

Unveiling the Impact of Allethrin-Related Mosquito Coil Exposure on Testicular Histology: Investigating the Protective Role of Vitamin C and withdrawal dynamics

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ABSTRACT

Introduction: Allethrin is a common pyrethroid, one of the major component of mosquito coils that is noxious. The aim of the current study is to see the protective effect of Vitamin C on an induced testicular damage by Allethrin as well as comparing it to withdrawal prognosis.

Material and method: The Wistar rats comprised of following groups: Group 1-control group, Groups II, III, and IV- exposure groups. Each group consisted of 12 rats. These were subjected to mosquito coil fumes continuously overnight for eight hours every day for a duration of twelve weeks. To demonstrate the effects of withdrawal, Wistar rats from group III were kept in an environment free from additional exposure for eight weeks after the 12-week exposure period. In addition, Group IV received Vitamin C.

Result: Group II exhibited significant alterations in the testicular architecture, including conspicuous shrinkage of seminiferous tubules, an increase in intertubular space, thickening and disruption of the basement membrane, decreased thickness of the germinal epithelium, sloughing of germinal cells into the lumen, reduced numbers and size of spermatogenic cells, retracted cytoplasmic processes of Sertoli cells, interstitial edema, lipid vacuolation, and deformation of Leydig cells. Withdrawal in group III showed some histopathological improvements, while group IV, treated with Vitamin C, demonstrated even more remarkable enhancements.

Conclusion: Exposure of Wistar Albino rats to mosquito coils containing allethrin led to notable histopathological changes. However, these adverse effects can be ameliorated by incorporating daily antioxidant intake, such as Vitamin C supplementation.

KEY WORDS: Mosquito Coil, Allethrin, Testicular, Seminiferous Tubules, Vitamin C, Antioxidant.

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INTRODUCTION

Mosquito coil usage remains prevalent in many parts of the world as a primary method

for repelling mosquitoes and prevention. However, its widespread use has raised concerns due to its implication in various

adverse health effects. These effects may include respiratory irritation, allergic reactions, and potential neurotoxicity, prompting further investigation into its safety and potential alternatives for mosquito control vector-borne diseases. However, concerns have been raised regarding the potential health impacts of prolonged exposure to the chemicals emitted by these coils. Mosquito coil is a type of mosquito repellent product typically made from mixture of ingredients such as powdered wood, saw dust and type of insecticide. Allethrin is currently the most common pyrethroid and primary active ingredient in mosquito coils which is widely used to combat a range of mosquito family including *Aedes*, *Anopheles*, and *Mansonia* [1]. However, its widespread use has raised concerns due to its implication in various adverse health effects. These effects may include respiratory irritation, allergic reactions, and potential neurotoxicity, prompting further investigation into its safety and potential alternatives for mosquito control [2].

Burning a mosquito coil releases insecticides into the air through smoke, effectively deterring mosquitoes from entering room [3]. Burning these coils releases fumes that is composed allethrin coated submicron particles, heavy metals, and an array of vapours, including phenol O-cresol [4]. These when frequently employed overnight may lead to increased exposure to smoke particles and can cause substantial harm to different organs depending on their susceptibility [5]. Because rats share physiological similarities with humans and are readily accessible, we conducted an experiment with an aim to investigate the impact of allethrin-containing mosquito coils on the testis of an animal model, employing Wistar rats. Additionally, we examined the curative role of Vitamin C, as well as its withdrawal implications.

MATERIALS AND METHODS

Ethical review: Ethical approval for this study was taken from Animal Institutional Ethical Committee, of the institutes after taking all ethical aspect into consideration.

