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ORIGINAL ARTICLE

Ameliorative Role of Cinnamon Oil in Opposing Cyclophosphamide-induced Nephrotoxicity in the Adult Male Albino Rats "Histological and Histomorphometric Study"

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Abstract

Background: **Cyclophosphamide used commonly in chemotherapy in various types of cancers and also autoimmune diseases. But it had altered histological structures caused by oxidative stress. Cinnamon Oil has an antioxidant plus anti-inflammatory activity.**

Objective: **To determine the histological differentiation in the kidney of adult male albino rats exposed to cyclophosphamide plus role of co-treatment by cinnamon oil.**

Methods: **The study was done at Pharmacology Department Laboratory, Faculty of Medicine, Al-Azhar University between June and October 2022. Twenty-four male adult albino rats divided into 4 groups were involved in our study, each group is formed of six rats. Group I (control group) take normal diet, Group II treated with Cyclophosphamide only, Group III treated with Cinnamon Oil 10 days just after- Cyclophosphamide, Group IV treated with Cinnamon Oil 5 days previous and 5 days later Cyclophosphamide. The kidneys dissected then removed for illustration under light microscope and statistical study.**

Results: **Illustration under light microscope of the renal cortex belong group II showed shrunken glomeruli with widening of Bowman's space and inflammatory interstitial infiltration. There were dilatations in both proximal and distal convoluted tubules. The amount of collagen in glomeruli increased in the interstitial tissue stained by Masson's sections. Examination of group III and IV kidneys showed improvement in histological changes.**

Conclusion: **Intake of Cinnamon Oil caused recovery in structure. May reach parameters of the group I, also this improvement much better in group IV than group III.**

Keywords: Cinnamon oil, Cyclophosphamide, Kidney

1. Introduction

Cyclophosphamide (CP), is considered the ideal treatment of malignancies of various organs and autoimmune difficulties through enhancing number of dendritic cells or through transporting stem cells from bone marrow to lymphatic tissue so exaggerate immune response.[1–3]

Continuous administration of CP is restricted -even in case of its efficacy-due to the toxicity of so many organs developing from the formation of free radicals and oxidative stress.[4]

The Toxicity resulting from CP explained as it is inactive but its metabolites; phosphoramide, acrolein, mustardand, resulting from hepatic enzymes P450 system, bound with alkylation nucleophilic regions in DNA. The becoming cross-linking nucleobases stopped DNA replication also the synthesis of protein.[5]

The common cause of nephrotoxicity developed by CP due to high vascular perfusion also variation of concentration of solutes' in the urine. Glomerular plus tubular alteration, pyelonephritis, necrosis of papillae and hemorrhagic cystitis observed in rats

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induced by CP.[4] Secondary metastasis in the bladder and also the kidney was side effect in cases suffering from non-Hodgkin's lymphoma treated by CP.[6]

The free radicals and oxidative stress presence in the cells leading to a misbalance between pro-oxidants and antioxidants.[7]

Cinnamon oil known by its anti-inflammatory and antioxidant performances.[8] The mechanism of that due to the role of cinnamon oil in increasing the reuptake of hepatic Low-Density Lipoprotein (LDL) and decreasing the enzyme 3-Hydroxy 3-Methyl Glutaryl co-enzyme A (HMG-CoA) reductase.[9]

Cinnamon oil had also very important therapeutic outcome in pleiotropic effects-having multiple effects from a single gene for being strong antioxidant and used for the prevention of inflammatory responses.[10]

So, this work designed to evaluate the possible role of cinnamon oil in the prevention and treatment of the possible renal histological alterations induced by CP.

2. Material and methods

2.1. Animals

Twenty-four adult male albino rats weighing 200–250 gm were used. All rats housed in cages under normal standard environmental and laboratory environment and also free access to water and food.

2.2. Experimental design

Four groups (6 rats each); Group I (control group): no medications and received standard diet only.

