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Royal Jelly, A Super Food, Protects Against Celecoxib-Induced Renal Toxicity in Adult Male Albino Rats

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Abstract

Background: Celecoxib is a COX-2 nonsteroidal anti-inflammatory drug (NSAID). It is widely used for the treatment of osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis.

Objective: This study aimed to explore the effect of long-term administration of celecoxib on kidney of male albino rats, and to study the potential effect of treatment discontinuation on such tissues. The study also examined the alleged ameliorative effect of royal jelly (RJ).

Methods: Fifty, male albino rats were divided into 5 equal groups; 10 each. Group 1: rats received no drug (control group). Group 2: rats received celecoxib (50 mg/kg/day, orally for 30 successive days). Group 3: rats received celecoxib (50 mg/kg/day, orally) and royal jelly (300 mg/kg/day, orally) for 30 successive days. Group 4: rats received celecoxib for 30 successive days, then rats were left untreated for another 30 days. Group 5: rats received celecoxib and RJ for 30 successive days, then rats were left untreated for another 30 days.

Results: Long-term celecoxib administration caused significant elevation in kidney function tests, with ameliorative effects of RJ against celecoxib-induced renal toxicity.

Conclusion: Long-term celecoxib administration caused renal toxicity in male albino rats, with ameliorative effects of RJ.

Abrege

Contexte: Le célécoxib est un anti-inflammatoire non stéroïdien (AINS) inhibiteur de COX-2. Ce médicament est largement utilisé pour le traitement symptomatique de l'arthrose, de la polyarthrite rhumatoïde et de la spondylarthrite ankylosante.

Objectifs: Cet essai visait à examiner l'effet d'une administration à long terme de célécoxib sur les reins de rats albinos mâles, à étudier les possibles effets de l'arrêt du traitement sur ces tissus et à vérifier l'effet d'amélioration allégué de la gelée royale.

Méthodologie: Cinquante rats albinos mâles ont été répartis en cinq groupes égaux (10 rats par groupe). Groupe 1 (groupe témoin): rats n'ayant reçu aucun médicament. Groupe 2: rats ayant reçu du célécoxib (50 mg/kg/jour, par voie orale pendant 30 jours consécutifs). Groupe 3: rats ayant reçu du célécoxib (50 mg/kg/jour, par voie orale) et de la gelée royale (300 mg/kg/jour, par voie orale) pendant 30 jours consécutifs. Groupe 4: rats ayant reçu du célécoxib pendant 30 jours consécutifs, puis laissés sans traitement pendant 30 jours supplémentaires. Groupe 5: rats ayant reçu du célécoxib et de la gelée royale pendant 30 jours consécutifs, puis laissés sans traitement pendant 30 jours supplémentaires.

Résultats: L'administration à long terme de célécoxib a entraîné une augmentation significative des tests de la fonction rénale; la gelée royale a montré des effets d'amélioration contre la toxicité rénale induite par le célécoxib.

Conclusion: L'administration à long terme de célécoxib a provoqué une toxicité rénale chez les rats albinos mâles contre laquelle la gelée royale a montré des effets protecteurs.

Keywords

celecoxib, kidney, renal, royal jelly, toxicity

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Introduction

Celecoxib is a COX-2 nonsteroidal anti-inflammatory drug (NSAID).[1] It is widely used for the treatment of osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis.[2,3] Celecoxib is a prospective alternative for traditional NSAIDs as it possesses anti-inflammatory, antipyretic, and analgesic properties with excellent gastrointestinal safety profile.[4] Celecoxib's side effects still need to be further researched. Studies on celecoxib's impact on the kidneys produced a wide range of findings. While some research verified that celecoxib is safe for the kidneys,[5] other studies even went as far as to claim that celecoxib has a protective impact on the kidneys.[6] On the contrary, some research suggests that celecoxib may be hazardous to the kidneys.[7]

Royal jelly (RJ) is a nutrient-rich source of bioactive chemicals that are crucial to numerous biological activities,[8] including antibacterial, antioxidant, anti-inflammatory, anticancer, anti-hyperlipidemic, cardio-protective, and hepatorenal-protective health benefits.[9] The renal protection is assumed to be facilitated by free radical scavenging, antioxidant capacities, and anti-apoptotic activation of RJ.[10,11]

The stark discrepancy between the findings of several studies addressing celecoxib's impact on the kidney served as a strong impetus for us to conduct this investigation in an effort to shed as much light as possible on the situation as feasible. This study was created to examine the effects of long-term celecoxib administration on renal tissue in male albino rats and to investigate the potential effects of treatment termination, with a focus on the potential effects of RJ administration.

