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Evaluation of the changes induced by tramadol and the possible protective effect of vitamin C on the kidney

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Abstract. – OBJECTIVE: Addiction is a widespread public health problem despite all efforts to prevent and treat it. Over the past few years, tramadol abuse has been sharply increasing in Middle Eastern countries. This research aims to identify the tramadol-induced histological changes in rat kidneys and any potential protective effects of vitamin C on these changes.

MATERIALS AND METHODS: This is an experimental study conducted at Prince Sattam bin Abdul Aziz University. Thirty-three adult albino rats were randomly divided into three groups. Control, Tramadol, and vitamin C groups. The Tramadol group received 25 mg/ Kg a day of tramadol orally via gastric gavage for three weeks. In the vitamin C + tramadol treated group, 100 mg/Kg/b.wt of vitamin C was administered intravenously to the animals 30 minutes before receiving the same dose of tramadol

RESULTS: Specimens from the kidney of every rat were excised for histological examination by the light and electron microscope. Tramadol damage to the kidney's glomeruli and proximal and distal convoluted tubule hypertrophy were among its long-term harmful consequences. When vitamin C was added to tramadol, the distal and proximal convoluted tubules, and the renal glomeruli, improved.

CONCLUSIONS: When vitamin C was given to the tramadol group, the drug's harmful effects on the kidney were reduced.

Key Words:
Tramadol, Vitamin C, Kidney.

Introduction

Opioids are a class of drugs mostly used to treat moderate to severe pain[1]. The naturally occurring opiates are derived from opium, the sticky fluid found inside the immature capsule of the opium poppy's blooming head. In Southeast Asian populations worldwide, opium is still mostly consumed by smoking. Traditional oral pharmaceutical opium formulations include paregoric and tincture of opium[2]. Opioids are a wide range of chemicals that can bind to one or more opioid receptors (mu, k, and d), which are mostly found in the central nervous system and gastrointestinal tract, with morphine-like pharmacological activity either agonistic or antagonistic[3].

Tramadol was first created in 1962 and has been marketed as a pain reliever since 1977[4,5]. It is a racemic chemical, and its enantiomers and metabolites have physiological effects that affect the way the compound behaves[6]. Tramadol is a centrally-acting opioid analgesic that shares structural similarities with morphine and codeine. It is frequently and successfully used to treat both acute and ongoing pain[7].

The liver metabolizes tramadol extensively by demethylation, oxidation, and conjugation (glucuronidation and sulphation). There are now 23 identified metabolites[8]. Metabolites of O- and N-desmethyl, including di- and tri-desmethyl compounds, are produced[9].

When using tramadol for longer than a few weeks or months, especially in the event of high doses, drug addiction and dependence may arise[10,11]. Tramadol's usage as a suitable substitute in patients who exhibit drug-seeking behavior, as well as its long-term use for the treatment of pain, are debatable[9]. There was a greater chance of hospitalization and mortality when tramadol was used in comparison to not utilizing it[12].

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Similar to other opioids, tramadol can cause physical opioid dependence with continued use, and its termination can result in withdrawal syndrome[6].

Numerous investigations[13,14] have demonstrated a connection between prolonged opioid use and elevated reactive oxygen species generation (ROS)[13]. Superoxide (O_2) radical is scavenged by vitamin C's water-soluble antioxidant properties, which also serve to defend against oxidative stress. Vitamin C is known to increase bacterial defense, improve microcirculatory blood flow, preserve endothelial barriers, and restore vascular reactivity to vasoconstrictors[14]. The objective of the study is to determine the histological changes of rat kidneys induced by tramadol and the possible protective effect of vitamin C on these changes.

Materials and Methods

Our work was conducted in accordance with the PSA University's Al-Kharj Ethical Committee's guidelines for the use and care of animals in research (IF2/PSAU/2022/03/22284). A Tramadol tablet containing 225 milligrams of tramadol hydrochloride was used (HCL). The tablet was purchased from Mina Pharmacy in Cairo, Egypt. Each ml of water contains 10 mg of tramadol after each tablet has been dissolved in 22.5 ml of distilled water (225 mg/22.5 ml). By gastric gavage, the comparable dose for rats was 0.18 mg every day. Memphis Co. (Amerya, Cairo, Egypt) sold a 500 mg tablet of vitamin C, also known as L-ascorbic acid, for use in Cairo, Egypt's pharmaceutical and chemical industries. After each tablet was dissolved in 50 ml of distilled water (500 mg/50 ml), each milliliter of water contained 10 mg of vitamin C. By gastric gavage, the comparable dose for rats was 2.7 mg daily. We used 33 adult, albino rats of both sexes, aged 8 to 10 weeks, weighing 200 to 250 grams. The animals were from PSA University's experimental facility. They were kept in air-conditioned, climate-controlled spaces with roomy wire mesh cages, a regular day/night cycle, free access to clean water, and commercial rat food. The Institutional Animal Ethics Committee's recommendations were followed in all studies.

