Original Article

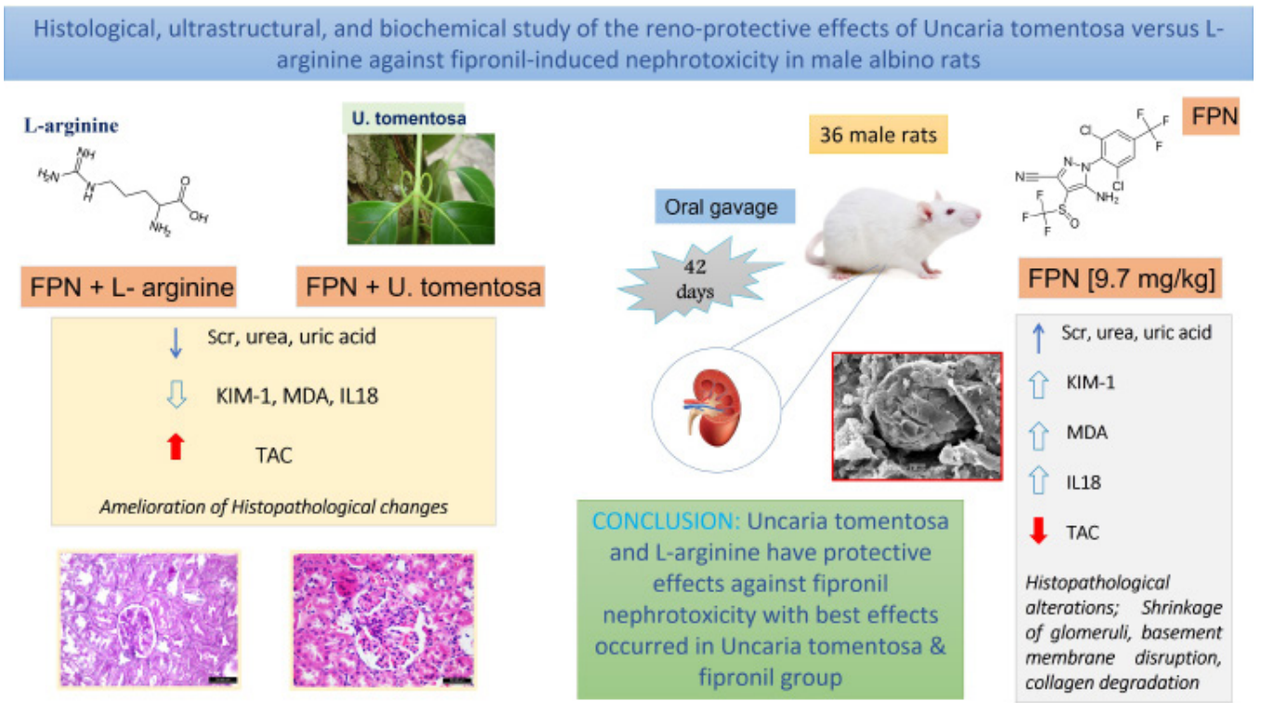
Histological, Ultrastructural, and Biochemical Study of the Reno-Protective Effects of *Uncaria Tomentosa* and L-arginine Against Fipronil-Induced Nephrotoxicity in Male Albino Rats

Nema Soliman[1,2], Marwa A. El-Beltagy[3], Heba M. A. Abdelrazek[4] and Sahar Galal Gouda[1]

[1]Department of Histology and Cell Biology, Faculty of Medicine, Suez Canal University, [2]Center of Excellence in Molecular and Cellular Medicine, Suez Canal University, [3]Biochemistry, [4]Physiology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

ABSTRACT

Introduction: Fipronil induces vulnerable effects on the kidney causing nephrotoxicity via oxidative stress. The purpose of this study was to compare the protective effects of *Uncaria tomentosa* versus L-arginine against fipronil induced nephrotoxicity.
Material and Methods: Thirty-six male adult rats were randomized into six groups (n=6) and received the following treatments via gavage for 42 days; group I- had distilled water, group II- *Uncaria tomentosa* (250 mg/kg of 20% U. tomentosa extract), group III- L-arginine (200 mg/kg of 20% L-arginine), group IV- fipronil (9.7 mg/kg; 1/10 of LD50), group V- fipronil & *Uncaria tomentosa*, and group VI- fipronil & L-arginine in the same doses as mentioned before. We assessed renal activities of renal malondialdehyde (MDA), total antioxidant capacity (TAC), and IL-18, as well as relative kidney weight, serum creatinine, uric acid, urea, and kidney injury molecule-1 (KIM-1). We examined the general kidney architecture, glomeruli, proximal and distal tubules, basement membranes, and collagen fibers. Ultrastructurally, the renal corpuscles and proximal tubules were evaluated by scanning and transmission electron microscopes.
Results: Fipronil treatment induced a significant ($P<0.05$) increment in serum biochemical measures, renal MDA, IL-18, and a considerable decrease in TAC. There was a significant ($P<0.05$) increase in the percentage of shrunken glomeruli, basement membrane disruption, collagen degradation, and capsular adhesions. We observed ultrastructurally degenerated mitochondria and fragmented microvilli of the proximal tubules. *Uncaria tomentosa* and L-arginine markedly reversed the changes induced by fipronil treatment.
Conclusion: It was concluded that *Uncaria tomentosa* and L-arginine were protective against nephrotoxicity by fipronil, the group receiving *Uncaria tomentosa* and fipronil provided better protection.



Graphical abstract

DOI: 10.21608/ejh.2023.176998.1827

Received: 08 January 2023, **Accepted: 21** March 2023.

Key Words: Capsular adhesions, fipronil, nephrotoxicity, total antioxidant capacity, *uncaria tomentosa*.

Corresponding Author: Sahar Galal Gouda, PhD, Department of Histology and Cell Biology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt, **Tel.**: 01095337169, **E-mail:** sahar.galal@med.suez.edu.eg

ISSN: 1110-0559, Vol. 46, No. 1, March 2023

INTRODUCTION

Exposure to pesticides such as fipronil can lead to nephrotoxicity. Fipronil (FPN), the novel and selective pesticide, belongs to the new class known as phenylpyrazoles[1]. It became increasingly popular for pest management and crop protection. Products made from fipronil go by various trade names (Over n' OutTM, ICON 6.2FSTM, Choice, Chipco®, and Teck Pac)[2,3].

Owing to the incorrect or frequent use of fipronil, which leads to soil and water contamination, there is a growing possibility that fipronil could have negative impacts on both humans and animals by affecting the antioxidant defense mechanism[4]. Oxidative stress induces damage to cellular macromolecules and mitochondria, resulting in necrotic or apoptotic cell death[5].

Combating oxidative damage is a useful target for novel treatments to prevent diseases, involving L-arginine, a crucial component of triggering the antioxidant mechanism[6,7]. L-arginine is a metabolically versatile amino acid that contributes in the production of nitric oxide. Exogenous L-arginine has various pharmacological benefits when taken in doses greater than average dietary intake, according to several experimental and human studies[8,9].

It's debatable whether L-arginine has a role in renal disorders. Most of the data showed improvement in pathology and renal function in animals with glomerulonephritis, obstructive nephropathy, and acute ischemic renal failure receiving L-arginine[10,11].

During the last two decades, a lot of interest has been given to exogenous antioxidant supplementation and the antioxidant capability of plants. *Uncaria tomentosa* (cat's claw) has grown in popularity due to its ability to stimulate the immune response and fight inflammation, oxidative damage, and cancer[12]. *Uncaria tomentosa* has at least 50 compounds, among which about 35 are exclusive to this species, including alkaloids, glycosides, sterol fractions, tannins, flavonoids, phenols, and related beneficial compounds[13].

Uncaria tomentosa improved fipronil-induced hepatotoxicity and oxidative stress in rats through suppression of NF-κB[14]. It reduced inflammation associated with osteoarthritis through inhibition of TNFα[15]. Moreover, it might inhibit neurodegenerative changes and improve human Alzheimer's disease and physiological aging[16].

In contrast, other studies point out that herbal therapies may have inherent risks, and the kidney could be particularly susceptible to toxic consequences. By inducing apoptosis, herbs may cause nephrotoxicity.[17]

Both *Uncaria tomentosa* and L-arginine remain challenging. So, determining *Uncaria tomentosa*'s and L-arginine protective effects against nephrotoxicity was the goal of the current study in male rats using biochemical, histological, histochemical, and ultrastructural techniques.

MATERIALS AND METHODS

Animals

Thirty-six adult male albino Wistar rats, 3-4 months age, each weighing 210-230 grams, purchased from the College of Veterinary Medicine's Animal House at Suez Canal University in Egypt. Rats were kept in cages with sawdust floors and had free access to food and water on a regular basis in a temperature-controlled environment (25°C). This study's protocol received approval. Everything was completed according to the guidelines. no. 2019013, established at Suez Canal University's school of veterinary medicine.

