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Research Article/Özgün Araştırma

Effects of ethyl 7-hydroxy-2-imino-2H-chromene-3-carboxylate, a synthesized coumarin derivative, on bisphenol A-induced kidney toxicity

Sentezlenen bir kumarin türevi olan etil 7-hidroksi-2-imino-2H-kromen-3-karboksilatın'ın Bisfenol A ile indüklenen böbrek toksisitesi üzerine etkileri

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Abstract

Aim: In this study, it was aimed to investigate the protective effect of the synthesized coumarin ethyl 7-hydroxy-2-imino-2H-chromene-3 carboxylate (CM) against the renal toxicity of Bisphenol A (BPA).

Materials and Methods: The CM molecule was synthesised through the reaction between 2,4-dihydroxybenzaldehyde and ethyl cyanoacetate. Experiment was conducted in four groups of rats: control, BPA, CM, and BPA+CM. Total Antioxidant Status (TAS), Total Oxidant Status (TOS), and Malondialdehyde (MDA) levels in kidney tissue were evaluated. serum samples were analyzed for Total-Native thiol and kidney function test parameters.

Results: The BPA-treated group exhibited significant decreases in TAS and thiol levels, increases in TOS and MDA levels. However, these side effects were significantly reduced with CM.

Conclusion: results obtained in this study indicate that CM molecule has a protective effect against BPA induced kidney toxicity

Keywords: Bisphenol A; Coumarin; Kidney; Antioxidant; Oxidative stres.

Öz

Amaç: Bu çalışmada sentezlenen kumarin etil 7-hidroksi-2-imino-2H-kromen-3 karboksilatın (CM) Bisfenol A'nın (BPA) renal toksisitesine karşı koruyucu etkinliğinin araştırılması amaçlandı.

Gereç ve Yöntem: CM molekülü, 2,4-dihidroksibenzaldehit ve etil siyanoasetat arasındaki reaksiyon yoluyla sentezlendi. Hayvan denemeleri için ratlar dört gruba ayrıldı: kontrol, BPA, CM ve BPA + CM. Böbrek dokusunda Toplam Antioksidan Durum (TAS), Toplam Oksidan Durum (TOS) ve Malondialdehit (MDA) seviyeleri değerlendirildi. Serum örnekleri Total-Native tiyol ve böbrek fonksiyon testi parametreleri açısından analiz edildi.

Bulgular: BPA ile tedavi edilen grupta TAS ve tiyol düzeylerinde önemli düşüşler, TOS ve MDA düzeylerinde ise artışlar görüldü. Ancak CM ile bu yan etkiler önemli ölçüde azaldı.

Sonuç: Bu çalışmada elde edilen sonuçlar CM molekülünün BPA kaynaklı böbrek toksisitesine karşı koruyucu etkiye sahip olduğunu göstermektedir.

Anahtar Kelimeler: Bisfenol A; Kumarin; Böbrek; Antioksidan; Oksidatif stres.

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Introduction

BPA, prevalent in polycarbonate plastics utilized in the food industry, can migrate into consumable items when they come into contact with food and beverages.

Thorough investigations have conclusively shown that individuals might unknowingly subject themselves to the harmful impacts of chemicals such as BPA through the consumption of contaminated products.[1-3]

The liver, renowned for its function in detoxifying harmful substances, and the kidneys, tasked with metabolite excretion, are notably vulnerable to the toxic effects of these compounds in the body.[4,5]

Under normal physiological conditions, the equilibrium of oxidant molecules is meticulously maintained through the ongoing activity of antioxidants. However, in the context of BPA-induced cytotoxicity, a rapid increase in the production of reactive oxygen species (ROS) is observed.[3] Antioxidants play a crucial role in shielding cells from oxidative damage. However, the effectiveness of enzymes and proteins tasked with converting oxidants into harmless molecules falls short of meeting the required capacity.[6] This disruption leads to the build-up of pro-oxidants, triggering a series of events linked to phenomena such as DNA damage, inflammation, and cellular deterioration, particularly in the kidneys.[7] Therefore, maintaining redox equilibrium and mitigating reactive oxygen species (ROS) levels become crucial tactics for alleviating the detrimental impacts of kidney damage induced by toxic substances such as BPA.[8] In this context, the supplementation of antioxidants plays a critical role in fortifying the body's defense mechanisms against these toxic effects, thereby safeguarding kidney health.

