Original Article

The Effect of Exposure to Fipronil on the Structure of Adult Albino Rat Renal Cortex and Possible Role of Gallic Acid

Sally S. Anwer and Noha A. Rashed

Department of Human Anatomy and Embryology, Faculty of Medicine, Assiut University, Assiut

ABSTRACT

Introduction: The Kidney is highly specialized and sensitive organ to oxidative stress. Fipronil as an insecticide has a toxic effect on different organs. Gallic acid is one of the most abundant phenolic acids in the plant kingdom. It is a colorless or slightly yellow crystalline compound, with extensive application in the food and pharmaceutical industries.

Aim of the Work: To detect the effect of fipronil on the structure of adult albino rat renal cortex and also, to identify the role of gallic acid as an ameliorative substance.

Materials and Methods: A total number of 40 adult albino rats weighting (200-250) gm were used. They divided into 4 groups: control group, fipronil received group, fipronil and gallic acid received group and gallic acid received group. The rats received treatment (fipronil at a dose 10 mg/kg body weight orally and gallic acid at a dose 10 mg/kg dissolved in saline orally by an intragastric tube daily) for 28 days. Then, they were scarified and their kidneys were manipulated for light and electron microscopy histological examination and Immunohistochemistry study. Morphometric and statistical studies were also performed.

Results: The present study showed that fipronil causes destruction in the cells if renal cortex at the glomeruli, proximal and distal convoluted tubules. Morphometric results revealed statistically significant decrease in the mean number of epithelial cells in proximal convoluted tubules in fipronil received group as compared with control group. Most of destructive changes are improved by gallic acid.

Conclusion: Concomitant administration of gallic acid could reduce the side effects caused by fipronil on rat kidney.

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Key Words: Fipronil; gallic acid; immuno-histochemistry; kidney; ultrastructural study.

Corresponding Author: Noha A. Rashed, PhD, Department of Human Anatomy and Embryology, Faculty of Medicine, Assiut University, Assiut, **Tel.**: +20 10 1564 0083 **E-mail:** noharashed@aun.edu.eg

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INTRODUCTION

Fipronil is an innovative phenyl pyrazole selective pesticide which has been gaining popularity as an effective way of controlling pests and safeguarding crops. There is an increasing chance that fipronil may have adverse effects on the antioxidant system in both humans and animals' defensive technique due to improper or overuse of fipronil, which contaminates soil and water[1]. Inhibiting the suppressive reaction of gamma aminobutyric acid (GABA) leads to toxic effects of fipronil by reaching GABAA-regulated chloride channels[2]. Target organs for fipronil toxicity include the liver, brain and kidney as examples for the organs with rich blood supply[3]. This insecticide is metabolized and cleared by the liver and the kidneys. The sensitivity of these tissues can be attributed to the unbalance between oxidative stress and antioxidant capacity induced by fipronil[4]. A mechanism of toxicity can be explained by the changes occur after exposure leading to modifications in enzymatic (GPx, SOD, and CAT) and nonenzymatic (GSH) antioxidants[5]. Studies reported that fipronil suppress respiratory chain of mitochondria and calcium balance, oxidative and nitrosative stress, furthermore destruction of amino acids leading to devastating effects on liver, brain and kidneys[1].

Owing to their role in biotransformation of fipronil, the kidneys are sensitive target organs of insecticide toxicity. Oxidative stress is reported after the vulnerability to low amount of fipronil. Fipronil causes the attack of oxidants on lipid (LPO) which produce lipid peroxyl radicals, hydroperoxides, and different oxidation products inducing oxidative stress in rats leading to pathological lesions in the kidney[6].

Polyphenyl category products which presented naturally include gallic acid and its imitative are plenteous in food beverages like green tea and red wine. It is believed to have anti-inflammatory, anticancer and antioxidant properties[7]. Many considerations, for instance the number, site of bounding and reciprocal positions of hydroxyls on the aromatic ring affect the efficacy of these antiradical and antioxidant reactions of Gallic combinations. Gallic acid reduces the demand of excess sugar so that the oxidative stress is reduced[8]. The ability of inhibiting cellular damage is considered as a protective function of gallic acid. This happens by inhibiting reactive oxygen species, producing programmed cancer cell death, increasing glutathione peroxidase (GPX) expression, and mitigating free radicals' existence[9,10].

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Despite the previous studies on Fipronil have concentrated on its effect on various tissues, however, there is limited information about its effects on the histological, immunohistochemical and morphometric aspects of kidney of Albino rat. Therefore, this study intended for detecting the effects of fipronil on the kidney of adult Albino rat and to appraise the co-administration of gallic acid role in protection against these structural changes in it.

MATERIAL AND METHODS

Chemicals

We obtained Fipronil in the form of solution (Fipromex® 20% EC, MAC-GmbH, Company, Germany) from a local pesticide market, Egypt. Gallic acid was purchased from Solaribo Chemical Reagent with purity 99%.

Experimental Animals

The study included 40 adult male albino rats weighting (200-250) gm. The rats were obtained and housed in fine wood bedded cages at the animal house of Assiut University. The animals were kept under optimal appropriate conditions with (12/12) hours light/dark cycle at optimal temperature (25 ± 5) ºC. Food and water ad libitum were given. The experiment was carried out after the approval of the Scientific Research Ethics Committee of the Faculty of Medicine of Assiut University (local approval number: 04-2022-300001) that follow the recommendations of the National Institute of Health Guides for Laboratory Animals Care and Use.

Experimental design

Rats were randomly divided into four groups (ten rats in each one).

Group 1 (Control group): was given saline only.

Group 2 (Fipronil-received group): The rats of this group received fipronil at a dose 10 mg/kg body weight dissolved in saline. The selected dose of fipronil was 1/10 of oral LD50[3].

Group 3 (Fipronil and gallic acid received group): The rats of this group received fipronil at a dose 10 mg/kg body weight in concomitant with the use of gallic acid at a dose (10 mg/kg) mixed in saline[3,11].

Group 4 (Gallic acid received group): The rats of this group received gallic acid (10 mg/kg) dissolve in saline[11].

All treatments were given orally via intragastric tube once daily for 28 days

At the end of the 28 days post administration, all rats were anesthetized by breathing ether and the wall of their chest opened. Intracardiac perfusion started in the left ventricle by 0.9% saline and 4% paraformaldehyde solution after ligation of the descending aorta. The perfusion continued until the right atrial venous return became clear. Rat kidneys were removed and preserved in 10% neutral buffered formalin for subsequent histopathological and immunohistochemical analyses[12].

Histological study

Light microscopic study

The cortices of right kidneys were selected for light microscopic study and cut into small pieces then rapidly fixed in 10% formaldehyde solution for 48 hours. The specimens were placed in ascending alcohol grades for dehydration. Clearing was done by xylene. Then, milted paraffin was used for embedding the specimens. Lastly, the blocks of paraffin were cut into 5 mm thickness slices and stained by hematoxylin and eosin to demonstrate the general histologic structure and Sirius red to assess collagen fibers[13]. The optical microscope (OLYMPUS CX31-Japan) was used for examining and photographing the stained slides. This was done at the Department of Human Anatomy and Embryology, Faculty of Medicine, Assiut University.

Electron microscopic study

The cortices of left kidneys were chosen for electron microscopic study and dissected into very small pieces (≈ $1mm^3$) then rapidly fixed using 4% cold glutaraldehyde for twenty-four hours. The fixed specimens were washed 3-4 times in phosphate buffer (PH 7.2) for 20 minutes each. Then, the specimens were put in 1% Osmium tetroxide for 2h. Then, the samples were washed four more times in the same buffer. Dehydration was done by butting the specimens in ascending grades of alcohol for 2 hours. Finally, embedding in Epon araldite mixture was performed and semithin sections (0.5 micron) and ultrathin sections (50 nm in thickness) were done by the ultra-microtome. The semithin sections were stained with toluidine blue. The ultrathin sections were stained with lead citrate and uranyl acetate[14]. The transmission electron microscope (Joel-JEM-100 CXII, Joel; Tokyo, Japan) at Assiut University Electron Microscopic Unit was used for photographing the ultrathin sections.