Experimental Rats: 48 male wistar rats in good

health, weighing between 250 and 300 gram two to three months old, were used in this investigation and divided into equal groups that were randomly categorized as I-IV. We obtained the rats from an animal house. No prior calculation of sample size was performed; designating the study animals relied upon the maximum number of available healthy male albino rats that met the specified age and weight criteria provided by the animal house. Upon obtaining the rats, they underwent a two weeks of adaptation during which they were provided with a pellet diet meeting U.S. National Research Council standards. They were housed in consistent environmental conditions with proper aeration, proper light and temperature. Food and water were available ad libitum, and hygiene and care followed CPCSEA (Committee for the Purpose of Control and Supervision of Experimental rats) guidelines. Rats were housed in cages with a maximum of four rats per cage. Four groups were created each comprising 12 rats. Group I (control group) rats were not subjected to fumes from mosquito coil. Group II rats were subjected to fumes from mosquito coils for 8 hours overnight for 12 weeks. Group III (withdrawal group) were exposed to mosquito coils for 8 hours overnight for 12 weeks followed by eight weeks without any exposure. Group IV rats were orally supplemented with vitamin C at a dose of 20 mg/kg body weight once a day for a period of 12 weeks, for same period of exposure to mosquito coil smoke. [6] One 500 mg pill of vitamin C (Tablet Limcee by Abbott Healthcare Pvt Ltd, India) was dissolved in ten milliliters of water to create a aqueous solution. Rats in experimental group IV were given a freshly made solution orally with the use of a feeding canula.

Mosquito coil exposure

The commonly available mosquito coil from brand Mortein was used. Mosquito repellent coil brand- Mortein Power Guard - Manufactured by- Lotus Household Products Pvt. Ltd. Marketed by- M/s Reckitt Benckiser (India) Ltd. Its consisted of 0.1% w/w d-trans allethrin.

The rats in experimental groups II, III, and IV were housed in cages made of wire mesh with

small gaps that allowed mosquito coil smoke to enter the cage and fill the whole space, giving the rats the maximum amount of exposure to the fumes. After 12 weeks rats from all the group except group III were euthanized by placing the rats in a desiccators containing cotton soaked in diethyl ether. Group III rats were euthanized after an additional 8-week period during which they had no exposure to the experimental condition. The rats were positioned on their backs with all four limbs spread out, securely pinned to a wax-coated dissection tray. Rats were fixed in vivo and testis was removed for histological profile. Paraffin Sections, 5mm in thickness, were sliced and stained using hematoxylin and eosin.

RESULTS

Group I: Under light microscopy testis exhibited cut sections of seminiferous tubules in different planes. (Fig.1). The seminiferous tubules were closely packed. Each tubule was surrounded by fibroelastic connective tissue(fig1) and lined by a stratified cuboidal epithelium which consisted of two distinct population of cells- (1) Cells undergoing various phases of spermatogenesis, with spermatogonia, having spherical nuclei next to basement membrane (Fig.2). (2) Visible Sertoli cells, which are non-spermatogenic cells that feed and sustain developing spermatozoa. Intertubular space consisted of connective tissue cells and Leydig Cells (Fig.2).

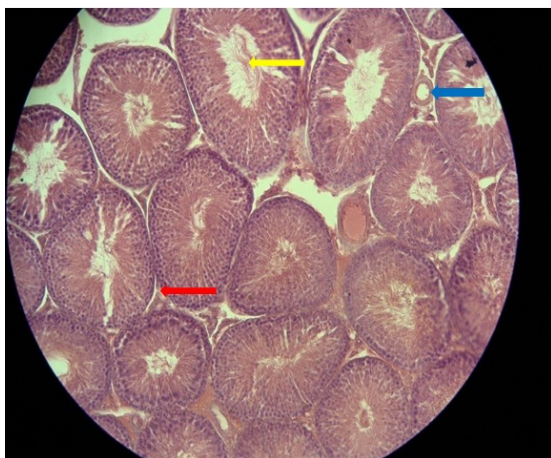


Fig. 1: Photomicrograph showing closely packed seminiferous tubules with normal spermatogenesis. Lumen filled with tail of spermatozoa (yellow arrow). Blood vessel in intertubular space (blue arrow) with minimal intertubular spaces between them (red arrow). Group I, H&E(200x)

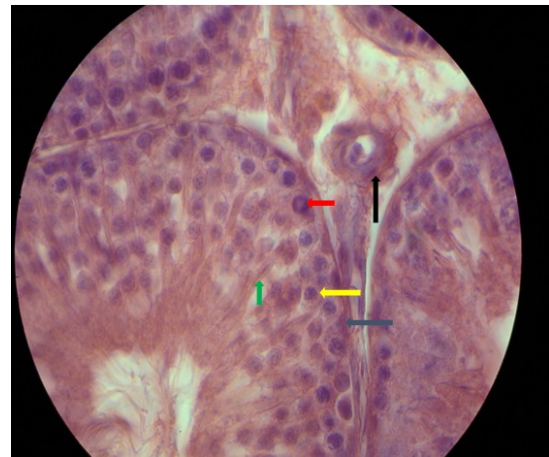


Fig. 2: Photomicrograph showing spermatogenic series of cells i.e. spermatogonia (red arrow), spermatocytes (yellow arrow), spermatids (green arrow); Sertoli cells (blue arrow); group of Leydig cells clumped around blood vessels (black arrow). Group I, H&E(1000x)

