

# Histological Changes in the Cerebellum of Albino Rats after Inhalational Exposure to Pyrethroid Based Mosquito Repellent

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## ABSTRACT

**Background:** There has been an ever increasing use of liquid vaporizers as residential insecticides to get rid of the problem of mosquito and as a concern shown towards prevention of increasing cases of vector borne diseases. Adverse impact of these chemicals are many including giddiness, nausea, headache, body ache, lethargy and dizziness but the current research on the safety of these chemical compounds is market driven and proper histological studies that can establish their toxic effects on cerebellar cortex which acts as higher centre of coordination, balance and learning are rare. Accordingly the present study was planned to look into the claim of safety of these inhalational compounds and to establish the correlation, if any between pyrethroid based mosquito repellent inhalational use and the histological insult to the cerebellum of Albino rats. **Methods:** Total of twenty albino rats were marked into groups marked as control and experimental. The exposure of experimental group was carried out to 3.2% w/v prallethrin vapours for total of 12 hours in a day and continued for 180 days. The Albino rats in control group were put in similar surroundings but without exposure to any mosquito repellent. The albino rats were killed after completing exposure of 180 days. The rats brain was dissected and Cerebellum was taken out. Tissue processing and sectioning done and finally stained using haematoxylin, eosin and thionin stains. **Results:** Outer molecular and inner granular layer of cerebellum showed areas of degeneration with disruption and decreased density of cells in Purkinje cell layer. **Conclusions:** The findings of the study do confirms that mosquito repellents given by inhalational route leads to toxic insult as evident in this study on Albino rats on long term exposure of 180 days as shown by histological alterations in the sections of cerebellar cortex of rat CNS.

**Key words:** Mosquito repellent, inhalation, neurotoxicity, albino rats, cerebellar cortex

## INTRODUCTION

Prallethrin, a type-I pyrethroid is a chief constituent in insecticidal formulations intended for indoor use in developing countries<sup>[1,2]</sup> in the form of powders, sprays, impregnated papers, mats electro evaporators and coils<sup>[3]</sup> to control mosquitoes, cockroaches and other insects. Their use has risen dramatically during the last 10 years in India

and other developing countries due to high insecticidal and low mammalian toxic effects.<sup>[1,4,5,6,7]</sup> As a consequence of its extensive use for agricultural and domestic purposes, there has been a concern among public regarding the routine use of this pyrethroid. Prallethrin is regularly used with its maximal human exposure for prolonged periods for at least 8h/day<sup>[8]</sup> but there is a paucity of information concerning the effect of routine and long term use of the prallethrin based mosquito repellents in humans. As majority of the earlier studies are on small animals who received drug by oral, IV or intramuscular route and not by respiratory route, which is conventional route by which majority of population is exposed for several decades.

The cerebellum is the seat of coordination, fine movements, equilibrium, posture and balance. Any insult to the cerebellum is bound to be reflected in the above parameters that can be quantitatively measured and correlated with the histological findings as observed in our study. Therefore the present study was planned to look into the claim of safety of these inhalational compounds and to establish the correlation, if any between pyrethroid based mosquito

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repellent inhalational use and the histological insult to the cerebellum of Albino rats.

## METHODS

The current research was undertaken on Charles foster, adult Albino rats that weight around 100-150gms. The animals were provided laboratory diet (Lipton India Limited) and water ad-libitum. The permission from institute ethical committee was obtained and animals were handled, fed and taken care as per the ethics committee guidelines. The rats were placed under identical diurnal and temperature conditions. After recording their weights, the animals were numbered and marked and assigned a group- experimental or control.

### Group 1-Experimental group

### Group 2-Control group

The plastic cages of size 360mm x 220mm x 140mm with many holes were used for the housing of these animals. Exposure to liquid mosquito repellent was carried out inside a closed room (1800mm x 2400mm) according to the method of Sinha et al<sup>8</sup>. The exposure of Albino rats was carried out to 3.2% w/v prallethrin vapours for 12 hours every day for a total duration of 180 days. The animals in control group were housed under identical conditions but without any exposure to 3.2% w/v of chemical compound, prallethrin using inhalational vapours. The Institutional animal ethics committee provided necessary permission to carry out the present study. proper guidelines for handling and experimentation was followed.

