Original Article

The Light Microscopic Study of Effect of Exposure and Withdrawal of Pyrethroid and Herbal Based Mosquito Vaporizers On the Kidney of Male Wistar Albino Rat

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Abstract

Background: Pyrethroids, organochlorines, organophosphates, and carbamates constitute important active ingredients of available mosquito repellents in the Indian market, in which pyrethroids are found very effective and safe. The natural compounds produced by plants can interfere major metabolic pathways, thus causing death of the insects. The aim of present study was to know the effect of exposure and withdrawal of transfluthrin and herbal based mosquito vaporizers on kidney of male albino rats. **Material and Methods:** We have taken kidney of 26 well preserved male albino wistar rats and divided in to three groups. Control group: Six rats; not exposed to any mosquito repellents. Experimental group 1- 12 rats: divided in subgroup 1A, the pyrethroid Exposure group; and subgroup 1B, the Pyrethroid withdrawal group. Experimental Group 2 – Eight rats: divided in subgroup 2A, the herbal Exposure group and 2B, the herbal withdrawal group. **Results:** The kidney of pyrethroid vaporizer exposed group showed gross changes in their histology than the herbal based mosquito vaporizer exposure group. Also, some of the normal histological features were recovered after withdrawal of chemicals and reversal was better in herbal based mosquito vaporizer. **Conclusion:** Histological findings of present study suggests that pyrethroid (Transfluthrin) based mosquito repellents are harmful to the kidney. Herbal based mosquito repellent also showed damage though the severity was mild as compared to pyrethroid. Reversal changes were much better in herbal withdrawal group than the pyrethroid withdrawal group.

Keywords: Pyrethroid, Transfluthrin, Herbal, Mosquito repellent, Kidney, Withdrawal.

Received: 15 August 2026 **Revised: 25 August 2026** **Accepted: 12 September 2026** **Published: 22 September 2026**

Introduction

Pyrethrins are present as natural substance in the flowers of Pyrethrum species.[1] The synthetic pyrethroids derived from these pyrethrins are widely used as a crucial components of available mosquito repellents. They are proven to be least toxic as these are rapidly neutralized during metabolic processes inside the human body. The ion transport through the membrane of nerve axons is interfered by these pyrethroid that eventually causes muscular paralysis and kills the insects.[2,3] According to WHO data, the consumption of active component of pyrethroids in the ongoing extensive vector control programmes alone is about 520 tonnes annually in the world.[4]

People use personal protective measures in several forms to prevent mosquito borne diseases. But the extensive use of common mosquito repellents causes potential toxicity on various mammalian vital organs and tissues. Studies have been found that the animals exposed to pyrethroid exhibit histopathological changes along with various physiological disturbances.[5]

Now a days, herbal mosquito repellents are becoming popular among the population. The secondary compounds produced by plants showed insecticidal properties thus provides a new sources of natural insecticides.[6,7] Some of these Secondary compounds are in the form of alkaloids, terpenoids, different types of phenols, flavonoids, and variety of other chemicals.[8] These chemicals usually kill the insects by inhibiting the metabolic pathways, or interfere with the life-cycle of the insect.[9] Although, the herbal based mosquito repellents are certified but systematic studies of its organotoxicity in comparison to synthetic pyrethroids based repellents is not much available, so we have focused to unveil the toxicity of herbal based mosquito vaporizers on kidney in comparison to the pyrethroid toxicity.

Materials and Methods

Study place and duration: This study was conducted in the Department of Anatomy in collaboration with the Department of Pharmacology, King George's Medical University, UP, Lucknow during August 2016 to September 2017.

Ethical clearance: It was obtained from Institutional Animal Ethics committee (IAEC), King George's Medical University.

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DOI:
10.21276/amit.2026.v13.i3.1062

How to cite this article: Pandey M, Pankaj AK, Manik P, Rani A, Tomar S. The Light Microscopic Study of Effect of Exposure and Withdrawal of Pyrethroid and Herbal Based Mosquito Vaporizers On the Kidney of Male Wistar Albino Rat. Acta Med Int. 2026;13(3):310-313.

Sample size: The present study was carried out on various formalin fixed viscera from 26 male wistar albino rats.

Inclusion Criteria

Kidneys from well preserved male wistar albino rats exposed to pyrethroid and herbal based mosquito vaporizers were taken for study.

Exclusion Criteria

Kidneys of male albino wistar rats not well preserved with distorted morphology were excluded from the present study.

Study Design: Well-preserved kidneys of 26 Male albino rats with, were divided into 3 groups: Control Group, Experimental Group I and Experimental Group II. The later two groups were further divided in to A and B Subgroups.

