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Histological Assessment of Selenium Protective Effect Against Diclofenac Sodium-Induced Nephrotoxicity in Rats

Suhair Albassit[1,*], Shaza Al Laham[1] and Ahmad Al-Manadili[2]

[1]*Pharmacology and Toxicology Department, Faculty of Pharmacy, Damascus University, Damascus, Syria*

[2]*Oral Histopathology Department, Faculty of Dentistry, Damascus University, Damascus, Syria*

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*Corresponding Author
E-mail: suhairalbassit@gmail.com

Abstract:

Drug-induced nephrotoxicity is a matter of global significance, with widespread implications for human health. Non-steroidal anti-inflammatory drugs (NSAIDs), such as Diclofenac Sodium, are widely used but have been associated with numerous adverse effects, including those on the gastrointestinal tract, liver, and kidney. Selenium, an essential element in mammalian biology, has demonstrated antioxidant properties that enable it to antagonize the effects of free radicals. The present study aimed to investigate the potential protective effect of Selenium against Diclofenac Sodium-induced nephrotoxicity in rats. The study sample consisted of 32 male albino Wistar rats and was randomly divided into 4 groups: Control group, Diclofenac group (Dic group) (administered at a dose of 50 mg/kg I.M., for 3 days), Selenium group (Se group) (administered at a dose of 1 mg/kg I.P., for 8 days), and Diclofenac plus Selenium group (Dic + Se group). Rats were sacrificed on the 9th day. The results were analyzed with a significance level of $p<0.05$. The findings revealed significant tubular and glomerular injuries in the Diclofenac group compared to the control group. However, there was no statistically significant improvement observed in the Dic + Se group. In conclusion, the results of this study suggest that Selenium affords partial protection against Diclofenac Sodium-induced nephrotoxicity, but it is not sufficient to provide complete protection.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are globally prescribed for the management of pain, fever, and inflammation, with an estimated use of >30 million per day [1]. NSAIDs exert their pharmacological actions by inhibiting the activity of Cyclooxygenase (COX) enzymes. This inhibition results in the blockage of prostaglandin synthesis. Non-selective NSAIDs can inhibit both COX-1 and COX-2. COX-1 is a constitutively expressed enzyme in several tissues, which plays essential physiological roles, such as maintaining renal blood flow, protecting the gastric mucosa, and regulating platelet aggregation. In contrast, COX-2 is an inducible enzyme that responds to pro-inflammatory cytokines and growth factors. The adverse effects associated with the inhibition of COX-1 have limited the use of non-selective NSAIDs [2, 3].

According to reports, the usage of NSAIDs has been associated with up to 36% of drug-induced acute kidney injury (AKI) cases and up to 7% of all AKI cases [4]. The risk of developing AKI due to NSAID usage is higher in older individuals and those with comorbidities [5]. NSAID consumption can negatively impact renal hemodynamics, and induce direct cytotoxicity and injury mediated by allergic reactions, ultimately leading to AKI. In addition, NSAIDs may cause acute interstitial nephritis (AIN) [6].

Diclofenac (Dic) is a non-selective NSAID and a derivative of phenylacetic acid. Its potency in inhibiting COX-2 is greater than that of COX-1. Moreover, diclofenac can reduce the production of leukotrienes and inhibit phospholipase A_2 (PLA_2), which further supports its high potency [7, 8]. This drug is indicated for conditions such as osteoarthritis (OA), rheumatoid arthritis (RA), juvenile idiopathic arthritis, ankylosing spondylitis, migraine, dysmenorrhea, and mild to moderate pain [7-9].

Apart from AKI, Diclofenac has been also associated with several adverse effects, including an increased risk of stroke, acute myocardial infarction, immune-mediated hepatotoxicity, and gastrointestinal complications, which are the most frequently encountered [10]. Long-term use of diclofenac can cause a dose-dependent reduction in glomerular filtration rate (GFR) [11]. Furthermore, diclofenac and its reactive metabolites can cause mitochondrial dysfunction, resulting in the depletion of adenosine triphosphate (ATP), and the generation of reactive oxygen species (ROS), ultimately leading to oxidative stress and lipid peroxidation. As a consequence, Deoxyribonucleic acid (DNA) fragmentation and apoptosis may occur [11-13].