Coumarins, derived from both natural and synthetic sources, have been the focus of extensive research due to their significant contributions to human health. These compounds are renowned for their diverse properties, encompassing antimicrobial, anticoagulant, antifungal, antineoplastic, and antioxidant characteristics.[9] The incorporation of various substituents at different positions within the chemical structure of coumarins has greatly expanded their physicochemical and pharmacological attributes. This diversification has not only broadened their therapeutic potential but has also rendered them indispensable in the pharmaceutical industry.[10] Of particular note is the ability of coumarins to accommodate substituents at positions 1-8 of the core ring, with positions 4-8 being particularly noteworthy. Research for the biosynthesis of coumarin derivatives has consistently centered around these positions, revealing the capacity of coumarins to regulate pathological processes frequently linked to inflammation and infection.

These findings underscore the immense therapeutic versatility of coumarins.[10,11]

In this study, considering the bioactive properties associated with coumarin derivative, we synthesized the molecule ethyl 7-hydroxy-2-imino-2H-chromene-3-carboxylate (CM). The primary objective of our research is to investigate the antioxidant potential of this synthesized iminocoumarin compound in mitigating kidney damage induced by BPA. Through this exploration, we aim to test the potential of a coumarin derivative as a protective agent against toxic insults, further emphasizing its role in preserving and safeguarding kidney health.

Materials and Methods

Animals and experimental procedure

28 adult (aged 2.5 months) male Wistar albino rats (250±10 g body weight) were obtained from the Department of Animal Experiments. Ethical approval for this study was obtained from the Department of Animal Experiments Local Ethics Committee of Adıyaman University (Decision No: 2022/072). The welfare and living conditions of the animals used in the research fully complied with ethical standards. The 28 rats were divided into four groups: Control (n=7), BPA (n=7), CM (n=7) and BPA + CM (n=7). CM was administered to the animals in distilled water at a dose of 20 mg/kg orogastrically.[12] BPA was dissolved in distilled water, and the animals were given 20 µg/kg orogastrically.[13] During the application, in order to prevent possible complex formation

between BPA and coumarin, BPA and coumarin exposures were applied at different time intervals. The exposure of BPA was made between 09:00 am and 10:00 am every other day for 30 days. CM administration was performed between 04:00 pm and 05:00 pm every other day for 30 days. The rats were housed under a standard light/dark schedule (12-h light/12-h dark cycle) at constant temperature (21 ± 1°C) and humidity (55 ± 5%) with free access to pelleted food and fresh tap water. The rats were sacrificed at the end of day 30. For biochemical analyses, kidney samples were stored at –80°C until the assays were performed. The rest of the analytical reagent grade chemicals and reagents were acquired from commercial supliers.

Synthesis of CM molecule

The coumarin-derived compound was synthesized according to the previously reported method.[14] All chemicals and anhydrous solvents were purchased from Sigma-Aldrich, and TCI and used without further purification. Melting points (mp) were determined with SMP30 melting point apparatus in open capillaries and are uncorrected. FT-IR spectra were recorded by using Perkin Elmer Spectrum 100 FT-IR spectrometer. Nuclear Magnetic Resonance (1H-NMR and 13C-NMR) spectra of molecule was recorded using a Bruker 300 MHz spectrometer in DMSO-d6 and TMS as an internal standard. A mixture of 2,4-dihydroxybenzaldehyde (1) 10 mmol (1.38 gr) and ethyl cyanoacetate (2) 10 mmol (1.13 gr) was dissolved in 20 ml of Tetrahydrofuran (THF) and a few drop of piperidine was added. The reaction mixture was stirred at room temperature for 24 hours. The product was precipitated from the reaction mixture and collected by filtration. It was recrystallized from water/acetone (1/4) solvent. Compound (CM) was isolated as a yellow solid (Figure 1). Yield: %94 (2.19 gr). mp=219-220, lit, mp=220-221°C; FT-IR ($\upsilon_{max,}cm^{-1}$): 3310 (O-H or N-H, broad),3120-3005 (aromatic C-H), 2986-2934 (Aliphatic C-H),1690 (C=O),1620 (C=N), 1564-1486 (C=C), 1275 (C-O-C) ; ^{1}H-NMR (300MHz,DMSO-d_6)δ(ppm): 1.29 (3H, t, *J*=7.2Hz,-CH_3), 4.26 (2H, q, *J*=7.2Hz, -CH_2-), 6.43 (2H, m, aromatic), 8.15 (1H,d, *J*=6.6Hz, aromatic), 8.55 (1H,s), ^{13}C-NMR (75MHz, DMSO-d_6)δ(ppm): 14.6, 62.2, 94.2, 102.7, 109.6, 111.0, 117.7, 130.4, 148.7, 162.1, 163.8, 165.5;