Immunohistochemistry

Immunohistochemical study for caspase-3 was done on cortices of right kidney to evaluate the apoptotic state. Phosphate buffered saline was used for washing renal sections for 5 minutes. Then, they were treated with an antibody to caspase-3 at a dilution of 1:200 (In*vitro*gen; Sweden AB; Stockholm, Sweden) overnight at 4°C. Then, they were washed and incubated with an antibody to secondary goat-anti-rabbit (1:500) (In*vitro*gen; Molecular Probes; Eugene, Oregon, USA) for 1 hour at room temperature. Finally, following a 15-minute incubation period in 3,3-diaminobenzidene, the slides were counter-stained with Mayer's haematoxylin, dehydrated and mounted using dibutyl phthalate in xylene (DPX)[15].

Morphometric Study and Statistical Analysis

Morphometric study was performed on Toluidine blue stained sections using the mean number of cells in the proximal convoluted tubules as an example for the effect of fipronil on the renal cortex per an area of 14.380 μ^2 at

magnification × 400 in all studied groups were measured using a computerized image analyzer system (Leica Q 500 MCO; Leica, Wetzlar, Germany) connected to a camera which is linked to a Leica universal microscope. From 10 sections of each group, ten non-overlapping fields were randomly chosen for measuring the number of epithelial cells of proximal convoluted tubules. The magnification used was X400 for cell numbering. SPSS (Inc., Chicago, Illinois, USA) version 21.0 was used for the statistical analysis and measuring mean, standard deviation (SD) and *p-value*. One-way analysis of variance (ANOVA) test followed by post-hoc Tukey test was performed. The results were expressed in the form of mean ± SD. We considered the *p-value* < 0.05 as statistically significant.

RESULTS

Histological Results

(A) Light microscopic results

1-Hematoxyline and eosin-stained results

Group 1 (Control group): Renal cortex sections of adult control group were examined. The examination revealed that the cortex is composed of glomeruli, proximal and distal convoluted tubules. The glomeruli consisted of a capillary tuft encircled by Bowman's space between the parietal and visceral layers of the capsule (Figure 1a) The proximal convoluted tubules were composed of cuboidal epithelial cells with its characteristic brush border (Figure 1a). Also, the distal convoluted tubules contained epithelial cells which were low cuboidal (Figure 1a).

Group 2 (Fipronil received group): Renal cortex sections of this group were examined after hematoxylin and eosin staining. The examination of glomeruli reveled fragmented capillary tuft, contracted glomeruli and ill -defined parietal layer of Bowman's capsule (Figure 1b). Cells in the proximal and distal convoluted tubules both showed vacuolated cytoplasm and pyknotic nuclei (Figure 1b). The lumen of some of tubules contained cellular debris as evidence of desquamation (Figure 1b).

Group 3 (Gallic acid and fipronil treated group): Renal cortex examination of this group was nearly normal having normal Bowman's space and well - defined parietal layer of Bowman's capsule (Figure 1c). The proximal and distal convoluted tubules' epithelial cells have prominent nuclei and nucleoli except some cells which had darkly stained nuclei (Figure 1c). There was blood extravasation in the interstitial space in between the renal tubules.

Group 4 (Gallic acid received group): Renal cortex sections' examination seemed to be like the control group (Figure 1d).

2-Sirius red - stained results

Group 1 (Control group): Renal cortex sections of adult control group were examined. The examination revealed the normal distribution of collagen fibers in the renal cortical tissue. The fibers arranged in relation to parietal and visceral layers of Bowman's capsule (Figure 2a). Furthermore, the fibers surrounded distal as well as proximal convoluted tubules (Figure 2a).

Group 2 (Fipronil received group): Renal cortex sections of this group by Sirius red staining were examined. The examination showed fibrosis in the renal cortical tissues as evidenced by excessive deposition of collagen fibers in relation to glomeruli and tubules (Figure 2b).

Group 3 (Gallic acid and fipronil treated group): Renal cortex of this group was examined. The examination showed apparent reduction in collagen deposition in the renal cortical tissue with administration of gallic acid and fipronil (Figure 2c).

Group 4 (Gallic acid received group): The examination of cortical tissue of the kidney by using Sirius red stain appeared to be like the control group (Figure 2d).

3- Toluidine blue-stained results

Group 1 (Control group): Upon examining the semithin sections of the control group stained with Toluidine blue, it was observed that the glomerulus consisted of a tuft of capillaries encircled by Bowman's capsule which was differentiated into parietal and visceral layers of epithelial cells, with Bowman's space situated between them (Figure 3a). The lining cells of both proximal and distal convoluted tubules had well defined outline and prominent nuclei (Figure 3a). The proximal convoluted tubules' epithelial cells had apical brush border and basal striations (Figure 3a).

Group 2 (Fipronil received group): In Fipronil received group, the glomerular cells appeared to be destructed with darkly stained nuclei with widening of Bowman's space (Figure 3b). Furthermore, the cells of proximal convoluted tubules showed disintegration as cytoplasmic vacuolations, indistinct borders and dark nuclei (Figure 3b). Also, the cells of distal convoluted tubules were destructed as evidenced by rarified cytoplasm with indefinite nuclei (Figure 3b). There was cellular debris inside the tubular lumen and inflammatory infiltrates in between the tubules (Figure 3b).

Group 3 (Gallic acid and fipronil received group): Examination of semithin sections of this group demonstrated improvements in the cortical tissue of the kidneys in the form of well distinct outline of glomeruli (Figure 3c). Proximal and distal convoluted tubules' epithelial cells showed prominent nuclei and nucleoli (Figure 3c). There was a residual degeneration in cells of renal cortical tubules in the form of dense nuclei and vacuolated cytoplasm (Figure 3c).

(B) Electron Microscopic Results

Group 1 (Control group): On examinations of ultrathin sections of glomeruli in the renal cortex of control group revealed normal architecture in the form of glomerular basement membrane, endothelial cell, podocyte with foot processes and mesangial cells (Figure 4a). The proximal

convoluted tubules' epithelial cells in control group revealed prominent nucleus and well-defined brush border (Figure 5a). The cytoplasm possessed mitochondria, resorption vacuoles and free ribosomes (Figure 5a). The distal convoluted tubules of this group had basal striations with rounded nucleus (Figure 6a). The cytoplasm contains numerous longitudinally arranged mitochondria with pinocytotic vesicles (Figure 6a).

Group 2 (Fipronil received group): The examination of glomerulus under electron microscope in this group showed thickening in the glomerular basement membrane (Figure 4b). The endothelial cell had contracted nucleus with chromatin condensed peripherally (Figure 4b). The cytoplasm was vacuolated with cellular debris inside the capillary lumen (Figure 4b). The foot processes of podocyte couldn't be observed (Figure 4b). The nucleus of proximal convoluted tubules in this group showed peripheral chromatin condensation (Figure 5b). The apical microvilli were damaged, and the cytoplasm contained vacuolations, destructed mitochondria and lysosomes (Figure 5b). The distal convoluted tubules' epithelial cells showed thickened basement membrane(Figure 6b). The nucleus had irregular outline (Figure 6b). The cytoplasm had vacuolations and destructed mitochondria (Figure 6b). The cell membrane was interrupted with herniation of the membrane towards the lumen of tubule (Figure 6b).

Group 3 (Gallic acid and fipronil received group): Examination of glomerulus in this group revealed that the glomerular basement membrane appeared to be normal (Figure 4c). The podocytes had foot processes (Figure 4c). The capillary lumen contained cellular debris (Figure 4c). The endothelial cells showed ghost like bodies (Figure 4c). The proximal convoluted tubules epithelial cells showed identifiable basal infoldings with thickening in the basement membrane (Figure 5c). The nucleus had uniformly distributed chromatin and the brush border seemed to be intact. The cytoplasm had mitochondria, resorption vacuoles and areas of cytoplasmic vacuolations (Figure 5c). The distal convoluted tubules' epithelial cells had basal infoldings (Figure 6c). The nucleus is present with prominent nucleolus and diffusely arranged chromatin.