In recent years, there has been a growing trend in scientific research focused on food supplements and medical herbs, with numerous studies aiming to identify treatments that can modulate human diseases [14]. Several natural antioxidants and plant extracts are effective in protecting the kidney from diclofenac-induced damage, including Carvacrol, Caffeine, Cinammoun aqueous extract, Aframomum melegueta seeds extract, Hesperidin Methyl Chalcone, Chlorophyll and vitamin D_3 [15-20]. Additionally, a new series of compounds, namely 5-substituted-1,3,4-thiadiazole-2-thiols (3a-m) and 5-substituted-2-(3,4,5-tri hydroxyphenyl)-1,3,4-oxadiazoles (3n-z), was synthesized and assessed for its *in vitro* antioxidant activities. It is noteworthy that some of these compounds exhibited promising *in vitro* potential as novel antioxidants, which may be beneficial for the development of new antioxidant agents in the future [21, 22].

Selenium (Se) is a micronutrient, an essential constituent of selenoproteins such as glutathione peroxidase (GPx), and plays a critical role in antioxidant defense systems. Selenium has been suggested to exhibit hormetic effects as it can be toxic at high doses but has beneficial impacts at very low doses [23]. The range between deficiency, essentiality, and toxicity is narrow [24]; therefore, the recommended dietary allowance for selenium is 55µg per day [25]. Adequate selenium intake is important in preventing various diseases, particularly those related to thyroid hormones, fertility, skeletal muscle health, inflammatory-based diseases, and some neurodegenerative disorders [23]. Sodium Selenite, an inorganic selenium compound, has anti-proliferative activities against different types of cancer cells [26].

Selenium has renoprotective activity against nephrotoxicity induced by several chemicals [27-30] and has been effective in preventing ischemic renal failure [31]. However, the efficacy of selenium in preventing AKI and associated morbidity and mortality after off-pump coronary bypass graft surgery is controversial, as a recent article reported conflicting results [32]. For this reason, further investigations are needed to clarify the potential protective efficacy of selenium.

RESEARCH OBJECTIVES

Based on the aforementioned background, in this study, we aimed at investigating the potential protective effects of Selenium in a rat model of Dic-induced nephrotoxicity.

MATERIALS AND METHODS

Drugs

- Nephroprotective drug: Sodium Selenite (Se) was obtained from Proto Chemical Industries (India), and dissolved in a normal saline solution of 0.9%.
- Nephrotoxic drug: Diclofenac Sodium (Dic) 99.92% was obtained from Zhongbao Chemical (China), and prepared as an aqueous solution of 4% (w/w), using Propylene Glycol (PG) and Polyethylene Glycol 400 (PEG 400) in a ratio 2:1. Stock Solutions were prepared each day just before administration. Analytical-grade chemicals were used in our study for all other procedures.

Animals Care

A total of 32 adult male Wistar Albino rats (weighing 133-274 g) were acquired from the animal house colony of the Scientific Research Center (Damascus, Syria) and used in the present study. The rats were kept in plastic cages, in a well-ventilated room, and maintained under controlled conditions (temperature: 25 ± 4 C, and a natural photoperiod of 12-hour light/dark cycle). They were allowed to have free access to drinking water and standard rat chow. All the experimental procedures were performed following the general guidelines for the care and use of laboratory animals issued by the Faculty of Pharmacy, Damascus University.

Experimental Design

After 7 days of acclimatization, the animals were distributed randomly into 4 groups (each containing 8 rats) and subjected to treatments, the dosage of diclofenac and Selenium were selected based on the previous articles [9, 28, 33, 34].

- Control group (Control): served as the negative control, it was given physiological saline 0.9% (1 ml/day) intraperitoneally (I.P.) for 8 successive days and an equal volume of diclofenac's vehicle intramuscularly (I.M.) from day 6 to 8.
- Toxic group (Dic): received saline (1 ml/day) I.P. for 8 successive days and started to receive 50 mg/kg/day of diclofenac I.M. from day 6 to 8.
- Se group (Se): served as the positive control, it was treated with sodium selenite only at a dose of 1 mg/kg/day I.P. for 8 successive days.
- Preventive group (Dic+Se): was injected with Se (1 mg/kg/day) I.P. for 8 successive days and diclofenac (50 mg/kg/day) I.M. from day 6 to 8, it should be noted that Se was first administered, followed by diclofenac within 1 hour of Se administration [35, 36].

During the study, one fatality was recorded in the group treated with diclofenac and Selenium (Dic + Se group). On the final day of the experiment, day 9^{th}, all rats were weighed and euthanized under diethyl ether anesthesia. Subsequently, the abdominal cavity of each rat was opened, and their kidneys were carefully dissected, weighed, washed with 0.9% saline, and placed on blotting paper to remove any blood. The left kidneys were then processed for histopathological examinations.