Control Group: Kidneys of 6 male albino rats, those were not exposed to mosquito vaporizer fumes.

Experimental Group I: Kidneys of 12 male albino rats (divided in subgroup I A and subgroup I B; each subgroup contains kidneys of 6 male albino rats).

Subgroup I A: Contains kidneys of male albino rats exposed to chemical-based mosquito vaporizer (Transfluthrin 0.88% w/w) for 8 hours /day, 6 days in a week for 12 weeks.

Subgroup I B: Contains kidneys of male albino rats those were given exposure of chemical-based mosquito vaporizer (Transfluthrin 0.88% w/w) for 8 hours/day, 6 days in a week for 12 weeks and further kept for 4 weeks to see the reversal changes.

Experimental Group II: Kidneys of 8 male albino rats (divided in subgroup II A and subgroup II B; each subgroup contains organs of 4 male albino rats).

Subgroup II A: Kidneys of male albino rats exposed to herbal based mosquito vaporizer for 8 hours/day, 6 days in a week for 12 weeks.

Subgroup II B: Contains kidneys of male albino rats those were exposed to herbal based mosquito vaporizer for 8 hours/day, 6 days in a week for 12 weeks and further kept for 4 weeks to see the reversal changes.

Products detail:

Chemical Based Mosquito Vaporizer: Good Knight Refill (Vaporizer containing 0.88% transfluthrin, Reg. No. CIR-48,420/2004; manufactured by Godrej Consumer Products Ltd.) and herbal based Mosquito Vaporizer Mosrelief Refill Vaporizer (Vaporizer containing Cymbopogon citrius*, Bhutika,oil: 6%, Azardirachta indica**, Nimba oil: 6%, Eucalyptus Globulus, Tailaparna, oil : 6%, Exciepients: OS, *API, **AFI; Mff. Licence No. AUS920; manufactured by Strategi, Plot No.:50, Part 1, 4th Phase, KIADB industrial area, Mular-563130, Kolar distt., Karnataka, India.) were used in our study.

The routine Histological procedure i.e. fixation, clearing, paraffin embedding and sections of 5 µm thick were cut by rotary microtome and stained with Hematoxylin and Eosin stain. Slides were prepared for histopathological observation under Light microscope.

The Light microscopic examination of sections of kidneys was done to evaluate and compare the histological changes. Observation of slides was done under 10X, 40X, and 100X by Kyowa Trinocular Research Microscope. Microphotograph of different tissues were taken for documentation by digital camera (DSC-WX80/RC E32, manufactured in China by Sony corporation, 1-7-1 konan, Minto-KU, Tokyo 108-0075, Japan, month of import April 2014).

Results

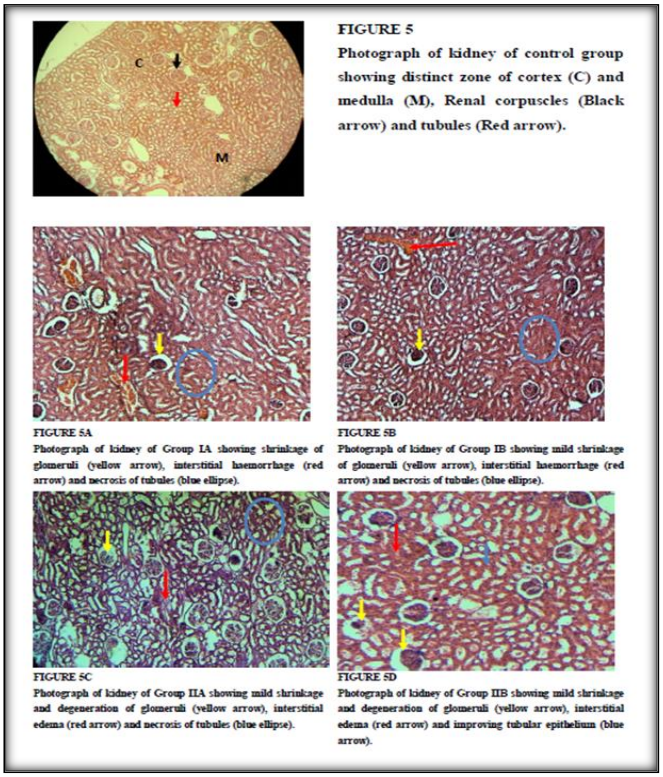

FIGURE 5
Photograph of kidney of control group showing distinct zone of cortex (C) and medulla (M), Renal corpuscles (Black arrow) and tubules (Red arrow).