(C) Caspase-3 immunohistochemically stained results

Upon analysis of the control group's caspase-3 immunohistochemically stained sections we found weak immunoreactivity response towards Caspas-3 (Figure 7a). While in the group that received fipronil only, there was strong positive reaction as an intense brown coloration especially in relation to renal tubules and glomeruli (Figure 7b). In third group which received both gallic acid and fipronil, there was moderate immune reaction in relation to tubules and glomeruli (Figure 7c).

(D) Morphometric results

Mean number of cells in the proximal convoluted tubules.

There was a statistically significant decrease in the mean number of cells in the proximal convoluted tubules in the renal cortex observed in fipronil treated group in comparison with the control. There was no statistically significant difference in proximal convoluted tubules' cells mean number in the treated group with gallic acid and fipronil in comparison with the control (Table 1, Histogram 1).

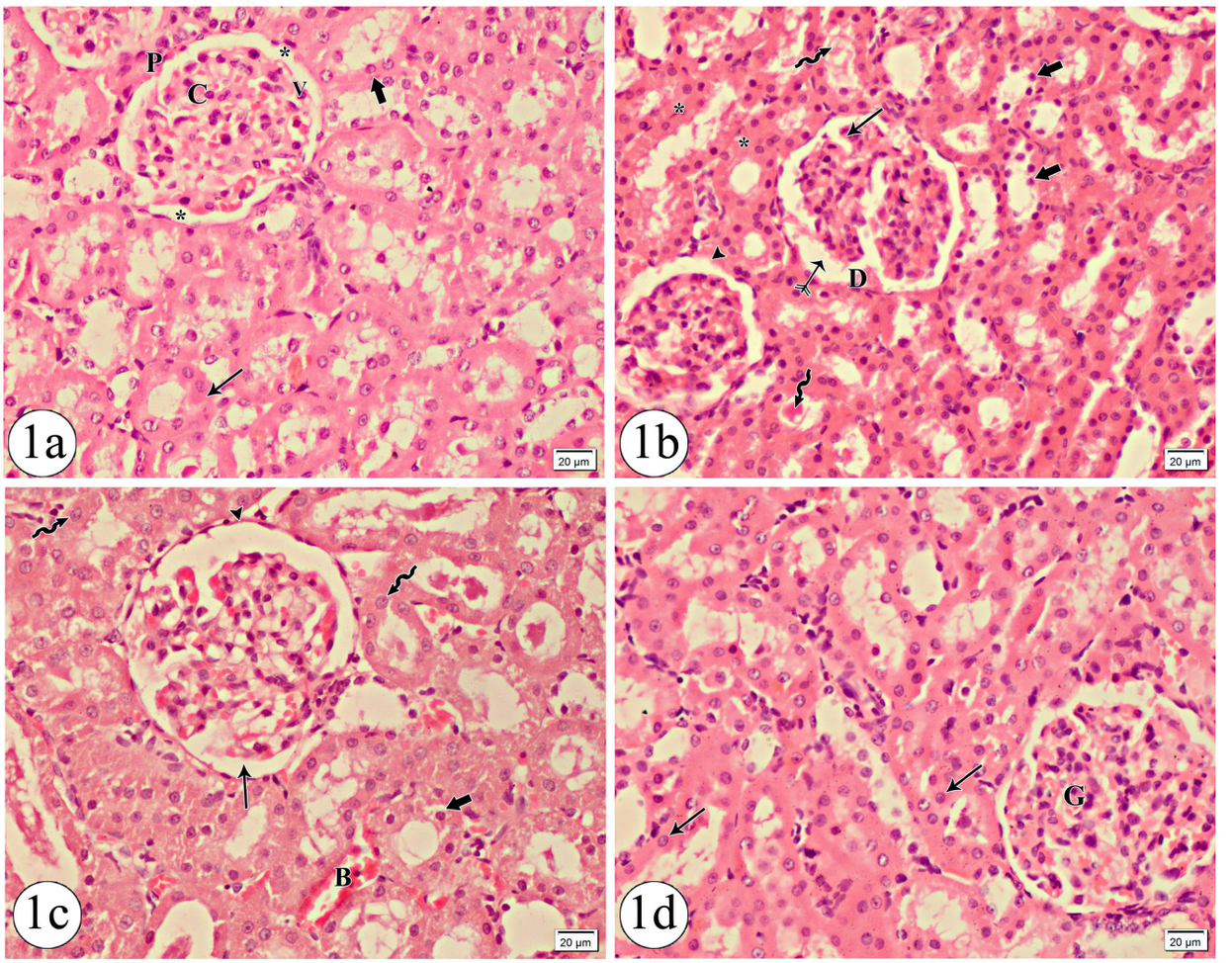


Fig. 1: photomicrographs of an adult albino rat's renal cortex section.
(1a) The control group displays the typical normal architecture of cortical structures. The glomerulus is composed of capillary tuft (C) with normal arrangement of cells of parietal layer (P) and Visceral layer (V) of Bowman's capsule. There is clear Bowman's space (star). The epithelial cells of proximal convoluted tubules are cuboidal in shape (thin arrow) with apical brush border (arrowhead). The cells of distal convoluted tubules are low cuboidal in shape (thick arrow). (1b) Treated group with fipronil showing disturbed cell architecture of glomeruli in the form of fragmentation of glomerular capillaries (thin arrow). There is an indistinct parietal layer of Bowman's capsule (arrowhead) with shrinkage of glomeruli and widening of Bowman's space (tailed arrow). Nearby are areas of necrosis in the glomeruli replaced by eosinophilic debris (D). The cells of tubules are showing vacuolations in cytoplasm with pyknotic nuclei (thick arrow). Some tubules are showing the cell debris due to desquamation of cells (wavy arrow). Note: There is absence of space between tubules (stars). (1c) Treated group with gallic acid and fipronil showing apparent decrease in the Bowman's space (thin arrow) as compared with previous group. There is a distinct Parietal layer of Bowman 's space (arrowhead). Many of the cells of renal tubules have prominent nucleus and nucleolus (wavy arrows). Other cells have vacuolated cytoplasm with darkly stained nuclei (thick arrow). Note: There is extravasation of blood that can be observed (B). (1d) Treated group with gallic acid only showing that structure of glomerulus (G) is like that of control with normal appearance of renal tubular cells (arrows).

(Hematoxylin and Eosin stain, X400)