The body weight was measured weekly and the water consumption was assessed daily.

Rats in the experimental group were exposed to mosquito repellent vapours daily for 12 hours and continued for total of 180 days. At the end of exposure (after 180 days) the animals (experimental and control) were killed and sections prepared from cerebellar cortex (grey matter) to detect the neuro-histological changes, if any. Staining reagents used for the histological preparations i.e. Haematoxylin Eosin and thionin were obtained from Department of Anatomy J.N. Medical College.

## RESULTS

### Cerebellar Cortex:

#### Haematoxylin and Eosin stained sections

X100 Magnification –The control sections showed normal trilaminar structure of cerebellum. All the layers showed well stained nuclei, orientation of purkinje layer is maintained. The experimental tissues showed trilaminar structure of cerebellum with areas showing degeneration in outer molecular and inner granular layer, disruption of Purkinje cell layer, and vacuoles in white matter (Figure 1.1 & 1.2).

X400 Magnification – Control rats showed the regular arrangement of Purkinje cell layer. The experimental animals showed disruption of normal Purkinje layer at interphase of outer molecular and inner granular layer (Figure 1.3 & 1.4).

### Thionin stained sections:

X100 Magnification – The control tissues showed intact trilaminar cortical structure with well stained nuclei. The experimental specimens showed disruption of Purkinje cell layer and increase density of nuclei in granular layer (Figure 2.1 & 2.2).

X400 Magnification – The control sections showed well stained nuclei in granular layer and regularly arranged Purkinje cell layer. The experimental specimens showed irregular spacing of Purkinje, increase in the density of nuclei in granular layer and separation of molecular layer from Purkinje cell layer ( Figure 2.3 & 2.4).



Fig 1.1: Cerebellum, control. H&E (X100)

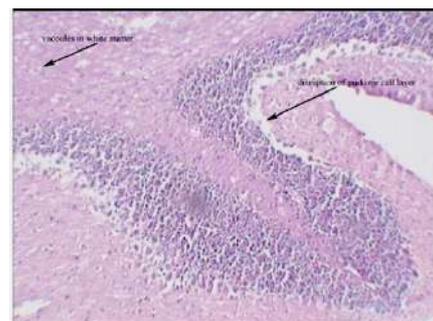


Fig1.2: Cerebellum, exp. H&E(X100)

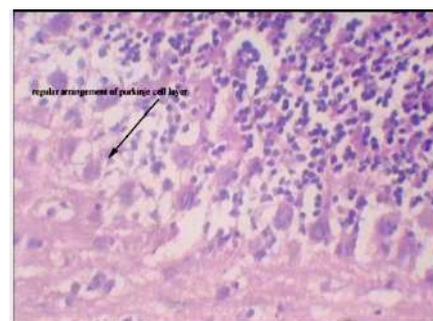


Fig1.3: Cerebellum, control, H&E(X400)

## DISCUSSION

Histological parameters are considered as an evidence of tissue insults by toxic compounds. Hence to study the neurotoxicity of any drug the assessments of histological damage have been conducted. Tauer et al<sup>[9]</sup> found that Phenytoin alters Purkinje cell axon morphology. Also Ohmori et al (1999) studied the developmental neurotoxicity of Phenytoin on granule cells and Purkinje cells in mouse cerebellum.