Group II: There was apparent shrinkage of seminiferous tubules, and they were loosely packed with markedly reduced diameter. The surrounding basement membrane was thickened and disrupted. There was marked detachment of germinal epithelium from basement membrane as well as sloughing of cells within the lumen were seen (fig3). Empty spaces were present in between germ cell lines (fig 4). Some tubules showed degeneration of cell towards lumen and at few places there was maturation arrest. There were apparently lesser number of sperms in the lumen with most tubules completely absent with sperm tails (fig 4). Sertoli cells appeared to retract their cytoplasmic processes with basally placed irregular nucleus(fig5).

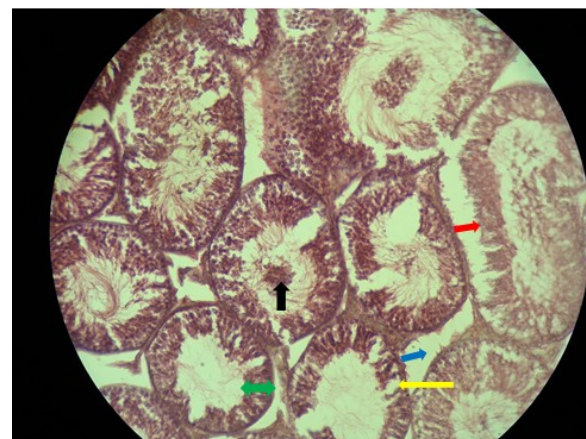


Fig. 3: Photomicrograph showing sloughing of spermatogenic cells in the lumen (black arrow), vacuolations between the germ cell lines(yellow arrow) shrinkage of seminiferous tubules with increased intertubular spaces (blue arrow), basement membrane detachment(red arrow),and reduced thickness of germinal epithelium(green arrow). Group II, H&E(200x)

At some place they were rounded off and floated into the lumen. Interstitial spaces showed homogenous materials may be due to oedema with collapsed blood vessels. The cell boundaries of Leydig cells were poorly defined (fig.5).

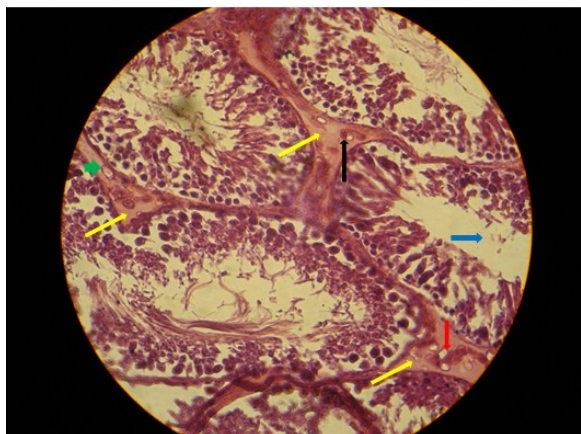


Fig. 4: Photomicrograph showing lipid vacuolation (red arrow), indistinct cellular boundaries of Leydig cells (black arrow), collapsed blood vessel (green arrow), empty lumen (blue arrow) and marked interstitial oedema (yellow arrow) Group II, H&E (400x)

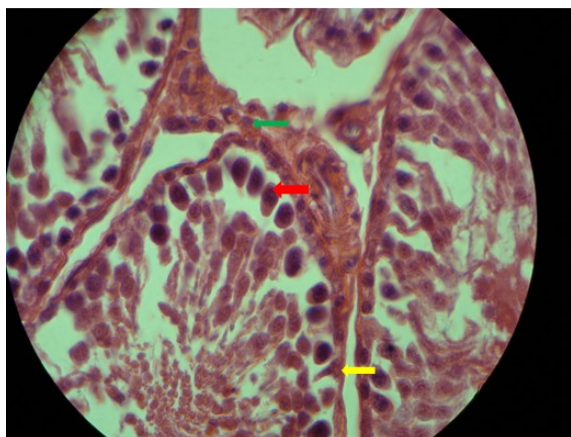


Fig. 5: Photomicrograph showing reduced number of elongated spermatids, predominantly dark spermatogonia (red arrow), Leydig cells with indistinct cellular boundary (green arrow) Sertoli cell with retracted process and nucleus away from basal lamina (yellow arrow). Group II H&E (1000x)

