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Effects of Passiflora foetida on the Number of Purkinje Cells in the Cerebellum of Albino Rats

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Abstract. *Permot (Passiflora foetida) is one type of plant that has been used as a mosquito coil. Besides being effective at killing Aedes aegypti mosquitoes, mosquito coils made from permot leaf extract are also effective at killing German cockroaches and house flies. Until now there have been no reports stating the side effects of using permot leaf extract in humans. This study was designed to demonstrate the harmful side effects of inhalation of smoked mosquito coils made from permot leaf extract on the cerebellar cortex of adult male rats. Thirty-five adult male rats were used in this study. Rats were divided into 7 groups, namely group 1 as a negative control (without exposure), group 2 as a positive control (transfluthrin exposure 3000 ppm), groups 3 to 7 were treatment groups of permot leaf extract with graded doses (500 ppm, 1000 ppm, 2000 ppm, 3000 ppm, and 4000 ppm), and was treated for 12 weeks. All rats were sacrificed in a timely manner and cerebellar specimens were taken, prepared and observed using a light microscope. Previously, rats were weighed periodically to determine the effect of toxicity. The results showed that exposure to mosquito coil smoke made from 3000 ppm Transflutrin and 4000 ppm permot leaf extract caused weight loss in rats. Likewise on the weight of the rat cerebellum. The effect of decreasing the number of Purkinje cells also occurred in the group exposed to mosquito coils made from Transflutrin 3000 ppm and permot leaf extract 4000 ppm. Therefore, the safe and effective dose of mosquito coils made from permot leaves is less than 4000 ppm, because exposure to doses of more than 4000 ppm can cause degenerative changes in the cerebellar cortex.*

Keywords: *cerebellum, mosquito coil, permot leaf, Purkinje cells, rats*

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INTRODUCTION

Synthetic pyrethroids such as Transfluthrin have been widely used in recent years because they are considered safer and have low toxicity in mammalian experimental animals such as mice and rats, unlike other types of pyrethroids which still have a high level of toxicity in mammals (Rehman et al., 2014; Cham et al., 2016). Synthetic pyrethroid insecticides like transfluthrin are safer to use than pyrethrins. Pyrethrin like transfluthrin compound itself is obtained from flower seed extract (*Chrysanthemum* spp). The advantage of using synthetic pyrethroid insecticides is that they are more stable in light and air than

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pyrethrins. Rodriguez et al. (2016) stated that insecticides made from synthetic pyrethroids were the most widely used in households and agriculture. Insecticides made from pretroids are neurotoxic, that is, they act on voltage-gated sodium channels in neurons, causing depolarization and hyperexcitation of the nervous system. The activity of the acetylcholinesterase enzyme is another potential target for pyrethroid action in insects (Harrill et al., 2008; Syed et al., 2016). However, the effect of pyrethroids on neurotransmitter release may be either dual excitatory or inhibitory, or it may be due to both (Rodríguez et al., 2016).

Although synthetic pyrethroids have been considered harmless to mammals, some severe problems have been reported. Cases of poisoning in humans appear to be limited to intentional or accidental overexposure. Synthetic pyrethroids turn out to be highly neuroactive substances when the nervous system is directly accessible (Rodriguez et al,. 2016)

One of the medicinal plants suspected of containing active ingredients that can be used as bioinsecticides is the permot plant (*Passiflora foetida*) (Susilowati, 2016). Permot is part of Indonesia's plant that grows wild and is found in abundance. Permot plants can be easily found in fields, rice fields, gardens, or growing vines between the main plants that are deliberately planted, fenced, and also propagated on the walls so that these plants are usually cleaned, burned or simply thrown away. Permot leaf extract has the main chemical composition of alkaloids, phenols, flavonoids, and cyanogenic compounds, as well as fatty acids (Patel et al., 2011). Susilowati (2017) stated that mosquito coils made from permot leaf extract were effective in killing *Aedes aegypti* (> 90%) at a dose of 2165 ppm.

