wen et al., 2012). When ROS, such as superoxide ($O_2^-$), hydrogen peroxide ($H_2O_2$), and hydroxyl radicals (OH), are produced more than antioxidant defenses, oxidative stress creates an unbalanced condition. Since $O_2^-$ is unstable, super oxide dismutase (SOD) will quickly change it into the more stable and membrane-permeable form of $H_2O_2$ (Griendling et al., 2000).

Sánchez et al. (2015) noticed that the MDA level had increased shortly after the radiation. Malondialdehyde (MDA) levels were likely raised by the interaction of OH, a byproduct of water radiolysis, with the polyunsaturated fatty acids found in the phospholipid component of cellular membranes subsequent to ionizing radiation exposure (Parihar et al., 2006). The reduction in GSH content is most likely the result of its greater use to counteract the excess of free radicals produced in the body following ionizing radiation exposure (Srinivasan et al., 2007). The current investigation demonstrated clear irradiation-induced oxidative stress effects, as seen by higher kidney malondialdehyde (MDA) and protein carbonyl (PCO) levels, as well as decreased total thiol (tSH), reduced glutathione (GSH) content, and superoxide dismutase SOD activity. Abareshi et al. (2017) suggested that diminished tSH and SOD tissue levels were linked to ACE-enhanced Ang II production which reveals raised ROS. The formation of protein carbonyl can be a key sign of protein dysfunction caused by oxidative stress (Alou-El-Makarem et al., 2014). Bromelain treatment ameliorated the oxidative stress indices to approximately normal levels. This effect can probably be attributed to the bromelain’s ability to scavenge ROS and act as an antioxidant through ACE inhibition. Akaras et al. (2023) noticed that bromelain inhibited paracetamol induced lipid peroxidation, elevated antioxidant levels and lessened tissue damage.

Radiation directly up-regulates Tumor necrosis factor-α (TNFα) is a “master regulator” of cytokine production and is crucial in inflammatory disorders (Parameswaran and Patial, 2010). Also, nuclear factor kappa B (NF-κB) is activated in response to many stimuli. TNF family cytokines are well-known inducers of NF-κB activity (Hayden and Ghosh, 2014). Interleukin-1β (IL-1β), is a powerful pro-inflammatory cytokine that regulates other inflammation-related molecules and contributes to radiation-induced fibrosis (Liu et al., 2006).

The present data demonstrated that irradiated rats displayed a rise in the kidney’s TNF-α, NF-κB and IL-1β. These results are in good agreement with previously published observations that irradiation stimulates the up-regulation of inflammatory cytokines (Radwan and Hasan, 2019; Galal et al., 2024). AngII signaling enhances inflammation, fibrosis, and cellular hypertrophy, which contribute to disease pathophysiology (Bennion et al., 2015b) as well as expression of proinflammatory proteins such as NF-κB and TNF-α (Goel et al., 2018).

This research detected that bromelain diminished kidney TNF-α, NF-κB, and IL-1β levels in irradiated rats. These findings could be attributable to bromelain›s anti-inflammatory effect and inhibition of ACE/AngII signaling, a key target for reducing radiation-induced injury (Robbins and Diz, 2006). Previously detected, bromelain reduced inflammation in rat peripheral neurons by inhibiting TNF-α, IL-1β, and NF-κB (Bakare and Owoyele, 2021).

Several kidney disorders have been linked to elevated levels of hyaluronic acid (HA) in renal tissue and serum in both experimental models and human samples (Honkanen et al., 1991; Stridh et al., 2012). Moreover, Yagmur et al. (2012)

found that circulating HA levels were linked to compromised kidney parameters estimated (glomerular filtration rate (eGFR) and cystatin C). In view of the fact that HA plays a role in controlling the progression of renal fibrosis and inflammation, the elevation in its levels in the blood could be a renal extracellular matrix (ECM) characteristic happening throughout the development and progression of renal disease (Clotet-Freixas and Konvalinka, 2021). Additionally, as proposed by Pecoits-Filho et al. (2003), seeing that HA is primarily eliminated by the kidney, circulating levels of this biomarker could indicate renal impairment. Type III procollagen (PCIII) has also been identified in adult patients with varying stages of chronic kidney disease (CKD) (El Ghoul et al., 2010). Other investigations have shown that elevated urine and serum PCIII levels are linked with advancing stages of CKD and interstitial renal fibrosis in both children and adults (Jianguo et al., 2014; Taranta-Janusz et al., 2019). The current study found that serum levels of hyaluronic acid (HA), laminin (LN), and Type III procollagen (PCIII) increased significantly in irradiated rats, which could be attributed to fibrosis advancement. This was consistent with other investigators (Hu et al., 2018). On the other hand, HA, LN, and PCIII levels declined dramatically in the bromelain-treated group. Our results were supported by Pothacharoen et al. (2021).

Furthermore, hydroxyproline (HYP) is a key component of collagen and is considered a sensitive indicator of fibrosis (Bennion et al., 2015a). In the present evaluation, the renal HYP content of irradiated rats was significantly increased, indicating the development of fibrosis, which was verified in a prior study (Zheng et al., 2014; Hasan et al., 2020). Interestingly, bromelain decreased HYP compared to the irradiation group, which could be related to its anti-fibrotic properties. NF-kB activates TGF-β1, a major fibrogenic molecule involved in renal fibrosis. Attenuating NF-kB may prevent fibrosis development and reduce renal HYP content (Goel et al., 2018).

In another stage of the study, sections of kidney tissue were taken for pathological examination to observe the effects of radiation on kidney tissue revealing hypercellularity of glomerular tufts and narrowing of Bowman's space. Renal tubules showed swelling of the tubular epithelial lining, followed by constriction and blockage of the tubular lumen by albumin and cellular casts. In previous studies, glomerular atrophy, hemorrhage and abundant necrosis were detected in kidney tissues after irradiation (Hasan et al., 2020)¯. Treatment with bromelain significantly reduced these damages. These conclusions were backed by (El-Demerdash et al., 2020; Akaras et al., 2023).

Despite promising results, this study has limitations. Data from preclinical models may not accurately represent the complexity of actual renal injury. Future studies should involve clinical trials with diverse patient populations to validate the therapeutic potential of bromelain.

## Conclusion

In summary, many pieces of evidence presented in this study showed that bromelain was effective at delaying the development of kidney damage in irradiated rats. This possible impact could be ascribed to its anti-oxidative, anti-inflammatory and anti-fibrotic properties proven by biochemical and histological assessment. In consequence, this research revealed that bromelain might be a promising remedy for diminishing the associated renal implications generated by radiation exposure by suppressing the RAAS system in radiotherapeutic patients. Still, future researches are required to provide conclusive results on this issue.

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**Disclosure statement**

The author(s) disclosed no possible conflicts of interest.

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**Data availability Statement**

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

**Author Contributions**

The study's inception and design were collaborative efforts by all authors. Materials preparation, data gathering, and analysis. Shereen Mohamed Galal completed the initial draft of the paper. The final manuscript was read and approved by all writers.

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