The animals were separated into three groups, each of which included eleven rats. Each rat in Group I (the control group) did not receive any medication for a week. In Group II (tramadol group) each rat received for three weeks 25 mg/Kg a day of tramadol orally via gastric gavage[15]. In Group III (vitamin C + tramadol treated group) each rat had 100 mg/Kg/b.wt of vitamin C for 3 weeks administered intravenously 30 minutes before receiving the same dose of tramadol as in the second group, for a period of four weeks. Each tramadol group rat was not given any medication for two weeks. By making a median abdominal incision on the specified dates, the kidneys were removed from the rats, preserved in 10% buffered formalin, and then processed for paraffin slices. Then, ether inhalation anesthesia was administered to the rats. To get ready for paraffin sectioning, all specimens were kept in 10% formol saline solution for three days. The kidney was removed right away and left to cure for three days in 10% formal saline solution. The specimens were completely dehydrated before being treated with ethyl alcohol at ascending concentrations for a total of three hours after a day in which they were treated with 70%, 90%, and finally, absolute alcohol. The samples were benzene-cleared for 24 hours. The cleaned samples were immersed three times for an hour each in paraffin wax. After that, the samples were firmly embedded in paraffin wax. A rotatory microtome was used to slice the paraffin blocks into serial transverse sections, each at a thickness of 7u. On an albumenized glass slide, five paraffin portions were inserted in succession.

Hematoxylin and eosin (H&E) stain were alternately applied to the successive slides made from each specimen. Using Masson's trichrome dye, collagen fibers were made visible. For the goal of detecting neutral mucopolysaccharides, use Periodic Acid Schiff. The Allied Health Science College at PSA University handled the preparation of the specimens for the electron microscope and the examination. It was carried out with a 60 kv Zeiss EM transmission electron microscope. The specimen holder received the grids. The field was first located using low magnification (×1,000), and then the cells were examined, and the result was assessed using higher magnification. We took pictures, had them developed, printed, and then we looked at them.

Statistical Analysis

Using SPSS software Version 16 (SPSS Inc., Chicago, IL, USA), statistical analysis of measurements of the mean diameters of the glomeruli, proximal and distal convoluted tubules was performed. The mean and SD (standard deviation) were used to represent the variables. Finally, the significance was evaluated in accordance with the p-value ($p<0.05$ was considered significant).

Results

Morphological Changes in the Control Group

Examination of renal H&E-stained cortical slices by light microscope revealed the renal corpuscles, distal and proximal convoluted tubules in the cortex. A glomerular capillary tuft and Bowman's capsule encircled each renal corpuscle. The capsule was made up of a visceral layer covering the glomerulus capillaries and a parietal layer of simple squamous epithelium. There was the Bowman's (urinary) gap between the two decks of the capsule. The simple cuboidal epithelium lining the proximal convoluted tubules gave them their narrow lumen. Large, spherical nuclei and acidophilic cytoplasm were features of the cells. Compared to the proximal tubules, the distal tubules had broader lumen and less acidophilic cytoplasm. Examination of the renal cortical sections of the control group revealed a strong apical and basal membrane of the proximal and distal tubule cells, as well as the brush boundary of the proximal tubules, demonstrating a positive Periodic Acid Schiff (PAS) reaction. Compared to proximal tubules, distal tubule cells showed a broader lumen and lighter staining. A significant positive PAS reaction was visible in the basal membrane of the parietal layer of the Bowman capsules and glomerular tufts (Figure 1A). Masson Collagen fibers were distributed normally in the capsule wall, peritubular, and around the blood vessels within the renal cortex in trichrome-stained slices from the kidneys of control rats (Figure 1B).