Several previous studies have been conducted on the toxic effects of permot leaf extract in mosquito coils in non-target experimental animals, such as mice and carp. The histopathological picture of exposure to mosquito coils made from permot leaf extract at a dose of 500 ppm to 3000 ppm did not show damage to the liver and kidney tissues of mice, but a dose of 4000 ppm could cause cell necrosis (Susilowati, 2016). Susilowati's research (2017) stated that exposure to mosquito coils made from permot leaf extract up to a dose of 3000 ppm did not cause damage to the blood cells of mice, but exposure to a dose of 4000 ppm caused damage to the blood cells of mice. However, there has never been a neurotoxicity test for mosquito coils made from permot leaf extract on the histopathological features of the cerebellum and the number of Purkinje cells. This study aimed to evaluate the neurotoxicological effect of mosquito coils made from permot leaf extract on histopathological changes in the cerebellum and Purkinye cell number in rats.

MATERIALS AND METHODS

This research was conducted at the Biology laboratory of the Faculty of Medicine and Health, Krida Wacana Christian University, Jakarta, from July 2021 - September 2021.

Permot Leaf Extraction

Permot leaves (Fig. 1) were cleaned of impurities, dried at room temperature to avoid the damage of secondary metabolites by direct sunlight. Permot leaves were cut into small pieces with a knife, then crushed with a blender until forming powder. The powder was macerated with 70% ethanol for 3-4 days at room temperature to remove all the compounds contained in the powder. After 3-4 days of filtering, the resulting maserate was accommodated in an erlenmeyer. The filtering was repeated several times until the maserate became clear. The maserate was concentrated

using a rotary evaporator so that the solvent evaporates, and separate the compound from the solvent (Patel et al., 2011).

Making Mosquito Coils

Based on Susilowati (2013) and preliminary research to determine the effective dose, a graded dose of mosquito coils made from permot leaf extract was produced. The ingredients for the mosquito coils consisted of coconut shell and permot leaf pulp as a filler. The dough was made by mixing all ingredients with tapioca flour as adhesive (5% of the total weight) to become a thick dough. The weight of the particles used for one test sample of mosquito repellent was 52.24 g and the weight of the adhesive was 2.6 g. The dough was then put into a circular worm-shaped mold and dried in the sun for a while. The mosquito coil was then separated from the mold and was forged by a pressure of 25 kgThe resulting product was dried until a moisture content of about 12%.

Research Stages

Before the treatment, the rats were acclimatized for 2 weeks by being given food and drink ad libitum. Total of 35 male rats Balb B strain weighing 150-165 g were grouped into 7: negative control group (without exposure), positive control group (3000 ppm Transflutrin exposure), permot leaf extract treatment group with graded doses (500 ppm, 1000 ppm, 2000 ppm, 3000 ppm, and 4000 ppm). Exposure to mosquito coils was given for 12 weeks, 8 hours per day (Armalina et al., 2021). After 12 weeks of treatment, all rats were weighed and then anesthetized using pentobarbital. Head surgery of rats for all groups were conducted using brain forceps to bulge the head, the brain was carefully removed, the cerebellum was cut and weighed. The cerebellum was put into the fixative solution and histological preparations were made using the standard method and stained using Hematoxylin and Eosin (Ramaswamy & Dayasagar, 2017). Cerebellum histological preparations were used to see the histopathological changes that occurred and the number of Purkinje cells.

Statistical Analysis

The research data obtained was analyzed using one way Anova (SPSS series 25 program). If the results of the one way Anova test were significant ($p < 0.01$), the test was continued with LSD. In addition, the data displayed in the form of mean ± standard deviation.

RESULTS AND DISCUSSION

The results on rats expose to mosquito coils made from Transflutrin 3000 ppm and multidose permot leaf extract were divided into changes in body weight, changes in cerebellum weight, and Purkinje cell number (Table 1).

Body Weight

The study showed significant results ($p<0.05$) on body weight changes in the positive control group (3000 ppm transfluthrin) and the treatment group for mosquito coils made from permot leaf extract 4000 ppm. (Table 1). These could be due to the active chemicals contained in the mosquito coil causing a lack of food intake by mice, so weight gain was not as good as the control group and the less concentrated treatment group. The low weight gain in this study was similar to the previous studies conducted by Yadav et al (2021), the assessment suggested that inhalation of pyrethroid-based mosquito vaporisers fumes have toxic effects, reflected as low weight gain following subchronic exposure.

