DOI:10.24041/ejmr2020.7

PROTECTIVE ROLE OF VITAMIN C AGAINST TOXIC INDOOR POLLUTANTS RELEASED FROM PYRETHROID BASED MOSQUITO COIL ON A RAT MODEL: A HISTOPATHOLOGICAL STUDY

Raghvendra Singh*, Heena Singh, Ranjana***, Punita Manik***,**
Rakesh Kumar Diwan*, Sangeeta Kumari*****

*Department of Forensic Medicine & Toxicology**, Department of Anatomy**
Era's Lucknow Medical College & Hospital, Sarfarazganj Lucknow, U.P., India-226003*
Autonomous State Medical College, Bahraich**
*Department of Anatomy****
King George's Medical University Lucknow, U.P., India-226003***

Received on : 15-01-2020
Accepted on : 04-02-2020

Address for correspondence
Dr. Heena Singh
Department of Anatomy
Autonomous State Medical College, Bahraich.
Email: dr.singhheena@gmail.com
Contact no: +91-8009108884

ABSTRACT

Mosquitoes are the deadliest animals in the world because of their ability to carry and spread disease to humans and thus causes millions of deaths every year. India is struggling every year to get rid of deadly diseases caused by these mosquitoes like malaria, chickungunya and dengue etc. Pyrethroid based mosquito coils (PBMC) are most common used mosquito repellents in India because of its effectiveness, low cost and easy availability. This study is an attempt to delineate the effect of inhaling mosquito coil smoke on the histopathological changes in heart, lung and kidney of albino wistar rats along with possible protective role of vitamin C. A total of thirty-six (36) adult albino rats of the Wistar strain are randomly divided into three groups of twelve rats in each. Group I served as control (no exposure to mosquito coil smoke). Group II and III received mosquito coil smoke 8 hours a day, 7 days a week for 12 weeks. Group III rats are co-administered with oral supplementation of Vit. C (20 mg/kg body weight) once in a day for the same time period. At the end of each experimental period, rats are sacrificed, organs are removed, and formalin preserved for histopathological study. Each specimen is examined under 5x and 10x for any histological changes. These findings suggest that inhaling mosquito coil smoke cause significant histological damage in rats' lung and kidney but least effect on their heart. this study also demonstrate the protective effect of vitamin c in group III rats. Pyrethroid based mosquito repellents are toxic to our organ system so there should be vigorous effort to replace them with other nontoxic methods. Vitamin C has a protective effects against the damage caused by these Pyrethroid based mosquito coil (PBMC), so proper supplementation should be given to affected individuals.

KEYWORDS: Pyrethroid based mosquito coil, Histopathological changes, Vitamin C supplementation.

INTRODUCTION

Mosquitoes are the deadliest animals in the world because of their ability to carry and spread disease to humans and thus causes millions of deaths every year. India is struggling every year to get rid of deadly diseases caused by these mosquitoes like malaria, chickungunya and dengue etc. To understand the stakes, look no further than the capital, New Delhi, where this summer an outbreak of chikungunya affected thousands with fever and joint pain. This was soon followed by the seasonal dengue epidemic, a more severe virus that can be fatal. If we look towards the other part of the country where dengue, malaria, Japanese encephalitis, chikungunya etc. have their terrible presence, people are suffering both physically and mentally from these mosquitos borne diseases. People are so terrified in some state like in rural Maharashtra that they coin term jahreela malaria for these mosquitos borne diseases. Even in cases of simple viral fever, patients pressurize the doctors to investigate them for malaria and dengue and the condition is such devastating that if the treating qualified clinician refuses to investigate for malaria and dengue in absence of signs and symptoms, patients keep switching from one doctor to another until they reach