FIGURE 5A
Photograph of kidney of Group IA showing shrinkage of glomeruli (yellow arrow), interstitial haemorrhage (red arrow) and necrosis of tubules (blue ellipse).

FIGURE 5B
Photograph of kidney of Group IB showing mild shrinkage of glomeruli (yellow arrow), interstitial haemorrhage (red arrow) and necrosis of tubules (blue ellipse).

FIGURE 5C
Photograph of kidney of Group IIA showing mild shrinkage and degeneration of glomeruli (yellow arrow), interstitial edema (red arrow) and necrosis of tubules (blue ellipse).

FIGURE 5D
Photograph of kidney of Group IIB showing mild shrinkage and degeneration of glomeruli (yellow arrow), interstitial edema (red arrow) and improving tubular epithelium (blue arrow).

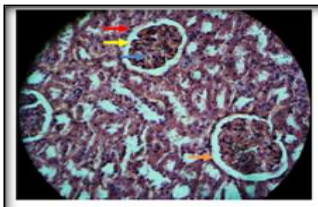

FIGURE 6
Photograph of kidney of control group showing visceral (yellow arrow) and parietal layer (red arrow) of renal corpuscles, glomerulus (blue arrow), Bowman space (orange arrow).

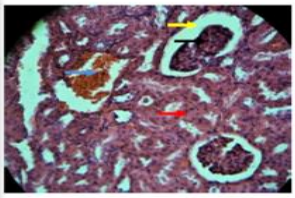

FIGURE 6A
Photograph of kidney of Group IA showing shrinkage of glomeruli (black arrow), glomerular haemorrhage (blue arrow), increased Bowman space (yellow arrow) and necrosis of tubules (red arrow).

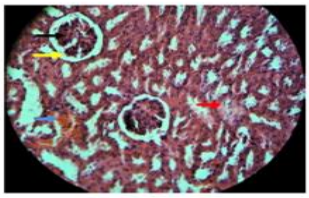

FIGURE 6B
Photograph of kidney of Group IB showing mild shrinkage of glomeruli (black arrow), mild haemorrhage (blue arrow), decreased widening of Bowman space (yellow arrow) and extensive necrosis of tubules (red arrow).

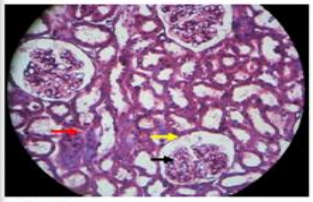

FIGURE 6C
Photograph of kidney of Group IIA showing vacuolation of glomeruli (black arrow), mild increase in Bowman space (yellow arrow) and Interstitial edema (red arrow).

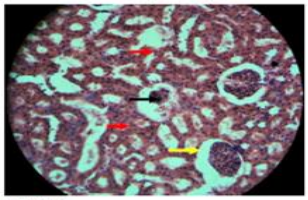

FIGURE 6D
Photograph of kidney of Group IIB showing shrunken glomeruli (black arrow), mild increase in Bowman space (yellow arrow) and recovery of most of the tubules (red arrow).

Histological findings of kidney

Control group: Histological findings of the kidney of control group showed two distinct areas; An outer darker staining region, the cortex and an inner lighter staining region, the medulla [Figure 5].

Bowman's capsule showed well defined intact parietal layer of simple squamous epithelium along with intact visceral layer. The cluster of blood vessels was clearly visible in the glomeruli. Numerous tubules, sectioned in various planes were observed adjacent to the renal corpuscles. At higher magnification(100X) the simple cuboidal epithelium with brush-border and small, irregular lumen was well evident in proximal convoluted tubules. The distal convoluted tubules of the nephrons showed lightly stained cuboidal cells with no brush-border [Figure 6]. The thin segment of loop of Henle (simple squamous epithelium), the proximal convoluted tubules and distal convoluted tubules were well seen in medulla.

At still higher magnification 100X of a small area of the kidney cortex showed adjacent tubules, and juxtaglomerular apparatus at vascular pole of renal corpuscles.

Group Ia (pyrethroid exposure group): There was gross disruption of visceral and parietal cell layer of Bowman's capsule. Shrunken and hemorrhagic glomeruli leading to increased Bowman's space. There was extensive sloughing of epithelial cells of renal tubules. At places the lumen of proximal convoluted tubules was devoid of brush borders and the lumen size of proximal convoluted tubules & distal convoluted tubules was almost same. Intertubular spaces were showing severe congestion and haemorrhages at some areas of cortex [Figure 7]. Thinning of epithelial lining of collecting tubules along with haemorrhages in medulla was remarkable. Juxtaglomerular apparatus at vascular pole of renal corpuscles were degenerated from some areas.