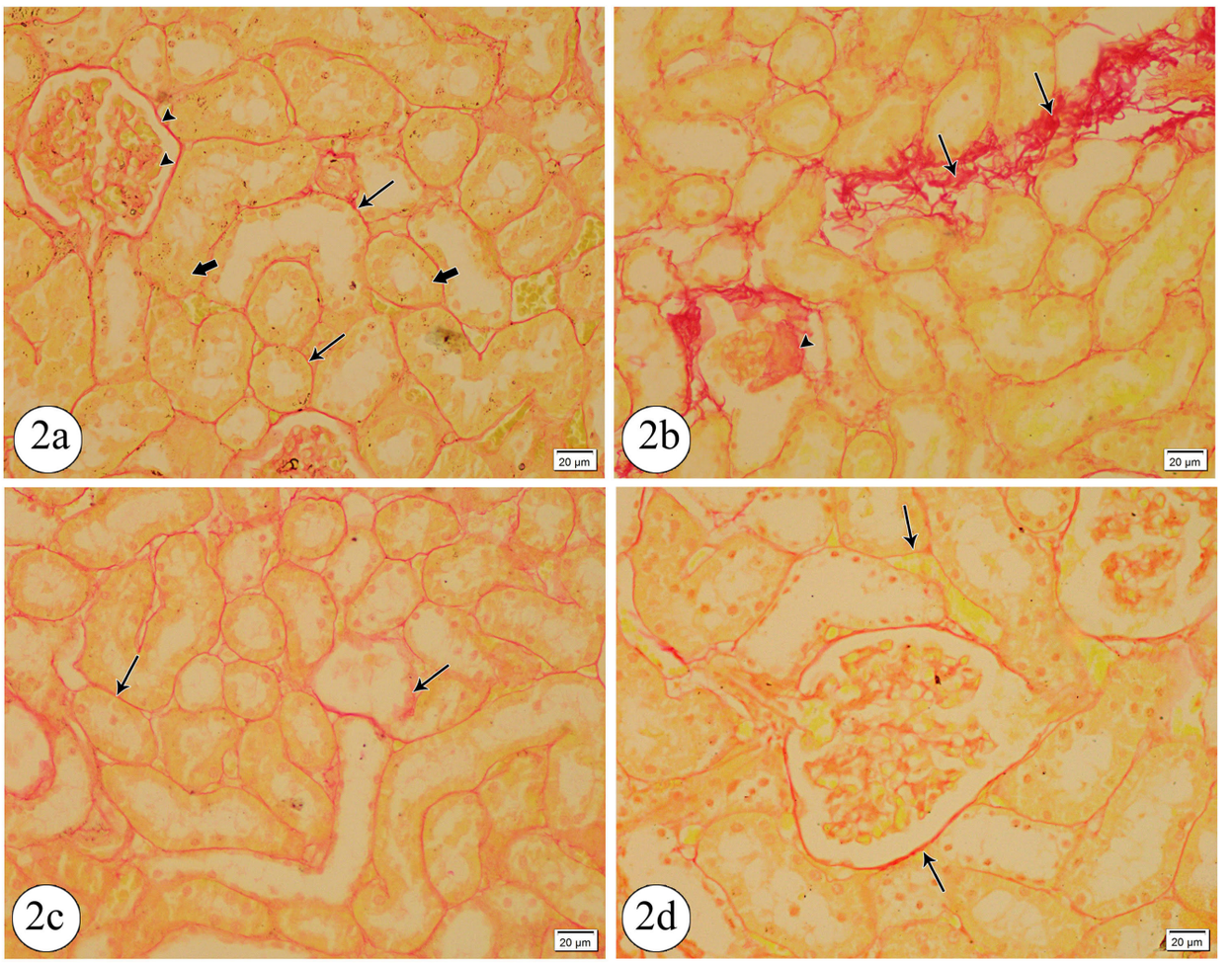


Fig 2: photomicrographs of an adult albino rat's renal cortex section.
(2a): The control group displays the red-colored collagen fiber arrangement along the parietal and visceral layers of the Bowman's capsule (arrow heads). Also, the collagen fibers surround the outline of renal tubules (thin arrows). The cytoplasm of renal tubular cells appeared yellow in color (thick arrows). (2b) Treated group with fipronil showing extensive deposition of collagen fibers in areas of destructed renal tubules (arrows). Also, there is deposition of collagen fibers inside glomeruli (arrowhead). (2c) Treated group with gallic acid and fipronil showing apparent reduction in collagen deposition in the renal cortical tissue (arrows) as compared with second group. (2d) Treated group with gallic acid only showing the distribution of collagen fibers as control group (arrows) (Sirius red stain, X400)

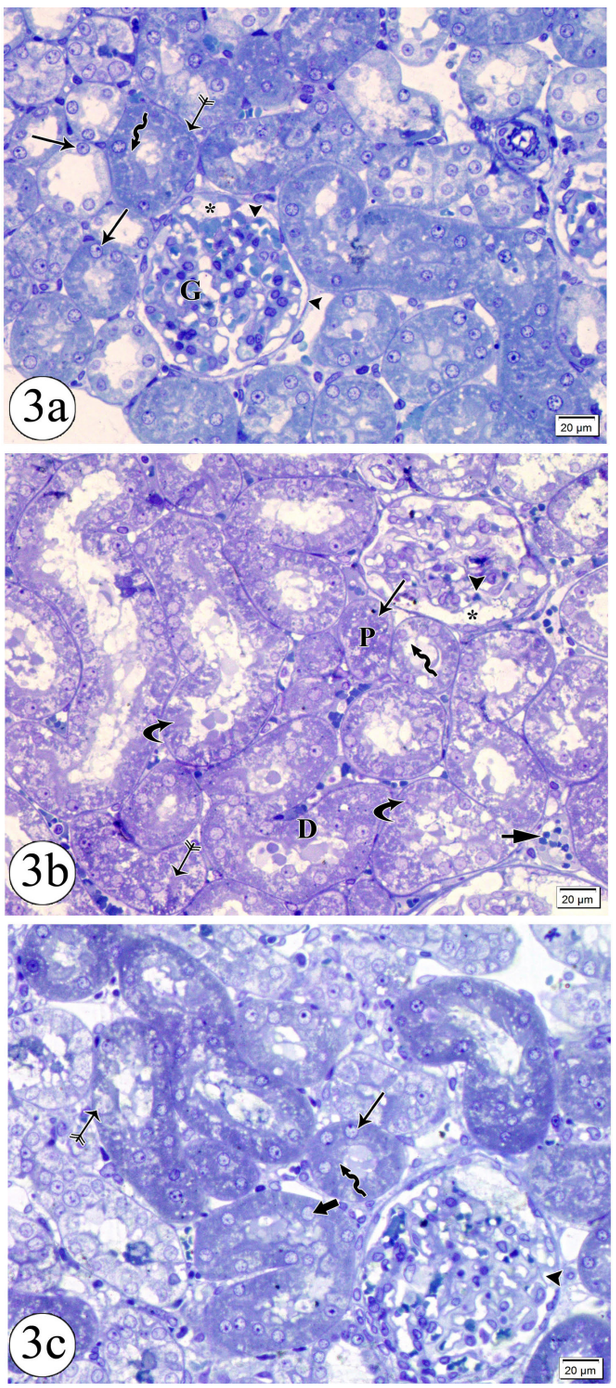


Fig 3: photomicrographs of semithin sections from an adult Albino rat's renal cortex.
(3a) Control group showing normal structure of glomerulus (G) with well-defined parietal and visceral layers of Bowman's capsule (arrowheads). There is well defined Bowman's space (star). The epithelial cells lining both proximal and distal convoluted tubules appear with well-distinct outline with prominent nuclei (arrows). The epithelial cells of proximal convoluted tubules showing apical brush border (wavy arrow) and basal striations (tailed arrow). (3b) Treated group with fipronil showing that the glomerular capillary tuft contains cells with darkly stained nuclei (arrow head). There is widening in Bowman 's space (star). In the proximal convoluted tubules (P), the cells have ill -defined borders with obliteration of tubular lumen (arrow). Some of cells have cytoplasmic vacuolations with shrunken nucleus (wavy arrow). The cells of distal convoluted tubules (D) showing cloudy swelling of cells with cellular debris inside the lumen (tailed arrow). Many of cells in distal convoluted tubules have destructed rarified cytoplasm with absent nuclei (curved arrows). Note the presence of inflammatory infiltrates in between tubules (short arrow). (3c) Treated group with gallic acid and fipronil showing distinct outline of glomerulus with hypercellularity of glomerular cells (arrow head). The epithelial cells in the proximal convoluted tubules have well prominent nucleus and nucleolus (thin arrow). There is well apparent brush border (wavy arrow). The distal convoluted tubules have regular well-defined outline (thick arrow) with cells have prominent nuclei. Some of cells in distal convoluted tubules have cytoplasmic vacuolations with dense nucleus (tailed arrow).