Group II, receiving CP: intraperitoneally single dose of CP in the dose of 200 mg/kg body weight then left for 10 days.

Group III: intraperitoneal injection of CP in a single dose of 200 mg/kg body weight then received Cinnamon oil orally by gastric tube in a dose of 10 mg/kg body weight for 10 days.

Group IV: received Cinnamon oil orally by gastric tube in the daily dose of 10 mg/kg body weight for five days, then received intraperitoneal injection of CP in a single dose of 200 mg/kg body weight on day 6, then given Cinnamon oil orally by gastric tube in the dose of 10 mg/kg body weight for 5 days also.

At the end of the experiment, the rats were sacrificed by decapitation and the kidneys were excised.

2.3. Chemicals

CP was obtained from Misr Pharmacies (Cairo, Egypt) as powder in the form of 1 gm vials.

Cinnamon oil was commercially available 'natural Cinnamon oil', from El Captain Company (CAP PHARM).

At exact time, rats anesthetized by ether inhalation. Dissection of kidneys gently was done. And the specimens prepared to following:

Light microscopic studies: the kidneys were fixed in 10% formal saline then dehydrated in ethanol then embedded in paraffin. Sections were cut at 5 μm thick from Paraffin mounted on slides and stained with.

Haematoxylin and eosin (H&E) stain for general histological structure.[11]

Masson's trichrome stain for identification of collagen fibers.[11]

Histomorphometric studies[12]

The data prepared by Leica Qwin 500 image analyzer coloured monitor computer, hard disc of personal computer IBM connected to microscope under control of Leica Qwin 500 software.

Using measuring field, the mean percent of collagen fibers showed in Masson's-stained of kidney sections was measured.

2.4. Statistical analysis

Values showed as mean, confidence intervals values and Standard deviation (SD). Data were collected for exploring normality by using test of Kolmogorov-Smirnov. This results with Kolmogorov-Smirnov test showed that data normally managed (parametric data), so, One way analysis of variance (ANOVA) used to compare between the groups.

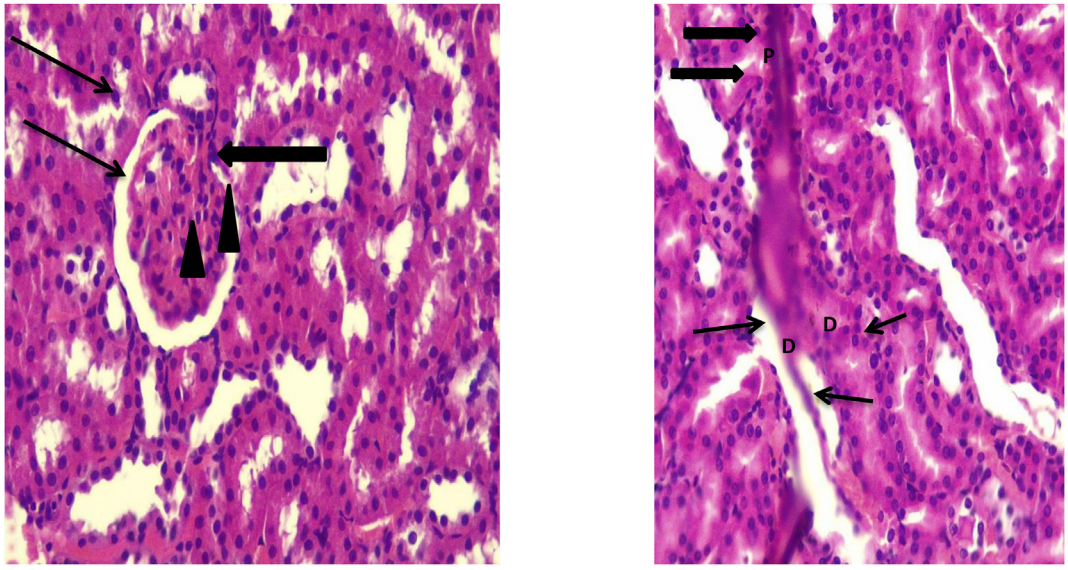
The significance level set at $P \leq 0.05$. Statistical analysis performed with SPSS 18.0 (Statistical Package and Scientific Studies, Inc., Chicago, IL, USA) for Windows.[12] This study was done at the Department of Pharmacology, Faculty of Medicine, AL-Azhar University from June 2022–October 2022.