Determination of Kidney Weight/ Body Weight Ratio%

The kidney weight/ body weight ratio was calculated using the following formula [37]:

Kidney Weight/ Body Weight Ratio = {Rat Kidneys' Weight (g) / Body Weight (g)} × 100

Tissue Section Preparation and Histopathological Assessment

The renal slices were prepared as previously described by Bancroft and Layton [38], left kidneys were sectioned longitudinally into two halves, then preserved in 13% buffered formalin for 24 hours. The samples were dehydrated by dipping in serial ascending dilutions of Ethanol (70%, 80%, 100%), cleared in Xylene. After that, they were placed in Paraffin wax at 56° C for 24 hours. The obtained paraffin blocks were cut at a thickness of 5 µm using a microtome device (Leica, RM2155, Germany). The specimens were mounted on glass slides for the deparaffinization process, then stained with Hematoxylin and Eosin dye (H&E) for histopathological observation. Sections were viewed under a light microscope at different magnifications by a pathologist who was blinded to the

treatments, photomicrographs were taken by a camera attached to the microscope. At least 10 non-overlapping, indiscriminately chosen fields for each slide were analyzed.

The morphological alterations were determined using a semi-quantitative evaluation system as formerly published criteria [39, 40]. The degree of renal injury severity was graded on a scale of 0-4 (0: normal appearance (no damage), 1: mild <10% of sections, 2: moderate 10-25% of sections, 3: severe 25-50% of sections, 4: very severe >50% of sections) considering the presence of tubular dilatation, vacuoles in tubular cells, tubular degeneration, medullary congestion, interstitial edema, glomerular pathology including shrinkage of glomerular tuft with an increase of Bowman's (urinary) space and glomerular necrosis [27, 41]. The study also examined hemorrhage, inflammatory cell infiltration, coagulation, and congested vessels in the cortical interstitial tissue, where the presence of these lesions had a score of 1, and their absence had a score of 0 [42 43].

Statistical Analysis

The statistical analyses were carried out using GraphPad Prism version 8.0.1. For the parametric data, values are expressed as mean ± standard error of the mean (SEM). After checking the normality of distribution, the difference between groups was calculated by one-way analysis of variance (ANOVA) followed by LSD (Least Significant Difference) when comparing two different groups. For the non-parametric categorical data, the level of significance between groups was detected by the Kruskal-Wallis test followed by the Mann-Whitney U test. The binary categorical data were analyzed by Fisher's exact test. Abnormally distributed data were treated like non-parametric data. Statistical Significance is established when the p-value is less than 0.5.

RESULTS

In the current study, the NSAIDs-nephrotoxicity model was induced by injecting diclofenac. Administration of diclofenac alone (Dic group) markedly increased the kidney weight-to-body weight ratio compared with the control group (p=0.0004). Meanwhile, this percentage was not reduced in the animals pretreated with Se (Dic+Se group) (p>0.05). There was no statistically significant difference between groups control and Se (p>0.05). As shown in Table **1** and Figure **1**.

Table 1: Selenium Effect on Kidney Weight/ Body Weight Ratio in Diclofenac-Induced Nephrotoxicity. Data are Expressed as mean ± SEM

Groups	Kidney Weight/ Body Weight %
Control Group	0.5529 ± 0.0116
Dic Group	0.7275 ± 0.0479
Se Group	0.5571 ± 0.0133
Dic + Se Group	0.7296 ± 0.0368

