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RESEARCH ARTICLE

The efficacy of oleuropein against non-steroidal anti-inflammatory drug induced toxicity in rat kidney

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Abstract

Indomethacin is generally used in clinical therapeutics as a non-steroidal anti-inflammatory drug. However, its use has been limited due to the gastrointestinal and renal toxic effects of this drug. These toxic effects were associated with not only the inhibition of prostaglandin synthesis but also drug-elevated oxidative stress. To ameliorate these toxicities, natural antioxidants can be used as an alternative and/or combination therapies. Therefore, the current study was conducted to assess the renoprotective effects of oleuropein against indomethacin-induced renal damages. Male Sprague-Dawley rats were pretreated with oleuropein (75, 150, and 300 mg/kg), and then treated with indomethacin (25 mg/kg). To evaluate kidney function, serum blood urea nitrogen, uric acid, and creatinine were measured. In addition, prostaglandin E_2, tumor necrosis factor-alpha, endothelial nitric oxide synthase, caspase-3, oxidant/antioxidant status, and 8-Oxo-2′-deoxyguanosine levels were determined for the antioxidative and anti-inflammatory effects of oleuropein. Tissue sections were also histopathologically assessed. The biochemical and histopathological analysis proved the toxic effects of indomethacin on kidney. However, the pretreatment with oleuropein (300 mg/kg) protects kidney from indomethacin-induced damages. Our study proved that prior administration of oleuropein has renoprotective activity against indomethacin-associated toxicities.

KEYWORDS

8-OHdG, eNOS, histopathologic assessment, oleuropein, prostaglandin E2

1 | INTRODUCTION

Indomethacin is a methylated indole acetic acid derivative NSAIDs and commonly used in clinical therapy as anti-inflammatory, analgesic and anti-pyretic drugs.[1] Its extensive utility is arisen from the rapid absorption, rapid and efficient crossing blood brain barrier.[2] Despite of its common use, it has adverse effects including gastrointestinal and kidney toxicities. The studies on the mechanisms of these adverse effects stated that they are mainly caused from the inhibition of prostaglandin synthesis via the suppression of cyclooxygenase activity.[3] In addition, the indomethacin-elevated oxidative stress and reduced endothelial nitric oxide synthase activity have been demonstrated to contribute to the pathogenesis of this drug-induced kidney injuries.[4]

Since the oxidative stress play a role in indomethacin-induced renal injury, alternative and/or combination therapies including natural antioxidant agents such as olive oil can be used to prevent the effects of oxidative stress produced. It is very well known that olive oil phenolic compounds have anti-bacterial hepatoprotective, anti-microbial, antioxidative, and anti-virus properties.[5,6] Oleuropein is a major polyphenolic compound present in olive-tree leaves, fruits (olives), and olive oil.[7] The protective effects of this compound against different agents-induced tissue damages including stomach and kidney have been shown in several studies.[8,9]

We have previously investigated the protective effects of oleuropein on Indomethacin-induced gastric ulcer rat model via histopathological and biochemical analysis (unpublished data). This study indicated that 25 mg/kg dose of Indomethacin administration caused gastric ulcer formation in rat through pathological injuries and elevated oxidative stress. However, pretreatment of oleuropein at the different doses prevented the formation of these effects. Moreover,

Ahmadvand and his co-workers indicated that oleuropein was able to ameliorate oxidative stress induced renal damages in diabetic rats.[10] This ameliorative property was attributed to the antioxidative and antiatherogenic activities of oleuropein. In a recent study, the ameliorative effects of aqueous olive leaf extracts on kidney functions were also demonstrated in diabetic pregnant mice and their foetuses.[11] Despite of these ethnopharmacological studies, the protective efficiency of this compound in indomethacin-induced renal injury remains to be elucidated. Therefore, our study was aimed to assess the protective effect of oleuropein on renal damages induced by indomethacin. To evaluate its renoprotective effects, histopathological analysis of samples were performed. Total antioxidant capacity (TAC), total oxidant status (TOS), endothelial nitric oxide synthase (eNOS), tumor necrosis factor-alpha (TNF-α), prostaglandin E_2 (PGE_2), and caspase-3 were measured. Additionally, 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels, a sensitive marker of oxidative DNA damage was obtained.