3. Results

3.1. Light microscopic results

3.1.1. H&E-stained sections

Group I (control group): the cortex was seen with rounded glomeruli, each one was surrounded by normal (narrow) capsular space with a single layer of flat squamous cells internally, parietal layer of Bowman's capsule (Fig. 1A). The (PCT) proximal



(A) (B)

Fig. 1. A photomicrograph of rat kidneys control group: (A, B) showing: (A) Rounded glomeruli (thick arrows), each glomerulus was surrounded by a narrow capsular space (arrow heads) lined with flat squamous cells of parietal layer (thin arrows) of Bowman's capsule. (B) The proximal convoluted tubules (P) with narrow lumen and rounded vesicular nuclei near the base of the cell (thick arrows). The distal convoluted tubules (D) have wide lumen and lined by simple cuboidal cells with rounded nuclei (thin arrows). (A) (H&E, X200). (B) (H&E, X400).

convoluted tubules containing narrow cavity and lined by few hardly differentiated cells containing rounded nuclei, vesicular and basally located. The (DCT) distal convoluted tubules containing wide cavity and lined by multiple cells and nuclei was rounded (Fig. 1B).

By using Masson's trichrome stain, the control rats kidneys containing thin parietal layer of Bowman's capsule also containing thin tubular basement membrane and few collagen in interstitial tissue (Fig. 4 A).

Group II (CP group): the rats kidneys showed congested glomeruli and large capsular space (Fig. 2). Both convoluted tubules showed wide lumen also their inner lining cells revealed vacuolation with pyknotic or fragmented nuclei. Also areas of hemorrhage around glomeruli also noticed (Fig. 2).

By using Masson's trichrome-stained sections revealed focal areas denoting increased interstitial collagen fibers (Fig. 4B).

Group III: in some rats' kidney specimens, there were wide glomeruli also wide capsular spaces which widely separated also intraglomerular congestion noticed. Moderate dilation of both proximal and distal convoluted tubules (Fig. 3 A&C).

In Masson's trichrome-stained sections revealed increase in the interstitial collagen fibers (Fig. 4C).

Group IV: glomeruli and surrounded capsular space were nearly normal with slightly intraglomerular congestion. Also mild dilation of both proximal and distal convoluted tubules (Fig. 3B&D).

In Masson's trichrome-stained sections minimal collagen fibers formation (Fig. 4D).

3.2. Results of histomorphometric study

3.2.1. Collagen fibers mean area percent

The highest mean value recorded in group II, then group III, followed by group IV, and the least mean value in group I. ANOVA test showed that differences between groups was statistically significant ($P = 0.00$). (Table 1), (Fig. 5).

4. Discussion

CP is an effective cytotoxic agent [5]. CP itself inactive but it's metabolism by the microsomal cytochrome P450 as hepatic factor, resulting active metabolites; acrolein and aldophosphamide mustard. These metabolites the main cause of CP toxicity due to the production of free radicals, oxidative stress and Reactive Oxygen Species (ROS). Metabolites also act with essential cell molecules like proteins, membrane, DNA and lipids forming functional and structural alterations.[13] CP nephrotoxicity is very common due to most of CP metabolites excreted in urine.[4,14] On the other hand, some researchers reported that CP not show various adverse effect on the kidney because acrolein and metabolite not nephrotoxic.[15] In our present study shrunken glomeruli with subsequent dilatation of

