

## HISTOPATHOLOGICAL CHANGES IN CEREBELLUM OF ALBINO RAT EXPOSED TO SYNTHETIC PYRETHROID AND OCIMUM OIL

V. Annu and R. Sudesh\*

Department of Zoology, Maharshi Dayanand University, Rohtak, Haryana, India

\* Author of correspondence: [sudesh\\_zoology@rediffmail.com](mailto:sudesh_zoology@rediffmail.com)

### ABSTRACT

This study presented histopathological changes in the cerebellum of albino rats. Twenty rats of both sexes weighing between 3-4 months were used for this study. Three groups, I, II and III, were made for study. Group III served as a control with no treatment administered. Rats in groups I and II were allowed to inhale synthetic pyrethroid, transfluthrin and herbal, Ocimum oil for 90 days. After 30 and 90 days, rats were sacrificed, and sections of the brains were processed for histopathology. Results revealed vacuolation in the molecular layer, depletion of Purkinje cell layer, and degeneration of granular layer in the cerebellum was observed in rats allowed to inhale synthetic pyrethroids. There were no such alterations seen in the cerebellum of rats allowed to inhale Ocimum oil. Therefore, it was concluded that synthetic pyrethroid-based mosquito repellent is more hazardous to the health of the test animals than herbal Ocimum oil mosquito repellent.

**Keywords:** Cerebellum, Transfluthrin, Ocimum Oil, Pyrethroids

### INTRODUCTION

Pyrethroid insecticides are generally used in the field, houses, etc., to get protection from insects like mosquitoes, etc. Because of their importance, new insecticides have been synthesized, which are found to be less toxic for mammals and less resistant to an insect than other insecticides (Das *et al.*, 2003). The main insecticides which are frequently used in a household are mosquito coils, liquid vaporizers, and aerosol (Krieger, 2003). Many studies indicated that synthetic pyrethroids harm the brain of animals like they induced neurotoxicity in rats. Further, their expansion may also cause central nervous system toxicity in infants (Briassoulis, 2001; Sudakin *et al.*, 2003). Many studies indicated the depleting effect of synthetic pyrethroids in liquid vaporizers on cerebellum rats (Saim Hasan *et al.*, 2012; Rodríguez *et al.*, 2016). Besides synthetic pyrethroids, *Ocimum sanctum* (Tulsi), a herbal insect repellent, has healing and medicinal properties (Sunanda and Sandhya, 2018). The oil of tulsi has been found to have anti-stress and anti-anxious properties due to the presence of ethanolic compounds in them, as found in certain studies (Edris and Farrag, 2003). Due to the wide application of osmium oil and harmful effects of synthetic pyrethroid present experiment was designed to observe the effect of synthetic pyrethroids, transfluthrin 0.88% w/v, and Ocimum oil, vapors on albino rat cerebellum for a period of 90 days.

### MATERIALS AND METHODS

**Animals:** Experimental Albino rats (3-4 months) from the animal house of DFSAH, LUVAS, Hisar, Haryana, were housed in different polypropylene plastic cages and acclimatized under laboratory conditions with 12 hr. light/dark conditions for ten days. They were fed on a standard diet twice a day and given water *ad libitum*. After getting clearance from the institutional animal ethical committee experiment was started.

**Experimental design:** Animals were divided into three groups- Group I and Group II acted as experimental groups, and group III was taken as a control group. In Group I, eight animals were allowed to inhale mosquito repellent containing synthetic pyrethroid transfluthrin. Further animals of this Group

were divided into two subgroups with four animals in each. One subgroup was allowed to inhale synthetic mosquito repellent for 8 hrs./day (Shrivastva *et al.*, 2006), and the Other one was exposed for a period of 18 hrs./day (Gupta *et al.*, 1999). The methodology of Group II, where Ocimum oil was used as mosquito repellent, remained the same as that of Group-I.

**Histopathological studies:** The animals were sacrificed after 90 days, and the brain of rats was fixed in 10% formalin for 48 hrs. So that the tissue becomes immobilized and the cells get fixed without losing their original morphology and shape. After fixation, the brain tissue was allowed to clean under running tap water at room temperature for more than 2 hours to remove the extra fixative absorbed by the tissue as it can cause hindrance in the staining process. After washing, cut the tissue section into roughly small pieces of L.S and V.S as needed accordingly. Dehydration is done by subjecting the tissue section in ascending order of 30%, 50%, 70%, 90%, and 100% alcohol for 10 to 20 minutes. As dehydration removes the excess water from the tissue section and prevents the tissues from putrefaction. After dehydration, the tissue was cleared with alcohol, xylene, and paraffin wax; after this process, the section was standardized in paraffin wax by embedding. The tissue sections of 5microns in size were cut by using a rotary microtome. The sections were stained with Harries hematoxylin and eosin. Observations were made using a light microscope, and photographs were taken with an automatic photomicrographic system.