The glomerular capillaries in the renal cortex of the control group were revealed by electron microscopy to be lined with fenestrated endothelial cells and protected by the cell bodies of podocytes. The major processes of the podocytes gave rise to numerous secondary processes, also known as pedicles, which terminate on the capillary basement membrane. The pedicles of podocytes were revealed to have filtration slits. The proximal convoluted tubules (PCTs) and distal convoluted tubules (DCTs) were lined with cubical cells that had rounded euchromatic nuclei. Many longitudinally oriented mitochondria were observed at the basal region of the cells between the basal cell membrane infoldings. Moreover, some lysosomes were discovered in the tubules' cytoplasm. Whereas few or no microvilli were visible in DCTs, numerous lengthy microvilli were found extending from the luminal surface of PCTs (Figure 1C-D).

Cortical renal slices from the tramadol group stained with H&E. from tramadol-treated rats showed extensive changes in renal glomeruli and tubules compared to the control group. Examples of renal glomerular changes include large Bowman's gap and atrophied glomerulus with collapsed tuft. While some glomeruli displayed lobulation and disintegration, other glomeruli were reduced. Cellular infiltration, a reduction in the number of podocytes, the absence of capillaries, and other signs of damage were present. Mesangial cells appeared normal (Figure 2A). The renal tubular changes include degeneration of tubules with obstructed lumen. The proximal convoluted tubules showed vacuolation of cytoplasm, loss of nuclei, and destruction of their brush border. The distal convoluted tubules were also dilated. When compared to the control group, periodic acid Schiff (PAS) analysis of the renal cortical sections from the tramadol-treated group showed a considerable depletion in the PAS-reactivity in the kidney tissue components.

The glomerulus and its surroundings were shown to have a noticeable increase in collagen fibers in Masson Trichrome stained kidney sections from tramadol users. After receiving tramadol medication, the amount of collagen fibers deposited around the proximal and distal convoluted tubules was slightly enhanced (Figure 2B-D).

Electron microscopic examination of the renal cortex from rats treated with tramadol showed prominent Renal glomerular and Tubular changes. The thickening glomerular basement membrane (GBM) and the destruction of capillary lumens by endocapillary hypercellularity and hypertrophy are examples of renal glomerular alterations (Figure 2C).

The vitamin C group was subjected to treatments with tramadol and vitamin C light microscopical stained with H&E. showed that most of the glomeruli restored their normal pictures. Several glomeruli had dilated urine spaces and were lobulated.

Tramadol and vitamin C-stained slices of the kidneys were examined under light microscopy. The showed comparison between the renal corpuscle and renal tubules' fine and distributed collagen fibers to the control group is challenging, since vitamin C inhibits the growth of fibrous tissue that surrounds glomeruli and renal tubules (Figure 3D). The basement membrane, renal tissue, and brush border of the renal tubules are all visible in the PAS stain as being PAS-positive (Figure 3A-B).