a quack, who is ready to investigate and prescribe them treatment for malaria and dengue, which is actually not required. Various preventive measures were prescribed by directorate of national vector borne disease control, directorate general of health services and ministry of health & family welfare, government of India but the common preventive measures used by the people is pyrethroid based mosquito coils. Mosquito coil is a relatively cheaper and easy to use insecticide and can even be used in remote and rural areas, which are totally deprived of basic amenities like electricity and proper drainage. Majority of people living in Asia and Africa continents are devoid of healthier way of prevention against these mosquito bites. The annual worldwide consumption of these insecticidal products is in the billions of units (1). These mosquito coils are slow-burning devices which emit smoke containing pyrethroid insecticides, each coil burns for several hours and are used near persons, requiring protection against mosquitoes in order to repel mosquito, a vector of widely distributed diseases. So, these mosquito coils lead to elevated exposure smoke containing small particles (1µm), metal fumes and vapors that (2) may reach the alveolar region of the lung (3). Researchers have also found that the gas phase of mosquito coil smoke contain carbonyl compounds like formaldehyde and acetaldehyde that can produce strong irritating effects on the upper respiratory tract of children and their parents (4). In low and middle income areas where use of mosquito coil is overwhelmed to counter mosquito bites, children and adults are at a great risk so this study is perform to delineate the toxic effect of parathyroid based mosquito coil on heart, lungs, kidney of albino wistar rat. Although many studies have been performed separately on testis, brain, lung, kidney and on spleen but this study also demonstrate the possible protective role of vitamin C or antioxidants from ill effect of pyrethroid based mosquito coil.

MATERIAL & METHOD

A total of thirty-six (36) adult albino rats of the Wister strain of the age between 2-3 months weighing between 250-300 grams were obtained from the animal house of Indian Institute of Toxicology and Research. After procurement of rats they were allowed to acclimatize for 2 weeks and were fed freely on standard pellet diet 5gm/rat/day. Relatively constant environmental conditions were maintained with proper aeration and good source of light (12hours light-12hours dark and 24 $\pm$ 6^{0}C). Food and water were provided ad libitum. Rats were kept in proper hygienic condition and care was delivered in accordance with the guidelines given by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

Ethical and Legal Aspects

Ethical approval for this study was obtained from Animal Institutional Ethical Committee via reference number 66/IAH/Pharma-14 after taking all ethical aspect into consideration

Animals were randomly divided into three groups of twelve rats in each group

1) Group I - A total of 12 rats and served as control (no exposure to mosquito coil smoke)
2) Group II - A total of 12 rats with 8 hours exposure to mosquito coil smoke 7 days in a week for 12 weeks.
3) Group III- A total of 12 rats with 8 hours exposure to mosquito coil smoke 7 days in a week for 12 weeks along with oral supplementation of ascorbic acid (vitamin C) in the dose of 20 mg/kg body weight once in a day for the same time.

Animals from experimental group II and III were kept in a room of dimension 9.5 feet×9feet×9feet having proper cross ventilation. Rats could inhale pyrethrin based mosquito coil smoke by burning it for 8 hours (9:00 AM -5:00 PM). Each coil measured 15 cm in diameter and 12 gm in weight and contained 0.1%w/w of d-trans allethrin.

Treatment with ascorbic acid

A fresh aqueous solution of vitamin C was prepared by dissolving one vitamin C tablet of 500 mg in 10 ml of water. Freshly prepared solution was orally administered with the help of feeding canula to rats of experimental group III at dose level of 20mg/kg wt. (twice as the human recommended dose of 10 mg/kg body weight) (5).

Animal Sacrifice and Sample Collection

Rats were sacrificed after 12 weeks of exposure period. Rats were anaesthetized by using chloroform. Thereafter they were spread on their back and all four limbs and were held by pinning them on the wax coated dissection tray. Next the incision was made on the thoracoabdominal part. through three way stop cock, rats were perfused with normal saline followed by10% formalin. After perfusion heart, lungs and kidney were removed and were preserved in 10% formalin and sent for the histopathological analysis.

Preparation of slide and examination

Appropriate tissue samples were collected from each organ and after tissue processing and sectioning, slides were prepared which were stained using hematoxylin and eosin. Each slide was then examined first under 5x then finally under 10x for any histological changes.