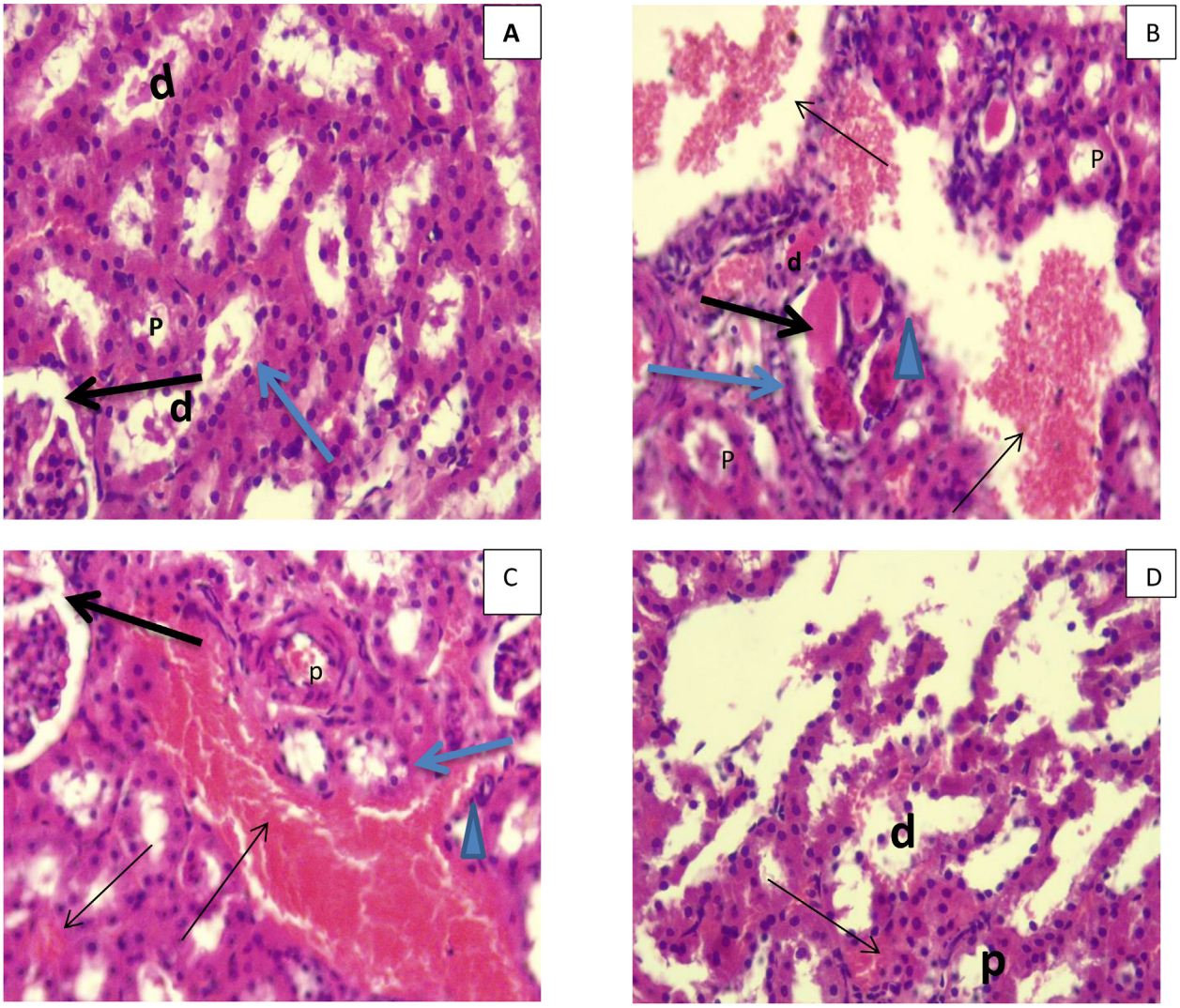


Fig. 2. A photomicrograph of rat kidneys (group II), displaying shrunken glomeruli with intraglomerular congestion and wide capsular space (black arrows). The proximal convoluted tubules (P) and the distal convoluted tubules (D) have wide lumen and showing vacuolizations (blue arrows) with pyknotic nuclei (arrows heads). Note areas of hemorrhage (thin arrow) in (2b) (H&E, X400).