(Toluidine blue stain, X400)

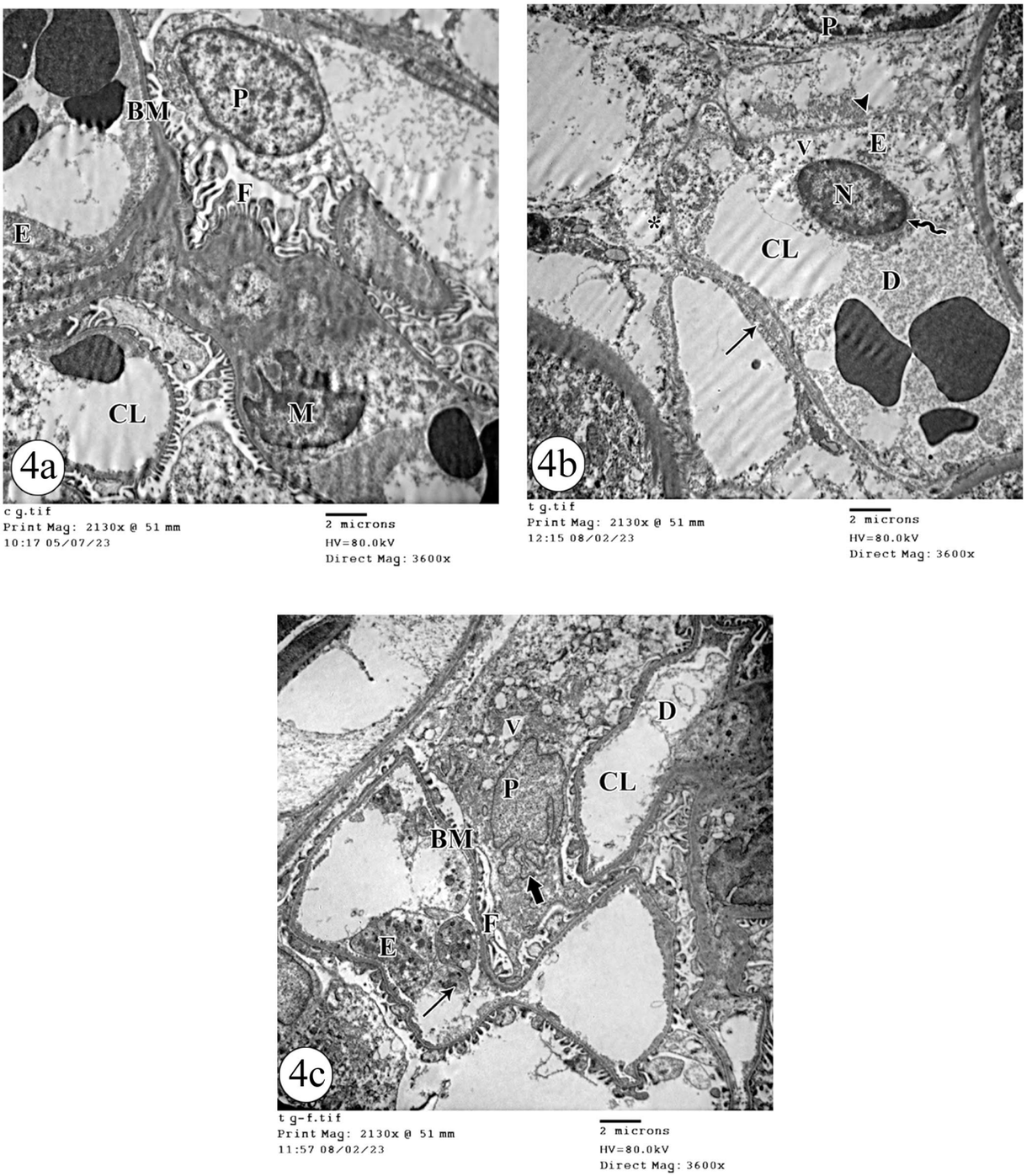


Fig 4: Electron photomicrographs of a section in the glomerulus in the renal cortex of adult Albino rat.
(4a) Control group showing the architecture of the glomerulus composed of the glomerular basement membrane (BM), endothelial cell (E), podocyte (P) with foot processes (F) and mesangial cells (M). Note the presence of capillary lumen (CL). (4b) Treated group with fipronil showing thickening in the glomerular basement membrane (arrow) and discontinuity of it in some regions (arrowhead). The endothelial cell (E) has contracted nucleus (N) with peripheral chromatin condensation (wavy arrow) and the cytoplasm shows vacuolations (V). The capillary lumen (CL) contains cell debris (D). The foot processes of podocyte (P) are absent (star). (4c) Treated group with gallic acid and fipronil showing the glomerular basement membrane (BM) appeared to be normal. The capillary lumen (CL) still contains cellular debris (D) and endothelial cell (E) has ghost like bodies (thin arrow). The podocytes (P) show the presence of foot processes (F) as compared with treated group with fipronil only. The nucleus of podocyte shows marked irregularity in its membrane (thick arrow) with the presence of cytoplasmic vacuolations (V).

(TEM X3600)