Group III: In group III, which is a withdrawal group, the diameter of seminiferous tubules appeared to improve but basement membrane was disrupted at some places (fig7a). Thickness of germinal epithelium was normal. Empty spaces were seen inside at few tubules. Sperm tails in the lumen were evidently less in comparison to group I but more than the group II (fig8a). Spermatogonia were reduced in number and most of the cells were of dark type. Primary spermatocytes were loosely arranged. No intracytoplasmic vacuolations were seen. Spermatids were seen with distinct cell boundaries. Sertoli cells with retracted cytoplasmic processes with basally placed irregular nuclei were seen. Sperm heads at the apices of Sertoli cells were more than in group II. Interstitial spaces were increased but not much as in group II (fig6a). Slight oedema were seen. Leydig cells were smaller in size.

Group IV: Seminiferous tubules exhibited near-normality in diameter and were loosely packed. Surrounding basement membrane were less thickened (fig7b). Thickness of germinal epithelium was normal. Vacuolations were absent in by and large all the seminiferous tubules. Sperm tails flooded the lumen, though less in number as compare to group I (fig8b). Elongated spermatids were increased in number with their tails filling the lumen of seminiferous tubules. Sertoli cells were normal in appearance having branched cytoplasmic processes with basally placed nucleus. Intertubular spaces were near normal (fig6b). Cell boundaries of interstitial cells of Leydig were indistinct. (fig8b).

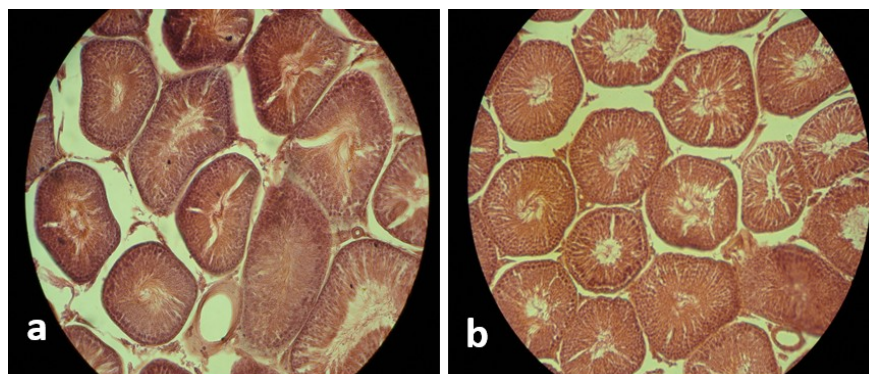


Fig. 6: Photomicrograph of seminiferous tubules showing: -improvement in the spermatogenic activity -reduction in the empty space between germ cell lines -reduction in the intertubular space. Improvement in all the features more marked in Group IV than Group III. Fig 6a- group III. Fig 6b- group IV, H&E (200x).