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Singh et al. (2009) stated that subchronic exposure to pyrethroid-based mosquito vaporiser fumes adversely affected the body weight of exposed group rats. The body weight of an organism reflects its overall state of metabolism and the capability to maintain its normal growth and development. The tentative hypothesis for weight gain reduction that happened in this study may be due to decreased absorption or utilisation of food and gastrointestinal disturbances caused by altered function of hydrolytic enzymes of small intestine by pyrethroid exposure. Another study reported that the oral administration of single dose of pyrethroid-based pesticide resulted in significant changes of intestinal enzymes activity that may result in serious disturbances in the intestinal food uptake (Khamrakulova, 2012). The study conducted by Sangha et al. (2011) helped explain decreased absorption of food in pyrethroid exposed rats as they observed that there was hypertrophy of goblet cells, necrotic changes, infiltration, and congestion in the duodenum

Table 1. Changes in body weight, cerebellum weight and the number of Purkinje cells in rat after 12 weeks of treatment

Group	Initial Weight (g)	Final Weight (g)	Body Weight Difference (g)	Cerebellum Weight (g)	Number of Purkinje Cell (x10^3 cell)
1	159.8	197.6	19.09 ± 2.54	0.5060 ± 0.008	387.40 ± 1.140
2	160.4	184.6	13.07 ± 1.87*	0.4854 ± 0.009*	370.80 ± 1.924*
3	160.6	194.2	16.76 ± 2.58	0.5050 ± 0.007	389.40 ± 1.517
4	163.4	197.4	17.20 ± 1.89	0.5058 ± 0.008	387.00 ± 1.225
5	161.8	196.8	17.76 ± 1.50	0.5038 ± 0.007	386.40 ± 1.949
6	161.4	193.8	16.72 ± 1.40	0.5032 ± 0.004	382.20 ± 1.789
7	162.6	187.4	13.21 ± 1.87*	0.5022 ± 0.009	380.20 ± 3.033*

Note: (1) control group (without exposure), (2) Transflutrin 3000 ppm, (3) Permot 500 ppm, (4) Permot 1000 ppm, (5) Permot 2000 ppm, (6) Permot 3000 ppm), (7) Permot 4000 ppm, *) significant at P<0.05

Cerebellum Weight

In addition to a low weight gain of mice, the cerebellum weight of mice in exposed groups was also reduce in a dose-dependent manner. Mice exposed to mosquito coil smoke made from 3000 ppm Transflutrin had the lowest cerebellum weight (Table 1). Research conducted by Sayim et al. (2005) stated a significant decrease in relative brain weights of the animals treated with cypermethrin in all doses was determined ($p < 0.05$). Changes in the cerebellar weight of mice in the 3000 ppm transflutrin-based mosquito coil exposure group were associated with changes in the histopathology of the mice's cerebellum.

Cerebellar Histopathological Changes

Histological preparation of the cerebellum cross-section of control group mice showed that the cortex consisted of an outer molecular layer, a normal inner granular layer and a Purkinje cell layer with normal nuclei. The granular layer is filled with dense cells, unlike the molecular layer which is composed of a large number of unmyelinated fibers. Mice exposed to mosquito coil smoke made from Transflutrin 3000 ppm and permot leaf extract 4000 ppm showed a significant increase in the density of cells in the cerebellar cortex and a decrease in the number of Purkinje cells.

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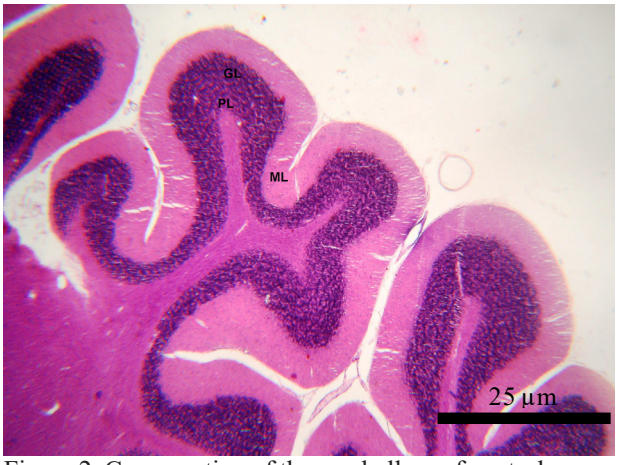