the Bowman's space could be analyzed by oxidative stress that indicated by oxidative markers in the kidney tissues.[16,17] Moreover, literatures informed that kidney was sensitive to numerous xenobiotics toxicants, due to high vascular perfusion and differences of solutes' concentration in urine. Another studies told that the cause of toxicity was arteriolopathy that affect the afferent glomerular arteriole which lead to secondary ischemia.[18] In the current work, the kidney of rats exposed to CP showed dilatation of renal tubules due to fact that both tubules especially the proximal ones metabolically active and susceptible to hypoxia. Also some authors said that CP produced damage of DNA because interaction of DNA with acrolein. The tubular changes of nephrotoxicity opposed in our work by vacuolization of cytoplasm and loss the brush border of apices of proximal convoluted tubules. These results explained by reuptake of large molecules into the proximal tubular cells so induced enhancement of osmotic pressure across the plasma membrane; the transportation of water to the cells which now became vacuolated and swollen.[19] CP cytotoxicity caused some degenerative changes in the lining epithelium of the tubules like apoptosis CP produced oxidative stress through forming links in DNA-protein and DNA-DNA so caused damage of DNA.[10] Cellular infiltration of inflammatory cells was detected in our study due to resulting ischemia which led to production of mediators causing interstitial infiltration of inflammatory cells.[20] Congestion of glomeruli was reported as dysfunction of endothelium resulted from nitric oxide production and oxidative stress produce vascular