2 | MATERIAL AND METHODS

2.1 | Chemicals

Indomethacin (Endol 25 mg; 25 cap.) were purchased from DEVA Holding A.S. (Istanbul, Turkey). Oleuropein (HPLC grade ≥ 98%) and all chemicals were purchased from Sigma-Aldrich International (St. Louis, Missouri).

2.2 | Animal experimental protocols and rat ulcer model

Male Sprague-Dawley rats, weighing 250 ± 20 g, were purchased from Ataturk University's Experimental Animal Laboratory of the Medicinal and Experimental Application and Research Center (ATADEM). All rats were housed under controlled light (12 hours dark/12 hours light cycle) and temperature (20°C-23°C) conditions with free access to standard chow and water. This study and procedures were performed in accordance with national guidelines for the use and care for laboratory animals and were approved by Ataturk University's local animal care committee (No. 104, February 6, 2015). Rats were fasted 24 hours prior to administration of each of control and tested compounds and had access to water during fasting. The doses of oleuropein and indomethacin and their administration way were determined based on the previous studies.[12,13] Indomethacin was orally given to rats (25 mg/kg), followed by oleuropein (at different doses). Rats were randomly divided into 5 groups (n = 7 in each group): normal control (group I); indomethacin (25 mg/kg) treated rats (group II); indomethacin-induced rats treated with oleuropein (75 mg/kg; group III); indomethacin-induced rats treated with oleuropein (150 mg/kg; group IV); indomethacin-induced rats treated with oleuropein (300 mg/kg) treated rats (group V). Oleuropein (1 ml/rat) was given orally (by gavage) before 10 minutes from induction of ulcer by oral dosing with indomethacin (0.5 ml). After 6 hours the rats were anesthetized using sevoflurane (3.5 vol% and 30% oxygen: 70% N_2O). Blood and renal tissues were harvested for further analysis.

2.3 | Blood urea nitrogen, uric acid, and creatinine determination

Blood samples were collected from abdominal aorta and centrifuged to isolate serum, plasma, and then separated into aliquots and stored at −80°C until analyzed. Blood urea nitrogen (BUN), uric acid (UA), and serum creatinine (Cr) were measured by an automatic biochemical analyzer 7600 (Hitachi, Tokyo, Japan) to evaluate the alteration of renal function.

2.4 | Tissue preparation and homogenization

Before biochemical assays, kidney tissues were weighed, broken down into very small pieces, and placed in empty glass tubes. 1 ml of 140 mM KCl solution per gram of tissue was added to each tube, and then all tissues were homogenized in a motor driven homogenizer. The homogenate was centrifuged at 2800g for 10 minutes at 4°C. The resulting supernatant was used for the determination of TAC, TOS, eNOS, TNF-α, PGE_2, caspase-3, and 8-OHdG levels.

2.5 | Determination of oxidative stress biomarkers and biochemical parameters

The commercially available ELISA kits were used according to the manufacturers' instructions: TAC and TOS kits (Rel Assay Diagnostics, Gaziantep, Turkey), Rat TNF-α kit (Biolegend San Diego, California), caspase-3 activity kit (Beyotime Institute of Biotechnology, Haimen, China), PGE_2 Express kit (Cayman Chemical Company, Ann Arbor, Michigan), eNOS kit (Korain Biotech, Junjiang International Bldg., Shanghai, China), 8-OHdG kit (Elabscience Biotechnology Co., Ltd., Hubei, China) were used for biochemical assays.

2.6 | Histological injury assessment

Kidney tissues were removed aseptically from all the groups were cut into small pieces and samples were fixed in 10% formaldehyde solution. The tissue sections (5 μm) were mounted on glass slides and stained with haematoxylin-eosin (HE) and periodic acid-Schiff (PAS) for evaluating the kidney structure. Five pieces of pathological sections were prepared from each rat. Each stained section was semi-quantitatively evaluated under light microscope (Olympus BX51 microscope with a magnification of ×200) by a histologist blinded to the treatment group.

2.7 | Statistical analysis

The data are presented as the mean ± SE. Statistical significance was determined by 1-way ANOVA test followed by Duncan multiple range test. (SPSS for Windows 18.0, SPSS Inc., Chicago, Illinois). The values indicated by the different letters (a, b, c, d, e) were significantly different from each other at $P < .05$.