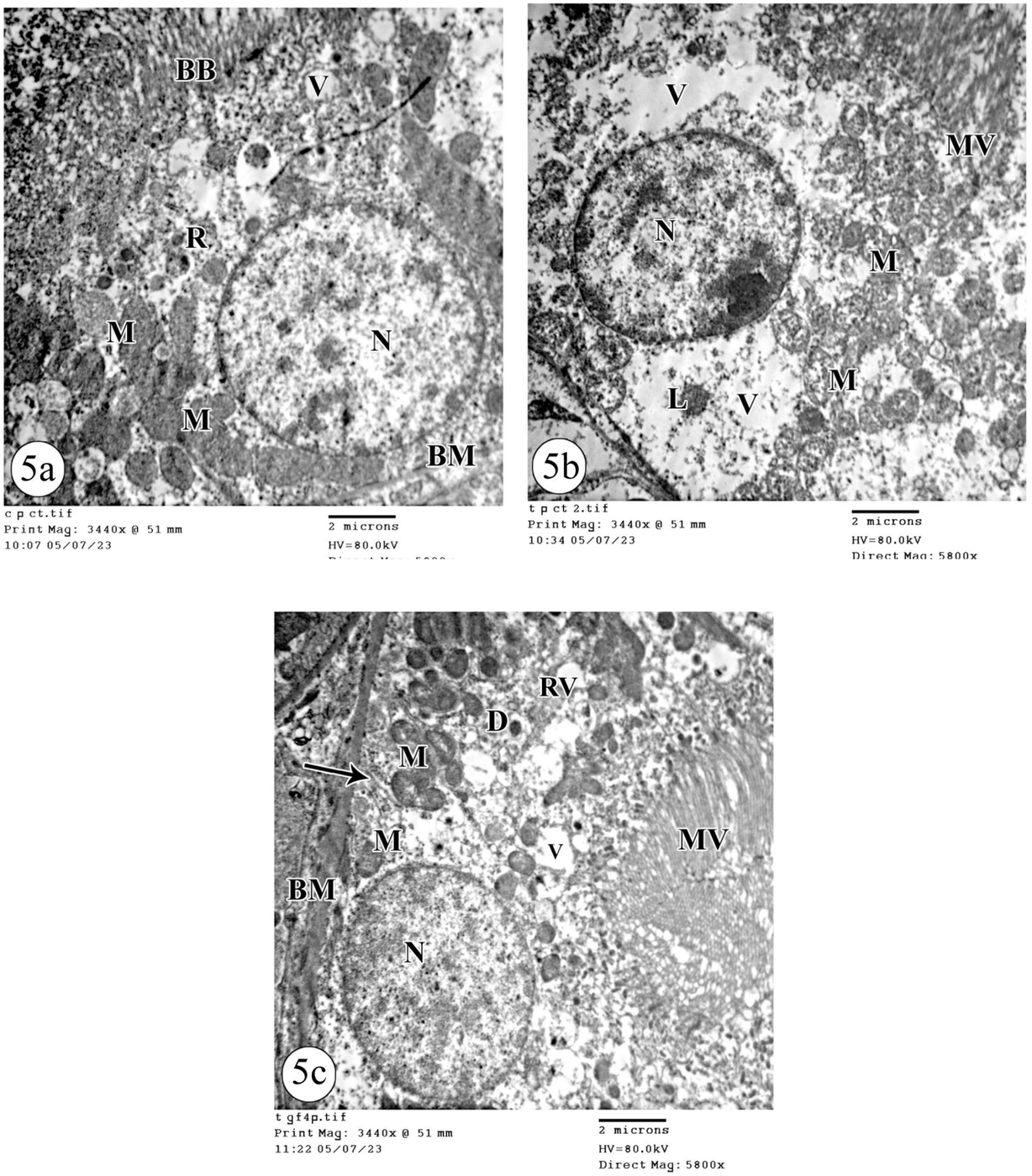


Fig 5: Electron photomicrographs displaying a portion of the proximal convoluted tubule (PCT) in the adult Albino rat renal cortex.
(5a) The control group demonstrates the basement membrane (BM) of the proximal convoluted tubule epithelial cells. The nucleus (N) has a regular outline and brush border (BB) is well evident. The cytoplasm contains resorption vacuoles (V) and numerous mitochondria (M) with identifiable cristae. Also, free ribosomes (R) are distributed all over the cytoplasm. (5b) Treated group with fipronil showing the nucleus (N) with the chromatin is condensed peripherally. There is destruction in the apical microvilli (MV) and the cytoplasm contains many vacuolations(V) and lysosomes (L). The mitochondria (M) appeared to be damaged due to destruction of its cristae. (5c) Treated group with Gallic acid and fipronil showing the presence of basement membrane (BM) of the epithelial cells which appeared to be thickened with identifiable basal infoldings (arrow). The nucleus (N) has uniformly distributed chromatin and the apical microvilli (MV) appeared to be intact. The cytoplasm contains mitochondria (M), resorption vacuoles (RV) and areas of cytoplasmic vacuolations (V). There are many dense bodies (D).

(TEM X5800)

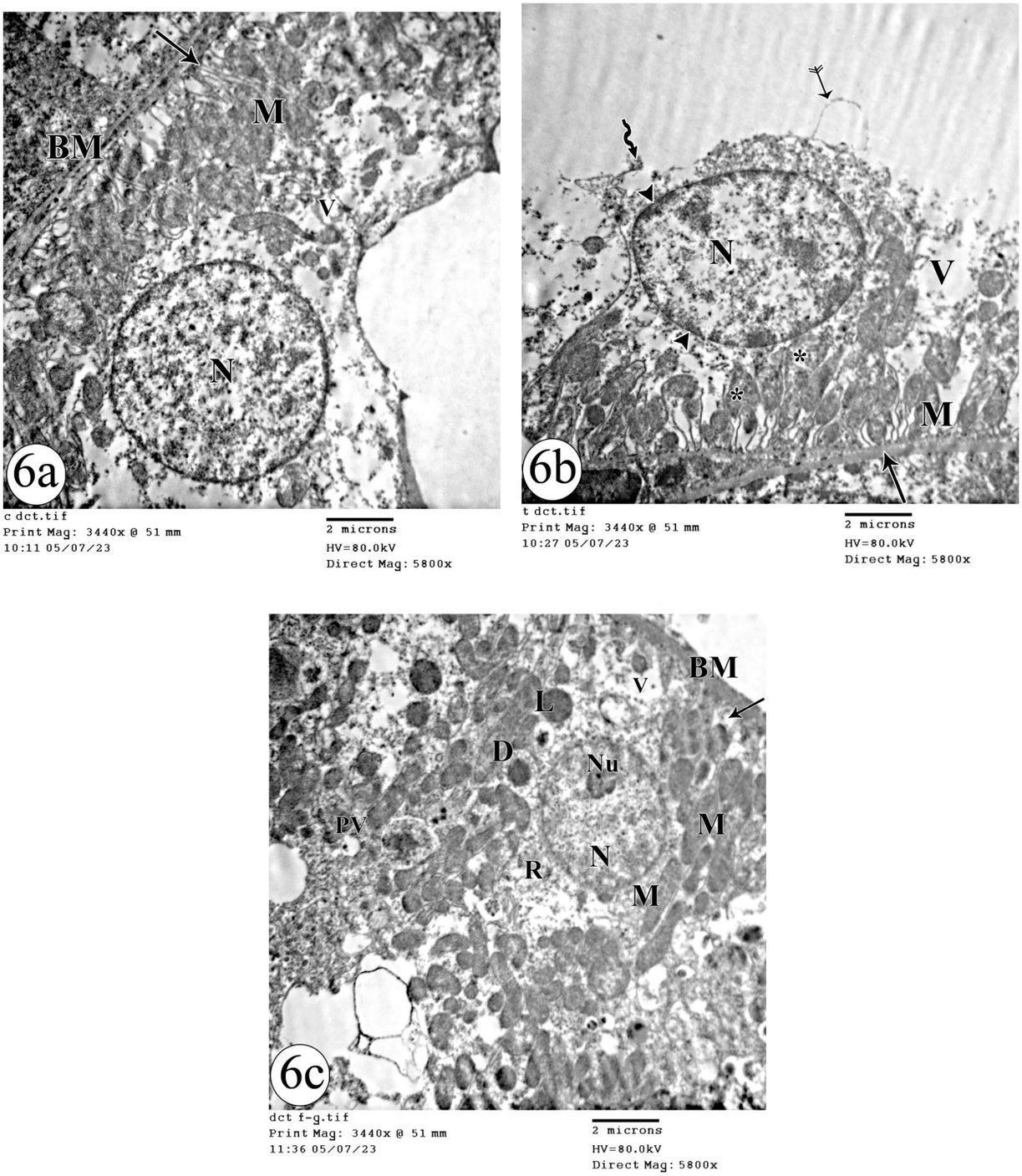


Fig. 6: Electron photomicrographs displaying a portion of the distal convoluted tubule (DCT) in the adult Albino rat renal cortex.
(6a) Control group showing the epithelial cell resting upon basement membrane (BM) with characteristic basal infoldings (Arrow). The nucleus (N) is rounded and euchromatic and the cytoplasm contains numerous longitudinally arranged mitochondria (M). There are pinocytotic vesicles (V). (6b) Treated group with fipronil showing the epithelial cell with thickened basement membrane (arrow). The nucleus (N) has irregularly distributed chromatin (arrow head). The mitochondria appeared to be destructed (M) and in some areas are swollen (star). The cytoplasm has areas of vacuolations (V) and the cell membrane is interrupted (wavy arrow) with herniation of its luminal border (tailed arrow). (6c) Treated group with gallic acid and fipronil showing the epithelial cells of DCT resting upon the basement membrane (BM). The nucleus (N) is present with prominent nucleolus (Nu) and diffusely arranged chromatin. The mitochondria (M) are distributed basally but some of them are destructed (arrow). The cytoplasm also contains pinocytotic vesicles (PV), free ribosomes (R) containing a few vacuolation areas (V), dense bodies (D) and lysosomes (L).