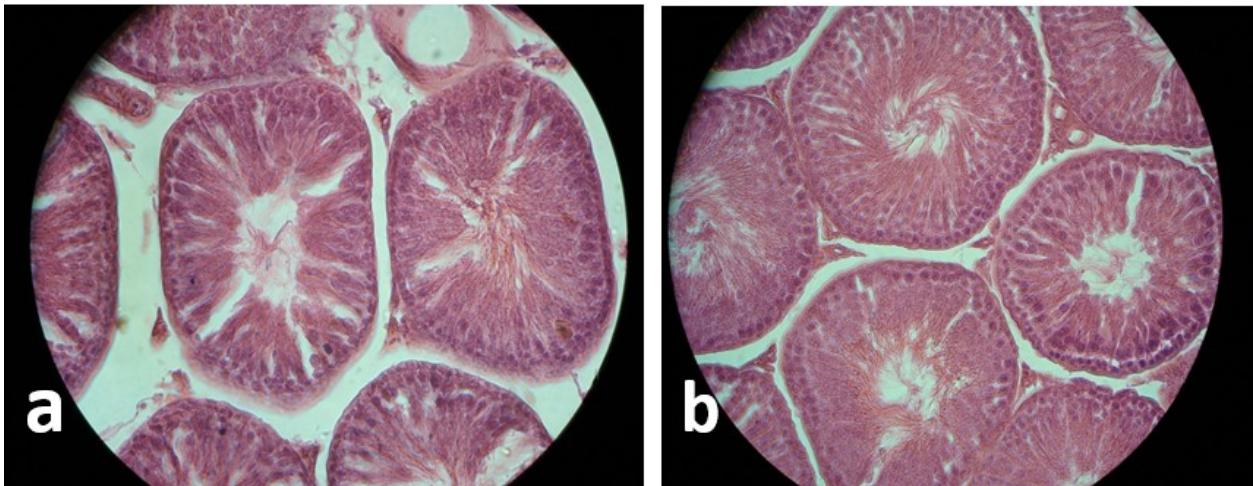


Fig. 7: Photomicrograph of seminiferous tubules showing: -improvement in the height of germinal epithelium, Normal basement membrane, detachment of germinal epithelium at few tubules, improvement in features more marked in Group IV than Group III. Fig 7a-group III, Fig7 b- group IV, H&E(400x)

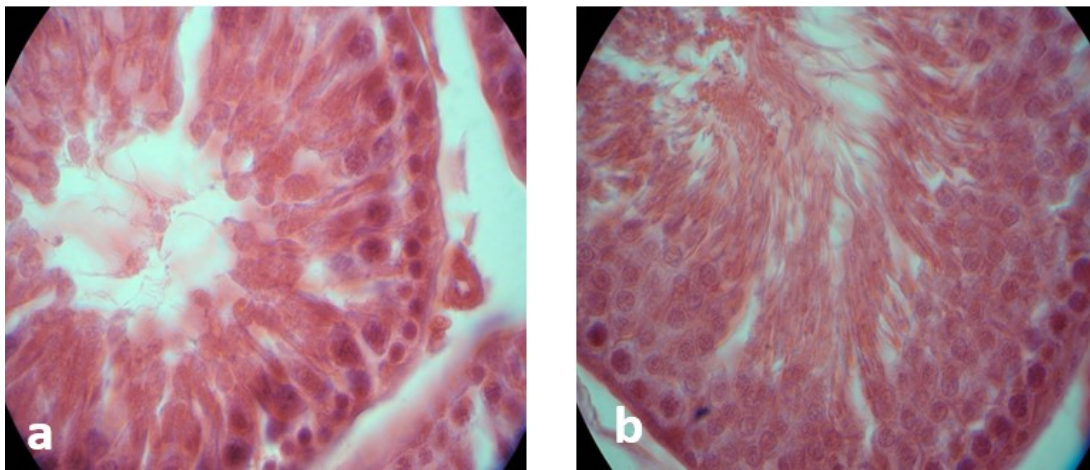


Fig. 8: Photomicrograph of seminiferous tubules showing: -improvement in the size and number of germ cell, improvement in number of elongated spermatids, improvement in above features more marked in Group IV than Group III. Fig 8a- group III ,Fig 8 b- group IV, H&E(1000x)

DISCUSSION

Pyrethroid is an insecticide of choice in current scenario. Pyrethroid deleteriously affects many tissues of insects, rodents, and other mammals. Numerous investigations have demonstrated the adverse effects of these pyrethroids on male germ line and testicular health [7].

The histological profile of group II demonstrated that seminiferous tubules were considerably smaller and loosely packed. There was thickening and disruption of the adjacent basement membrane at various sites. Similar alterations were seen in the testis of Wistar rats subjected to pyrethroids tetramethrin via inhalation, reporting aberrant seminiferous tubules with many vacuoles [8].

In another study in which rats were treated with deltamethrin, there was thickening of

basement membrane leading to collapse and distortion of seminiferous tubules. Distorted and atrophic seminiferous tubules were also observed in rats treated with cypermethrin orally [9].

The conceivable justification for decrease in the size of seminiferous tubules and irregularities in basal lamina could be attributed to fibrosis. Fibrosis results from increase amount of collagen fibers either from overproduction or by decreased phagocytosis. This may also result in thickened basement membrane. Shrinkage of seminiferous tubules can also be because of endocrine disruption which may cause reproductive system to atrophy [10].