3 | RESULTS

3.1 | Biochemical results

3.1.1 | Oleuropein decreased serum levels of BUN, UA, and Cr in rats after indomethacin treatment

The rats in indomethacin group displayed significant deterioration of renal function, as reflected by remarkably increased levels of BUN, UA, and Cr compared with that of control group. The oral administration of oleuropein in indomethacin + oleuropein (except for 75 mg/kg) group reduced the BUN, UA, and Cr values that showed a decrease in kidney damage. Moreover, the high dose of oleuropein provided a significant improvement in the functional parameters of kidney (Table 1).

3.1.2 | Oleuropein protected against cytotoxicity, oxidative stress, inflammation caused by indomethacin and prevented the apoptosis

As shown in Figures 1A,B, 2A,D, and 3, all the biochemical parameters of renal tissue samples except PGE_2 and TAC were increased significantly in indomethacin group as compared with the control group. In comparison to the indomethacin group, indomethacin + low dose oleuropein group (75 mg/kg) was not statistically significant in terms of all biochemical parameters. The increasing dose of oleuropein (150 mg/kg) provided a slight reduction in TOS, eNOS, TNF-α, caspase-3, and 8-OHdG levels. However, the results of the indomethacin + high dose of oleuropein group (300 mg/kg) showed a significant difference from that of indomethacin group. This group was the treatment group and the protective activity of oleuropein was significant by reversing the measured biochemical parameters.

3.1.3 | Oleuropein administration attenuated renal pathological injury

Kidney sections stained with HE and PAS revealed that indomethacin resulted in a typical tubular injury characterized by pronounced degeneration of tubular architecture, tubular vacuolization, tubular dilatation, inflammation, congestion and amyloid deposits. And these histological changes in the high dose oleuropein-treatment group were less severe than that of indomethacin group (Figures 4A-H and 5A-E). Generally, the kidney histology of this group was similar to the control group.

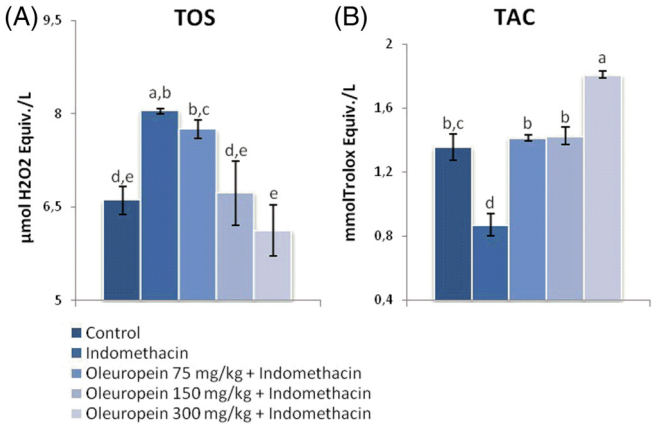

FIGURE 1 The effects of oleuropein on kidney. A, TOS and B, TAC levels after treated with Indomethacine. The bars shown by different letter are different from each other at a level of .05. TAC, total antioxidant capacity; TOS, total oxidant status [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

The drug toxicity can cause serious cellular degenerations. The increase in the number of people with kidney diseases is taken into consideration, the search for alternative treatments with fewer side effects is vital, as well as the demand for foods or plant-based products that can help to protect the kidney. In this context, the use of new therapeutic strategies based on natural plant products may be useful to provide higher efficacy, minimal toxicity and a wider therapeutic perspective for effective medications than existing pharmaceuticals.[14]

Indomethacin-induced toxic alterations in several organs have been previously reported, the induction of reactive oxygen species (ROS) formation and depletion of antioxidant defenses have also been shown in previous studies.[15] Moreover, oxidative stress mostly served as a non-specific contributor in various organ disorders. Thus, the detailed mechanisms of indomethacin-induced toxicity still need to be explored in specific organ diseases.[16] In this study, we firstly examined the regulation of eNOS, TNF-α, caspase-3, and PGE_2 levels in renal apoptosis following the indomethacin treatment. According to our study, indomethacin caused severe renal damages and it also increased the TOS levels as well as the contents of eNOS, TNF-α, and levels of caspase-3 (all $P < .05$). In addition, treatment with indomethacin (25 mg/kg) caused a significant ($P < .05$) reduction in PGE_2 levels compared to the control group. In this manner, the significant

TABLE 1 Effect of oleuropein treatment on levels of kidney function markers after treated with indomethacin