Figure 2. Cross section of the cerebellum of control group mice (x40, HE). Note: The cerebellar layer is composed of three layers with normal thickness and number of cells, molecular layer (ML), Purkinje cell layer (PL), granular cell layer (GL)

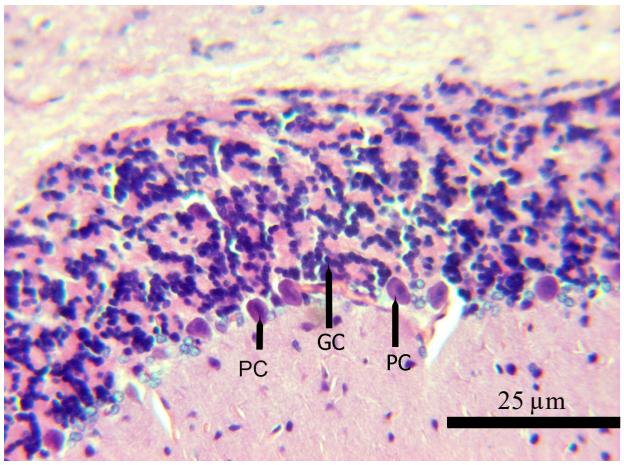

Figure 3. Cross-section of the cerebellum of control group mice (x450, HE). Notice: Purkinje cell nucleus looks round and solid (PC), granule cells (GC)

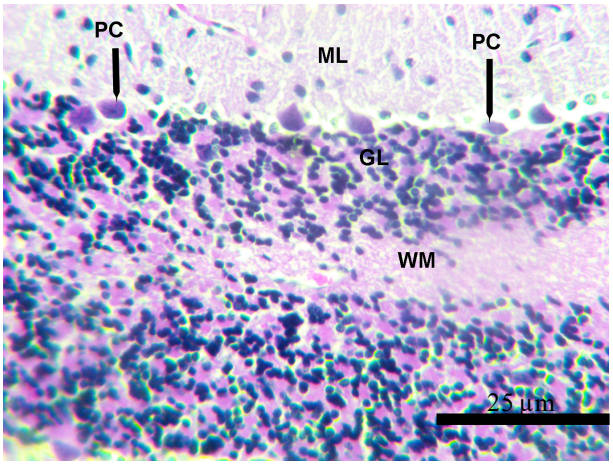

Figure 4. Cross section of the cerebellum of mice exposed to permot leaf extract at a dose of 4000 ppm (x450, HE) showing an increase in white matter thickness (WM) compared to the control group, a nucleus of the Purkinje cell changes shape to become flat and even damaged (PC, arrows), without typical changes in the granular architecture of the cell layer (GL) and molecular layer (ML)

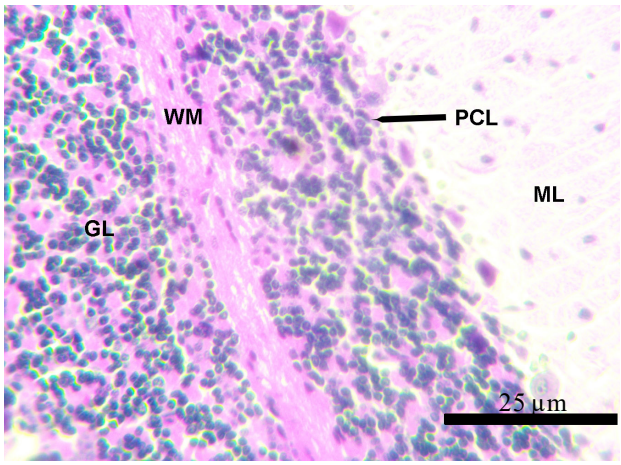

Figure 5. Cross section of mice exposed to Transflutrin 3000 ppm (x450, HE), showing a decrease in the thickness of the granular cell layer (GL), a decrease in the number of cell bodies in the molecular cell layer (ML), decrease in Purkinje cell layer thickness (PCL), and loss of white matter (WM)