Materials and Methods

Experimental Animals

Fifty, apparently healthy, male albino rats weighing 200 to 230 gm, randomly divided into 5 equal groups; each of 10 rats. They were kept in orderly cages with walls made of wire-bottomed galvanized metal. A healthy food and unrestricted access to clean drinking water were provided. They had a 7-day acclimatization period before starting the study.

Experimental Setting and Design

This study was conducted in the Lab animal unit, Pharmacology department, Faculty of Veterinary Medicine, Zagazig University, in the period from March 2023 to May 2023. All steps and procedures in the study were carried out following Zagazig University Institutional Animal Care and Use Committee regulations, with approval No. ZU-IACUC/2/F/522/2023.

Rats used in this study were allocated into the following groups:

Control group (Group 1, n = 10): Rats did not receive any medication. Rats were employed as the control group.
Celecoxib group (Group 2, n = 10): For 30 consecutive days, rats were given celecoxib (50 mg/kg/day, orally).[12]
Celecoxib + RJ group (Group 3, n = 10): For 30 consecutive days, rats were given celecoxib plus RJ (300 mg/kg/day, orally).[9]
Celecoxib recovery group (Group 4, n = 10): For 30 consecutive days, rats were given celecoxib, then rats were left untreated for another 30 days.
Celecoxib + RJ recovery group (Group 5, n = 10): For 30 consecutive days, rats were given celecoxib plus RJ, then rats were left untreated for another 30 days,

At the end, all group rats were euthanized and samples were taken.

Samples Collection and Preservation

Blood samples were collected using a 3 mL syringe directly from the ventricular puncture of rats into centrifuge tubes and left to clot for 15 minutes at room temperature, then centrifuged at 3000 rpm for 10 minutes to allow serum separation, which was then aspirated into cryovials and stored at −20°C for serum biochemical assays.

The kidney tissues of each animal were dissected and collected immediately, transferred in liquid nitrogen, and kept at −80°C for total RNA extraction used for the determination of apoptotic and anti-apoptotic mRNA expression levels using reverse transcription polymerase chain reaction (RT-PCR).[13]

Kidney Function Tests

Blood urea nitrogen (BUN)[14] and creatinine concentrations[15] were estimated via using commercially available Specific Pointe® Scientific Inc. colorimetric kits.

Oxidant/Antioxidant Status

Serum malondialdehyde (MDA) and superoxide dismutase (SOD)[16] were estimated via using commercially available kits supplied from Oxi Select™, USA.

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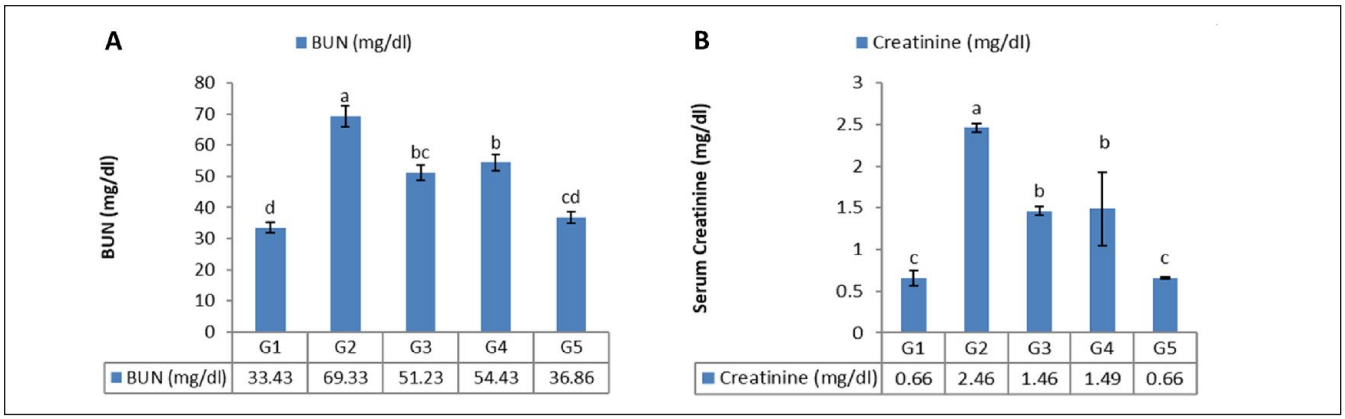