Groups	BUN (U/L)	UA (U/L)	Cr (U/L)
Control	22.42 ± 2.86^{a}	1.33 ± 0.30^{a}	0.20 ± 0.019^{a}
Indomethacin	95.68 ± 5.29^{d}	3.82 ± 0.28^{d}	0.97 ± 0.034^{d}
Oleuropein 75 mg/kg + indomethacin	93.81 ± 4.71^{d}	3.74 ± 0.20^{d}	0.91 ± 0.042^{d}
Oleuropein 150 mg/kg + indomethacin	54.29 ± 7.29^{c}	2.44 ± 0.34^{c}	0.86 ± 0.023^{d}
Oleuropein 300 mg/kg + indomethacin	29.68 ± 6.06^{a}	1.50 ± 0.19^{a}	0.32 ± 0.017^{a}

Data are presented as means ± SD ($n = 7$).
Abbreviations: BUN, blood urea nitrogen; Cr, serum creatinine; UA, uric acid.
[a,b,c,d]The groups in the same column with different letters are statistically significant ($P < .05$).

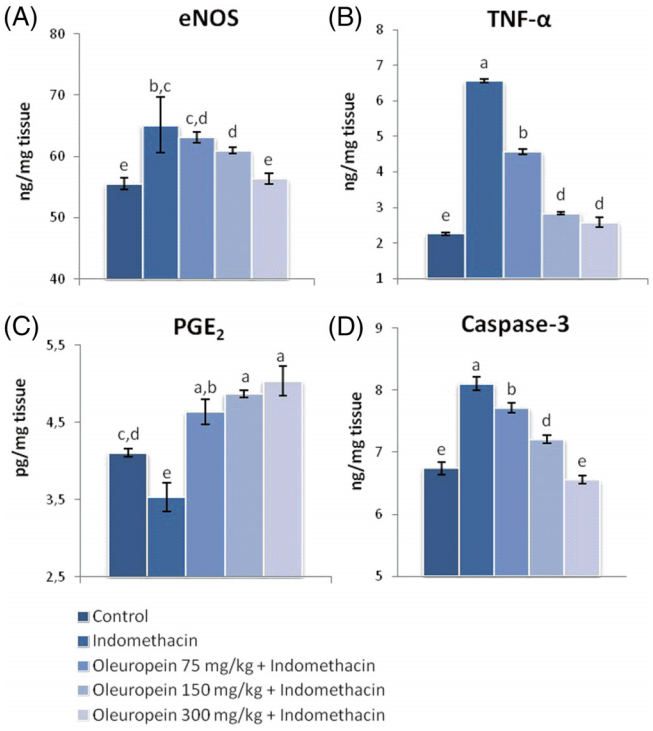

FIGURE 2 The effects of oleuropein on kidney. A, eNOS; B, TNF-α; C, PGE_2; and D, caspase-3 levels after treated with Indomethacine. The bars shown by different letter are different from each other at a level of .05. eNOS, endothelial nitric oxide synthase; PGE_2, prostaglandin E_2; TNF-α, tumor necrosis factor-alpha [Color figure can be viewed at wileyonlinelibrary.com]

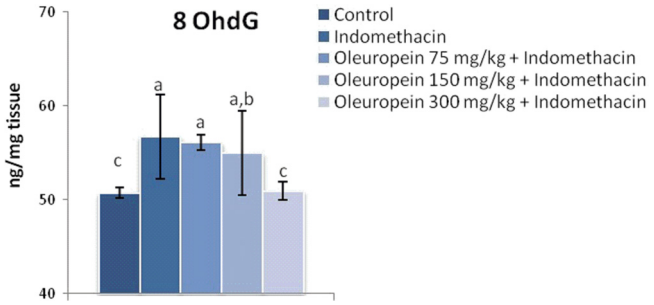

FIGURE 3 The effects of Oleuropein on kidney 8-OHdG level after treated with Indomethacine. The bars shown by different letter are different from each other at a level of .05 [Color figure can be viewed at wileyonlinelibrary.com]

toxicological effects of indomethacin were detected along with all the parameters investigated.