RESULTS

Normal histological and microscopic features observed in the lungs (Fig 1.1), kidneys (Fig 2.1) and heart (Fig 3.1) in control group (Group I)

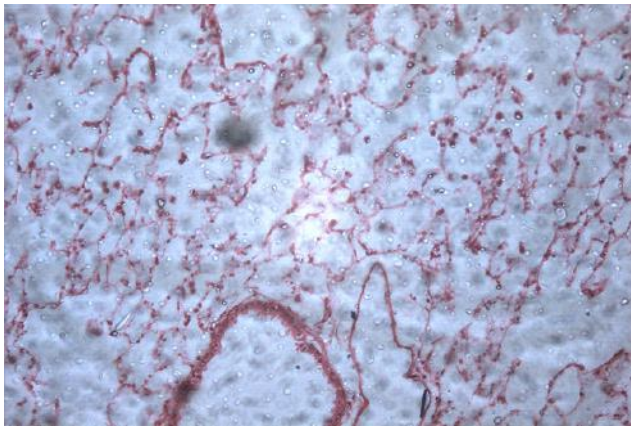

Fig 1.1 Photomicrograph of lung of control rat. Group I, H&E(100x)

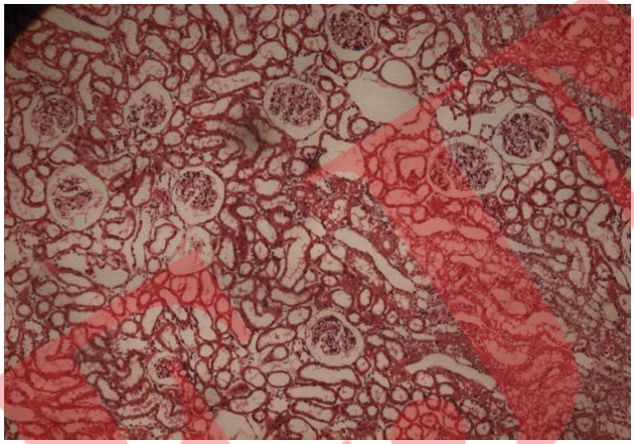

Fig 2.1 Photomicrograph of Kidney of Control Group. Group I, H&E(50x)

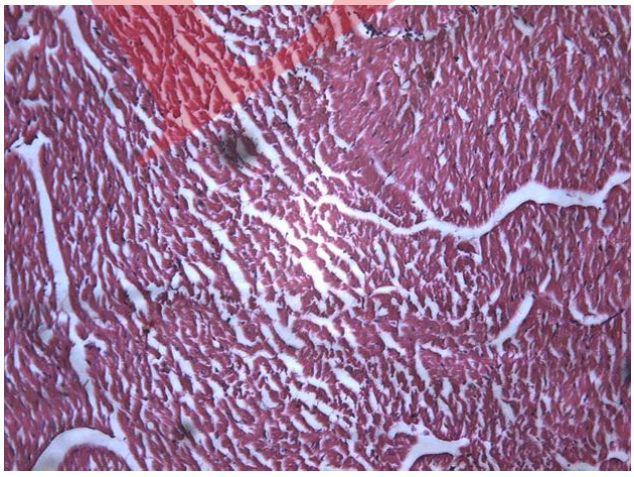

Fig.3.1 Photomicrograph of Heart of Control Group. Group I, H&E(100x)

Effect on lungs

Pyrethroid toxicity in lungs (Fig 1.2) in Group II:

Edema present (++) and Congestion (++) present all over lung alveoli and interstitium. Mononuclear infiltrate in interstitium or inflammatory infiltrate in interestitium (+++). Some bronchi show necrotizing effect with an increase no of alveolar macrophage in interstitium.