**Table 1: Exposure of synthetic and herbal pyrethriod to albino rats**

Time of Exposure hrs/day	Synthetic pyrethroids group-I	Ocimum oil group-II	group-III Control
	No. of animals	No. of animals	No. of animals
8	4	4	-
18	4	4	-
0	-	-	4

## RESULTS AND DISCUSSION

Present study evaluated long-term inhalation of synthetic pyrethroid results in an adverse effect on the cerebellum of albino rats. After 30 days of exposure to synthetic pyrethroid, the Purkinje cell layer was found to be reduced in male and female rats. The granular layer and the appearance of the vacuole in the molecular layer were also observed in these animals' cerebellum (Fig. 2,6). This effect becomes more prominent in male and female animals after 90days (Figs. 3, 7).

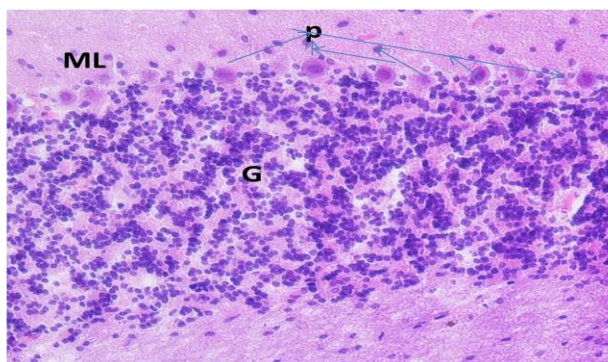


Fig 1. L.S. of The control-group rats brain section  
 •normal Purkinje cells (P),  
 •the densely populated granule cell layer (G) and  
 •the uniform molecular layer (ML) in the

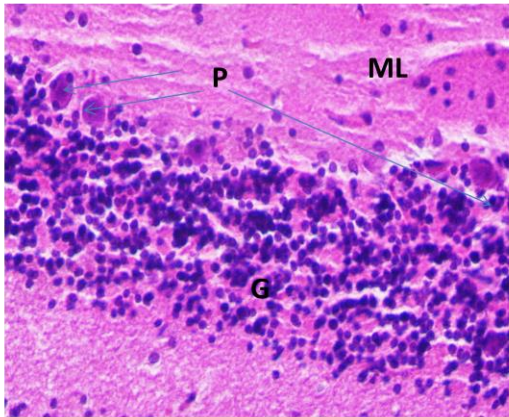


Fig 2. L.S. of Synthetic exposed male rats (30days) brain section  
 •Less number of Purkinje cells (P),  
 •Reduced granule cell layer (G) and  
 •Appearance of vacuoles in molecular layer (ML) in the

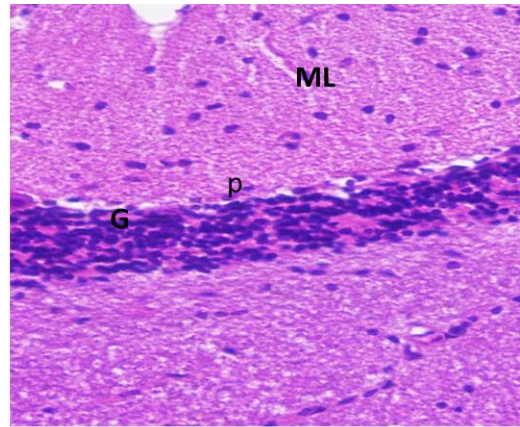


Fig 3. L.S. of Synthetic exposed male rats (90days) brain section  
 •Loss of Purkinje cells (P),  
 •Very much Reduced granule cell layer (G) and  
 •Appearance of vacuoles in molecular layer (ML) in the