(TEM X 5800)

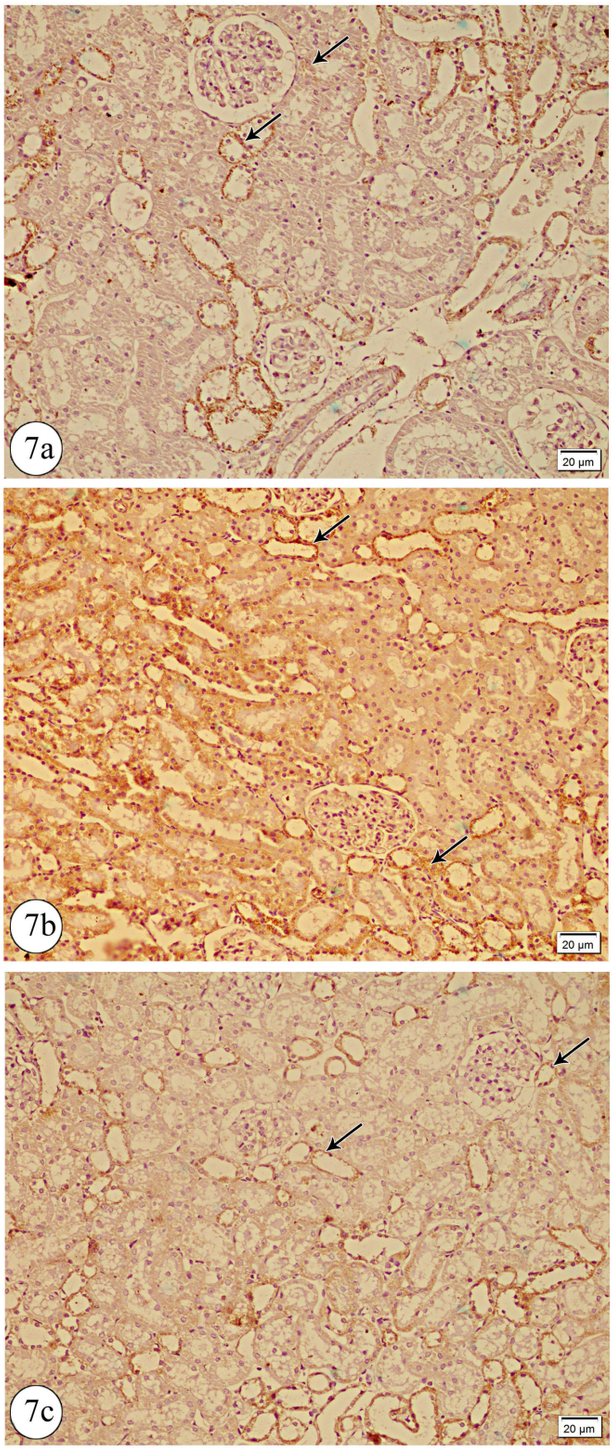
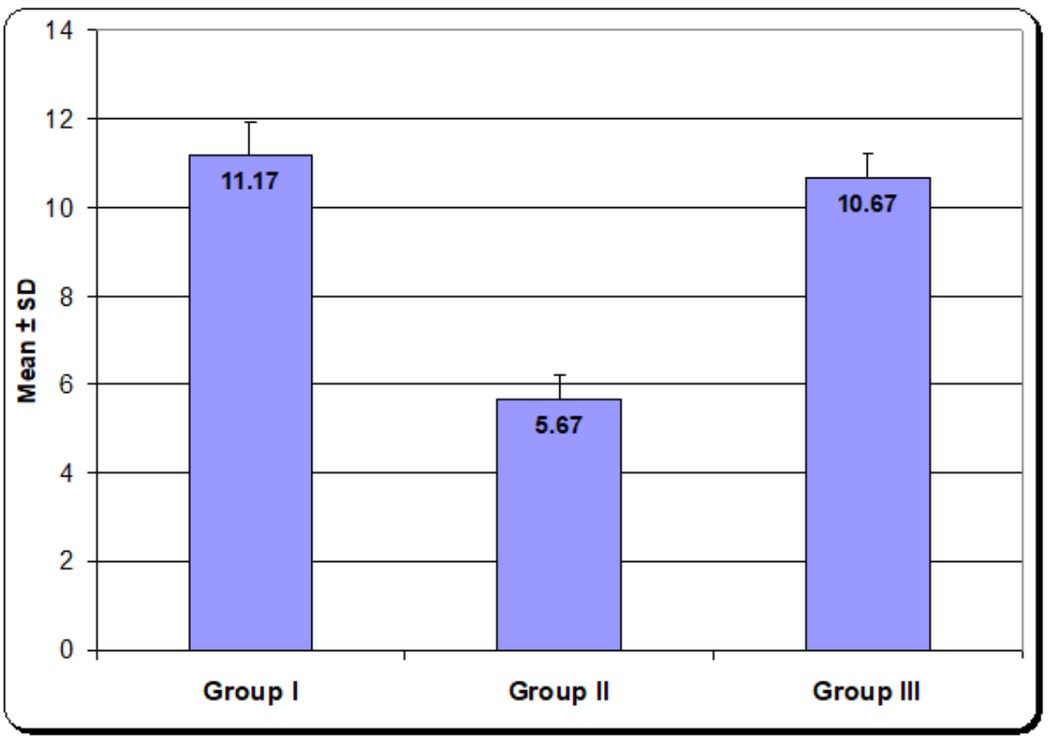


Fig. 7: A photomicrograph of anti Caspase -3 Immuno-stained sections in an adult albino rat's renal cortex
(7a) The control group displays low level of immunological response to caspase-3 in the form of very light brown coloration in relation to glomeruli and tubules (arrows). (7b) Treated group with fipronil showing strong immune reaction to caspase -3 mainly around the renal tubules (arrows). (7c) Treated group with fipronil and gallic acid displaying primarily the brown color surrounding the tubules (arrows) indicates a moderate immune response.
(Anti Caspase -3 Immunostaining ×200)

Table 1: Mean number of cells in proximal convoluted tubules of adult albino rat renal cortex

No. of cells	Group I	Group II	Group III	*P-value*[1]	*P-value*[2]	*P-value*[3]	*P-value*[4]
Mean ± SD	11.17 ± 0.75	5.67 ± 0.52	10.67 ± 0.52	0.000*	0.000*	0.173	0.000*
Range	10.0-12.0	5.0-6.0	10.0-11.0				

1: Comparison among all groups
2: Comparison between Group I and Group II
3: Comparison between Group I and Group III
4: Comparison between Group II and Group II
ANOVA test/ Post Hoc test



Histogram 1: Mean number of cells in proximal convoluted tubules of adult albino rat renal cortex

DISCUSSION

Fipronil is used close to humans as it is heavily utilizable to control insects on various crops and public health. Among the fipronil effects are the potential for both metabolic harm and oxidative stress incidence in albino rats[16]. For this research, the albino rat was the preferable animal due to the similarity in the anatomy and physiology of these animals to human[17].

The current study demonstrated Fipronil-induced alterations to histopathology and immunohistochemistry that were confirmed morphometrically on the renal cortex of the albino rat. The light microscopic findings following the administration of 10 mg of fipronil/kg/day once daily for 28 days in this study could be explained by oxidative stress damage that promotes inflammation of renal tissue. These findings were in harmony with Abd Eldaim and others[12] who stated that fipronil consumption caused promotion of the lipid peroxidation in *vitro* as well as causing oxidative damage to the tissues of rats' liver, kidney, and brain. Furthermore, the apparent destruction and shrinkage of glomeruli was in concomitant with Abdel-Mobdy and others[16] who stated that fipronil led to oxidative stress and histopathological changes in many organs.

Examination of glomeruli and both proximal and distal convoluted tubules in fipronil treated group showed fragmented capillary tuft with vacuolated rarified cytoplasm in relation to epithelial cells of tubules. This is may be explained by the overproduction of reactive oxygen species (ROS) as previous studies reported that oxidative stress was induced by acute fipronil consumption[18] and the state of antioxidant in animals was reduced[19]. Relatively, overproduction of ROS is one of the toxicity factors of pesticides, making the cells of the tubules more vulnerable to oxidative stress[18,20].

The excessive collagen fibers deposition in the renal cortex of the fipronil treated group in the present work could be explained by the possibility of tissue degeneration induction which is replaced by fibrosis. These results can be correlated with El-Ballal and others[21] who attributed the destructive changes in both kidney and liver following

exposure to fipronil to the disruption of the antioxidant/ oxidant balance.

The electron microscopic findings in the present study revealed changes in the cell organelles of the fipronil treated group which could be attributed to the generation of ROS. When ROS production is excessive, damage is done to cells organelles and change in the cytoskeleton of the cell. These results were in concomitant with Abd Eldaim and others[12] as they suggested that ROS production which was secondary to fipronil exposure might lead to destructive changes involving essential cellular components and structures such as proteins, DNA, and membranes.