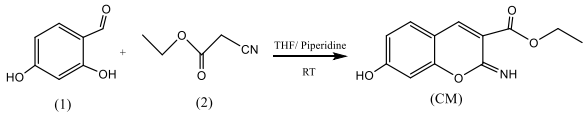

Figure 1. Synthesis of CM

Biochemical analysis

Kidney tissues were homogenized (BioSpec Products Mini- BeadBeater- 16, OK USA) in cold PBS (Phosphate Buffer Saline) (Ph 7.4) for biochemical analysis. Malondialdehyde (MDA) analysis was performed in the homogenate. The homogenates were then centrifuged at 5000 rpm and 4°C for 25 minutes to obtain supernatants. The supernatants were used for all other analyses. MDA levels were assessed as an indicator of lipid peroxidation using the method of Ohkawa et al.[15]. TAS and TOS in the supernatant and Total and Native Thiol concentrations in serum samples were determined using a spectrophotometer (microplate reader; Synergy H1). The ratio of TOS to TAS was considered as Oxidative Stress Index (OSI). In addition, urea, creatinine, sodium (Na), potassium (K), calcium (Ca), phosphorus (P), magnesium (Mg) levels in serum samples were measured with an autoanalyzer (Abbott Architecht C16000).

Histopathological evaluation

Upon completion of the experimental period, kidney tissues were excised from the animals and fixed in a 10% formaldehyde solution. Subsequently, the tissues underwent standard histological tissue processing, including dehydration through graded alcohol, clearing in xylene, and embedded in paraffin. Sections measuring 5 µm in thickness were obtained from the paraffin-embedded kidney tissue blocks. Hematoxylin and Eosin, as well as Masson's trichrome stains, were employed for histopathological examination. Images were captured using a microscope equipped with a Carl Zeiss Axiocam ERc5 digital camera attachment and were subjected to analysis and evaluation.

Statistical analysis of data

GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA) was used for statistical analysis. Shapiro-Wilk tests were used to assess the normality of the data distribution. The Kruskal-Wallis test followed by Dunn's post hoc test was used. Data are expressed as the mean ± standard deviation of the experiments. p-values less than 0.05 were considered to be statistically significant and are indicated as *p<0.05, **p<0.01 or ***p<0.001.

Results

Effects of CM on BPA-induced ROS formation

In the study, TOS, TAS, OSI, MDA, Total thiol, and Native thiol levels were evaluated to determine the oxidant-antioxidant balance in kidney tissue. It was observed that BPA administration decreased renal TAS, Total thiol, and Native thiol levels and increased TOS and MDA levels. As a result of the disruption of this balance in TAS and TOS, BPA caused an increase in OSI level. On the other hand, administration of CM to the BPA-treated group led to an improvement in the oxidant-antioxidant balance (Figure 2). These findings indicate that synthesized coumarin derivative compound showed a protective effect against BPA-induced renal toxicity.

In addition, the results of urea, creatinine, Na, K, Ca, P, Mg levels in serum samples are given in Table 1.