Figure 1. Effects of celecoxib (50 mg/kg/day, orally), royal jelly (300 mg/kg/day, orally), and treatments discontinuation on kidney profile in male albino rats. A: BUN (mg/dL), B: creatinine (mg/dL). Data are expressed as mean ± SD, n = 10/group.
Group 1 received no drug (control). Group 2 received celecoxib (50 mg/kg/day, orally), for 30 successive days. Group 3 received celecoxib (50 mg/kg/day, orally) and royal jelly (300 mg/kg/day, orally) for 30 successive days. Group 4 received celecoxib (50 mg/kg/day, orally) for 30 successive days, then rats were left untreated for another 30 days. Group 5 received celecoxib (50 mg/kg/day, orally) and royal jelly (300 mg/kg/day, orally) for 30 successive days, then rats were left untreated for another 30 days.

Apoptotic and Anti-Apoptotic Genes Expression

Apoptotic genes **(Bax)** and anti-apoptotic gene **(Bcl-2)**[13] were estimated via using commercially available kits supplied from TOPreal™ qPCR 2X PreMIX (SYBR Green with low ROX) (Cat. # P725, Enzynomics, Korea).

The primer sequences[13];

Bax: (Size: 109 bp, Accession no. NM_017059.2)

5′-CGAATTGGCGATGAACTGGA-3′ (forward)

5′-CAAACATGTCAGCTGCCACAC-3′ (reverse);

Bcl-2: (Size: 135 bp, Accession no. NM_016993.1)

5′-GACTGAGTACCTGAACCGGCATC-3′ (forward)

5′-CTGAGCAGCGTCTTCAGAGACA-3′ (reverse);

Gene expressions were measured using the below formula and Ct (2–ΔΔCt) (fold change) method:

$$\Delta\Delta Ct = (Ct_{target} - Ct_{reference})\ \text{test sample} - (Ct_{target} - Ct_{reference})\ \text{control sample}$$

Finally, considering the primer efficiency value of ~2, the gene expression level was determined as 1–ΔΔCt.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) program (version 26.0; SPSS Inc., Illinois) for Microsoft Windows® was used to statistically analyze the collected data. The means of various groups were compared using one-way analysis of variance (ANOVA). The in-between group comparisons were performed using Tukey honestly significant difference (HSD) post hoc analysis. A P value of .05 or less is regarded as significant.

Results

In the study rats, acute kidney injury was emerged by oral administration of celecoxib (50 mg/kg/day) for 30 consecutive days. This injury was allegedly characterized by a substantial rise in serum BUN and creatinine levels ($P < .05$), with RJ acting as an anti-nephrotoxic agent. When celecoxib and RJ were discontinued together, the effects of celecoxib's nephrotoxicity were greatly reduced, and the BUN and creatinine levels returned to normal (Figure 1).

Celecoxib administration (50 mg/kg/day, orally), for 30 successive days was claimed to cause oxidative stress (OS) the study rats as manifested by significant elevation of serum MDA and significant decline in SOD levels, with ameliorative effects of RJ against celecoxib-induced OS. Celecoxib discontinuation significantly diminished the celecoxib-induced OS effects, and normal oxidative enzyme levels were regained in the case of dual medications (celecoxib + RJ) discontinuation (Figure 2).

In the study rats, administration of celecoxib (50 mg/kg/day, orally) for 30 consecutive days was said to cause an apoptotic effect as evidenced by a significant increase in serum levels of the apoptotic gene Bax and a significant decrease in serum levels of the anti-apoptotic gene Bcl-2 in kidneys. Royal jelly was said to have protective effects against the celecoxib-induced apoptotic effect. Celecoxib withdrawal greatly reduced the celecoxib-induced apoptotic impact, and withdrawal of both drugs (celecoxib + RJ) restored normal expression of the apoptotic/anti-apoptotic genes (Figure 3).