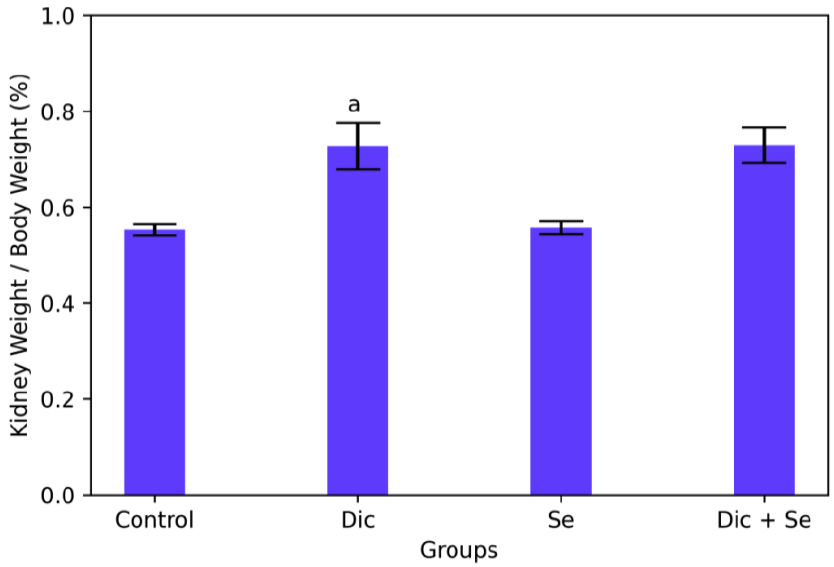

Figure 1: the Effect of Selenium on Kidney Wt/ Body Wt Ratio in Diclofenac-Induced Nephrotoxicity. Data are expressed as mean± SEM.

[a]significant value concerning the control rats (p<0.001). Dic: Diclofenac, Se: Selenium.

Histopathological Findings

The histological changes in kidney structures were recorded. In control and Se groups, the microscopic image showed a fairly symmetrical normal arrangement of kidney architecture, the cortical zone consisted of renal corpuscles and convoluted tubules, the renal corpuscle was composed of the glomerulus surrounded by the Bowman's capsule (Figures **2**, **3**), the renal tubules in the cortical and medullary portions appeared rounded or elliptical in cross-section with unobstructed lumens, the tubules were lined with a simple layer of cuboidal epithelium (Figures **2**, **3**). Some specimens revealed minimal histological changes in the tubules; and retraction of glomerular capillaries which were thought to be caused by the resection process. No presence of significant difference between the control and the Se groups ($p>0.05$).

The renal parenchyma in Diclofenac-only treated rats displayed structural abnormalities. Diclofenac induced profound acute tubular injury (especially proximal tubules) characterized by severe luminal ectasia ($p=0.0002$), moderate to severe vacuolization ($p=0.0003$), and mild to severe tubular degeneration, where the desquamated epithelial cells could be seen ($p=0.0006$) (Figures **2**, **3**). A moderate grade of glomerular injury was also observed, including

Table 2: Selenium Effects on Acute Inflammation, and Vascular Congestion in Cortical Interstitial Tissue. Values are Expressed as the Frequency of Injured Animals

Groups	Acute Inflammation		Significance	Vascular Congestion		Significance
	Injury	No Injury		Injury	No Injury	
Control Group	1	7		1	7	
Dic Group	6	2	*	7	1	*
Se Group	2	6		0	8	
Dic + Se Group	2	5		1	6	α

*$p<0.05$ compared to control group, $^{\alpha}$ $p<0.05$ compared to Dic group. Dic: Diclofenac, Se: Selenium.

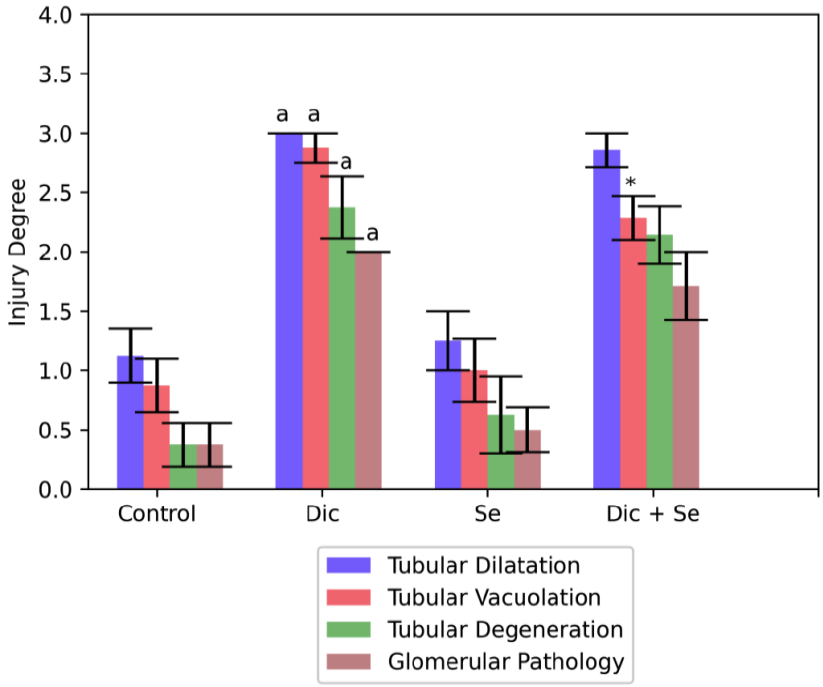

Figure 2: semiquantitative scoring of tubular dilatation, tubular vacuolation, tubular degeneration, and glomerular pathology. Data are expressed as mean ± SEM. ^{a} $p<0.001$ concerning the control group, * $p<0.05$ against Dic + Se group. Dic: Diclofenac, Se: Selenium.