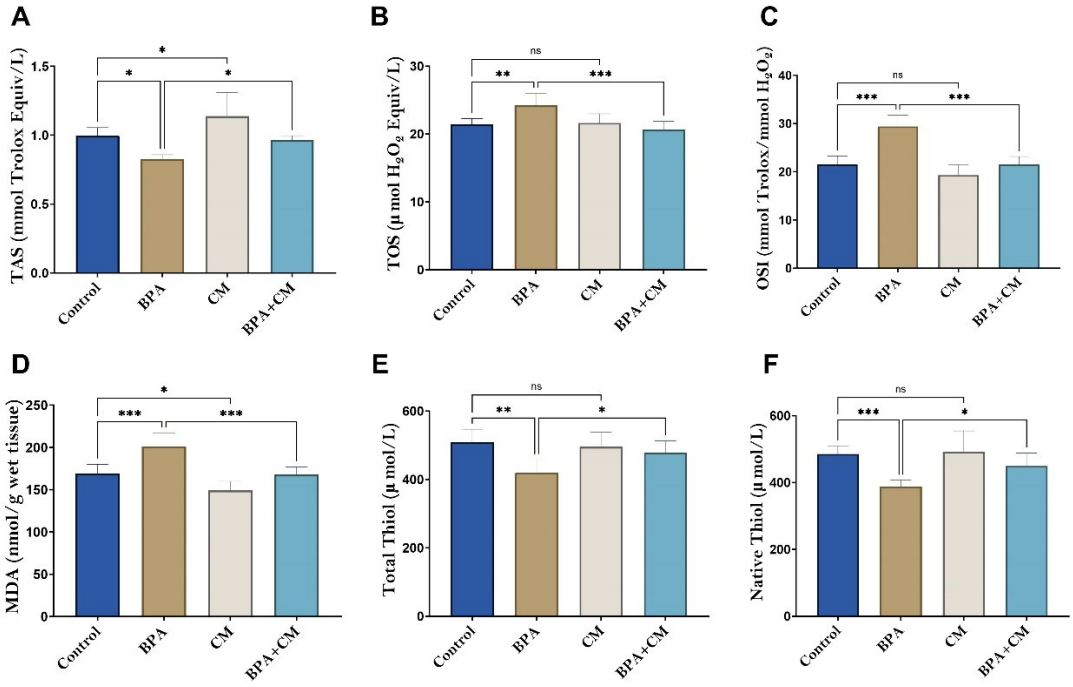

Figure 2. Oxidative stress parameters in kidney tissue.
Biochemical analysis results of kidney tissue in all groups were assessed. Levels of TAS (A), TOS (B), MDA (D), Total thiol (E), and Native thiol (F) were measured, and OSI (C) levels were calculated. The Kruskal-Wallis test followed by Dunn's post hoc test was used for statistical analysis. Data are expressed in mean ± SD. Statistical significance compared to the control group *$p<0.05$, **$p<0.01$ and ***$p<0.001$. Statistical significance compared to the BPA group #$p<0.05$, #$p<0.01$ and ###$p<0.001$.

Table 1. Kidney function test results

Parameters	Control	BPA	CM	BPA+CM
Urea (mg/dL)	56.57 ± 4.65	55.43 ± 5.88 ^{ns}	52.43 ± 5.56	55.43 ± 2.64
Creatinin (mg/dL)	0.40 ± 0.02	0.39 ± 0.01 ^{ns}	0.41 ± 0.03	0.43 ± 0.02
Sodium (mmol/dL)	137.4 ± 2.44	139.4 ± 1.51 ^{ns}	137.3 ± 2.29	134.1 ± 3.24
Potassium (mmol/dL)	9.06 ± 1.40	9.78 ± 0.57 ^{ns}	9.27 ± 1.25	10.00 ± 0.00
Calcium (mg/dL)	10.39 ± 0.45	11.11 ± 0.39 ^{ns}	10.53 ± 0.53	11.00 ± 0.58
Phosphorus (mg/dL)	11.46± 0.85	12.56 ± 1.52 ^{ns}	11.84 ± 1.59	14.37 ± 1.76
Magnesium (mg/dL)	3.57 ± 1.39	3.73 ± 1.20 ^{ns}	2.89 ± 0.51	3.33 ± 0.48

Histopathological evaluation of kidney tissue

The examination of kidney tissue involved assessment of both the cortex and medulla layers. In the cortex, various components including glomeruli, vessels, tubules, and interstitial areas were scrutinized, while in the medulla region, duct wall structures were evaluated. In the control and CM groups, glomerular structure, Bowman's space, tubular epithelial structures, duct wall epithelial structures, and lumens displayed a normal histological appearance (Figure 3A-3B and 4A-4B). Vascular wall structures appeared normal, and there were no signs of fibrosis in the interstitial area (Figure 5A- 5B).

In contrast, histological changes were evident in the BPA-administered group. These changes included capillary dilatation in glomeruli, reduction in Bowman's space, tubular defects, irregularly shaped glomeruli, and abnormalities in duct wall structures (Figure 3C and 4C). However, fibrotic alterations were not observed in the interstitial area (Figure 5C).