Chemicals and plant material

In this study, Zhejiang Yongnong Chem. Co., China's fipronil (Coach 20% SC) [5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-fluoromethylsulfinyl pyrazole] was used. A shipment of L-arginine was made from Ports now Co., USA. *Uncaria tomentosa* extract (10:1) with 1.5% oxindole alkaloids from Botanica Co. in New Zealand. We made an extract solution in distilled water at a concentration of 20% (w/v).

Experimental design

Thirty six Rats (n = 6) were split up into 6 groups after an adaptation period of one week and received the following treatments via gavage, once/day for 6 weeks.

Group I: distilled water (D.W.), made up the control

Group II (U.tomentosa group): 250 mg/kg of 20% U.tomentosa extract dissolved in D.W.[18]

Group III (L-arginine group): 200 mg/kg of 20% L-arginine dissolved in D.W.[19]

Group IV (Fipronil group): fipronil (1/10 of the LD50) is 9.7 mg/kg dissolved in D.W.[14,20]

Group V (Fipronil + U.tomentosa group): 9.7 milligram /kg of fipronil, and after one hour received 250 mg/kg of 20% U.tomentosa extract.

Group VI (Fipronil + L-arginine group): 9.7 mg/kg of fipronil + 200 mg/kg of 20% L- arginine after one hour.

Weekly, rats were weighed to adjust the previous doses according to body weight gain.

Blood sampling

After 42 days, anesthesia was provided to overnight fasting rats, and blood samples from the retro-orbital venous plexus were obtained using sterile plain tubes. To obtain sera, samples were centrifuged at 3000 rpm for 15 minutes after being left to clot for 15 minutes and being refrigerated for three hours. Sera were kept at -20ºC for further biochemical analysis: serum creatinine (SCr), uric acid, urea, and kidney injury molecule-1 (KIM-1).

Tissue collection and preparation

After blood collection, rats are decapitated, kidneys excised, rinsed with PBS, phosphate-buffered saline, to clean out any blood contamination, then dry with filter paper, weighed to determine Relative kidney weights. In order to prepare kidney homogenates for testing Total antioxidant capacity (TAC), malondialdehyde (MDA), and interleukin-18 (IL-18) levels, one kidney was frozen at -80 °C, the other kidney was cut sagittally into two halves; one fixed in 10% NPF and processed till 5 µm paraffin sections for subsequent tissue staining[18].

- Hematoxylin and eosin (H&E): (the renal architecture)

- Periodic Acid Schiff's (PAS): (the basement membrane and brush border).

- Masson's trichrome stain: (collagen fibers).

The other half of the kidney was immediately processed for electron microscopy, cut into small pieces (less than 1 mm in diameter) and fixed in 2.5% glutaraldehyde (pH 7.3) overnight at 4°C. Semi-thin sections (toluidine blue staining) with a thickness of 0.5 µm and ultra-thin sections (50 nm) were prepared[21]. These sections were examined using scanning and transmission electron microscopy (JSM-5500LV and JEOL-JEM 1010, respectively) at Al-Azhar University's Regional Center for Mycology and Biotechnology (RCMB).

Biochemical evaluation

Using Bio Merieux kits, serum creatinine, urea, and uric acid were measured (France). We assessed kidney injury molecule-1 (KIM-1) utilising Cell Biolabs, Inc. (USA)[22].

Renal Malondialdehyde (MDA) and Total Antioxidant Capacity (TAC):

Making use of the kit, (Cat. No. K739-100 from BioVision in the USA) of a wavelength (= 532 nm, renal MDA concentrations were calorimetrically assessed as an indication of lipid peroxidation. The steps were all followed exactly as the manufacturer had instructed[23].

TAC in the renal homogenate was determined using a commercial colorimetric kit (Ref O.X. 20-4100, LDN, Germany) in accordance with the directions provided by the manufacturer at absorbance 450 nm[24].

Estimation of renal level of Interleukin-18 (IL-18):

The renal IL-18 level was determined[25] using a commercial Rat IL-18 (Interleukin-18) Kit for ELISA (Cat. No.ER0036) and the manufacturer's instructions (R&D Systems, USA).

Histopathological analysis

Using a Leica DM1000 microscope and Leica DFC425 digital camera, Germany, H&E-stained kidney sections were examined and imaged to look for normal cortical architecture, shrunken glomeruli, widening of Bowman's space, congested blood vessels, inflammatory infiltrates, and disorganisation with loss of cellular architecture of convoluted tubules, both proximal and distal. The following variables were evaluated quantitatively: the glomerulus, Bowman's space, and renal corpuscle mean diameters (in micrometres) in H&E-stained sections and the proportion of glomeruli that are abnormally small. The mean colour area % of collagen fibres and mucopolysaccharides, of sections stained with PAS and Masson's trichrome, respectively. Software called Image J was used for quantitative analysis. Three sections per animal and five high power fields (HPF)/section were measured.

Statistical analysis

Analyzing variance in one direction ANOVA was employed in statistically examined the parametric data, followed by "Tuckey" post hoc test. *P value* less than 0.05 was used into account while evaluating the results. Kruskal-Wallis's test was used for non-parametric data. The significant difference in the frequency of aberrant renal corpuscles was examined using a Chi-square test. Calculations were made using SPSS (IBM Corp., Armonk, NY, version 21.0).

RESULTS

Relative kidney weight

As shown in Table 1, fipronil treatment (in group IV) caused significant ($P \leq 0.05$) decline in the mean value of relative kidney weight compared with the control group. As opposed to the fipronil group, groups (V) and (VI) had significantly ($P \leq 0.05$) improved.

Biochemical results

As shown in Table 1, the mean serum levels of creatinine, urea, uric acid, and KIM-1 increased significantly ($P \leq 0.05$) in fipronil group compared with the control, whereas groups V and VI showed statistically significant ($P \leq 0.05$) decline.

Although total antioxidant capacity (TAC) is significantly reduced ($P \leq 0.05$) in the fipronil group in contrast to the control, MDA and IL-18 levels are significantly higher ($P \leq 0.05$) in the fipronil group. Groups V & VI showed a significant improvement (Table 2).

Histological results

Hematoxylin and Eosin (H&E) Stain

When sections from the control, Uncaria, and L-arginine groups (groups I, II, and III) were stained with HE, they showed normal renal corpuscles, proximal (P.T.) and distal (D.T.) convoluted tubules, and a minimal amount of the interstitial tissue characteristic of typical renal architecture. Each kidney corpuscle was a glomerulus surrounded by Bowman's capsule. The visceral layer (the podocytes) of the Bowman's capsule lined the glomerulus, while the parietal layer was lined by a simple squamous epithelium. Bowman's space is present between the two layers. The P.T. exhibited an acidophilic, high cubical cell lining and a narrow lumen. The D.T. has a broad lumen and a pale cubical epithelial lining (Figures 1A, B & C). The fipronil group (group IV) showed marked distortion of the renal cortical architecture. Most of the glomeruli were significantly ($P \leq 0.05$) shrunken and destroyed, and Bowman's space appeared to widen. Interstitial blood vessels and glomerular capillaries were congested and dilated. Some of the renal corpuscles had ruptured Bowman's capsule, while others showed capsular adhesions. Most of the P.T. and D.T. were destroyed and disorganized with loss of cellular architecture. Some tubules showed loss of their epithelial lining, others had cells with vacuolated cytoplasm and pyknotic nuclei. Some tubules showed sloughing of cells in their lumina (Figures 1 D, E & H). Fipronil + Uncaria group (V) and fipronil + L-arginine group (VI) showed fewer histopathological changes compared to the fipronil group. The P.T. and D.T. had some areas of loss of cellular architecture. However, the extravasation was more apparent in the fipronil + L-Arginine group (Figures1 F & G). Most of the renal corpuscles in groups I, II & III appeared normal (Figure 1 H). The percentage of abnormal renal corpuscles indicated a significant increase ($P \leq 0.05$) in fipronil group, whilst in the V & VI groups it showed a significant decrease (Figure 1 H).The glomerular diameter exhibited significant ($P \leq 0.05$) decrease in the fipronil administered sections [53.5µm ± 11.3] compared to control. Meanwhile, a significant improvement was noticed in groups V and VI [71.96 µm ± 11.1 & 76.5 µm ± 11.6 respectively] as opposed to the fipronil group (Figure 2 A). A strong positive correlation between the diameter of the renal corpuscle and the glomerular diameter was observed in the study groups (Figure 2B).