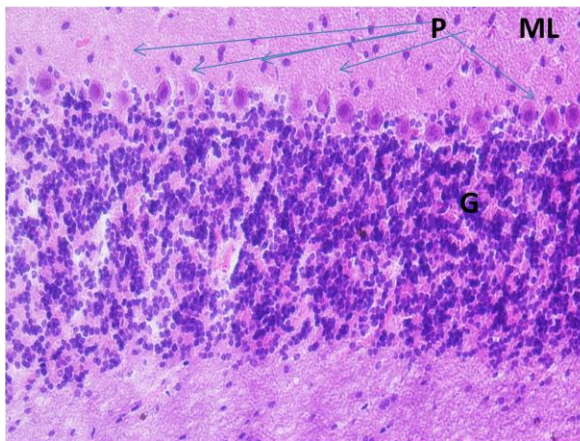


Fig 4. L.S. of herbal exposed male rats (30days) brain section  
 •Normal Purkinje cells (P),  
 •Intact granule cell layer (G) and  
 •Normal molecular layer (ML)

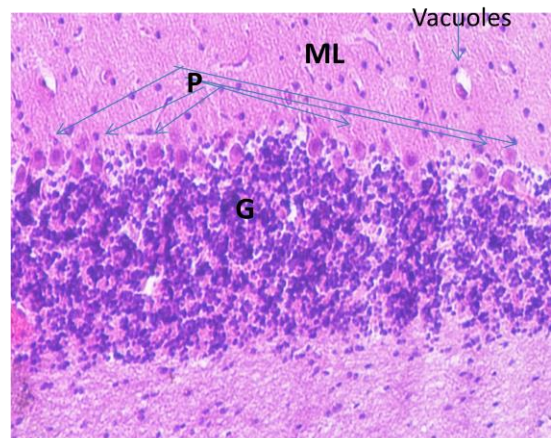


Fig 5. L.S. of herbal exposed male rats (90days) brain section  
 •Normal Purkinje cells (P),  
 •Intact granule cell layer (G) and  
 •Very less change in molecular layer (ML)

In animals of both sex exposed to Ocimum oil, normal Purkinje cells, a densely populated granular layer, and a uniform molecular layer was observed after 30 days (Fig 4,8). After 90 days, no adverse effect was observed in the cerebellum of rats except in the case of male rat cerebellum, where minor vacuoles appeared in the molecular layer (Fig 5). In females, all portions were remained normal after 90days of exposure to herbal Ocimum oil (Fig. 9). The result obtained from these experiments has shown that acute and chronic exposure to synthetic pyrethroids in mosquito repellent machines is more toxic to rats than

Ocimum oil. The toxicity of exposure depends on the period of exposure. The present study establishes that synthetic pyrethroids given by the inhalational route for a long period do cause neurotoxicity where degenerative changes were found out in the cerebellum.

Synthetic pyrethroid harms the cerebellum of the brain, and this fact is well supported by the present study in which albino rats were exposed to Synthetic pyrethroid through inhalation.  $\beta$ -cyfluthrin a synthetic pyrethroid, caused neurotoxicity as well as oxidative damage in the brain of Swiss albino mice at the tested dose levels, Rajawat *et al.*, (2019). Pathological effects were visible in granular cell layers, Purkinje cell layers, and molecular layers after 30 days of exposure. Report of several pieces of research by Nebeker *et al.*, (1992), Menegaux *et al.*, (2006), and Oluwatobi *et al.*, (2014) showed that exposure to synthetic pyrethroids had potential hazards in both man and animals and that prolonged exposure through inhalation caused changes in tissue architecture and brain malfunction. Manna *et al.*, (2004) and Khan *et al.*, (2009) observed congestion and hemorrhage in the brain of rats intoxicated with single and repeated doses of cypermethrin (synthetic pyrethroid), but the present study showed no such changes in the brain of albino rats were observed; this may be due to different routes of synthetic pyrethroid administration.

Pawar *et al.*, (2017) results indicated that 28 days of oral exposure to Lambda-cyhalothrin, a synthetic pyrethroid, causes oxidative damage to the brain of mice, which could be responsible for neurotoxicity. Administration with a high dose of fipronil resulted in histopathological changes-vacuolation in the molecular layer, necrosis of neurons, and loss of Nissl's substance in mice's cerebellum (Badgajar *et al.*, 2015). Pankaj *et al.*, (2019) observed that the Inhalation of pyrethroid and herbal mosquito vaporizers both cause neuronal degeneration and spongiosis in the spinal cord. Discontinuation of herbal vaporizer exposure for one month exhibits a significant reversal of injury compared to the withdrawal of pyrethroid-based mosquito vaporizer. Their results on synthetic pyrethroid are in favor of the present findings but against to Ocimum oil.