I-In the BPA + CM group, kidney tissue exhibited remarkably preserved glomerular structures, tubules, and duct walls (Figure 3D and 4D). No fibrosis was detected in the interstitial area (Figure 5D). Furthermore, there were no indications of inflammation or hemorrhage in any of the experimental groups.

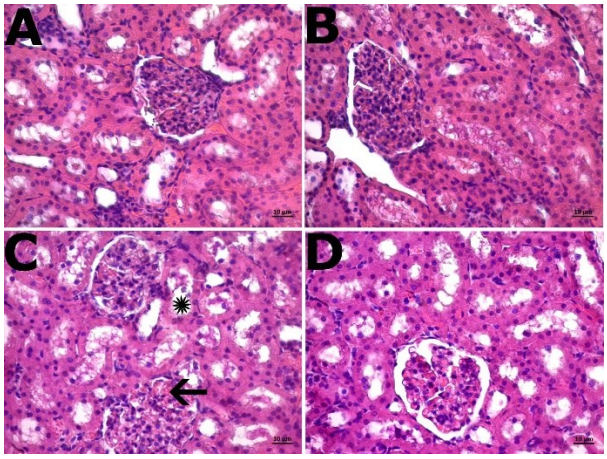

Figure 3. Light Microscopy Images (x40, Hematoxylin & Eosin Staining) of the Renal Cortex Region.
(A) Control group, **(B)** CM group, **(C)** BPA group, and **(D)** BPA + CM group. Asterisk indicates tubular defects, the black thick arrow denotes dilatation of glomerular capillaries, the black thin arrow indicates reduction in Bowman's space, the black arrowhead indicates irregularly shaped glomeruli. Healthy tissue appearance is observed in panels A, B, and D.

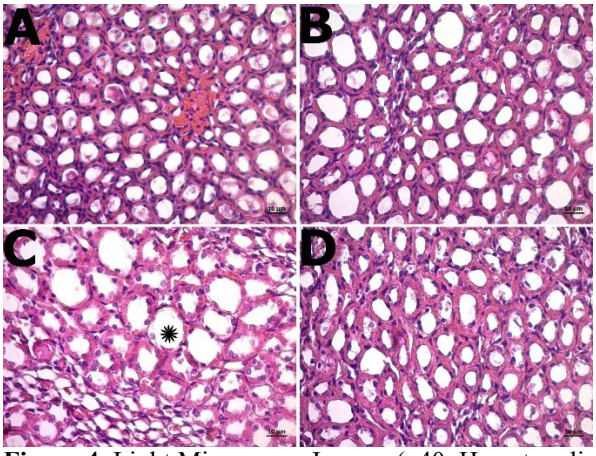

Figure 4. Light Microscopy Images (x40, Hematoxylin & Eosin Staining) of the Renal Medulla Region.
(A) Control group, **(B)** CM group, **(C)** BPA group, and **(D)** BPA + CM group. Asterisk indicates duct defects, and healthy tissue appearance is observed in panels A, B, and D.

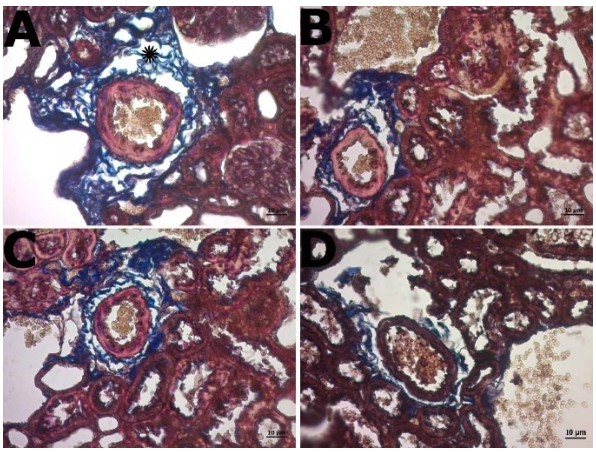

Figure 5. Light Microscopy Images (x40, Masson Trichrome Staining) of the Kidney Cortex Region.
(A) Control group, **(B)** CM group, **(C)** BPA group, and **(D)** BPA + CM group. Asterisk indicates connective tissue, and no fibrosis was observed in any of the groups.