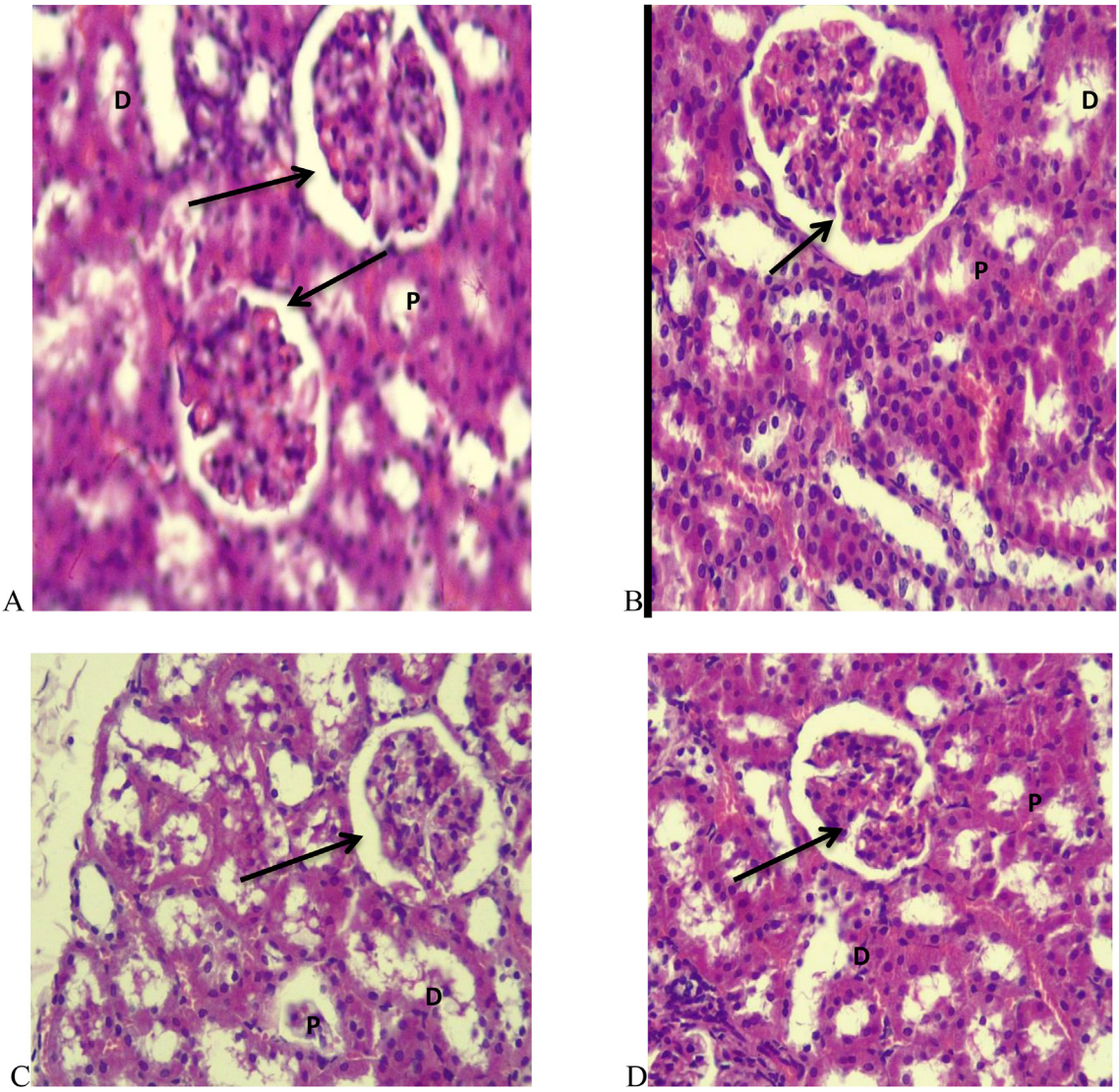


Fig. 3. A photomicrograph of rat kidneys, showing the glomeruli and the surrounding capsular space widely separated with intraglomerular congestion (black arrows). Moderate in (3 A&C) Group III to mild in (3B&D) Group IV. Dilatation of the proximal (P) and distal (D) convoluted tubules are seen moderate in (3 A&C) Group III and mild in (3B&D) Group IV (H&E, X400).

damage.[21] Interstitial fibrosis also was noticed in the investigated kidney specimens of group II may be due to increase both transforming growth factors and angiotensin II inducing fibroblast activation production of that had fibrotic properties thus increasing deposition and decreasing degradation of collagen fibers.[22] The increase in the interstitial fibrous tissue was supported by the histomorphometric results which depicted a statistically highly significant increase in the mean area percent of collagen fibers in CP-treated rats compared to the control group. CP had a pro-oxidant nature, resulting in the decrease in the activities of the antioxidant enzymes and increase in lipid peroxidation in a variety of organs in mice and rats.[23] GSH acted either as a non-enzymatic antioxidant by direct interaction of-SH group with Reactive Oxygen Species (ROS) or shared in the enzymatic detoxification reaction for ROS, as a cofactor or co-enzyme. The depletion of GSH content led to direct conjugation of CP and its metabolites with free or protein bound-SH groups inducing renal damage.[10] CP through exerting oxidative damage and lipid peroxidation, played an important role in the toxicity of many xenobiotics. The antioxidants as Superoxide Dismutase (SOD) increased in cells in response to the oxidative stress as a protective mechanism to eliminate xenobiotics. So, antioxidant system might be an adaptation to the oxidative stress and this explained the capacity of the testis antioxidant