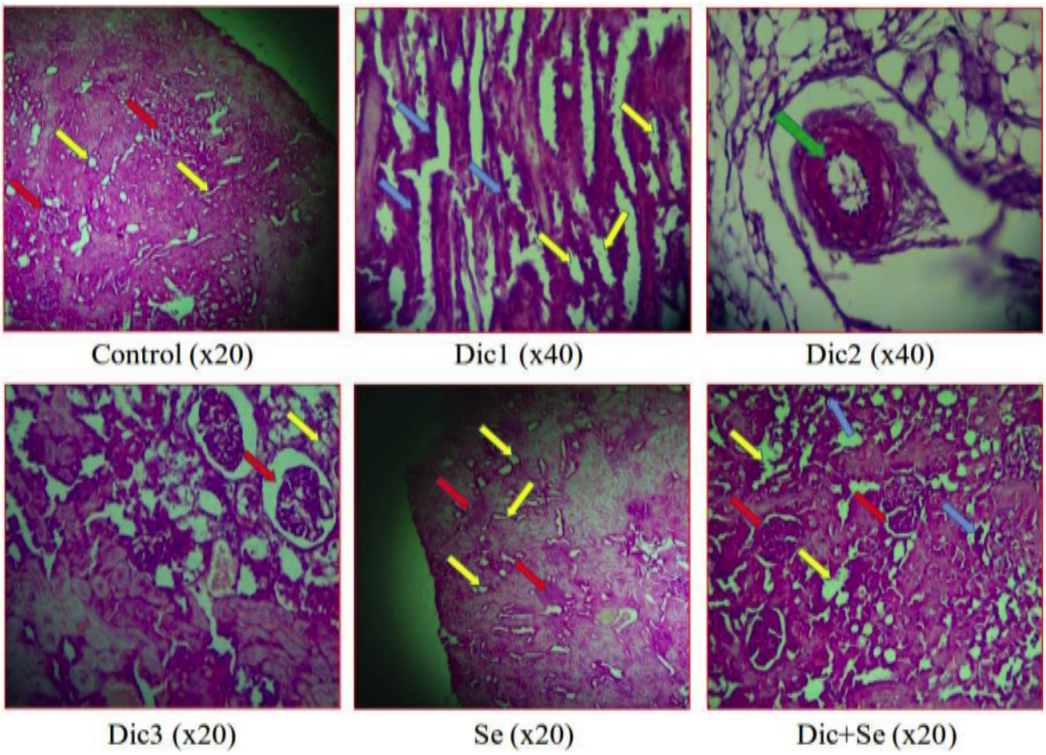

Figure 3: Photomicrographs of renal tissue sections stained with H&E. Control group showed normal histoarchitectural constituents, normal glomeruli (red arrows), and normal renal tubules (yellow arrows). Dic1, Dic2, and Dic3 represented the kidney section of a Dic-treated rat, Dic1: displayed degenerated tubules (yellow arrows) and infiltrating leukocytes (blue arrows), Dic2: indicated a congested blood vessel (green arrow), Dic3: showed a shrinkage of the glomeruli with increased Bowman's space (red arrow) and vacuolated tubules (yellow arrow). Se group displayed a normal appearance of glomeruli (red arrows) and tubules (yellow arrows). Dic + Se group exhibited slight enhancement in pathological parameters (red, yellow, and blue arrows) whereas no congested vessels were detected. Dic: Diclofenac, Se: Selenium.

widening of the Bowman's space and glomerular atrophy, while focal glomerular necrosis was detected in some glomeruli (p=0.0002) (Figures **2**, **3**). Infiltrating leukocytes were present in the interstitium of the majority of samples (p<0.05) (Figure **3**) (Table **2**) in addition to vascular congestion that was noted in cortical areas (p=0.01) (Figure **3**) (Table **2**).

The Dic + Se group exhibited a slight improvement in all histopathological parameters, but no significant differences were found according to that of the Dic group (p>0.05) (Figures **2**, **3**). Of note, the co-administration of Se markedly alleviated the toxic impact of Dic in respect of vacuolation (p<0.05) (Figures **2**, **3**) and cortical vessel congestion (p=0.01) (Figure **3**) (Table **2**). No signs of hemorrhagic areas, coagulative necrosis, medullary congestion, or interstitial edema in the tissue sections of all experimental groups.