Number of Purkinje Cell

Pyrethroid exposure has been reported to generate reactive oxygen species and the consequent oxidative stress in various tissues (Dubey et al., 2012; Dubeyet al., 2013; Fetoui et al., 2015). Observation of the cross-section of the cerebellum of mice in the treatment group for mosquito coils made from Transflutrin 3000 ppm showed a change in the structure characterized by the presence of black spots on the widened and degraded parts compared to the control group and the treatment

group of permot leaf extract up to a dose of 4000 ppm. This is in line with Xu et al. (2015), that rats exposed to Deltamethrin at a dose of 0.32 mg/kg/day also marked morphological changes in the size of the cerebellum section compared to the control group. These changes are the result of massive cell necrosis, inflammation, or apoptosis (Yuan et al., 2016) in the cerebellum caused by Reactive Oxygen Species (ROS) after being contaminated by synthetic pyrethroid-based insect repellent.

In this study, it was found that inhaling the vapors or smoke of mosquito coils made from Transflutrin 3000 ppm and permot leaf extract 4000 ppm could induce selective structural changes in the Purkinje cell layer of the cerebellar cortex, where the molecular and granular layers appeared more, or less normal. Some of the Purkinje cells shrank with indistinct nuclei, while others did not even exist. This finding coincides with the results of research by Asari et al. (2008), who reported that dermally applied pyrethroid-based insect repellent induces neuronal cell death and Purkinje cell loss. Sinha et al. (2004; 2006) revealed that exposure to even small doses of synthetic pyrethroids causes high concentrations of these chemicals in the nervous system, and reaches the blood-brain barrier, especially during the early developmental period resulting in deleterious changes in the nervous system and long-term functional disability.

In this experiment, it was found that exposure to pyrethroid smoke induces accumulation of neuroglial cells around the perikaryon of affected Purkinje cells. This is in accordance with Asari et al. (2008) who reported an increase in glial fibrillary acid protein immuno-staining indicating astrocyte hypertrophy in the cerebellum of adult mice treated with synthetic pyrethroids.

In normal nerve cells, it was found that the generation of an action potential by membrane depolarization involves the opening of the cell membrane sodium channels with a rapid increase in sodium influx. Thus, delayed closure of sodium channels increases cell membrane excitability. Pyrethroids were found to be soluble in the lipid phase of membranes and modify the characteristics of sodium channels in mammalian and invertebrate neural membranes to delay their closure and increase cell membrane excitability (Du et al., 2015; ffrench-Constant et al., 2016; Field et al., 2017).

Based on the results of the study using permot leaf extract in graded doses of up to 4000 ppm, histopathological changes in the cerebellum of mice were observed, such as narrowing of white matter, reduced cells in the molecular layer and damage to the nucleus of Purkinje cells. This can be caused by the main chemical composition in permot leaf extract that can inhibit the activity of the acetylcholinesterase enzyme. Based on Adewusi et al. (2010), AChE inhibition used parameters to classify the extracts analyzed were categorized into strong inhibitors (>50% inhibition), moderate inhibitors (30-50% inhibition) and weak inhibitors (<30% inhibition). Thus according to this study, permot leaf hexane extract can be considered as strong inhibitor of AChE.

Acetylcholinesterase (AChE) is responsible for degrading the neurotransmitter acetylcholine into choline and acetic acid, bringing the endpoint for cholinergic neurotransmission. This enzyme is distributed heterogeneously in the brain showing different activity depending on the part of the nerve (Khan et al., 2017). For this reason, AChE is the main target of a broad spectrum of compounds used as insecticides, neuroprotective agents, or therapeutic drugs for neurodegenerative diseases (Pohanka, 2011).

Soderlund (2010) stated that the tran-

sient permeability of nerve cell membranes to sodium ions is responsible for nerve action potentials which are the basic unit of information transfer in neurons. This transient change in permeability is mediated by voltage-gated sodium channels that cause proteins in cell membranes containing intrinsic sodium-selective ion pores to briefly open (activate) in response to depolarizing changes in the transmembrane potential and close (deactivate) by a mechanism independent of repolarization membrane. The simplest model for sodium channel function postulates three different channel states: closed or resting (available for activation), open, and not active.