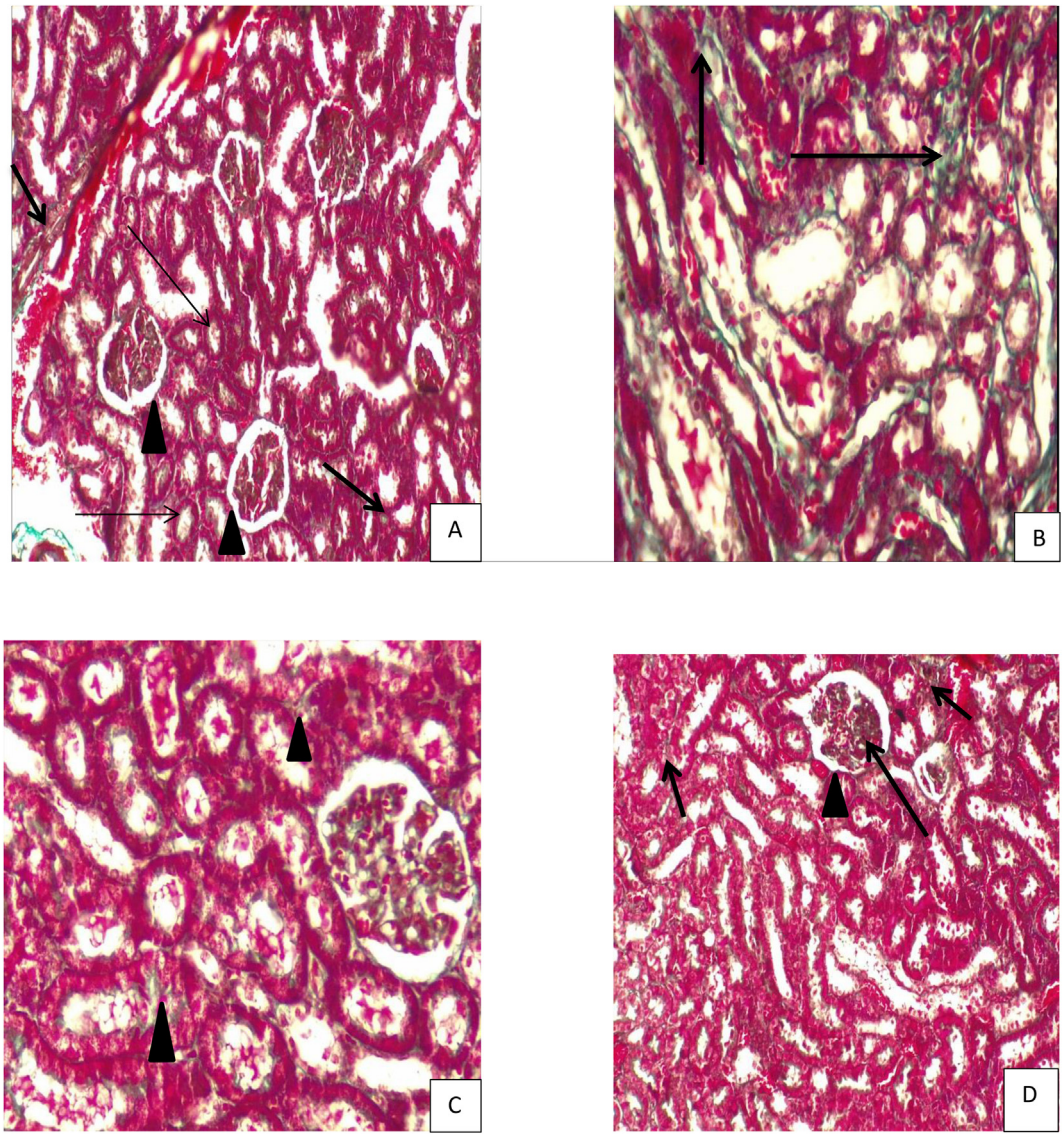


Fig. 4. A photomicrograph of rat kidneys (A, B, C, D): illustrating: (A) Control group thin parietal layer of Bowman's capsule (arrowheads) and thin tubular basement membrane (thin arrows). Minimal interstitial collagen tissue (thick arrows). (B) Group II, focal areas of increased interstitial collagen fibrous tissue content (arrows). (C) Group III, aggregate areas of collagen connective tissue (arrowheads) in the interstitium. (D) Group IV, limited collagen fibrous tissue in the interstitium (small arrows). Note apparently normal glomerulus (long arrow) with nearly normal capsular space (arrowhead) (Masson's trichrome X400).