Toluidine blue stain

Semithin sections of the control, Uncaria, and L-arginine groups (groups I, II, and III) displayed a conspicuous glomerular basement membrane (GBM) and a glomerulus made of a tuft of blood capillaries. Podocytes, endothelial cells, and mesangial cells all had visible nuclei. P.T. was lined with cubical cells that had apical brush borders and basal rounded vesicular nuclei. D.T. had pale, low-cubical cells lining it (Figures 3A, B & C). The glomerulus in the fipronil group (IV) was deformed, with dilated capillaries, merged GBM, and shrunken and distorted podocyte, mesangial, and endothelial cell nuclei. It was possible to see capsular adhesions between the glomerulus and the capsular epithelium (Figure 3D). P.T. displayed cells sloughing into the lumen, pyknotic nuclei, focal brush border loss, and vacuolated cells. D.T. exhibited exfoliated cells in the lumen and an irregular basal lamina (Figures 3 D & E).

Groups V and VI displayed less histological abnormalities, less congested glomerular capillaries, and a more prominent GBM in comparison to group IV. Endothelial, mesangial, and podocytes are almost normal. P.T. & D.T. are nearly intact (Figures 3 F & G).

Periodic Acid Schiff (PAS) stain

Basement membranes of the P.T., D.T., glomerulus, and capsular epithelium displayed a PAS-positive reaction (magenta colour) in the control, Uncaria, and L-arginine groups (groups I, II, and III). The P.T.'s brush border likewise displayed a PAS-positive reaction (Figures 4A, B & C). The glomerular basement membrane was not continuous in the fipronil group (group IV), and there were patches of adhesion with both the glomerulus and the parietal capsule. Additionally, the brush border of P.T. has areas of focal loss (Figures 4 D & E). The renal tubules' basement membranes and glomerulus in Fipronil & Uncaria group (V) displayed an intact brush border of P.T. and a positive continuous PAS- reaction (Figure 4F). The P.T. brush border showed focal loss in the Fipronil & L-arginine group (VI) (Figure 4 G).

The average percentage of PAS-positive areas significantly ($P \leq 0.05$) decreased in the fipronil group in contrast to the control group. Group V demonstrated a significant ($P \leq 0.05$) increase in PAS-positive reaction as compared to the fipronil group (Figure. 4 H).

Masson's Trichrome Stain

Sections stained with Masson trichrome; control, uncaria, and L-arginine groups (groups I, II & III) showed minimal amounts of collagen fibers around glomerular capillaries, the Bowman's capsule, and the renal tubules (appearing as green-stained streaks) (Figures 5A, B & C). The fipronil group (IV) showed fragmentation of collagen fibers within the glomerulus, disruption of Bowman's capsule and peritubular basement membrane (Figure 5D). Groups V & VI showed a nearly normal distribution of collagen fibers (Figures 5 E & F). The mean collagen fiber color was decreased significantly in the fipronil group in comparison to the control group ($P \leq 0.05$). Group V exhibited a significant improvement in collagen fibres compared with the fipronil group (Figure 5G).

Scanning Electron Microscopic results

The typical glomerulus was visible in scanning electron micrographs of the control, Uncaria, and L-arginine groups (groups I, II, and III), which exhibited Bowman's space separating it from the Bowman's capsule parietal layer (Figures 7 A, B & C). The fipronil group exhibited shrunken and distorted glomeruli surrounded by a wide Bowman's space (Figure 7D). Group V showed nearly normal glomeruli and Bowman's space (Figure 7E). Group VI showed distorted glomeruli compared to control (Figure 7F).

Transmission Electron Microscopic results

Photomicrographs of proximal tubular cells of the control group (group I) showed numerous apical long microvilli with spherical euchromatin nuclei, numerous vesicles and lysosomes. Basal infoldings and abundant elongated mitochondria arranged perpendicular to trilaminar basal lamina (Figure 8 A). Uncaria and L-arginine groups (groups II & III) were similar to control. The fipronil group showed shrunken nuclei with loss of the nuclear envelope, fragmented microvilli, large cytoplasmic spaces, few lysosomes, degenerated mitochondria, and loss of most basal folds (Figure 8B). Both groups V and VI showed improved ultrastructure compared to the fipronil group (Figures 8 C & D).

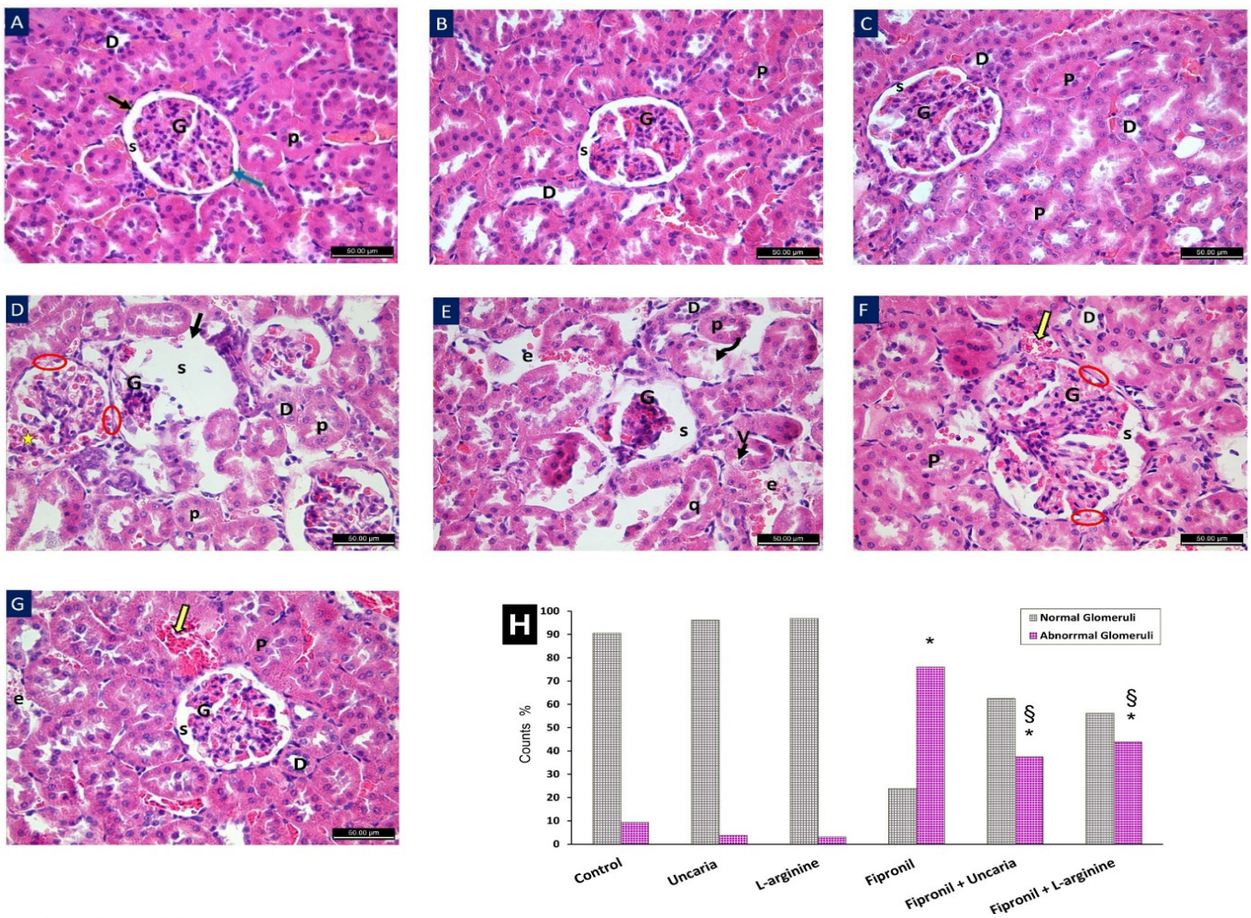


Fig. 1: (H&E x 400)
Photomicrographs of sections of renal cortex: A. The control group (group I) shows a normal architecture of the renal cortex, a renal corpuscle surrounded by proximal (P) and distal (D) convoluted tubules. The proximal tubule (P) has a narrow lumen lined with acidophilic high cubical cells with rounded vesicular nuclei. The distal tubule (D) has a wide lumen and is lined with pale cubical epithelium. The renal corpuscle contains the glomerulus (G) and is surrounded by Bowman's capsule. The parietal layer of Bowman's capsule is formed of simple squamous epithelium (black arrow), while the visceral layer is formed of podocytes (blue arrow) with Bowman's space in between (s). B & C. Uncaria group (II) and L-arginine group (III) are similar to control. D & E. Fipronil group (IV) showing a distorted architecture of the renal cortex; shrunken glomerulus (G), wide Bowman's space (s), discontinuity of Bowman's capsule (black arrow). Some renal corpuscles have capsular adhesions with the glomerulus (red circle) and congested glomerular capillaries (yellow star). There is also loss of tubular epithelium (curved arrow), sloughing into the lumen (q), vacuolation (V), and extravasation (e). F. Fipronil + Uncaria group (V) showing a near normal glomerulus (G) and Bowman's space (s), few adhesions (circle), and congested blood capillaries (yellow arrow) compared to fipronil group. G. Fipronil + L-arginine group (VI) showing near-normal renal corpuscle. There are congested blood capillaries (yellow arrow) and extravasation (e). H. Chart represents the frequency of abnormal renal corpuscles among study groups. Data were statistically analyzed by chi-square test. [*] significant versus the control group. [§] significant versus the fipronil group. *P-value ≤ 0.05*.