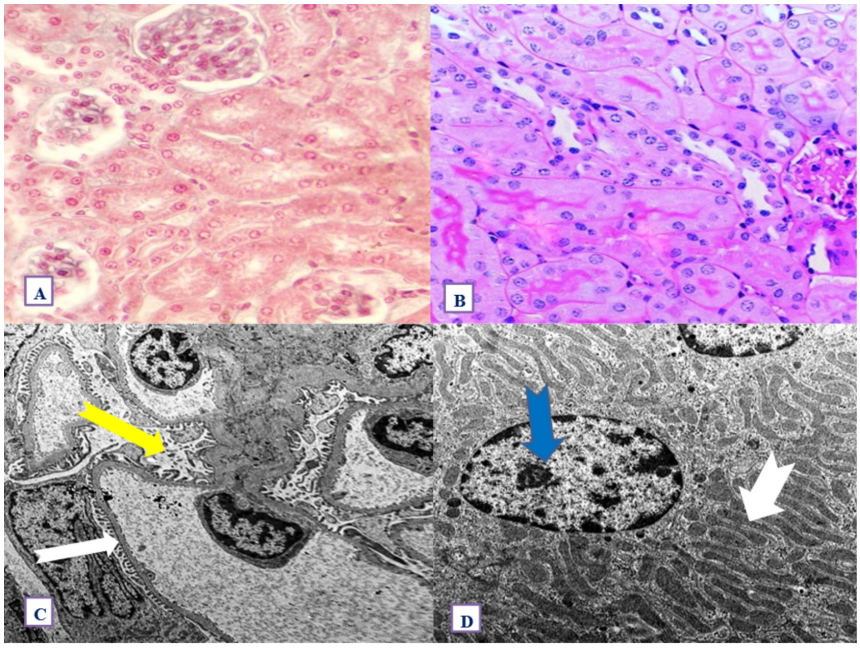

Figure 1. Different sections in the kidney from the control group. **A**, Masson T. stain showing a minimal amount of collagen around renal tubules (×400). **B**, PAS stain section in the kidney of adult albino rat (vitamin C group) showing PAS-positive materials magenta red color in the renal tissue, bowman's membrane brush border and basement membrane of the renal tubules and glomeruli (×400). **C**, E/M showing the glomerular filtration barrier, including glomerular basement membrane (white arrow), and fenestrated capillary endothelium (yellow arrow) (T.E.M. Mag. ×5,000). **D**, E/M showing normal nuclei of renal tubules with normal chromatin content (blue arrow) and normal distribution of mitochondria (white arrow) (×8,000).

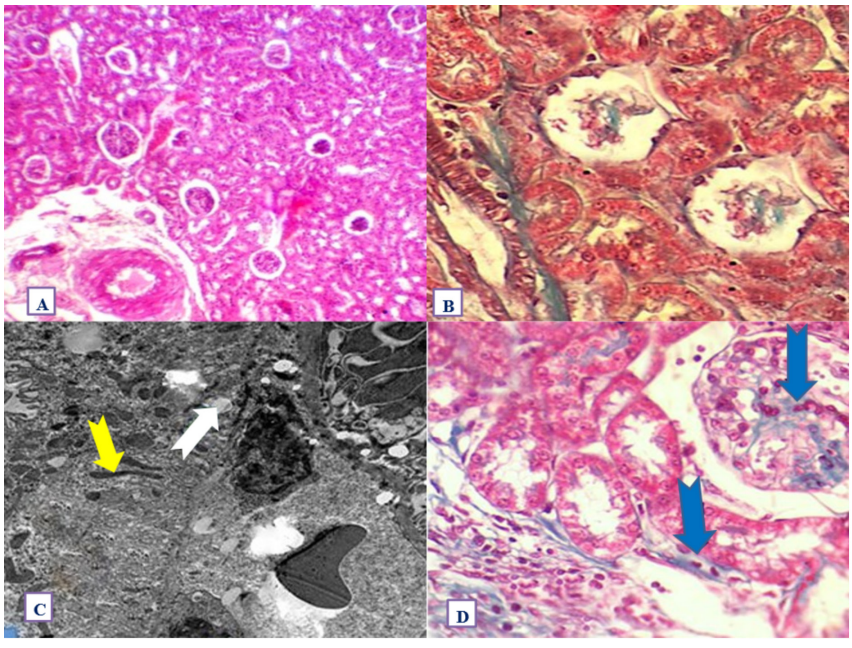

Figure 2. Different sections in kidney from tramadol group. **A**, H&E showing interstitial hemorrhage some glomeruli are atrophied (×100). **B**, Masson T stain showing a marked increase of the collagen fibers in interstitium and around Bowman's capsules and blood vessel and proximal convoluted tubule (×400). **C**, E/M showing the destruction of microvilli, cytoplasmic vacuolization (white arrow), irregular distribution, and destruction of mitochondria (yellow arrow) (×10,000). **D**, Masson T stain showing increased collagen fibers in the glomeruli, interstitium, and around and within the renal tubules (blue arrows) (×400).