Group Ib (pyrethroid withdrawal group): There was mild disruption of visceral and parietal cell layer of Bowman's capsule. Shrinkage as well as haemorrhages of glomeruli along with the increase of bowman space was less marked as compared to Group IA. The section showed extensive necrosis of epithelial cells of renal tubules at places. Intertubular spaces were evident congestion but hemorrhagic areas were not much prominent [Figure 8]. Medulla showed thinning of epithelial lining of collecting tubules and haemorrhages. Juxtaglomerular apparatus was found to be degenerated at places.

Group IIa (herbal exposure group): The parietal and visceral layer of Bowman's capsule were intact. Lymphocytic infiltration is present in glomerulus. Mild shrinkage of glomeruli exhibited little increase in bowman space. Tubular epithelium had lost its normal architecture [Figure 9]. Interstitial edema and degeneration of juxtaglomerular apparatus had been also observed. There was pronounced interstitial edema present in medulla of kidney of Group IIA.

Group IIb (herbal withdrawal): Noticeable disruption of parietal and visceral layer of Bowman's capsule was seen. Lymphocytic infiltration was present in glomerulus. There was mild shrinkage of glomeruli causing minute increase in bowman space. Tubular epithelium (PCT and DCT) had almost regained its normal architecture. Marked edema in addition to mild haemorrhage was observed in the intertubular space [Figure 10]. The juxtaglomerular apparatus also showed cellular details. comparable to control group. The medulla showed Loop of Henle with simple squamous epithelium.

Discussion

The kidney performs several essential functions in our body. Most importantly it removes the waste products produced endogenously in the body, controls volume status, regulates electrolyte and acid-base balance, and hormone secretion. Perazella, MA., et al., (2009) said that metabolism and excretion of exogenously administered therapeutic and diagnostic agents as well as environmental exposures are other major functions of kidney.[10] In its role as the primary eliminator of exogenous drugs and toxins, the kidney is vulnerable to develop various forms of injury. The damage caused by insecticide exposure has been a matter of concern for many years.

In the present study there was gross disruption of visceral and parietal cell layer of Bowman's capsule, increased Bowman's space, shrunken and haemorrhagic glomeruli. There was extensive sloughing and necrosis of epithelial cells of renal tubules. At places the lumen of proximal convoluted tubules was devoid of brush borders. Thus, the proximal convoluted tubules and distal convoluted tubules were exhibiting almost same lumen size. Thinning of epithelial lining of collecting tubules along with haemorrhages in medulla of pyrethroid treated kidney was remarkable. The observed results are in accordance with Majumder, S., et al.,(1994) who reported glomerular and tubular necrosis in broiler chicks treated with oral doses of fenvalerate.[11] Alden, CL., et al., (1991) have suggested that any process that interferes with the structural integrity of the glomeruli and renal tubules can cause nephrotoxic effect. In such cases, leakage of lysosomal enzymes may occur, thereby causing cell necrosis and renal damage.[12]

The results from the present experiment are also in agreement with the findings studied by Mamun, MAA., et al., (2014) on kidney tissue treated with cypermethrin. They observed shrinkage of glomeruli, necrosis of renal tubules, congested and dilated blood vessel, eroded Bowman's capsule and marked congestion of renal tubule, glomerulus along with haemorrhage.[13] Grewal, KK., et al., (2010) also reported cypermethrin intoxication resulting deleterious impact on the structure of the kidney tissues with shrinkage of glomeruli, necrosis of renal tubules, haemorrhage in the convoluted tubules in kidney tissues. Here, route of exposure (oral administration) and dose level (higher dose) was different from the present study but showing approximately similar results. These changes were not very well pronounced in pyrethroid withdrawal, herbal exposure and herbal withdrawal group as compared to pyrethroid exposure group.[14]

Conclusion

In this study we found remarkable damage of renal corpuscles and renal tubules in pyrethroid exposure group without much histological improvement after its withdrawal while herbal

repellent exposed group revealed minimal changes in the epithelial linings, renal corpuscles and renal tubules which does not show much improvement after the discontinuation of exposure (Group IIB). Thus, it can be inferred that both the groups of chemicals have toxic effect on mammalian tissues. Though the injury caused by synthetic pyrethroid was more pronounced nevertheless the detrimental effects of herbal mosquito repellents cannot be ignored. this necessitate to refine the present knowledge about the biological effects of transfluthrin and herbal based mosquito repellents as well as newer techniques must be establish to monitor and control its toxicity.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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