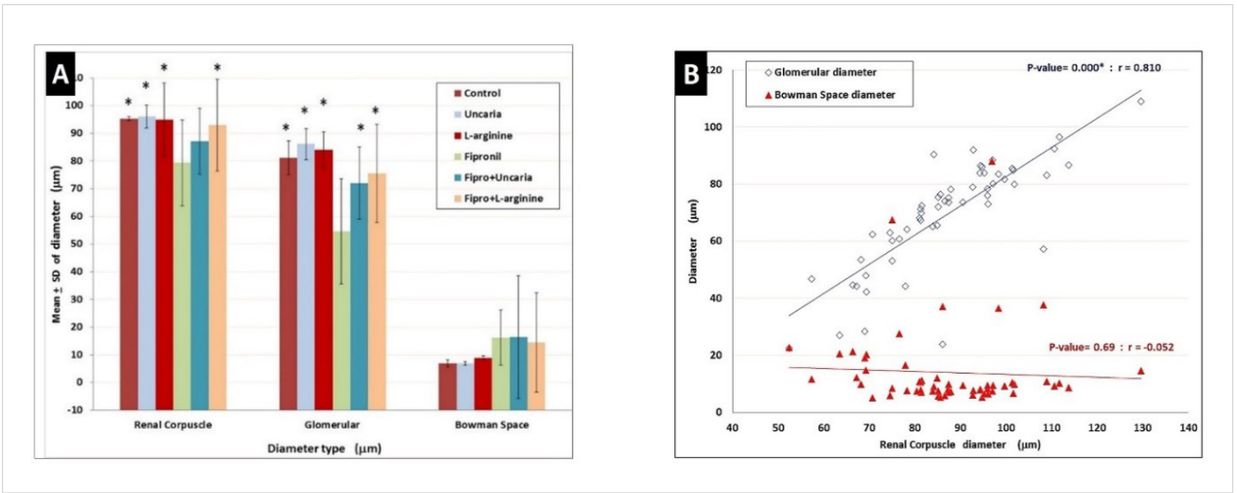


Fig. 2:

A. chart showing a comparison between study groups regarding renal corpuscle, glomerular, and Bowman's space diameter (µm). Data are presented as mean ± standard deviation. Statistical analysis is done by the Kruskal-Wallis test. [*] significant versus the fipronil group. *P-value ≤ 0.05*. B. Correlation between renal corpuscle diameter and both Bowman's space diameter and glomerular diameter. A statistically significant strong positive correlation (r=0.810) between renal corpuscle diameter and glomerular diameter ($p<0.05$), but nearly there was no correlation between Bowman's space diameter and renal corpuscle diameter (r=0.052), which was non-statistically significant ($p>0.05$).

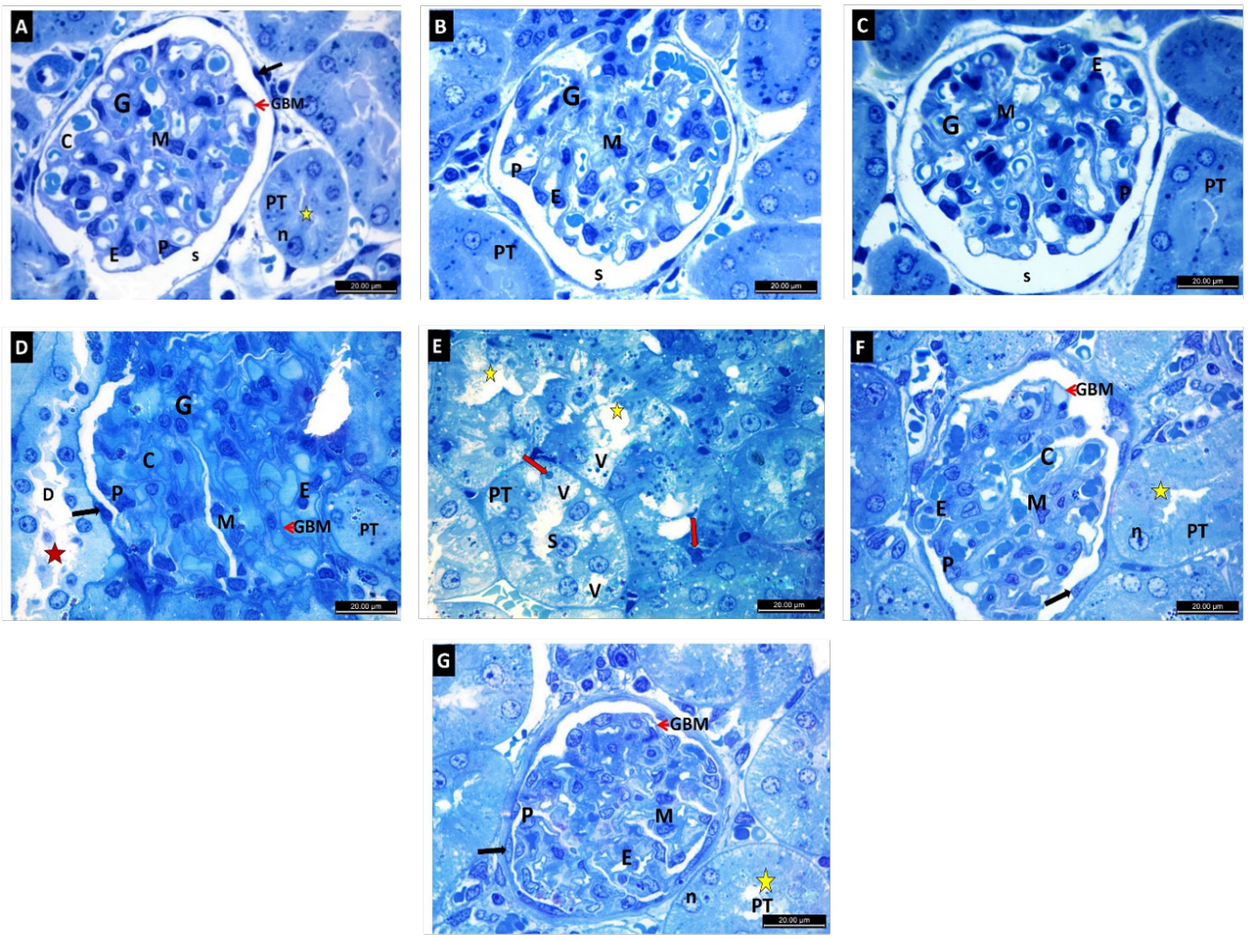


Fig. 3: (Toluidine blue x 1000)

Semithin sections of the renal cortex. A. Control group showing a glomerulus (G) formed of blood capillaries (C), with a prominent glomerular basement membrane (GBM). Notice the nuclei of the podocytes (P), endothelial (E), and mesangial (M) cells. The parietal layer of Bowman's capsule (arrow) is separated from the glomerulus by Bowman's space (s). The proximal tubule (PT) is lined with cubical cells with basally situated rounded nuclei (n) and apical brush border (yellow star). B & C. Uncaria group (II) and L-arginine group (III) respectively, are similar to control. D. Fipronil group (IV) showing shrunken distorted glomerulus (G) with irregular dilated capillaries (C) and fused GBM (red arrow). Shrunken and distorted nuclei of podocytes (P), mesangial (M), and endothelial (E) cells are noticed. There were also exfoliated cells (red star) in the lumen of the distal tubule. E. PT of group IV showing focal loss of brush border (yellow star), sloughing of cells (S), vacuolated cells (V), and pyknotic nuclei (red arrow). F. Fipronil + Uncaria group (V) showing near normal glomerulus with blood capillaries (C), GBM (red arrow), podocytes (P), endothelial (E), and mesangial (M) cells. PT with its brush border (yellow star) and cellular lining appeared normal. G. Fipronil + L-arginine group (VI) showing near normal glomerulus. The parietal layer of Bowman's capsule is thickened (black arrow).