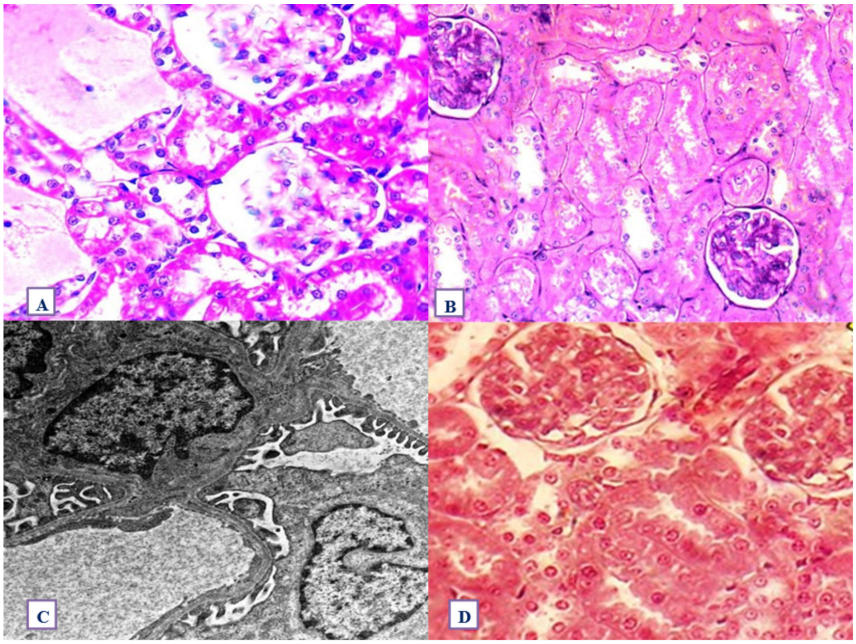

Figure 3. Different sections in the kidney from the vitamin C group. **A**, PAS stain showing PAS-positive materials in the renal tissue, basement membrane and brush border of the renal tubules (×400). **B**, PAS stain showing less strong positive PAS reaction seen in the apical and basal mem¬branes of the proximal and distal tubule cells as well as in the glomerular tufts comparable to the control (×200). **C**, E/M showing the podocyte cell with its nucleus and its primary and secondary processes (×10,000). **D**, Masson T stain showing minimal amount of collagen around afferent arteriole, normal capillary tuft of glomerulus (×400).

Electron microscopic examination of the renal cortex from rats treated with vitamin C demonstrated a well-maintained ultrastructure. The glomerular basement membrane, the lining endothelium of capillaries, and the podocytes' pedicle appeared intact to some extent.

The PCTs showed intact mitochondria, microvilli, and nuclei (Figure 3C). The DCTs also had a conserved ultrastructure. The renal tissue also showed healthy glomeruli still surrounded by some mononuclear inflammatory cells. There were no casts and the PCT tubules had normal nuclei and a noticeable brush boundary at the apical surface. Fewer localized structural alterations in the renal tissue than in the tramadol group. The interstitial tissue, as well as renal parenchyma, appeared with approximately normal histological structure.

The diameter of the glomerulus, proximal, and distal convoluted tubules in different groups was explained in Figure 4 and Table I.

Discussion

The findings about tramadol's effects on the kidney in the current study were in agreement with earlier findings[3], which demonstrated that tramadol-treated kidneys underwent extensive changes in the form of atrophied glomeruli, a large urinary gap, and degraded tubules.

Another renal investigation revealed that tramadol-treated rats had atrophy of the glomerulus with collapsed tuft, broad Bowman's gap, damaged tubules, cellular infiltration, and bleeding[16]. Prolonged tramadol toxicity resulted in degraded tubules, broad Bowman's gap, atrophied glomeruli with collapsed tufts, and cellular infiltration in the kidney[17]. This was explained by the toxicokinetic mechanism of tramadol, in which 30% of the drug is removed through the kidneys intact and the remaining 70% is changed by the liver into active metabolites. These metabolites that the kidney excretes harm cells and induce renal dysfunction[18].

In a previous study[19] tramadol's nephrotoxic effects were shown to include glomerular shrinkage with collapsed tufts, enlargement of Bowman's gap, tubular degeneration, cellular infiltration, and hemorrhage. Ultrastructural studies revealed a thickening of the basement membrane of the renal corpuscles. Another study[20] examined

Table I. Glomerulus, proximal, and distal convoluted tubules mean diameters in control and experimental groups are displayed (in pixels).

Diameter (In Pixels) Parameters	Control group	Tramadol group	Vitamin C group
Glomerulus (G)	782.12	411.33	499.77
Proximal convoluted tubules (PCT)	367.12	642.45	299.22
Distal convoluted tubules (DCT)	301.24	811.21	322.44

the harmful effects of cefotaxime on the liver and kidneys in rats, as well as any potential protective effects of vitamin C. It was evident that vitamin C was the most potent free-radical scavenger and antioxidant for preventing lipid peroxidation.

Also, after receiving tramadol for 30 days, histological signs of renal injury included glomeruli with collapsed tufts and broad Bowman's spaces, atrophic tubules, sloughing of tubular cells, cellular infiltration, and bleeding. These findings corroborated earlier studies[21] that found lipid peroxidation and the production of reactive oxygen species to be the main causes of tramadol-induced nephrotoxicity.