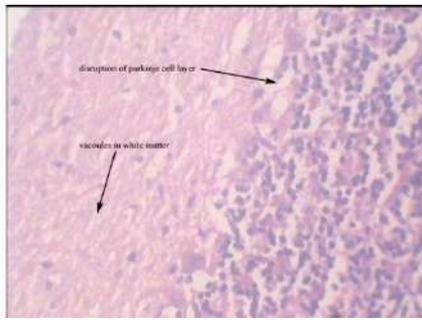


Fig1.4: Cerebellum, exp. H&E (X400)

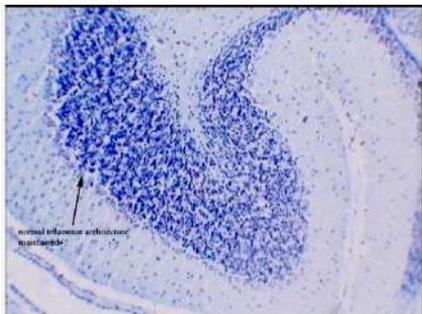


Fig 2.1: Cerebellum, control, Thionin (X100)

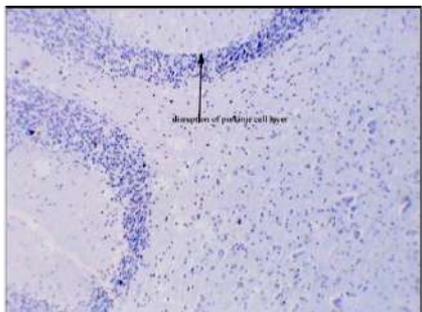


Fig2.2: Cerebellum, exp. Thionin (X100)

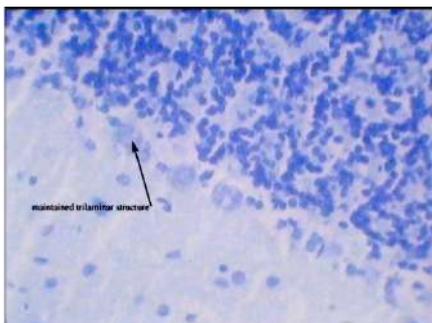


Fig 2.3: cerebellum, control, Thionin (X400)

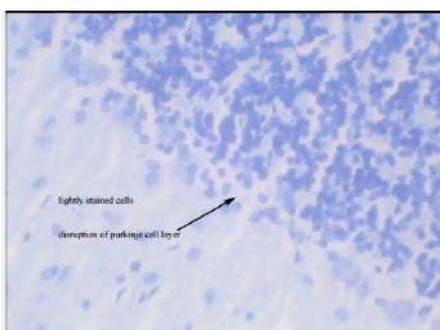


Fig 2.4: Cerebellum, exp. Thionin (X400)

deficiency and impaired maturation of Purkinje cells. Sections of the cerebellar cortex showed edema and degeneration of outer and inner layers and disruption of Purkinje cell layer (Fig. 1.2 and 1.4, 2.2 and 2.4) The Purkinje cell layer itself showed malalignment and decrease in number of Purkinje cells. The granular layer showed increase in cellular density and clumping of the nuclei (Fig. 2.2 & 2.4). The white bundle showed numerous small sized vacuoles in the experimental animals (Fig. 1.2 & 1.4).

Sinha et al<sup>[8]</sup> reported that body weight of rat pups exposed to pyrethroid containing mosquito repellent decreased significantly but no gross abnormality in behaviour was observed in rat pups and in dams. Tsuji et al<sup>[11]</sup> also reported lack of changes in brain muscarinic receptor and motor activity of mice after neonatal inhalation exposure to d-allethrin.

Till recently, pyrethroids were considered to be safe because of their short half-life and therefore widely used for mosquito control inside the house<sup>[8]</sup> but with reports of increasing incidence of pyrethroid neurotoxicity in the previous literatures reveals that the central nervous system is susceptible to the damaging effects of pyrethroid compounds. The present study also concludes that they are harmful to adults too. Hence their usage needs to be cautious.

## CONCLUSION

The findings of our study establish that pyrethroids given by inhalational route do cause neurotoxicity on chronic exposure as shown by degenerative changes in the histological sections of Cerebellum cortex.

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