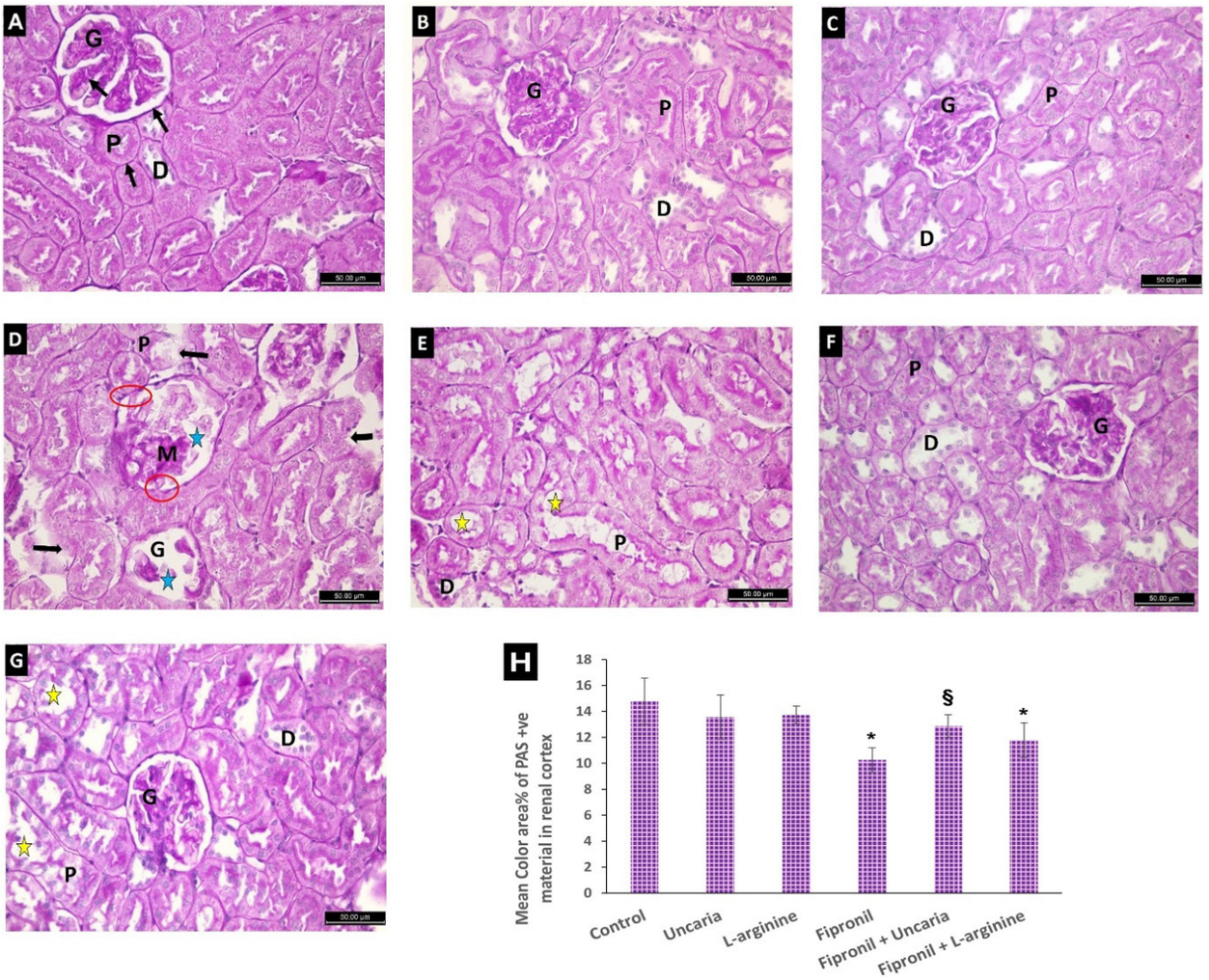


Fig. 4: (PAS x 400)

Photomicrographs of sections in the renal cortex. A. Control group (group I) showing PAS-positive reaction of the basement membrane (black arrow) of the glomerulus (G), proximal tubules (P), distal tubules (D), and Bowman's capsule. Proximal tubules (P) have a strong PAS-positive reaction along the apical brush border. B & C. Uncaria group (II) and L-arginine group (III) respectively, are similar to control. D. Fipronil group (IV) showing interrupted PAS +ve basement membrane of the glomerulus (blue star) and proximal tubules (black arrow). There are adhesions between the glomerulus and parietal layer of Bowman's capsule (red circle). E. Proximal tubules of group IV showing focal loss of brush border (yellow star). F. Fipronil + Uncaria group (V) showing near normal PAS +ve reaction of the basement membrane of the glomerulus and tubules and in the brush border of proximal tubules (P). G. Fipronil + L- arginine group (VI) shows focal loss of the brush border of the proximal tubules (yellow star). H. Chart represents the mean color area % of PAS-positive reaction in renal cortex among study groups. Data is presented as mean ± standard deviation. Statistical analysis is done by Tuckey post hoc test following ANOVA. [*] significant versus the control group. [§] significant versus the fipronil group. *P-value ≤ 0.05*.

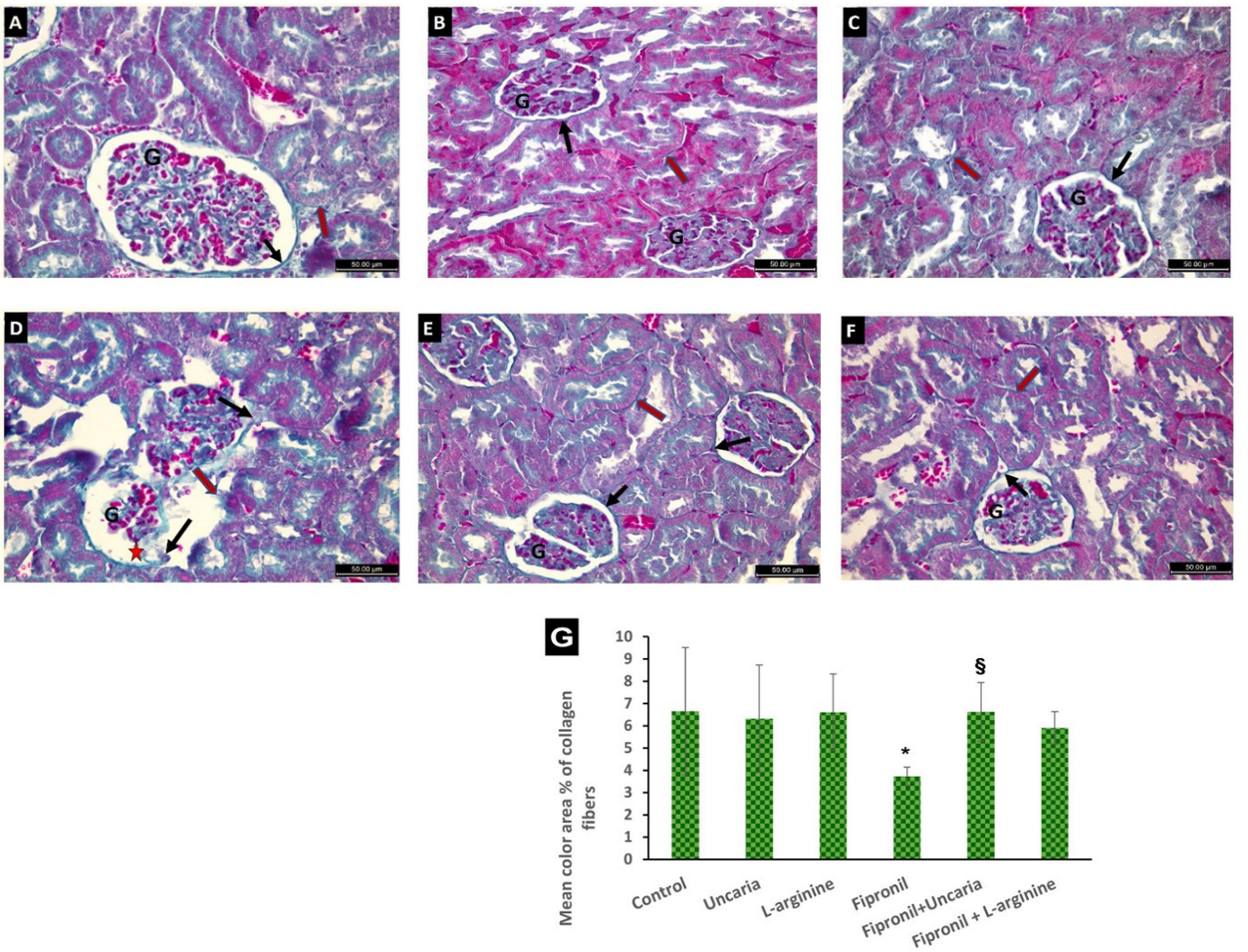


Fig. 5: (Masson's Trichrome x 400)

Photomicrographs of sections in renal cortex A. Control group (Group I) showing a minimal amount of collagen fibers in the wall of the proximal and distal tubules (red arrow), between glomerular capillaries (G), and in Bowman's capsule (black arrow). B & C. Uncaria group (group II) and L-arginine group (group III) respectively are similar to control. D. Fipronil group showing fragmentation of collagen fibers inside the glomerulus (star), interruption of Bowman's capsule (black arrow), and interruption of the basal lamina of renal tubules (red arrow). E & F. Both Group V & VI respectively showing nearly normal distribution of collagen fibers in the tubules (red arrow), between glomerular capillaries (G), and in Bowman's capsule (black arrow). G. chart represents the mean area % of collagen fibers in renal cortex among study groups. Data are presented as mean ± standard deviation. Statistical analysis is done by a Tuckey post hoc test following ANOVA. [*] significant versus the control group. [§] significant versus the Fipronil group. *P-value ≤ 0.05*