In this study, the examination of the epithelial cells of both proximal and distal convoluted tubules by the electron microscopy showed irregularly distributed chromatin in the nucleus with cytoplasmic vacuolations, damaged mitochondria, and destructed cell membrane. These results could be attributed to fipronil's direct toxic effect on the cells and cellular antioxidants declining which cause endogenous ROS to accumulate activating signaling pathways that resulted eventually in renal tubular cells death secondary to mitochondrial dysfunction. These findings were in agreement with Ortner and others[22] who found that endothelial dysfunction after fipronil exposure accompanied with a paradoxical rise in oxidative stress, particularly since antioxidant activity has decreased.

The Caspase-3 immunohistochemical results of fipronil received group showed that the cells of the tubules in the renal cortex had strong positive reaction. This finding can be explained by apoptotic activity of fipronil. Renal tissue damage may be explained by the expression of caspase-3 and Cox-2 proteins brought on by oxidative stress[4]. These results support prior research showing increased caspase-3 immune reactivity in the renal tissue of rats intoxicated with the drug[23]. Caspase-3 and Cox-2 are both in charge of the apoptotic process[24]. Proteolytic caspase mediates apoptosis and is necessary for the initiation, control, and completion of proteolytic processes[25]. Because it is the primary inhibitor of apoptosis, active caspase-3 as one of the proteolytic caspases is used to identify apoptotic cells[26].

This study revealed that mean number of cells in proximal convoluted tubules of adult albino rat renal cortex in the fipronil-treated group was statistically significantly lower than in the control group. The explanation of this finding is unbalanced state between prooxidants and antioxidants and the defect in the antioxidant activity which lead to free radical toxicity secondary to fipronil exposure. The oxidative injury can be caused by inadequate defense mechanisms against ROS. Lipid peroxidation is the most important type of ROS[27].

Using gallic acid concomitant with fipronil in this study revealed that the cells retained its regular outline with prominent nuclei and nucleoli with exception of some cells which had vacuolations in the cytoplasm. This is thought to be due to Gallic acid's antioxidant capacity and its ability in reduction of oxidized glutathione. These results were in agreement with Mansouri and others[28] as they stated that oral gallic acid administration resulted in a decrease in ROS levels in the neural tissues and a restoration of antioxidant enzyme activity in the cerebral cortex and hippocampus. Organs were protected against toxicity by using gallic acid. This is accomplished by lowering oxidative stress markers and increasing pro-inflammatory cytokines, like TNF-α, in the testis, liver, and heart[29]. Because of its antioxidant and anticholinesterase activities, gallic acid was found to have anti-amnesic activity against scopolamine-induced amnesia[30].

The group that received gallic acid with fipronil showed reductions in collagen fibers deposition in renal cortex which might be explained by ability of gallic acid as an antioxidant in scavenging free radicals caused by fipronil. These results were in consistent with Kazim and others[8] who proved that gallic acid prevented alcoholic liver damage by restoring the reduction in liver antioxidant activity. Additionally, superoxide dismutase (SOD) and catalase (CAT) activities were all normalized by gallic acid which also caused a raise the glutathione reductase (GR), glutathione peroxidase (GPx), and GSH levels[31].

The apparent improvements in ultrastructural examination in the third group that received both gallic acid and fipronil might be because of the potential impact of gallic acid as anti-inflammatory compound. These results were in agreement with the previous studies indicating that the consumption of gallic acid reduces inflammatory process and succeeding events leading to apoptosis in rats with polycystic ovarian syndrome and in rat ovaries exposed to the anticancer drug cisplatin[32].

In this work, Gallic acid's protective ability against the toxic effect of fipronil could explain the improvement in ultrastructure of proximal and distal convoluted tubules' cells. These findings were in concomitant with previous studies which indicated that antioxidant defense of the brain was enhanced by gallic acid stimulation which was considered as a useful progress in Parkinson's disease treatment[33].

Previous studies stated that gallic acid had the capability to hunt free radicals. Also, it has the ability to prevent oxidative stress caused by streptozotocin, lessen lipid peroxidation, and safeguard β-cells. This led to a drop in blood glucose and an increase in plasma insulin levels. Gallic acid's stimulant properties explain the increased insulin level[34]. Ahmadvand *et al*[35] confirmed that the rise in creatinine and urea levels that occur after ischemia reperfusion injury decreased after gallic acid usage.

The immunohistochemical examination of third group revealed moderate immune reaction to Caspase -3 indicating reduction of apoptosis in the renal cortical tissue. Asci *et al*.[36] specified that gallic acid had an effect in reducing the tubular necrosis and decreasing the amount of highly inflammatory markers expressed such as TNF-, after methotrexate-induced kidney destruction.

As for the morphometric results in our study, the mean number of cells in proximal convoluted tubules of adult albino rat renal cortex showed apparent increase as gallic acid is given in concomitant with fipronil when compared with second group that received fipronil only. This may be caused by activity of gallic acid in preventing the oxidative damage. These outcomes are consistent with Gu *et al*.,[37] who stated that in healthy cells, gallic acid reduced mitochondrial respiration in a dose- and time-dependent manner and exhibited strong antioxidant and anti-apoptotic properties, this lead to decrease in the oxidative stress.

CONCLUSION

Fipronil exposure caused structural destructive changes in the histology, ultrastructure, immunohistochemistry, and morphometry of the cortex of the kidney. Concomitant gallic acid administration could reduce the side effects caused by fipronil due to its antioxidant activity.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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الملخص العربى

تأثير التعرض للفبرونيل علي بنية قشرة الكلي لدي الفئران البيضاء البالغة و الدور المحتمل لحمض الغاليك

سالي سيد أنور ونهي أحمد راشد

قسم التشريح الادمي وعلم الأجنة - كلية الطب - جامعة أسيوط

مقدمة: الكلى عضو متخصص للغاية وحساس للإجهاد التأكسدي. فيبرونيل هومبيد حشري له تأثير سام على الأجهزة المختلفة. حمض الغال كأحد الأحماض الفينولية الأكثر وفرة في المملكة النباتية وهو عديم اللون أومركب بلوري أصفر قليلاً، يستخدم على نطاق واسع في الصناعات الغذائية والصيدلانية.

الهدف من البحث: الكشف عن تأثير الفبرونيل على بنية قشرة الكلى للفئران البيضاء البالغة وكذلك التعرف على دور حمض الغال كمادة محسّنة.

مواد وطرق البحث: تم استخدام عدد ٤٠ فأراً بالغاً بوزن (٢٠٠-٢٥٠) جرام. قسموا إلى ٤ مجموعات هم المجموعة الضابطة، ومجموعة الفبرونيل، ومجموعة فبرونيل وحمض الغاليك، ومجموعة حمض الغاليك. تلقت الفئران العلاج (فيبرونيل بجرعة ١٠ ملجم / كجم من وزن الجسم عن طريق الفم وحمض الغاليك بجرعة ١٠ ملجم / كجم مذاب في محلول ملحي عن طريق الفم بواسطة أنبوب داخل المعدة يومياً لمدة ٢٨ يوماً. ثم تم ذبحهم وتم استخدام كليتهم من أجل الفحص النسيجي بالمجهر الضوئي والإلكتروني والدراسة الكيميائية المناعية وكذالك الدراسات المورفومترية والإحصائية أيضا.

النتائج: أظهرت الدراسة الحالية أن الفبرونيل يسبب تدمير الخلايا في قشرة الكلى في الكُبَيْبَات الكلي والأنابيب الملتوية الدانية والبعيدة. أظهرت النتائج المورفومترية انخفاضا معتد به إحصائيا في متوسط عدد الخلايا الظهارية في الأنابيب الملتوية الدانية في مجموعة الفبرونيل مقارنة مع المجموعة الضابطة ومعظم التغييرات المدمرة يتم تحسينها بواسطة حمض الغال.

خلاصة البحث: الاستخدام المتزامن لحمض الغاليك يمكن أن يقلل من الآثار الجانبية التي يسببها الفبرونيل على كلية الفئران.