eNOS activity is consistent with earlier studies, which suggested a causal relationship between impaired endothelial activity and renal failure.[17] In our study, such oxidative effect could be accentuated by the oxidative damage induced by indomethacin and resulted in enhanced scavenging of NO released from endogenous (eg, vascular endothelial) or exogenous sources. Antioxidants-induced inhibition of vasoconstriction in rat kidney arteries is mediated by NO production induced by the activation of nitric oxide synthase. It is assumed that the plant based products had bioactive compounds such as antioxidants that acted on the eNOS levels, protecting them from the damage caused by indomethacin.[18] Therefore, the theory of increasing antioxidant capacity in renal cells to ameliorate the toxic effects caused by indomethacin was investigated in the present study. The present study showed the ability of oleuropein to inhibit endothelial eNOS levels effectively and increased the TAC levels in the oleuropein group compared with the indomethacin group. TOS values were significantly lower in the group which was administered with indomethacin + oleuropein compared to the group which only received indomethacin ($P < .05$). It is reported that oleuropein may prevent free radical formation by means of chelating metal ions that catalyzes ROS formation. Providing a hydroxyl group to suppress ROS formation is the way of its action.[19]

The other steps crucial for the pathogenesis of indomethacin involve the modulation of cell signalling via blocked- PGE_2 production, TNF-α production and endoplasmic reticulum stress.[20,21] Our results showed that oleuropein could relieve acute inflammation through inhibiting the activation of inflammatory mediators generated by indomethacin. Our results suggested that oleuropein might possess potential therapeutic activity to treat inflammation related disorders in the other NSAIDs-induced damages such as indomethacin. We obtained a striking finding that endogenous PGE_2 was essential for indomethacin-induced TNF-α production, suggesting a positive feedback loop. A positive feedback loop from TNF-α and back to PGE_2, which itself induced by TNF-α, is likely to be operating. In the current study, for the first time, it was clearly documented that apoptosis in renal tissue might therefore occur in the absence of PGE_2 response due to the inhibitory effect of PGE_2 on TNF-α production. It was also revealed from the caspase-3 data, nephrotoxic effects created by indomethacin administration can be limited with oleuropein. Apoptosis was the predominant mode of cell death caused by NSAIDs in renal cells. Interestingly, the relative potency of nonselective NSAIDs causing cell death correlates well with their relative potency in blocking PGE_2 production.[22] In our study, oleuropein treatment has anti-apoptotic effect, evident by its down-regulation related with the indomethacin induced over-expression of the renal caspase 3. Furthermore, the PGE_2 production was further stimulated by the high dose of oleuropein treatment in the indomethacin challenged rat. The treatment with oleuropein modulated this detrimental action of the drug and retained cell viability. A recent report demonstrated that indomethacin could induce renal apoptosis via an oxidative stress-related mechanism.[23] In the present study, 8-OHdG levels, which is a biomarker of DNA oxidative damage, significantly changed by indomethacin. However, from recently postulated point of view, the important roles of oxidative DNA damage in kidney injury lacks from the influence of indomethacin on 8-OHdG generation that clearly pointed out in the present study.[24,25] In our study, oleuropein suppressed the apoptotic indices as evidenced from 8-OHdG levels and histopathological studies. With oleuropein, there was a tendency of inhibition with clear dose-dependence on DNA damage. Although different in vitro systems have shown that olive oil phenols possess a potent antioxidant activity and prevent the reactive oxygen species-mediated cell injury.[26] However, there is a limited and contradictory evidence for such a protective role on DNA damage. Recently, oleuropein was demonstrated to be genotoxic by the micronucleus test.[27] Instead, we demonstrated that oleuropein may prevent the ROS-induced DNA damage in a high concentration range. The in vivo results of our study