DISCUSSION

NSAIDs have been linked to AKI, making them potential nephrotoxic agents [6]. Among NSAIDs, Diclofenac is widely used, and highly potent due to the diversity in its mechanisms of action [7]. As a result, there is a significant interest in understanding the pathways by which diclofenac induces nephrotoxicity [13].

This simple protocol has been implemented by earlier published studies [9, 34]. Animals in the Dic groups were injected with 50 mg/kg of Diclofenac Sodium I.M. for 3 consecutive days. Our research demonstrated that administration of diclofenac elicits kidney injury in rats, as evidenced by a significant increase in kidney weight-to-body weight ratio and remarkable histological damages.

The kidneys from the group treated with diclofenac were observed to be enlarged and swollen, with the kidney weight-to-body weight ratio increasing by 31.58% compared to the control group. This parameter is one of the indicators used in toxicological assessment [44]. As mentioned earlier, kidney swelling denotes organ inflammation, possibly caused by cytokines released due to diclofenac-induced AKI [18]. Our observations are consistent with previous studies

[14, 18 45]. In this context, Hickey *et al*. pointed out that Dic-exposed kidneys were pale and their fresh weights dramatically increased compared to controls [13]. Additionally, Owumi *et al*. showed an increased relative kidney weight following exposure to diclofenac sodium (10 mg/kg by gavage for 1 week) [46]. However, Anwar *et al*. verified a significant reduction in the kidneys' weight after a prolonged intake of diclofenac (10 mg/kg I.P. for 28 days) [17]. The differences in the findings may be due to differences in the doses, duration of treatment, and routes of administration used in these studies.

The present paper investigated the nephrotoxicity of diclofenac by examining the kidney tissue sections of rats under a light microscope. The results indicated that diclofenac application produced a pattern of acute tubular injury, particularly in proximal tubules. This injury was manifested by tubular distension, tubular vacuolation, and degeneration of the tubular epithelium. Glomerular lesions, congestion of renal vessels in the cortex, and signs of inflammation were also present. These findings are in agreement with previous publications which have also documented similar observations of kidney injury induced by diclofenac [9, 19, 47-49]. The study by Yasmeen *et al*. reported proximal tubule dilatation, degenerative changes in the lining epithelial cells of these tubules, where the nuclei of some cells appeared condensed, and congestion of blood vessels in the cortical area with few inflammatory foci [50]. In addition, Hashem *et al*. revealed that diclofenac administration at a dose of 50 mg/kg I.P. for 2 days exerted contraction of the glomeruli with the expansion of the urinary space, and some vacuolated and degenerated tubules were frequently seen [40]. While the findings of the current work and previously cited articles suggest that diclofenac can impinge on both the glomeruli and the tubules, other authors have proclaimed somewhat different observations. For example, Wood III *et al*. found that diclofenac did not affect the glomeruli but caused significant tubular necrosis and dilatation [51], whereas Mostafa *et al*. recorded that diclofenac did not impact the renal tubules but caused thickening of the glomerular membrane and massive areas of fibrosis [52]. It is important to note that these discrepancies in results depend on several factors such as study design, methodology, and interpretation of results.

Multiple mechanisms have been proposed to elucidate the histological abnormalities induced by diclofenac. Firstly, it has been postulated that the appearance of cellular vacuolations in the tubules is indicative of cellular stress and damage resulting from increased cell membrane permeability, which leads to water accumulation [53]. Consequently, renal intracellular osmolality rises, causing the tubules to dilate [54]. Tubular necrosis is caused by two primary factors: a direct toxic effect of a metabolite(s) or renal ischemia [55]. As the proximal tubules have more mitochondria to carry out their intensive metabolic functions and play a crucial role in solute and fluid reabsorption, they are more susceptible to any reduction in oxygen supply and mitochondrial dysfunction than other kidney structures [56].