The fibrosis of the surrounding myoid cells could also be the cause of the shrinkage of tubules. All the degenerative changes

observed may be primarily caused by increased oxidative stress and cellular toxicity. [11,12,13]

Germinal epithelium was disorganized along with sloughing of cells within the lumen. Exfoliation of the germ cell occurred because of the retraction of the cytoplasmic processes of Sertoli cells. In the systemic compartment, Sertoli cells act as mediators for every metabolic exchange and are specifically targeted by pyrethroids [14].

When rats are treated with pyrethroid, it led to redistribution of intracellular water and ion in Sertoli cells, causing retraction of cytoplasmic processes and finally shedding of germ cells [15]. Similarly, Sprague-Dawley rats that received deltamethrin treatment showed decreased testicular epithelial thickness in addition to apical sloughing and vacuolization [10]. Abnormal seminiferous tubules, marked reduction in spermatogenic cells with many vacuoles were also reported in a study in which albino rats were exposed to inhaling pyrethroids [8]. In another study rats exposed to a mosquito coil experienced vacuolization of the interstitium and hypospermatozoa production in the seminiferous tubules indicating degenerative changes with spermatozoa-free lumen [16].

Vacuolization, cell necrosis along with apical sloughing, degeneration of germ cells were also observed by in rats submitted to deltamethrin [17].

The current study's histopathological findings of group II also showed that most tubules had depleted germ layers due to the cessation of spermatogenesis, which resulted in a reduction in the thickness of the germinal epithelium. The majority of the primordial germ cell layer had both decreased in size and quantity. Failure to differentiate into germ cells or apoptosis brought on by oxidative stress could both contribute to the depletion of the germ cell layer [16,18].

Because of the high concentration of fatty acids in their plasma membrane, spermatogenic cells are at risk for oxidative insult from free radicals[19].

Cellular collapse and structural damage may

ultimately arise from lipid peroxidation of the cell membrane. Inevitably, abnormalities observed in testicular microstructure like germ cells and Leydig cells may attributed to peroxidation of unsaturated fatty acids on their plasma membranes. A notable decline in the light type cells suggested that maturation arrest occurred from the beginning. It was discovered that spermatogenesis was likely suppressed since apoptosis was restricted to the primary and secondary spermatocytes, basal germ cells, and Sertoli cell [20]. Decreased layers of spermatogenic cells and increased number of apoptotic cells were also seen in mice treated with fenvalerate in testes on exposure to pyrethroid [21].

In the group II the interstitial oedema and lipid vacuolations led to increase intertubular space. Deformed Leydig cells were also evident. Oedema has occurred due to rise in interstitial fluid, which is most likely the result of damage to the interstitial space. On repeated exposure to Deltamethrin, there was an edematous fluid buildup in intra and intertubular spaces [22]. Deformed Leydig cells were also reported in rats exposed to pyrethroid inhalation [23]. One probable explanation for Leydig cells atrophy could be localized vascular congestion and oedema.

In the current investigation, it was shown that vitamin C supplementation in group IV, resulted in an improvement in the aforementioned changes, demonstrating the function of vitamin C as an antioxidant agent. Rats subjected to oral cypermethrin plus Vitamin E exhibited marked improvement in seminiferous tubules and leydig cells [24]. Vitamin C can shield the testis, or any other organ, from the damaging effects of pyrethroid exposure by inhibiting lipid peroxidation and boosting the body's natural antioxidant defence system [25].

When group III rats were kept without being subjected to mosquito coil smoke to assess reversible changes, there was marked improvement in histological outcomes. Group IV showed a more pronounced improvement in comparison to group III. This indicates even more that the actions of pyrethroid are the source of these alterations, even though they

are transient. We were unable to locate any prior literature that discussed this form of categorization; thus a comparison could not be established.

CONCLUSION

The study demonstrated marked histopathological changes in wistar rats exposed to pyrethroid via inhalation of emissions produced by burning mosquito coil. While histopathological findings showed improvement in withdrawal group III, the enhancement was more pronounced in the group treated with Vitamin C (group IV) Hence Exposure to allethrin related mosquito coil can have deleterious health effects, which may be controlled by daily intake of antioxidants like Vitamin C in Wistar rats.

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Author Contributions

All the authors and co-authors equally contributed to the manuscript, right from the concept to publication draft.

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Conflicts of Interests: None

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