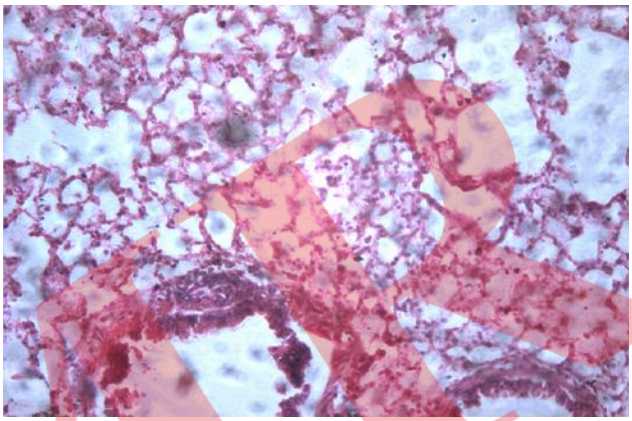

Fig 1.2 Photomicrograph of Lung of Exposure Group Showing Edema, Congestion Mononuclear Infiltrate in Interestitium Bronchi Shows Necrotizing Effect, Increase Number of Alveolar Macrophages in Interestitium. Group II, H&E(100x)

Effect of vitamin C supplementation on lungs (Fig 1.3) in group III:

Reduced edema and congestion in alveoli (+/±) and interstitium (+) in comparison group II. Interstitial infiltrates are also very less (±). There is reduced hypertrophy of bronchus without any necrotic change.

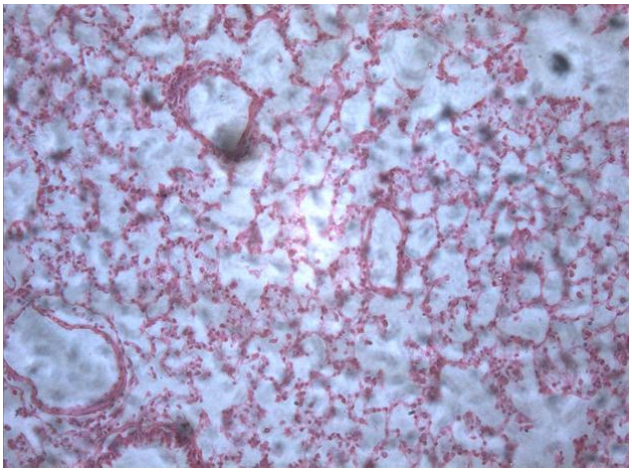

Fig 1.3 Photomicrograph of Lung of Exposure Group with Vitamin C Showing Edema and Congestion, Few Inflammatory Infiltrates and no Necrotic Changes in Bronchi. Group III, H&E(100x)

Effect on Kidney

Pyrethroid toxicity in kidney (Fig 2.2) in Group II:

In glomeruli, there is fusion of glomeruli tuft (+) with little bit congestion (+). Edema is present in tubule (hydronephrosis) and in interstitium. Infiltrates are also visualized in the interstitium (+). Blood vessels are congested.

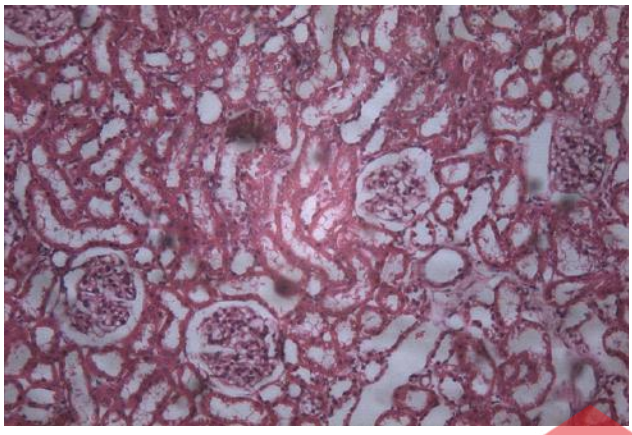

Fig 2.2 Photomicrograph of Kidney of Exposure Group Showing Fusion of Glomeruli Tuft with Little Bit Congestion and Edema, Inflammatory Infiltrates, Congestion in Blood Vessel. Group II, H&E(100x)

Effect of vitamin C supplementation on kidney (Fig 2.3) in group III:

Although protective effects are less marked in comparison to lungs but there is a clear difference between two slides. In glomeruli, there is minimal congestion & fusion of glomeruli tuft (±). Less amount of edema (+/±) is present in tubule and interstitium. Decrease interstitial infiltrate (+ or ±) in comparison to group II

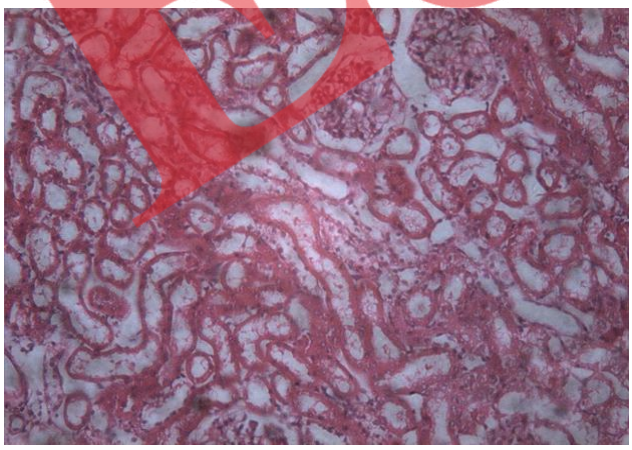

Fig 2.3 Photomicrograph of Kidney of Exposure Group with Vitamin C Showing Decrease Interstitial Infiltrate, Reduced Edema in Tubule and Interestium and Decreased Congestion & Fusion of Glomeruli Tuft. Group III, H&E(100x)

Effect on Heart

Pyrethroid toxicity in heart (Fig 3.2) in group II

In our study of 12-week duration, there is no major histopathological changes present except inflammatory infiltrates and few vacuolations were seen in the heart of group II. dilated blood vessels were also seen.

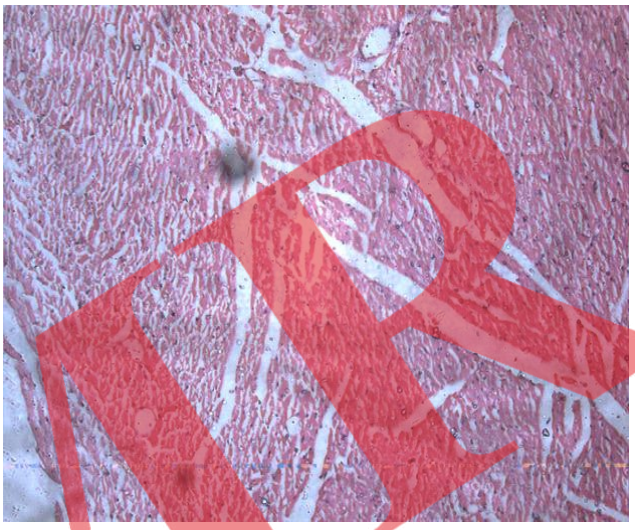

Fig 3.2 Photomicrograph of Hear of Exposure Group Showing Inflammatory Infiltrates and Few Vacuolations and Dilated Blood Vessels. Group II, H&E(100x)

Effect of vitamin C supplementation on heart (Fig 3.3) in group III

No major or minor changes are observed in this group. Vitamin C supplementation have provided full protection against these changes.

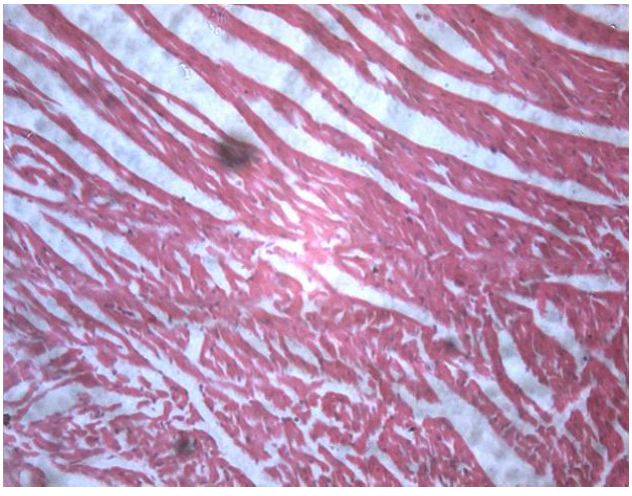

Fig 3.3 Photomicrograph of Hear of Exposure Group Showing Inflammatory Infiltrates and Few Vacuolations and Dilated Blood Vessels. Group II, H&E(100x)