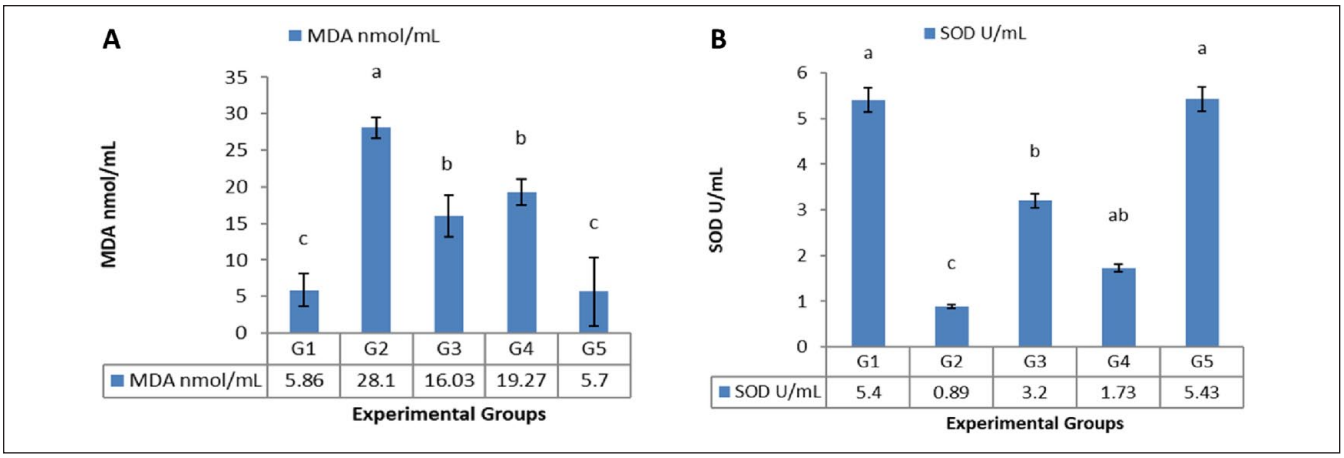

Figure 2. Effects of celecoxib (50 mg/kg/day, orally), royal jelly (300 mg/kg/day, orally), and treatments discontinuation on serum oxidative status profile in male albino rats, A: MDA (nmol/mL), B: SOD (U/mL). Data are expressed as mean ± SD, n = 10/group.
Group 1 received no drug (control). Group 2 received celecoxib (50 mg/kg/day, orally), for 30 successive days. Group 3 received celecoxib (50 mg/kg/day, orally) and royal jelly (300 mg/kg/day, orally) for 30 successive days. Group 4 received celecoxib (50 mg/kg/day, orally) for 30 successive days, then rats were left untreated for another 30 days. Group 5 received celecoxib (50 mg/kg/day, orally) and royal jelly (300 mg/kg/day, orally) for 30 successive days, then rats were left untreated for another 30 days.