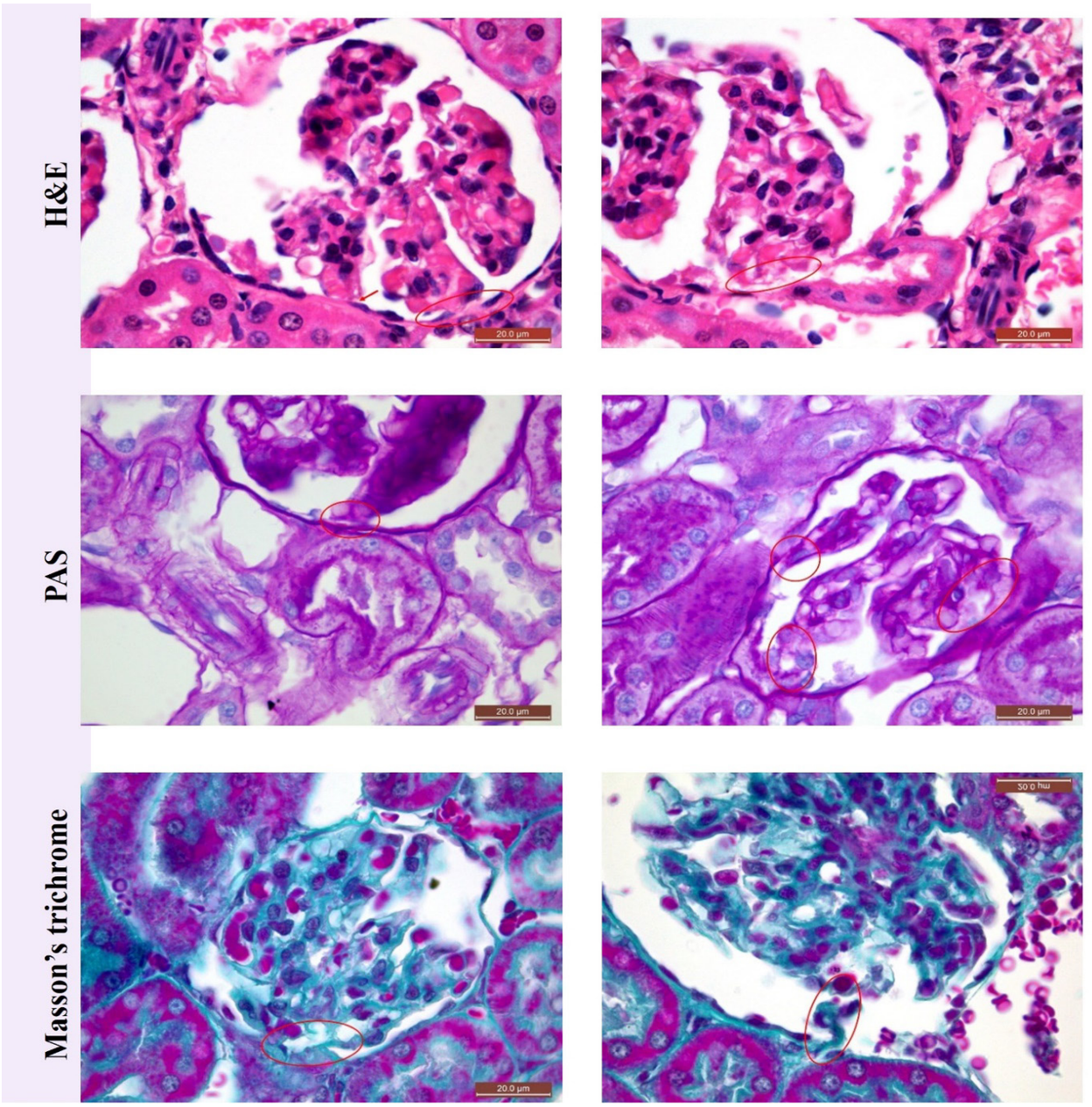


Fig. 6: Collection of Capsular adhesions with different stains in the fipronil group x 1000

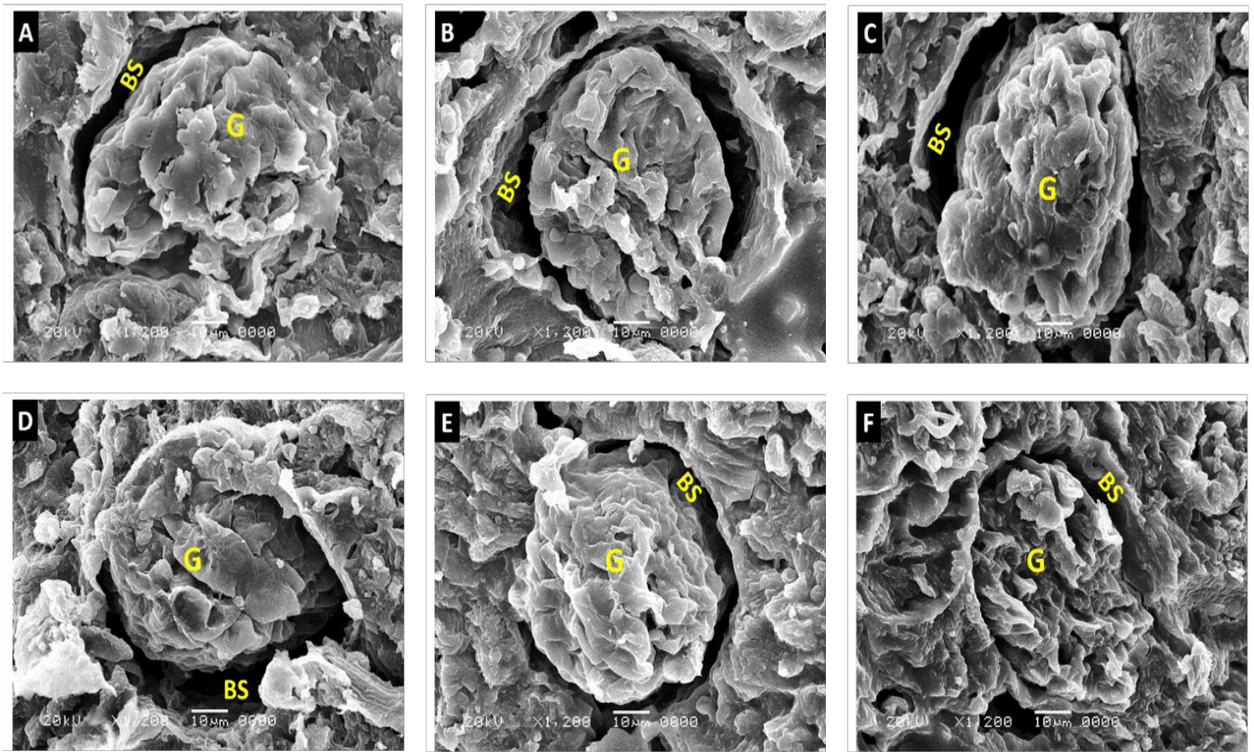


Fig. 7: (Scanning electron micrographs x 1200)

Scanning electron micrographs of the renal cortex. A. Control group (group I) showing normal glomerulus (G) separated from the parietal layer of Bowman's capsule by Bowman's space (BS). B & C. Uncaria group (II) and L-arginine group (III) respectively are similar to control. D. Fipronil group (IV) showing distorted, shrunken glomerulus (G) surrounded by wide Bowman's space (BS). E. Fipronil + Uncaria group showing near normal glomerulus and Bowman's space. F. Fipronil + L-arginine group showing distorted glomerulus compared to control group.