Reactive oxygen species are produced when tramadol is used, according to certain findings[22]. Oxidative stress is a state that is brought on by a specific kind of free radical termed ROS. Under these circumstances, the body's natural equilibrium between the generation of free radicals and antioxidants is upset, resulting in too many free radicals. It has been discovered that tramadol-induced oxidative stress contributes to the development of mitochondrial dysfunction. By damage to mitochondrial complex II and mitochondrial membrane, including damaged membrane pores, membrane collapse, and swollen mitochondria, excessive ROS generation brought on by tramadol treatment ultimately causes mitochondrial malfunction and injury[23]. So, it can be beneficial to employ techniques to lessen the damaging effects of medications like tramadol on the body's organs.

Conclusions

The toxic effects of tramadol on the kidney included glomerular damage and proximal and

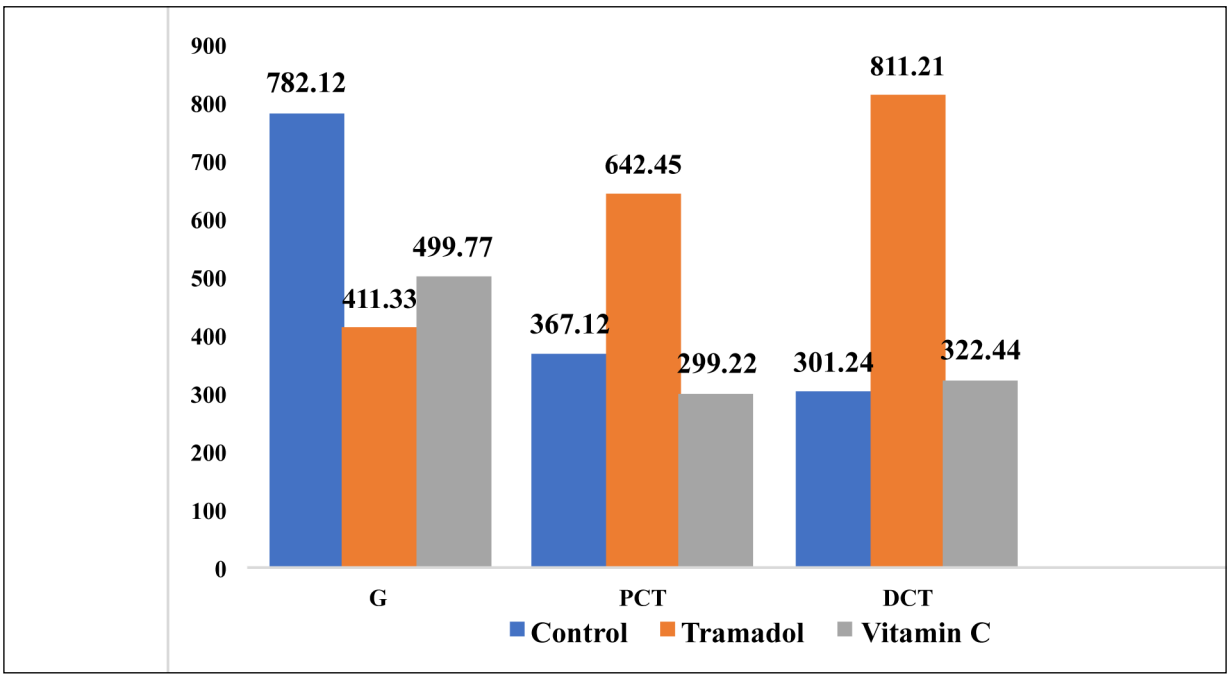

Figure 4. Glomerulus (G), proximal (PCT), and distal convoluted tubules (DCT) mean diameters in control and experimental groups are displayed (in pixels).

distal convoluted tubule enlargement. The proximal and distal convoluted tubules, as well as the renal glomeruli, were improved by tramadol when vitamin C was added to the drug.

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Authors' Contributions
All authors contributed to the research and/or preparation of the manuscript. Ali Hassan A Ali, participated in the study design and wrote the first draft of the manuscript. Ali Hassan A. Ali and Salman Bin Dayel collected and processed the samples. Mansour M. Alajmi participated in the study design and performed the statistical analyses. All of the authors read and approved the final manuscript.

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Conflict of Interests
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

Availability of Data and Materials
The data are available upon request from the authors.

Ethics Approval
All steps implemented in this study that included animal models were in compliance with the Ethics Committee of Prince Sattam bin Abdulaziz University Institutional Review Board (IF2/PSAU/2022/03/22284).

Informed Consent
Not applicable.

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