Dic-associated tubular necrosis is thought to be mainly caused by blocking the synthesis of prostaglandins in the afferent renal arterioles, which results in reduced renal blood flow (RBF), and ultimately leads to renal ischemia [50]. It is worth noting that diclofenac is primarily eliminated through renal excretion [57]. Upon metabolism, diclofenac's main metabolites are: diclofenac acyl glucuronides (DAG), 4-hydroxydiclofenac, 5-hydroxydiclofenac, 4-hydroxydiclofenac p-benzoquinoneimine, and 5-hydroxydiclofenac p-benzoquinoneimine [12]. Benzoquinones and acyl glucuronides are highly reactive and have the potential to bind to macromolecules such as DNA, lipids, and proteins, altering their functions and evoking oxidative stress [12, 58]. Mitochondria are considered diclofenac's prime target, and exposure to diclofenac has been shown to increase oxidative stress and DNA damage [13]. In particular, diclofenac induces calcium influx into the mitochondria, which increases their membrane permeability and causes mitochondrial dysfunction, releasing a proapoptotic protein called cytochrome C and activating the caspase cascade. This was supported by Hashem *et al*. who illustrated that diclofenac reduced mitochondrial viability and increased DNA fragmentation 40. Moreover, according to Huo *et al*., the interaction between DAG and the organic anion transporters (OATs) expressed on the basolateral membrane of renal proximal tubule cells may also contribute to diclofenac-induced nephrotoxicity [59]. DAG is a substrate of OAT 1/3 and can be taken up by these transporters, leading to its accumulation within the cells. When OATs become saturated with DAG, the proximal tubule cells may become more vulnerable to direct cytotoxicity from diclofenac [58].

Surveys have emphasized that diclofenac stimulates the movement of monocytes and macrophages, triggering pro-inflammatory cytokines production such

as TNF-α and IL-1β [60, 61]. High levels of these cytokines may rely on diclofenac-induced NF-κB activation [54]. Furthermore, it has been proven by Nouri *et al*. that exposure to diclofenac significantly activates the TNF-α expression in renal tissue [16], and it may cause mononuclear cell infiltration, promoting a long-lasting inflammatory process [62]. Taken together, these mechanisms may corroborate the deleterious impacts of diclofenac documented by our publication.

Selenium is a trace element that is essential for human health and is required in small amounts in the diet. It plays a role in various physiological processes, including the immune system, thyroid function, and antioxidant defense [63]. It is promising for mitigating numerous models of drug-induced nephrotoxicity [27, 29, 39]. In this investigation, we hypothesized that pretreatment with Se might have a beneficial effect against diclofenac-induced kidney injury. The dose of Se was based on other formerly accepted articles [28, 33]. Rats were supplemented with 1 mg/kg of Sodium Selenite I.P. for 8 consecutive days. Our study showed that there were no significant differences between the Se-alone group (positive control) and the control group regarding the kidney parameters that were analyzed, this suggests that the selected dose of Se is safe and did not adversely affect the rats' kidneys.

Surprisingly, the results obtained from the current research confirmed that Se only offered weak protection against diclofenac-induced nephrotoxicity in rats. While Se administration led to a noticeable recovery of vascular congestion in the cortical interstitium by 85.71%, and tubular vacuolization by 30.43%, there was only a slight amelioration in widened lumens, degenerated epithelium, distorted glomeruli, and infiltrating leukocytes by 16.6%, 21.05%, 25%, and 16.66%, respectively, compared to a diclofenac-treated group, and these changes were not statistically significant. Our findings are in accordance with former reports that also implied a partial ameliorative effect of Se on renal histopathology. For instance, Fransescato *et al*. disclosed that Se (2 mg/kg by gavage for 7 subsequent days) conferred partial protection, detected by decreased necrosis and casts formation in the Se + cisplatin group [64], and Fujieda *et al*. stated that Se (2 mg/kg by gavage 24h and 1h before cisplatin injection) diminished the tubular damage but did not provide total protection against cisplatin-induced nephrotoxicity [35], both studies attributed the lack of complete protection to the route of Se administration, the dose timing, or the dose amount. Similarly, corresponding to Ge *et al*., selenium nanoparticles exhibited the most notable protective effect on cadmium-induced nephrocyte lesions in chicken kidneys compared to other Se sources, but all Se sources displayed partial alleviation [65]. By the same token, Wan *et al*. certified that Sodium Selenite of a certain dose (0.63 mg/kg via drinking water for 42 days) possessed potential protective effects against a chicken chromium-induced nephrotoxicity model, due to the narrowed range between its essentiality and toxicity [44]. By contrast, our results disagreed with Owumi *et al*. who found that Se supplementation (0.125 mg and 0.25 mg) relieved the histoarchitectural defects in Se and diclofenac co-treated groups compared to diclofenac-only treated group [46]. Additionally, our observations diverged from those of Hasanvand *et al*. who asserted that Se injection (0.2 mg/kg I.P. for 2 weeks) considerably lessened eosinophilic casts, necrosis, and enlarged lumens of renal tubules in rats subjected to an ischemic injury [39]. The kidney weight-to-body weight ratio coincided with our histological findings, Se could not prevent kidney enlargement caused by diclofenac. Contrarily, AlBasher, *et al*. and Al-Brakati *et al*. signified that Se minimized the increased kidney weight and relative kidney weight in glycerol-injected rats [66, 67].