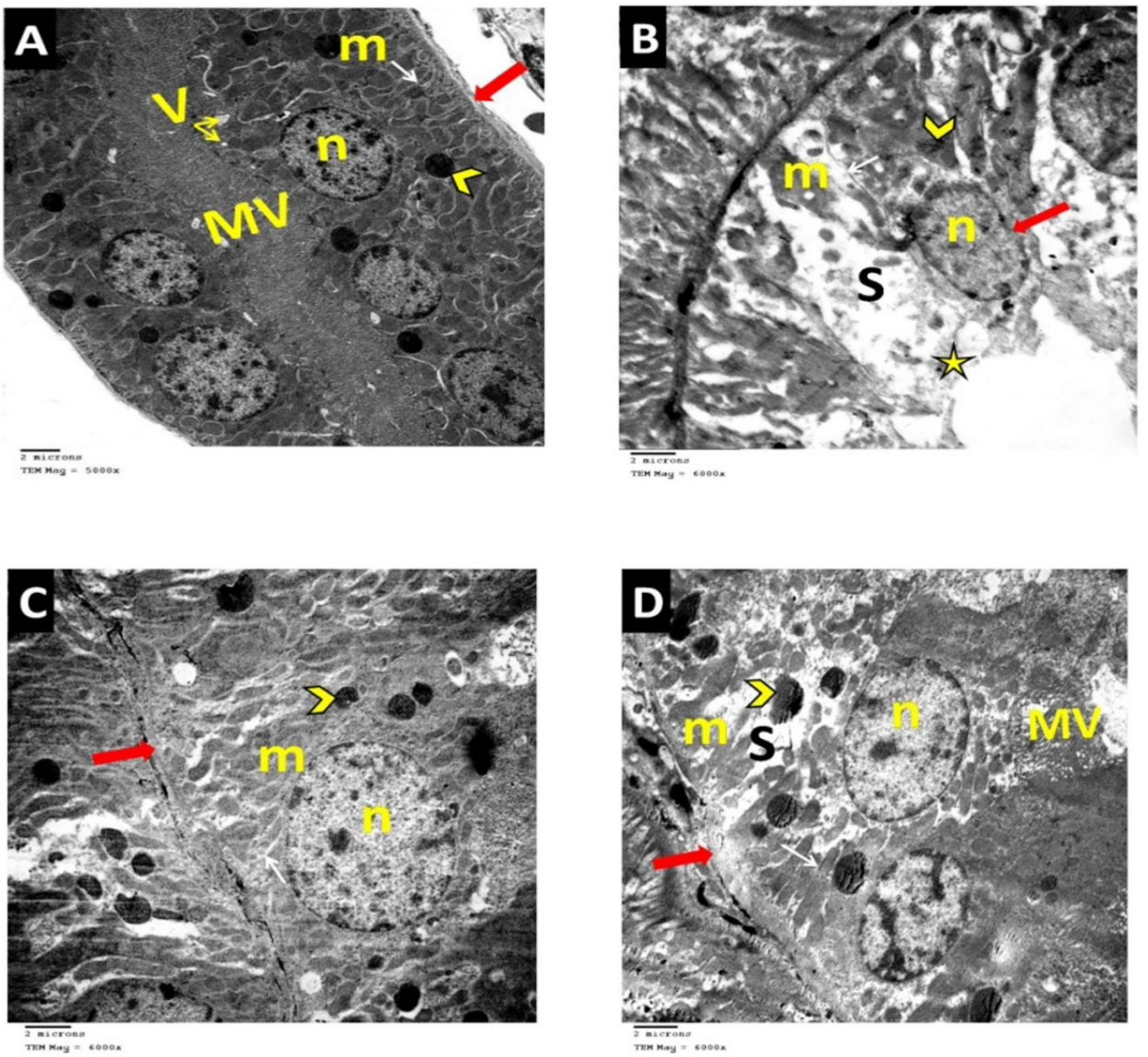


Fig. 8: (TEM x 6000 except A x 5000)

Photomicrographs of sections in renal cortex A. Control (Group I) showing proximal tubule cells with profuse long apical microvilli (MV), multiple vesicles (V), and lysosomes (arrowhead). Basal infoldings (white arrow) are apparent in most areas and contain abundant elongated mitochondria (m) arranged perpendicular to trilaminar basal lamina (red arrow). The nucleus (n) is spherical and euchromatic. B. Fipronil group showing fragmented microvilli (star), apparent wide spaces in the cytoplasm (S) and few remnants of lysosomes (arrowhead), degenerated mitochondria with no cristae (m). Basal infoldings are lost in most areas (white arrow). Shrunken nucleus (n) with loss of part of the nuclear membrane (red arrow). C. Fipronil + Uncaria group showing rounded, euchromatic nucleus (n), several mitochondria (m), few lysosomes (arrowhead) and basal infoldings (white arrow). D. Fipronil + L arginine group is showing distorted microvilli (MV), lysosomes (arrowhead) and basal lamina (red arrow) with spaces in the cytoplasm (S). Destructed mitochondria with no cristae (m), shrunken nucleus (n) with the preserved nuclear membrane, and less preserved basal infoldings (white arrow). Moreover, multiple lysosomes are seen

Table 1: Effect of *Uncaria tomentosa* and L-arginine against fipronil toxicity on relative kidney weight and serum biochemical analysis.

	Control	Uncaria	L-arginine	Fipronil	Fipronil + Uncaria	Fipronil + L-arginine
Relative kidney weight (g %)	0.26^{a}± 0.01	0.23^{a}± 0.01	0.27^{a}±0.00	0.09^{d}±0.02	0.11^{c}±0.01	0.22^{b}±0.03
KIM-1 (Pg/mL)	0.28^{c}± 0.00	0.27^{c}± 0.01	0.28^{c}±0.01	0.48^{a}±0.01	0.33^{b}±0.03	0.34^{b}±0.01
Creatinine (mg/dL)	0.80^{b}± 0.07	0.49^{c}± 0.06	0.77^{b}±0.07	1.24^{a}±0.13	0.82^{b}± 0.07	0.78^{b}±0.06
Uric acid (mg/dL)	$3.65^{b\,c}$± 0.18	3.13^{c}± 0.32	3.50^{bc}±0.28	6.23^{a}±1.23	4.07^{b}±0.26	4.40^{b}±0.43
Urea (mg/dL)	37.52^{c}± 0.98	39.82^{c}± 2.48	35.26^{c}±1.48	71.49^{a}±8.14	36.34^{c}±0.87	60.09^{b}±1.12

Values are presented as means ±SE.
Means in the same row with different superscripts (a-d) are significantly different (*P≤0.05*).

Table 2: Effect of *Uncaria tomentosa* and L-arginine against fipronil toxicity on malondialdehyde (MDA), total antioxidant capacity (TAC), and interleukin-18 (IL-18) levels in renal tissue homogenate.

	Control	Uncaria	L-arginine	Fipronil	Fipronil + Uncaria	Fipronil + L-arginine
MDA (nmol/g)	1.24^{c} ± 0.05	1.21^{c} ± 0.11	1.26^{bc} ± 0.08	1.79^{a} ± 0.06	1.42^{bc} ± 0.08	1.50^{b} ± 0.07
TAC (U/mg)	53.13^{ab} ±3.46	60.04^{a} ±3.19	55.35^{ab} ±1.69	37.38^{d} ±2.35	47.52^{bc} ±3.29	40.45^{cd} ±2.05
IL-18 (Pg/mL)	0.53^{c}±0.03	0.46^{d} ± 0.02	0.53^{c} ± 0.03	0.92^{a} ± 0.02	0.61^{b} ±0.02	0.69^{b} ±0.02

Values are presented as means ±SE.
Means in the same row with different superscripts (a-d) are significantly different (*P≤0.05*).

DISCUSSION

The kidney is a vulnerable organ to pesticide damage as it has a critical role in pesticide biotransformation. Fipronil, a relatively new, widely used pesticide, has an increasing concern due to its environmental and human health effects. Its excretion and accumulation in the kidney resulted in structural and functional changes localized mainly in the nephron's glomeruli, proximal and distal convoluted tubules[26,27].

According to the present study, rats treated with fipronil (9.7 mg/kg) for 6 weeks showed a considerable reduction in serum urea, uric acid, and creatinine levels, as well as a significant reduction in relative kidney weight. These results are consistent with other studies[1] reporting renal dysfunction following subchronic exposure to 10 mg/L drinking-water concentrations of fipronil for 45 days, as indicated by increased serum levels of uric acid and creatinine. Elevated uric acid levels may result from the breakdown of pyrimidines and purines, or from excess production or an inability to be excreted. Elevated blood creatinine levels indicate impaired renal function[1]. This may be associated with a significant increase in abnormally shrunken renal corpuscles, as shown by histopathological examination in the present study. In addition, the significant reduction in relative kidney weight may be related to the shrunken glomeruli, disrupted renal tubules, and degraded collagen fibers.

The group that received fipronil showed a substantial reduction in TAC and an increase in renal MDA levels, according to renal tissue homogenate. A significant by-product of lipid peroxidation is renal MDA[28]. The intensity of the cellular injury and membrane damage is indicated by its increase in the renal tissue, which represents oxidative stress and the generation of reactive oxygen species[29]. The considerable decrease in TAC is due to the elevated production of ROS, which affects the antioxidant system by disrupting the antioxidant enzymes activities[30,31].