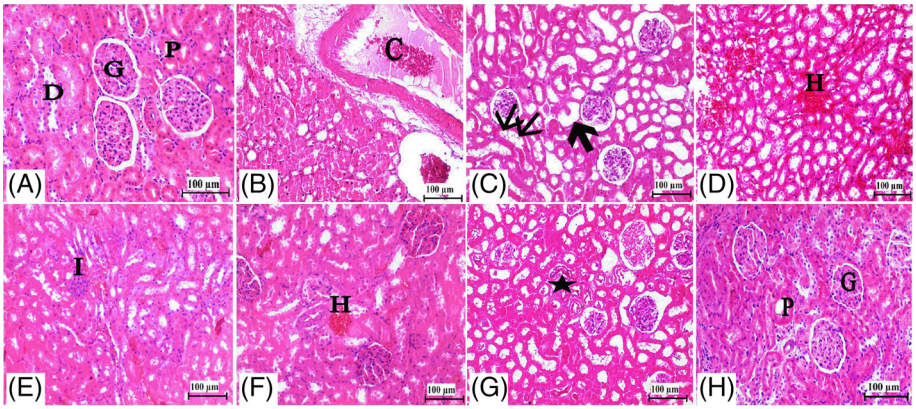

FIGURE 4 A, The control group with normal renal histology. G: Glomerulus, P: Proximal tubule, D: Distal tubule. B-E, Effects of indomethacin on renal tissue. Congestion (C), tubular dilatation (arrow), tubular vacuolization (double arrow), hemorrhage (H), infiltration (I). F-H, The renal tissue following 75, 150, and 300 mg/kg oleuropein treatment, respectively. H: Hemorrhage, decreased tubular degenerations (asterisk) and normal kidney histology at 300 mg/kg oleuropein dose. HE, Bar: 100 μm. HE, haematoxylin-eosin [Color figure can be viewed at wileyonlinelibrary.com]

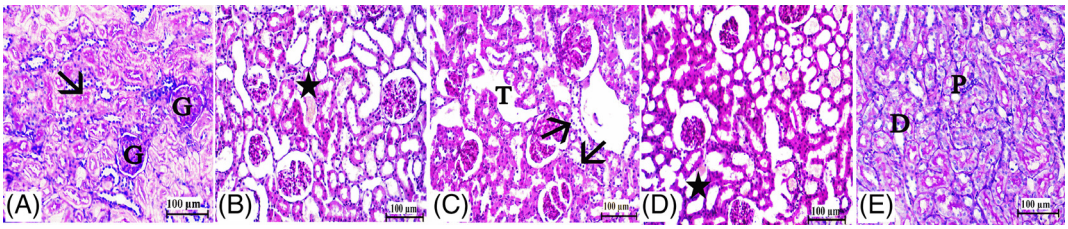

FIGURE 5 A, The control group with normal renal histology. Glomerulus (G), brush border (double arrow). B and C, Effects of indomethacin on renal tissue. Amyloidosis (asterisk), tubular dilatation (T), tubular epithelial degenerations (arrows). D, The renal tissue after 150 mg/kg dose of oleuropein treatment. Decreased amyloid accumulation (asterisk). E, The renal tissue similar to controls at the 300 mg/kg dose of oleuropein P: Proximal tubule, D: Distal tubule. PAS, Bar:100 μm. PAS, periodic acid-Schiff [Color figure can be viewed at wileyonlinelibrary.com]

suggested that oleuropein reduced 8-OHdG formation, at least partially, through endothelium-dependent vascular response and reduced the inflammatory molecules in macrophages.

Identification of NSAIDs that are effective as indomethacin with less side effects was highly important for the protection of renal functions.[28] In our study, oral exposure to indomethacin induced a significant increase in serum biochemical parameters in kidney tissue. However, oleuropein was quite effective in ameliorating the functional changes and in normalizing PGE_2 levels. In this study, we suggested that PGE_2 may a regulator of the functional response in the kidney and may provide a mechanistic basis for the protective effect of oleuropein and the deleterious effects of Indomethacin. It was reported that indomethacin was a prostaglandin inhibitor,[29] whereas, PGE_2 was a major determinant of renal vascular reactivity and renal function.[30] Thus, our study provides a good evidence supporting the value of PGE_2 determination on renal failure.

These findings suggested that the nephrotoxicity was one of the major side effects of the antiulcer drug indomethacin. The signaling mechanisms responsible for the indomethacin-induced cytotoxicity appeared to be multifactorial, involving the inflammation, eNOS production and 8-OHdG generation with oxidative stress. Once all the parameters were taken into consideration, oleuropein could protect against indomethacin-related kidney damages through activating PGE_2, antioxidative, antiapoptotic, and anti-inflammatory interactive mechanisms. Hence, this study showed a potent DNA damage preventive activity of oleuropein in renal tissue, providing a new evidence to support the possible role of this compound in the prevention of renal damages. This study concludes that to prevent Indomethacin-induced tissue injuries, the combination of oleuropein and indomethacin should be better than consumption of indomethacin alone. Further studies are required to confirm the use of oleuropein as a natural supplement for drug induced tissue toxicities.

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