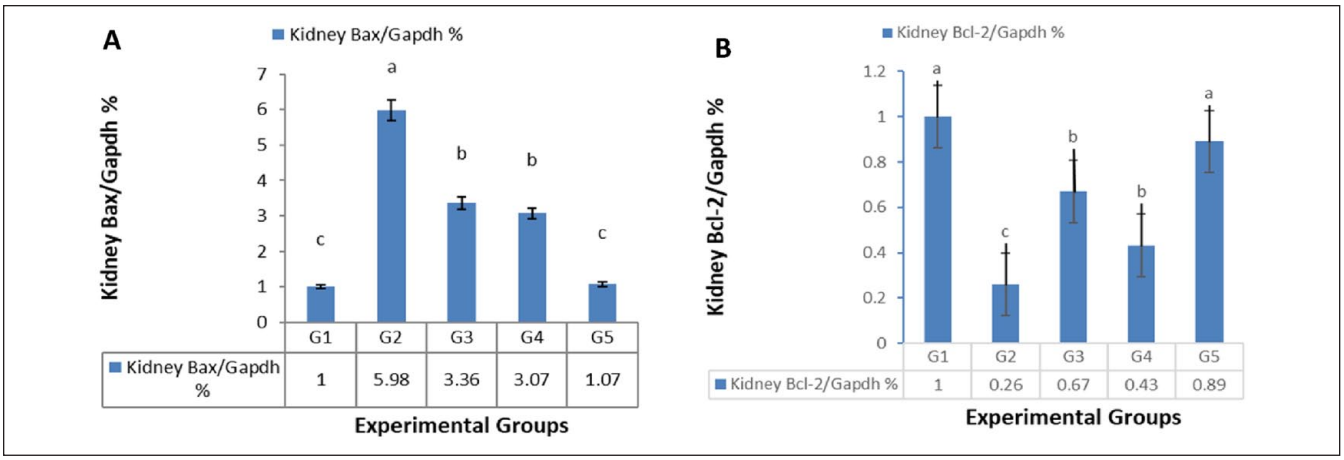

Figure 3. Effects of celecoxib (50 mg/kg/day, orally), royal jelly (300 mg/kg/day, orally), and treatments discontinuation on A: renal Bax (%), B: renal Bcl-2 (%). Data are expressed as mean ± SD, n = 10/group.
Group 1 received no drug (control). Group 2 received celecoxib (50 mg/kg/day, orally), for 30 successive days. Group 3 received celecoxib (50 mg/kg/day, orally) and royal jelly (300 mg/kg/day, orally) for 30 successive days. Group 4 received celecoxib (50 mg/kg/day, orally) for 30 successive days, then rats were left untreated for another 30 days. Group 5 received celecoxib (50 mg/kg/day, orally) and royal jelly (300 mg/kg/day, orally) for 30 successive days, then rats were left untreated for another 30 days.

Discussion

Celecoxib is a prospective alternative for the treatment of osteoarthritis and rheumatoid arthritis,[17] especially in individuals at high risk of gastrointestinal problems, due to its excellent gastrointestinal safety profile and long-term symptomatic improvement.[18]

In our study, celecoxib administration (50 mg/kg/day, orally), for 30 successive days caused acute kidney injury in the study rats with ameliorative effects of RJ against celecoxib-induced nephrotoxicity.

In fact, both COX-1 and COX-2 are expressed in the kidney.[19] COX-1, which acts primarily in the regulation of renal hemodynamics and glomerular filtration rate (GFR), is expressed mainly as a constitutive isoform in normal conditions.[20] On the other hand, COX-2, which is responsible for salt and water excretion, is expressed not only initially as an inducible isoform in the presence of damaging stimuli,[21] but also constitutively for ensuring the tubuloglomerular feedback, contributing to the establish homeostasis.[22] Therefore, the impact of blocking one or both of these enzymes on renal functions is the consequence of inhibiting

prostaglandin synthesis responsible for the maintenance of renal functions[23,24] which can result in acute renal failure. Moreover, there is the possibility that long-term administration of any NSAIDs can cause chronic renal failure in some patients.[25]

Selective COX-2 inhibitors, such as celecoxib, were developed, primarily, to produce the beneficial effects of NSAIDs, but spare the COX-1-mediated adverse events.[26] However, it has the same potential for adverse renal effects as traditional NSAIDs,[27] even in therapeutic doses,[28] especially in clinical situations associated with a renal impairment, such as sodium depletion, hypovolemia, cirrhosis, congestive heart failure, nephrotic syndrome, chronic kidney disease (CKD),[23] and old age.[29] Although there have been 2 cases of celecoxib-induced nephrotoxicity after 10 months of continuous usage, it has largely been also reported with short-term use, as fast as within 2 weeks.[30] Therefore, the same precautions in patients at risk for adverse renal effects probably apply to both the nonselective NSAIDs and COX-2 selective inhibitors.[19] The short duration of administration and close monitoring of renal functions, urine output, and fluid status are essential to avoid acute kidney injury because of celecoxib.[31,32]