Discussion

In clinical practice, several major conditions significantly impact kidney health, including acute kidney injury, glomerulosclerosis, diabetic nephropathy, pyelonephritis, and kidney stone formation. The development of these disorders is influenced not only by epigenetic and physical factors but also by environmental factors.[16] Among the potential environmental hazards to which our bodies are exposed daily are environmental toxicants such as smoking, alcohol, pharmaceuticals, agricultural toxins, heavy metals, acrylamide, and BPA.[17-19] Depending on the duration and extent of

exposure to these toxicants and an individual's inherent susceptibility, renal sensitization may ensue.

The liver plays a crucial role in activating enzymes to neutralize these toxic substances entering into the body through various sources, including food, beverages, drugs, and environmental pollutants. These enzymes encompass phase I and phase II enzymes involved in biotransformation, including cytochrome P450 enzymes, glucuronyl transferases, sulfotransferases, glutathione transferases, and acetyltransferases.[20] These enzymes play a pivotal role in metabolizing xenobiotics, facilitating the body's more efficient processing of toxicants and their conversion into hydrophilic metabolites for subsequent excretion via the kidneys. This fundamental detoxification and excretory function of the kidneys underscores their paramount importance in safeguarding overall body health.[21]

Oxidative stress stands out as one of the molecular consequences of exposure to toxic substances, and its impact on kidney health is substantial. Oxidative stress, with its high oxidative potential, is associated with proximal tubular toxicity in the kidneys.[22,23] This toxicity triggers mitochondrial dysfunction, impairing cellular energy production, and promoting the accumulation of pro-oxidant molecules within cells.[24] Numerous studies have demonstrated that BPA-induced kidney damage is closely linked to the development of oxidative stress. BPA-induced nephrotoxicity provokes the formation of reactive oxygen species (ROS) in cells, depleting critical components of the antioxidant defense system, including glutathione (GSH), catalase (CAT), Superoxide dismutase (SOD), and thiol-containing compounds.[25-27]

Under normal conditions, ROS are produced in the mitochondria of healthy kidney cells as byproducts of oxidative phosphorylation.[28] However, under pathological circumstances, oxidative stress arises due to increased free radical production, especially at specific sites such as complexes I and III in the electron transport chain, coupled with insufficient levels of antioxidant enzymes to scavenge these free radicals. This leads to mitochondrial membrane dysfunction, disrupting cellular energy balance, causing phospholipid and DNA damage, and impairing protein structures.[24,28] In our study, we explored the impact of the iminocoumarin compound synthesized in our laboratory on oxidative stress mechanisms to elucidate its potential against BPA-induced nephrotoxicity. We assessed the redox balance through TAS and TOS parameters to determine the iminocoumarin compound's effect. Our results revealed that the coumarin-derived compound strenghtened the antioxidant defense against renal toxicity by reducing tissue oxidant levels and increasing antioxidant levels.

Furthermore, we evaluated MDA levels to estimate lipid peroxidation, a contributing factor to tissue damage and dysfunction. We observed that BPA treatment led to increased MDA levels in kidney tissue, while iminocoumarin treatment reduced MDA levels. MDA level is used as a reliable biomarker of lipid peroxidation (LPO). Enzymes are affected by ROS and LPO production, thus SOD and CAT play a role in protecting cells against oxidative damage.[29,30] These findings imply that BPA administration enhances lipid peroxidation via free radicals, while iminocoumarin alleviates this effect. Additionally, we measured Total thiol and Native thiol to determine the levels of thiol compounds in the sample. These compounds possess antioxidant properties and neutralize the harmful effects of free radicals, converting them into harmless products for the cell. The results of our study revealed that the iminocoumarin compound increased serum thiol levels and thus enhanced antioxidant defense against kidney toxicity. In the study, TOS, TAS, OSI, MDA, Total thiol, and Native thiol levels were evaluated to determine the oxidant-antioxidant balance in kidney tissue. It was observed that BPA administration decreased renal TAS, Total thiol, and Native thiol levels and increased TOS and MDA levels.