In the current study, considerable histopathological affection was found in the rat renal cortex. The majority of the glomeruli were severely damaged and shrunken. Bowman's capsule and glomerular basement membrane discontinuity were seen together with congested interstitial blood vessels and glomerular capillaries. While some corpuscles exhibited adhesions between the glomerulus and capsular epithelium, others had ruptured Bowman's capsule. Collagen fibre fragmentation within the glomeruli and disruption of the basal lamina surrounding the renal tubules were observed. These results are comparable to another study[26] that gave mice fipronil (10 mg/kg) orally every day for 28 days. In a different experiment, rats given fipronil orally (19.4 mg/kg) for five days showed histological kidney lesions[32].

Fipronil is slowly metabolised, seven days after a single oral dose, 5-25% of orally administered fipronil is eliminated in urine whereas 45-75% is excreted in faeces. This data could help to clarify fipronil-induced nephrotoxicity[33]. Owing to entero-hepatic recirculation, where about 16 distinct derivatives of fipronil are present in bile, the clearance of fipronil metabolites is delayed[34]. Fipronil's delayed biotransformation causes the drug to accumulate in cellular compartments, impairing mitochondrial respiratory function and causing apoptotic cell death[35].

It's interesting that we observed capsular adhesions, also known as glomerular tip adhesions or Bowman's capsule adhesions, in the fipronil group. These are characterised as a continuity of the extracellular matrix of the glomerulus and Bowman's parietal layer[36]. Capillaries and the associated podocytes move peripherally, adhere to the parietal epithelium, and create a tight junction, which is how capsular adhesions are explained[37]. The initial committed lesion for focal segmental glomerulosclerosis is inevitably started by contact of podocytes to parietal cells, which leads to the creation of adhesions (FSGS). The course of IgA nephropathy may be influenced by the significance of glomerular tip adhesions, which is a predictive factor irrespective of the degree of interstitial injury to the kidney[38,39].

The majority of fipronil group's proximal and distal convoluted tubules were damaged, disordered, and lost their cellular architecture. While some tubules possessed vacuolated cytoplasm and pyknotic nuclei, others displayed cell sloughing in their lumina. Collagen fibres surrounding the renal tubules were found to be fragmented and to have a focal loss of the brush boundary. Proximal tubule cells had electron-dense broken mitochondria, rarefied vacuolated cytoplasm, fragmented microvilli, and lysosome remnants. The nuclei exhibited discontinuous nuclear membranes, were smaller than usual, and apically displaced. A considerable rise of various biochemical markers levels as KIM-1 in the blood and IL-18 in the tissues supported these histopathological alterations.

The most frequent reason of toxic acute kidney injury (AKI), according to reports from underdeveloped nations, is self-poisoning using pesticides. A biomarker called KIM-1 can spot early AKI. Additionally, toxic and nontoxic AKI both cause additional injury and IL-18 is required to prevent this[40]. It is advised to use KIM-1, a transmembrane glycoprotein, as a biomarker of renal proximal tubule insult because of its substantial and specific increase in renal proximal tubule injury. In addition, IL-18 is one of the mediators of ischemic injuries to the kidney and is broadly expressed as a proinflammatory cytokine in damaged proximal tubules where it is also involved in the immunological response[41,42 43].

Fipronil exposure damages mitochondria, which results in increased ROS and decreased cell energy. The removal of damaged mitochondria by cells by autophagy results in autophagic cell death, and the Cytochrome-c is released when mitochondrial membrane potential is lost causing the activation of caspase pathways, which induce the deletion of renal cells through apoptosis[44].

On the other hand, the groups treated with *Uncaria tomentosa* and L-arginine (group V and group VI, respectively) showed improved biochemical characteristics (serum creatinine, uric acid, TAC, MDA, KIM-1 and IL-18) and histopathology findings. In comparison to fipronil group, the proportion of abnormal renal corpuscles was noticeably reduced, there was less glomerular capillary obstruction, GBM continued, and podocytes, endothelial cells and mesangial cells were nearly normal. Bowman's space had fewer capsular adhesions and a greater increase in collagen fibers.

By increasing levels of pro-inflammatory cytokines and increasing nitric oxide (NO) production, L-arginine reduced the negative effects on endothelium and restored GFR[45]. Under conditions of oxidative stress brought on by renal failure, the primary factor controlling glomerular blood flow is NO[46,47]. To restore tubuloglomerular feedback mechanisms, IL-1, IL-6, and IL-8 upregulate it[48]. L-arginine additionally protects against the generation of peroxynitrite anions in renal glomeruli and removes superoxide anions directly from the body[47,49,50,51]. Additionally, L-arginine could reduce renal disease, and its treatment reduced the growth of interstitial fluid and collagen deposition in the obstructed kidney. In addition, L-arginine reduced the number of abnormal glomeruli and proteinuria in subtotal nephrectomized or diabetic rats[10].

Along with oxindole, indole alkaloids, and flavonoids, which are both antioxidants and anti-inflammatory substances, *Uncaria tomentosa* can repair damaged cells[12,52]. By blocking the activity of xanthine oxidase and/or free radical absorbers, antioxidant treatment reduces oxidative damage[53]. By removing ROS, active ingredients in *Uncaria tomentosa* may reduce lipid peroxidation. By inhibiting NF-β, Rats' oxidative stress and liver damage caused by fipronil have been found to be decreased by *Uncaria tomentosa* extract[14].

With continuous PAS-positive basement membrane, the P.T and D.T in Groups V and VI were almost completely intact. Group VI exhibited a greater degree of localised loss of the proximal tubules' brush border. All those findings were supported by ultrastructural analysis, which revealed that the proximal tubules had less damaged mitochondria, lysosomes, and basal lamina. We conclude by stating that the structural and functional effects of fipronil nephrotoxicity, including biochemical, histological, and ultrastructural changes, were alleviated by L-arginine and *Uncaria tomentosa*. The group receiving *Uncaria tomentosa* and fipronil showed better outcome.

ABBREVIATIONS

Kidney injury molecule-1 (KIM-1)

Malondialdehyde (MDA)

Interleukin-18 (IL-18)

Total Antioxidant Capacity (TAC)

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسه نسيجيه و بنيه اساسيه و كيميائيه حيويه للتاثيرات الوقائيه للانكاريا تومينتوسا و ال-ارجينين ضد السميه الكلويه المستحثه بالفبرونيل فى ذكور الجرذان البيضاء

نعمه سليمان، مروه احمد البلتاجى، هبه محمد عبد الرازق، سحر جلال جوده

المقدمه: يسبب الفبرونيل تاثيرات ضاره على الكلى مسببا سميه كلويه عن طريق الاجهاد التاكسدى. تهدف هذه الدراسه الى مقارنة التاثيرات الوقائيه للانكاريا تومنتوسا مقابل ال-ارجينين ضد السميه الكلويه التى يسببها الفبرونيل.

المواد و الطرق: تم اختيار سته و ثلاثين ذكورا من الجرذان البالغه و تقسيمها بشكل عشوائى الى ست مجموعات و تلقوا العلاجات التاليه عن طريق الفم لمدة 42 يوما: المجموعه الاولى- ماء مقطر، المجموعه الثانيه – انكاريا تومنتوسا (250 مجم \كجم من 20% مستخلص انكاريا تومنتوسا)، المجموعه الثالثه – ال-ارجينين (200 مجم \كجم من 20% ال-ارجينين)، المجموعه الرابعه – فبرونيل (9.7 مجم \كجم، 1\10 من LD50)، المجموعه الخامسه – فبرونيل + انكاريا تومنتوسا، لمجموعه السادسه – فبرونيل + ال-ارجينين، فى نفس الجرعات المذكوره سابقا.

قمنا بتقييم الانشطه الكلويه لمالونديالديهيد الكلوى (MDA) و القدره الاجماليه لمضادات الاكسده (TAC) ، و IL-18 ، و كذلك الوزن النسبى للكلى، و الكرياتين ،و حمض البوليك، و اليوريا، و جزىء اصابة الكلى1- (KIM-1). كما قمنا بفحص البنيه العامه للكلى و الكبيبات و الانابيب القريبه و البعيده و الياف الكولاجين. كما فحصنا الكريات الكلويه و الانابيب القريبه بواسطة المجهر الاكترونى.

النتائج: تسبب العلاج بالفبرونيل فى زيادة ($P<0.05$) القياسات البيوكيميائيه فى الدم، MDA الكلوى ، IL-18 ، و انخفاض كبير فى TAC. كما كان هناك زياده فى نسبة الكبيبات المنكمشه و تمزق الغشاء القاعدى و تكسير الكولاجين و التصاقات المحفظه. الفحص بالمجهر الالكترونى اظهر تدهور الميتكوندريا و تكسير الخملات الدقيقه للانابيب القريبه. انكاريا تومنتوسا و ال-ارجينين لهم تاثير وقائى ضد التغيرات الناتجه عن العلاج بالفبرونيل.

الإستنتاج: انكاريا تومنتوسا و ال-ارجينين وقائيين ضد السميه الكلويه المحدثه بالفبرونيل، المجموعه التى تلقت فبرونيل + انكاريا تومنتوسا وفرت حمايه افضل.