DISCUSSION

Mosquito repellents based on pyrethroid are widely used insecticides now a days to protect human against these vectors because of its effectiveness, low cost and easy availability. Since the inception of first pyrethroid pesticide, allethrin, was identified in 1949, the insecticidal activity of these synthetic pyrethroids was enhanced further to combat the developing resistance against them (6). The toxicological profile of these pyrethroids based mosquito repellents is very wide. On one side, the symptoms like allergy, irritation in skin & eyes, nausea, headache are so vague as often got misdiagnosed by treating physician (7-8) while on the other side various occupational and experimental studies suggest the downregulation of pyrethroids use only because of several clinical, biochemical and even neurological changes as a result of toxicological effects like oxidative stress, cholinergic dysfunction, defective organogenesis in early developmental period etc. (9-10). Hence there is an urgent need of several study design to evaluate the exact delineating effects as a result of factors like oxidative stress, cholinergic dysfunction etc. on certain vital organs like lungs, kidney and heart. The exposure of these eight hours in this present study is only to simulate the daily exposure of human beings in normal setting.

Inhalation being the most common route of exposure, make the lungs most susceptible organ. The lung tissue of exposed rats showed edema and congestion over alveoli and interestium. Mononuclear infiltrates were markedly present in interstitium, showing inflammatory changes. Some bronchi showed necrotizing effect with an increased number of alveolar macrophages in interstitium. These effects are produced due to inflammation occurring as a result of irritants released in burning coil like aldehydes, sulphates and polycyclic aromatic hydrocarbons such as acenaphthene, phenanthrene, benzo(a)pyrene (11). Several areas of consolidation, septal thickening in alveoli and destruction of bronchial epithelial wall are mainly due to inflammatory response against these pyrethroid based mosquito coils (12-13). As kidney receives 25% of total blood volume, it become more vulnerable to these toxic irritants produced by burning smoke, which are absorbed and circulated through blood. The chief function of the kidney is to process blood plasma and excrete urine, so it plays a vital role in the clearance and excretion of xenobiotics from the body. Other studies around the world also demonstrated consistent changes in kidney like edema in tubules and infiltrates in interstitium along with multifocal congestion; cystic dilatation in the medulla; proteinaceous casts within ducts, interstitial mononuclear cellular infiltration and widespread fibrosis (14-15). Heart tissues are also affected although changes are minimal and completely reversed by vitamin C supplementation. The toxic irritants released from pyrethroid based mosquito coil (PBMC) usually produce free radicals leading to the oxidative stress to tissue. Other researchers also postulated that reactive oxygen species, induced by toxins and smoke of PBMC, interferes with the antioxidant defense system, which would trigger inflammatory response in damage sites (13,16). That's why an antioxidant supplementation will prove to be beneficial in recovery from these PBMC related damages. So, in our study too, rats were supplemented with vitamin C to see its possible beneficial effects. The results of vitamin c supplementation were promising in our study. we found while somewhat normal appearance of heart tissue was detected in the exposed group that supplemented with vitamin C, reversal of changes in lung and kidney also occur to an extent.

CONCLUSION

Mosquito bites and the diseases related to it develops a kind of fear in peoples mind. This fear exposed them to these harmful pyrethrin based mosquito repellent due to their easy availability and cost effectiveness. This study will sensitize the law maker agencies to ban these toxic repellents and to promote use of herbal or non-toxic repellent methods like killing of mosquito larvae by use of kerosene or by fishes, uses of mosquito net etc. on larger scale. Vitamin C has shown a protective effects against the damage caused by these Pyrethroid based mosquito coil (PBMC), so proper supplementation should be given to affected individuals.