system to protect against oxidative damage caused by CP.[24] Cinnamon oil, acted as antioxidant and anti-inflammatory drug.[8,9] Thus, cinnamon oil protected cell structure against oxidative stress and inhibits the reduction of endogenous antioxidant enzymes.[10]

Furthermore, authors found that intake of cinnamon oil, as an antioxidant, 5 days before and 5 days

Table 1. Descriptive statistics and comparison of area percent in different groups (ANOVA Test).

	Mean	St. dev.	St. Error.	95% interval Lower bound	confidence for mean Upper bound	Min.	Max.	F	P
GI	40.60	1.38	0.43	44.45	46.42	43.16	47.41	533.386	0.000*
GII	70.42	1.87	0.58	69.17	71.84	66.62	72.53	533.386	0.000*
GIII	49.35	1.88	0.58	47.94	50.59	46.31	52.22	533.386	0.000*
GIV	46.37	1.32	0.40	45.56	47.38	44.42	48.32	533.386	0.000*

Significance level $P < 0.05$*: significant.

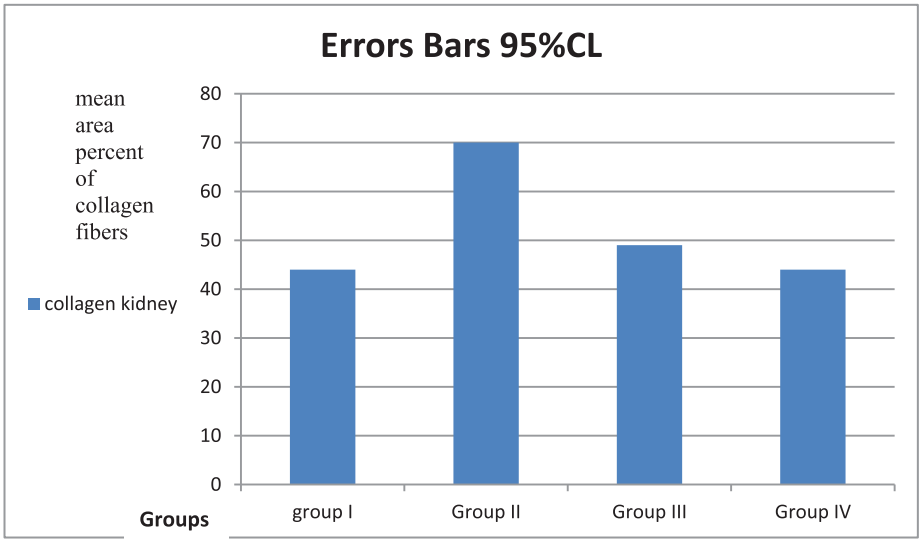


Fig. 5. Bar chart showing mean area of percent of different groups collagen fibers.

after administration of CP reduced apoptosis in the cells.[25,26] The exact mechanism of cinnamon oil in the treatment of oxidative stress is not fully detected. However, it could be explained as cinnamon oil decreased the lipid peroxidation thus removing the activity of the free radicals as supported by decreased DNA damage and cellular damage.[27] Cinnamon oil also inhibited lipid-independent isoprenoids thus improving the oxidative stress and inflammation.[28]The effect of cinnamon oil reduced hepatotoxicity by eliminating oxidative stress without affecting fertility and reproduction.[29]

From the present work, it is recommended to use CP with cinnamon oil.

5. Conclusion

Intake of Cinnamon Oil caused recovery in structure. May reach parameters of the group I, also this improvement much better in group IV than group III.

Disclosure

The authors have no financial interest to declare in relation to the content of this article.

Authorship

All authors have a substantial contribution to the article.

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Conflicts of interest

The authors have no conflict of interest to the content of this article.

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