The abundance and diversity of pyrethroid use contribute to the risk of exposure and adverse effects in the general population. The insecticidal action of pyrethroids depends on their ability to bind to and disrupt the voltage-gated sodium channels of insect nerves. Sodium channels are also important targets for the neurotoxic effects of pyrethroids. Other mammalian targets, particularly voltage-gated calcium and chloride channels, have been implicated as alternative or secondary sites of action for exposure to pyrethroid-based insecticides (Soderlund, 2012).

Venegas et al. (2014) revealed that when low concentrations of pyrethroids were added to cultured rat cerebellar Purkinje neurons, repeated discharges in nerve fibers and nerve terminals occurred with severe disruption of synaptic transmission, but this effect on cultured neurons could be reversed by washing with solution pyrethroid free. This implies that an alteration on the GABA receptors can affect the cerebellar circuits and the cerebellar cell population, which can induce a decrease in the Purkinje cell population and GABA dependent Cl- channels, as observed in the present study. A cypermethrin effect on GABA levels was showed in rats exposed to the insecticide to a single dose of 145 mg/kg, producing a significant decrease in the concentrations of the neurotransmitter (Manna et al., 2006b). In contrast, Srivastava et al. (2006) stated that there were no major side effects in adult rats suggesting the safe use of pyrethroid-based insect repellents.

CONCLUSION

The safe and effective dose of mosquito coils made from permot leaves was less than 4000 ppm, because exposure to doses of more than 4000 ppm can cause degenerative changes in the cerebellar cortex of mice.

AUTHOR CONTRIBUTION

R.P.S. and B.H. supervised all the process, carried out the experiment and wrote the manuscripstiting of the article.

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CONFLICT OF INTEREST

There is no potential conflict of interest during the research work.

REFERENCES

Adewusi, E. A., Moodley, N. &Steenkamp, V. (2010). Medicinal Plants with Cholinesterase Inhibitory Activity: A Review. *African Journal of Biotechnology,* 9(49), 8257-8276. DOI: 10.5897/AJB10.1129

Armalina, D., Witjahjo, B., Susilaningsih, N. (2021). Histopathological Changes in Liver, Kidney and Teratogenic Effects of Mice on Exposure to Mosquito Repellent. *Indonesian Journal of Medicine and Health (JKKI)*, 12(1):11-18. DOI: 10.20885/JKKI.Vol12.Iss1.art4.

Cham, E. Y. K., Tse, J. C. L., Chong, Y. K., Chen, M. L., Wong, O. F., Fung, H. T. (2016). A Case of Pyrethroid Poisoning with Clinical Presentation Mimicking Organophosphate Poisoning. *Hongkong J Emerg Med.* 23:47-51. https://doi.org/10.1177/102490791602300207.

Du, Y., Nomura, Y., Zhorov, B. S., Dong, K. (2015). Rotational Symmetry of Two Pyretroid Receptor Sites in the Mosquito Channel. *Mol Pharmacol,* 88, 273-280. DOI: 10.1124/mol.115.098707.

Dubey, N., Raina, R. & Khan, A. M. (2012). Toxic Effects of Deltamethrin and Fluoride on Antioxidant Parameters in Rats. *Fluoride,* 45, 242–246.

Dubey, N., Khan, A. M. & Raina, R. (2013). Sub-Acute Deltamethrin and Fluoride Toxicity Induced Hepatic Oxidative Stress and Biochemical Alterations in Rats. *Bulletin of Environmental Contamination and Toxicology,* 91, 334–338. DOI: 10.1007/s00128-013-1052-1.

Fetoui, H. (2015). Exposure to Lambda-Cyhalothrin, a Synthetic Pyrethroid, Increases Reactive Oxygen Species Production and Induces Genotoxicity in Rat Peripheral Blood. *Toxicology and Industrial Health,* 31(5), 433–441. DOI: 10.1177/0748233713475516

Ffrench-Constant, R. H., Williamson, M. S., Emyr Davies, T. G. & Bass, C. (2016). Ion Channels as Insecticide Targets. *Journal of Neurogenetics,* 30(3-4), 163-177. DOI: 10.1080/01677063.2016.1229781.

Field, L. M., Emyr Davies, T. H., O'Reilly, A. O., Williamson, M. S. & Wallace, B. A. (2017). Voltage-Gated Sodium Channels as Targets for Pyretroid Insecticides. *Eur Biophys,* 46, 675-679. DOI: 10.1007/s00249-016-1195-1.