Ethical & Legal aspects

We declare that the ethical approval for this study was obtained from Animal Institutional Ethical Committee, King George's Medical University via reference number 66/IAH/Pharma-14 after taking all ethical aspect into consideration.

Conflict of Interest

Author declare that there is no conflict of interest with any agencies.

REFERENCES

1. Krieger, R.L, T.M. Dinoff and X. Zhang. Octachlorodipropyl ether (S-2) mosquito coils are inadequately studied for residential use in Asia and illegal in the United States. Environmental Health Perspectives. 2003; 3: 12.
2. Liu, W.K. and S.E. Sun. Ultrastructural changes of tracheal epithelium and alveolar macrophages of rats exposed to mosquito-coil smoke. Toxicol. Lett. 1988; 41: 145-157.

3. Cheng V., H.R. Lee and C.S. Chen. Morphological changes in the respiratory system of mice after inhalation of mosquito-coil smoke. Toxicol. Lett. 1992; 62: 163-77.
4. Chang J.Y. and J.M. Lin. Aliphatic aldehydes and allethrin in mosquito-coil smoke. Chemosphere. 1998; 36: 617-624.
5. Kahn PK, Sinha SP. Antimutagenic efficacy of higher dose of Vitc. mutat. res. 1993; 298(3):157-161.
6. Bradberry SM, Cage SA, Proudfoot AT, et al. Poisoning due to pyrethroids. Toxicol Rev. 2005;24(2):93-106.
7. Sharma VP. Health hazards of mosquito repellents and safe alternatives. current science. 2001; 80:3.
8. Azizi, B.H.O. and R.L. Henry. The effects of indoor environmental factors on respiratory illness in primary school children in Kuala Lumpur. Int. J. Epidemiol. 1991; 20: 144-149.
9. Sinha C, Agrawal AK, Islam F, et al. Mosquito repellent (pyrethroid-based) induced dysfunction of blood-brain barrier permeability in developing brain. Int J Dev Neurosci. 2004; 22(1): 31-37.
10. Ansari RW, Shukla RK, Yadav RS, et al. Cholinergic dysfunctions and enhanced oxidative stress in the neurobehavioral toxicity of lambda-cyhalothrin in developing rats. Neurotox Res. 2012;22(4):292-309.
11. Liu W., J. Zhang J.H. Hashim J. Jalaludin, et al. Goldstein. Mosquito coil emissions and health implications. Environ. Health Perspect. 2003; 111:1454-1460.
12. A.F. Ayorinde, B.O. Oboh, O.A. Otubanjo, et al. Some Toxicological Effects of a Commonly Used Mosquito Repellent in Lagos State, Nigeria. Research Journal of Environmental Toxicology. 2014;8: 46-52.
13. Abdulla Al-Mamun M, Ataur Rahman M, Habibur Rahman M, et al. Biochemical and histological alterations induced by the smoke of allethrin based mosquito coil on mice model. BMC Clin Pathol. 2017;17(1):19.
14. Garba S.H., M.M. Shehu, A.B. Adelaiye. Histopathological and biochemical changes in the rats following exposure to a pyrethroid based mosquito coil. J. App. Sci. Res.2007; 3: 1788-1793.
15. Siddique N, Nazir S, Jabeen R. Mosquito Coil Smoke Inhalation Effects on Interstitium of Kidney of Albino Rats. P J M H S. 2015;9(2):672.
16. Idowu ET, Aimufua OJ, Ejowoke YO, et al. Toxocological effects of prolonged and intense use of mosquito coil in rats and its implication on malaria control. Int. J. Trop. Biol. 2013; 61(3): 1463-1473.

How to cite this article : Singh R., Singh H., Ranjana, Manik P., Diwan R.K., Kumari S. Protective Role Of Vitamin C Against Toxic Indoor Pollutants Released From Pyrethroid Based Mosquito Coil On A Rat Model: A Histopathological Study. Era J. Med. Res. 2020; 7(1): 40-45.