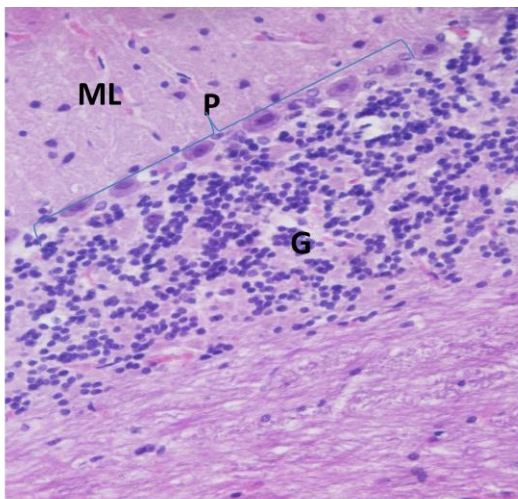


Fig 6. L.S. of Synthetic exposed female rats (30days) brain section

- Vacuoles appears in Purkinje cells (P) layer,
- Reduced granule cell layer (G) and
- Loose molecular layer (ML)

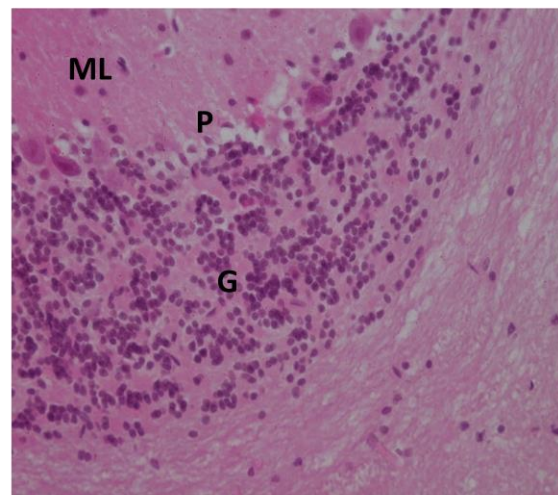


Fig 7. L.S. of Synthetic exposed female rats (90days) brain section

- disappearance of Purkinje cells (P) layer,
- Reduced granule cell layer (G) and
- Loose molecular layer (ML)

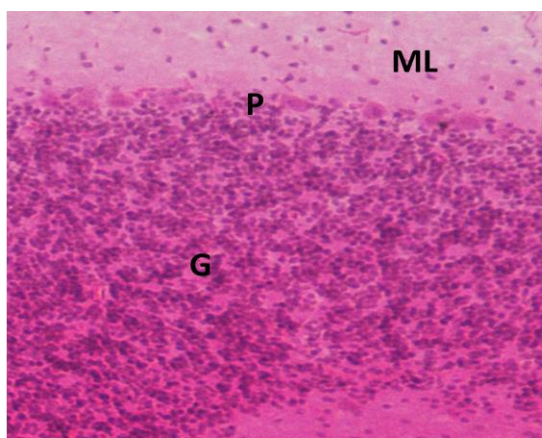


Fig 8. L.S. of Herbal exposed female rats (30days) brain section

- Normal Purkinje cells (P) layer,
- Normal granule cell layer (G) and
- Normal molecular layer (ML)

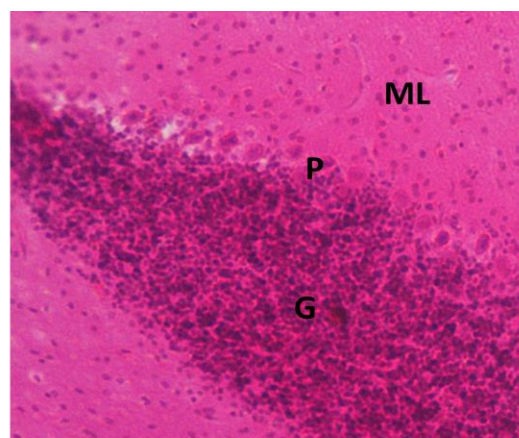


Fig 9. L.S. of herbal exposed female rats (90days) brain section

- Very less Vacuoles seen in Purkinje cells (P) layer,
- Normal granule cell layer (G) and
- Normal molecular layer (ML)

## CONCLUSION

The present findings confirmed that histopathological changes appeared in the cerebellum of albino rats who received synthetic pyrethroid. Further, this study showed that synthetic pyrethroid exposure to animals for a long time resulted in the loss of the Purkinje cell layer of the cerebellum in the albino rat. On the other hand, no such alteration in the cerebellum was observed in rats exposed to herbal *Ocimum* oil, so it can be considered that an herbal one is better than the synthetic one.

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