In general, antioxidants are known to attenuate nephrotoxicity by reducing inflammation and ROS generation. GPx is an oxidoreductase that is dependent on selenium and plays a key role in maintaining cellular redox balance [68] by scavenging H_2O_2 and organic hydroperoxides (ROOH) with the assistance of reduced glutathione (GSH) [24, 68]. The GPxs family comprises eight isoenzymes [68, 69], of which GPx-1 to GPx-4 contain selenocysteine as an integral component in their structure, while GPx-6 contains selenium in its catalytic site. GPx-5, GPx-7, and GPx-8 have normal cysteine instead of selenocysteine [68]. GPx-1, GPx-3, and GPx-4 were detected in the renal tissue [69]. GPx-1 is highly expressed in the kidneys and it is the first enzyme to be influenced by Se deficiency [70]. It has been assumed that GPx-1 has a renoprotective role against oxidative stress [69]. As previously observed, Se enhances GPx activity, GPx mRNA expression [33, 35, 65], and the activity of other antioxidant enzymes in the kidney, such as superoxide dismutase (SOD) and catalase (CAT) [65-67]. Owing to its effects on the antioxidant defense system and incorporation into GPx, Se can protect nephrocytes and ensure a condition of ROS homeostasis.

Sodium Selenite (Na_2SeO_3) is an antioxidant that requires bioconversion (reduction) in the organism before it can perform its function [30]. The reduction of selenite to selenide (from Se^{+4} to Se^{-2}) involves several steps. Firstly, selenite reacts with GSH to form selenodiglutathione (GS-Se-SG), then another molecule of GSH is also needed to reduce this compound to glutathioneselenol (GS-SeH) [68, 71], and lastly glutathione reductase (GR), with the consumption of nicotinamide adenine dinucleotide phosphate (NADPH), catalyzes the reduction of GS-SeH to hydrogen selenide (H_2Se), which is the bioactive form of selenium. However, increased production of ROS can lead to depletion of GSH in the cells, which may interfere with this bioconversion process and disrupt the antioxidant activity of selenite [71]. We suspect that selenite was not completely bioconverted in our experiment, leading to a negative impact on its bioavailability and efficacy.

If circulating GPx-1 or renal CAT can detoxify accumulating peroxides, kidney tissues may be protected against extended ROS formation. In a study by Shanu *et al*., it was found that Se increases GPx-1 expression in the renal tissue after rhabdomyolysis injury and reduces inflammation by inhibiting TNF-α expression and NF-κB activation; however, increased GPx-1 gene expression is not concomitantly associated with increased protein activity [72]. Another study assessing the impact of Se deficiency and Se supplementation following burn injury declared that GPx activity in red blood cells showed slow recovery after Se administration, while GPx activity in kidneys improved immediately [73]. We speculate that this finding may also explain why Se was not able to fully counteract kidney damage in our study.

Another key point is that Se is primarily distributed in the renal cortex [24] and plays a vital role in maintaining vascular tone in low-resistance arteries, such as renal arterioles [72]. It has been supposed that Se can help safeguard the balance between the formation of ROS and nitric oxide (NO), the most potent vasodilator, via the action of GPxs and thioredoxine reductases (TrxRs). It also decreases COX-2 activity, which influences the vascular tone [23]. This may clarify the conspicuous improvement in vascular congestion in the cortical interstitial tissue reported in this research.

CONCLUSION

This study sheds light on the adverse effects of diclofenac on kidney histology. The findings indicate that co-administration of 1 mg/kg of Sodium Selenite partially mitigates these toxic effects. These results have important implications for future research and could inform the development of novel treatments for kidney damage caused by diclofenac.

While our results are consistent with previous studies, further research is required to fully understand the underlying mechanisms of the protection provided by Selenium. To this end, it is recommended to explore the effects of varying doses and forms of Se, including organic compounds and nanoparticles, as well as varying administration durations. Additionally, the synergistic effects of Se in combination with other antioxidants should be also studied.

CONFLICTS OF INTEREST

The authors declared that there are no conflicts of interest related to this study.

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