Taken together, the results of the present study suggest that CM show antioxidant properties against the pro-oxidant effects of BPA. The antioxidant properties of coumarins are well documented. In particular, they have

the ability to scavenge free radicals and chelate metal ions. The antioxidant capacity of these derived molecules stems from their molecular structure.[31] The presence of groups such as hydroxy, acetoxy and methoxy in the structure of coumarin molecules affects the antioxidant capacity. Additionally, these groups in the molecule of coumarins are important for detoxification functions.[31] It has been reported that esculetin and scoparone-derived coumarins have a hepatoprotective effect in rat liver tissues, which have toxic effects with CCl_4. It has been stated that this effect of esculetin and scoparone coumarins is due to their structural properties.[32] The ameliorative effect of coumarin (1,2-benzopyrone) has been reported in rats with ferric nitrilotriacetate (Fe-NTA)-induced renal oxidative stress. It has been reported that while the level of MDA increases in the kidney tissues of rats exposed to Fe-NTA, the level of GSH and the activity of the GST enzyme decreased. It has been explained that coumarin (1,2-benzopyrone) corrects these negative effects.[31] In a rat study, it has been reported that 3-benzoyl-7-hydroxy coumarin molecule has a ameliorative effect on the antioxidant enzyme system, Al, Fe and Cu levels in rat liver tissues in which oxidative stress was created with $AlCl_3$.[33] Several studies have reported that coumarin molecules have antioxidant properties against various oxidative stress agents.[34-36] Coumarin-derived molecules such as fraxin, esculetin, grandivittin, agacillin, aegolinol benzoate and osthol have been reported to have free radical scavenging effects.[37]

Collectively, our results indicate that pro-oxidant molecules, heightened by BPA toxicity, causes damage in biomolecules such as lipids and proteins within kidney tissue. Furthermore, iminocoumarin emerges as an effective intervention against this nephrotoxicity, signifying its potential importance in protecting kidney cells from oxidative harm. When the effects of BPA on the kidney are examined histopathologically in the scientific literature; Hoque et al.[38] reported that BPA exerts deleterious impacts on histopathological parameters including association with renal injuries. Besides; Rahimi et al.[39] demonstrated that 100g/kg/day dose of BPA causes renal lesions such as dilation and propagation of glomeruli and degeneration of epithelium of proximal tubule. Furthermore; Shaimaa, et al.[40] pointed out that BPA has detrimental effects on the kidney. In this context; also our histopathological evaluations were conducted on cortex and medulla layers of kidney tissue.[40] In the cortex region, we scrutinized various components, including vessels, glomeruli, tubules, and interstitial spaces, while in the medulla region, we assessed duct wall structures. The presence of capillary dilatation in glomeruli, irregularly shaped glomerular structures, reduced Bowman's space, and abnormalities in duct wall structures in the BPA-treated group underscored the tissue damage induced by BPA. Türk et al.[41] pointed out that coumarin has reversed these changes significantly. Conversely, in the BPA and iminocoumarin-treated group, the absence of interstitial fibrosis and the preservation of vessel walls, tubular epithelium, and glomerular structure highlighted the protective effects of coumarin against the development of nephrotoxicity arising from BPA exposure.

Conclusion

Ongoing research endeavors are exploring the potential protective and therapeutic properties of compounds derived from diverse natural or synthetic sources, aiming to safeguard organ health and prevent associated ailments.[42-44] Prior investigations have unveiled the promising impacts of coumarin derivatives, whether extracted from botanical sources or synthesized, in mitigating tissue pathogenesis for the prevention of various diseases.[45,46]

Our study significantly contributes to this body of knowledge by highlighting the antioxidative potential of iminocoumarin in mitigating BPA-induced renal toxicity. The demonstrated capacity of the synthesized iminocoumarin compound to attenuate oxidative stress emphasizes the pivotal role of coumarin-derived compounds in ameliorating kidney diseases by counteracting nephrotoxicity induced by oxidative stress.

Ethics Committee Approval

Ethical approval for this study was obtained

from the Department of Animal Experiments Local Ethics Committee of Adıyaman University (Decision No: 2022/072). The welfare and living conditions of the animals used in the research fully complied with ethical standards.

Author Contributions

NBA and MMÜ conducted the biochemical analysis; NÜ wrote the paper; EA carried out histopathologic analysis; MK conducted coumarin synthesis and purification; RCB conducted the animal experiments; AÖ performed interpretation of biochemical analyzes; EMBY carried out tissue collection in animal experiments. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

Financial Disclosure

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Statements

These research results have not been presented anywhere previously.

Peer-review

Externally peer-reviewed.

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