The most frequent renal side effect associated with celecoxib administration is reversible renal insufficiency.[33-36] In the early 1900s, BUN quantification replaced urea and served as a superior renal function biomarker.[37] Serum creatinine supplanted BUN in the mid-1900s and remains the "gold standard" laboratory test for the assessment of kidney function and GFR until now.[38] As celecoxib treatment continues, it may cause acute renal injury[39,40] manifested by an abrupt reduction in kidney function: oliguria of 0.5 mL/kg/h for $>$ 6 hours, increase in serum creatinine of either $\geq$ 0.3 mg/dL, or 1.5-fold from baseline.[41] Progressive functional or structural deterioration in renal tissue throughout a period of months or years is known as CKD[42,43] manifested by a $<$ 60 mL/min per 1.73 m^2 reduction in glomerular filtration rate.[44,45]

The biochemical celecoxib-induced nephrotoxicity is attributed to OS where the balance between the generation of reactive oxygen species (ROS) and antioxidants defense is upset.[46] The OS pathophysiology is an increase in ROS levels with unbalanced extracellular antioxidant enzyme activity.[40,47]

In accordance with our findings, previous studies have demonstrated that the renal adverse effects of celecoxib are usually reversible. Most forms of acute renal failure from NSAID administration are short-term and reversible upon NSAID discontinuation.[48] Nonsteroidal anti-inflammatory drug administration for the short term for up to 6 weeks may preserve the chance for recovery; however, there has previously been no study to test the reversibility of renal adverse effects after long-term NSAID use.[49] Renal function regained following ceasing celecoxib medication after 2 weeks.[50]

Royal jelly increased renal COX-2 protein expression and prostaglandin E2 (PGE-2) renal content, providing protection against celecoxib toxicity. It decreased iNOS protein expression, renal myeloperoxidase (MPO) levels, and increased ROS scavenging, inhibiting inflammatory and pro-inflammatory responses, reducing kidney damage and oxidative damage.[9] Prior studies described the protective effects of RJ against cisplatin-induced,[51,52] doxorubicin-induced,[53] ethylene glycol-induced,[11] diclofenac-induced,[9] tyrosine kinase inhibitor-Induced,[54] fluoride-induced,[55] valproic acid-induced,[56] and CCl4-induced[57] renal toxicities in rats.

In our study, celecoxib administration (50 mg/kg/day, orally), for 30 successive days caused OS in the study rats as manifested by significant elevation of serum MDA and significant decline in SOD levels, with ameliorative effects of RJ against celecoxib-induced OS.

Oxidative stress is a balance shift between oxidant production and elimination through the antioxidant defense system.[58] Oxidative stress-mediated molecules are metabolites derived from ROS[59] and reactive nitrogen species (RNS).[60] Reactive oxygen species/reactive nitrogen species are produced from endogenous sources like immune system activation, inflammation, and mental stress[61,62] while exogenous sources include air pollution, water pollution, alcohol, and ultraviolet radiation.[63] Antioxidants are the body's primary defense against OS, inhibiting the oxidation reaction of molecules that produce free radicals.[64] They can be internally synthesized (endogenous) or externally supplied through foods like RJ, which acts as direct ROS scavengers and increase the antioxidant enzyme activities.[65] Endogenous antioxidant defenses involve a network of antioxidant enzymatic and non-enzymatic molecules.[66] Primary antioxidant enzymes involve SOD, catalase, and several peroxidases. Malondialdehyde is the end- product of polyunsaturated fatty acids oxidation in cellular membranes, thus, it acts as a dependable marker of OS.[67] and non-enzymatic molecules involve vitamins (A, C, E, and K), enzyme cofactors (Q10), and minerals (Zn, Mn, Cu, Se, etc).[68]

Previous studies show that rats treated with celecoxib exhibit altered oxidant/antioxidant status, leading to excessive generation of free radicals, which cause disease development, peroxidation, and tissue destruction.[69-72] In addition, previous studies have confirmed the beneficial and antioxidant effects of RJ,[73-75] with in vitro administration inhibiting pro-inflammatory cytokines and suggesting anti-inflammatory and antioxidant properties.[76] These properties are attributed to free radical scavengers, inhibiting lipid peroxidation and cytochrome P450 expression, and reducing lipid peroxidation.[77-79]

In our study, celecoxib administration (50 mg/kg/day, orally), for 30 successive days induced an apoptotic effect in the study rats as manifested by a significant elevation of renal tissue apoptotic gene (Bax) and a significant decline in

renal tissue anti-apoptotic gene (Bcl-2), with ameliorative effects of RJ against the celecoxib-induced apoptotic effect.

Apoptosis is a genetically determined process that involves the elimination of cells, regulated by 2 main proteins: caspases and the Bcl-2 family.[80] Caspases control cell degradation with minimal effect on surrounding tissues,[81] while Bcl-2 consists of anti-apoptotic and pro-apoptotic members. Anti-apoptotic members, such as Bcl-2 and Bcl-XL, sequester caspases or prevent the release of mitochondrial apoptogenic factors responsible for caspases activation, while pro-apoptotic members, such as Bax and Bak, trigger caspases by inducing the release of these factors.[82]

Apoptosis is a physiological process that selectively eliminates individual cells without harming the entire organ.[83] It involves cell shrinkage, pyknosis, cytoplasmic and nuclear condensation, chromatin cleavage, apoptotic bodies formation, and phagocytosis.[84] Necrosis, on the other hand, is a passive, accidental cell death triggered by external factors or disease.[85,86] The main morphological changes associated with necrosis include cell swelling, cytoplasmic vacuole formation, distended endoplasmic reticulum, cytoplasmic bleb formation, condensed, swollen, or ruptured mitochondria, disrupted organelle membranes, swollen and ruptured lysosomes, and ultimately disruption of the cell membrane.[87] Apoptosis is usually normal and beneficial, and can also occur as a protective process.[88]

In accordance with our findings, previous studies have confirmed celecoxib-induced apoptosis. Celecoxib causes apoptosis by causing the loss of the mitochondrial transmembrane potential, the release of cytochrome c and apoptosis inducing factor (AIF), and the activation of caspase-9 and caspase-3. In addition, the anti-apoptotic protein Bcl-2 was reduced in abundance whereas the pro-apoptotic protein Bax was enhanced by celecoxib. The data showed that mitochondria-dependent signaling, not PPAR/NF-B signaling, was the mechanism through which celecoxib triggered apoptosis in mouse liver cancer cells.[89] In another study, celecoxib-induced apoptosis in 5-fluorouracil-resistant gastric cancer cells through protein kinase B (PKB) inhibition,[90] which is a key component of the phosphatidyl-inositol-3 kinase (PI3K) intracellular pathway that exerts a pivotal role in regulating cell proliferation, survival, and metabolism.[91] Celecoxib-induced apoptosis in glioblastoma tumor cells, the primary malignant tumor of the brain, via suppressing CIP2A/PP2A/Akt signaling axis.[92]

Several previous studies confirmed the concept of the beneficial and anti-apoptotic effect of RJ against cisplatin-induced hepatorenal toxicity,[52] nicotine-induced testicular injury in mice,[93] doxorubicin-induced nephrotoxicity in male albino rats,[10] and hydroxyurea-induced hepatic injury in rats.[94] Moreover, RJ decreases the expression of the apoptotic gene (MMP-9) responsible for bladder cancer in humans.[95]

The limitation of our study was the relatively small sample size, study performing on male rats only excluding the female rats, and focusing on a single dose of medication. These issues should be addressed in future studies.

Conclusion

Long-term celecoxib administration caused renal toxicity in male albino rats, with ameliorative effects of RJ against celecoxib-induced oxidative and apoptotic stress.

Ethics Approval and Consent to Participate

Animals were not exposed to unnecessary pain or stress and animal manipulation was performed with maximal care and hygiene. Zagazig University Scientific Research and Publications Ethics Committee approved the study. All steps and procedures in the study were carried out following Zagazig University Institutional Animal Care and Use Committee regulations, with approval no. ZU-IACUC/2/F/ 522 /2023. All methods were carried out in accordance with relevant guidelines and regulations. It was performed according to the recommendations of Good Clinical Practice and the Declaration of Helsinki (2013).

Consent for Publication

Not applicable.

Availability of Data and Materials

All relevant data are included in this published article.

Author Contributions

All authors are responsible for the concept and design of the study; N.Z., and H.K. contributed to data acquisition; H.N statistical analysis; N.Z., H.K., and H.N. interpreted the results; H.N analyzed the data and drafted the manuscript. All authors critically revised the manuscript, approved the final version to be published, and agreed to be accountable for all aspects of the work. All authors contributed to the creation of the manuscript.

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None.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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