Harrill, J. A., Li, Z., Wright, F. A., Radio, N. M., Mundy, W. R., Tornero-Velez, R. & Crofton, K. M. (2008). Transcriptional Response of Rat Frontal Cortex Following Acute *In Vivo* Exposure to the Pyrethroid Insecticides Permethrin and Deltamethrin. *BMC Genomics,* 9, 546. DOI:10.1186/1471-2164-9-546.

Khamrakulova, M. (2012). Enzymatic Activity of the Intestine in Effect of Pesticides of Pyrethroid Group. *Medical and Health Science Journal,* 10, 62-66. DOI:10.15208/mhsj.2012.12.

Khan, A. M., Raina, R., Dubey, N. & Verma, P.K. (2017). Effect Effect of Deltamethrin and Fluoride Co-Exposure on the Brain Antioxidant Status and Cholinesterase Activity in Wistar rats. *Drug and Chemical Toxicology,* 1-5. DOI: 10.1080/01480545.2017.1321009.

Manna, S., Bhattacharyya, D., Mandal, T. & Dey, S. (2006a). Neuropharmacological Effects of Deltamethrin in Rats. *J Vet Sci,* 7, 133-136. doi: 10.4142/jvs.2006.7.2.133.Patel, S. S., Soni, H., Mishra, K. & Singhai, A. K. (2011). Recent Updates on the Genus Passiflora: A Review. *Int J Res Phytochem Pharmacol,* 1(1), 1-16.

Pohanka, M. (2011). Cholinesterases, a Target of Pharmacology and Toxicology. *Biomed Pap Med. Fac Univ Palacky Olomouc Czech Repub,* 155, 219–229. doi: 10.5507/bp.2011.036.

Ramaswamy, A. S. & Dayasagar, P. (2017). A Study of Xylene Free Hematoxylin and Eosin Staining Procedure. *Annals of Advanced Medical Sciences (AAMS),* 1(1), A16-A21. DOI: 10.21276/AAMS.1772.

Rehman, H., Aziz, A. T., Saggu, S., Khurshid, A. A., Mohan, A. & Ansari, A. A. (2014). Systematic Review on Pyrethroid Toxicity with Special Reference to Deltametrin. *India Journal of Entomology and Zoology Studies*, 2(5), 01-06.

Rodríguez, J. L. (2016). Effects of Exposure to Pyrethroid Cyfluthrin on Serotonin and Dopamine Levels in Brain Regions of Male Rats. *Environmental Research,* 146, 388-394. DOI: 10.1016/j.envres.2016.01.023.

Sangha, G. K., Kaur, K., Khera, K. S., Singh, B. (2011). Toxicological Effects of Cypermethrin on Female Albino Rats. *Toxicology International*, 18(1), 1-8. DOI: 10.4103/0971-6580.75844.

Sayım, F., Yavasoglu, N. U. K., Uyanıkgil, Y., Aktug, H., Yavasoglu, A. & Turgut, M. (2005). Neurotoxic Effects of Cypermethrin in Wistar Rats: a Haematological, Biochemical and Histopathological Study. *Journal of Health Science,* 51(3), 300–307. DOI:10.1248/JHS.51.300.

Singh, A. K., Saxena, P. N. & Sharma H. N. (2009). Sress Induced by Beta-Cyfluthrin, a Type-2 pyrethroid, on Brain Biochemistry of Albino Rat (*Rattus norvegicus*). *Biology and Medicine,* 1(2), 74-86.

Sinha, C., Agrawal, A., Seth, K., Chaturvedi, R. & Shukla, S. (2004). Mosquito Repellent (Pyrethroid-based) Induced Dysfunction of Blood-Brain Barrier Permeability in Developing Brain. *Int J Dev Neurosci,* 22(1), 31-7. DOI: 10.1016/j.ijdevneu.2003.10.005

Sinha, C., Seth, K., Islam, F., Chaturvedi, R. & Mathur, N. (2006). Behavioral and Neurochemical Effects Induced by Pyrethroid-Based Mosquito Repellent Exposure in Rat Offsprings During Prenatal and Early Postnatal Period. *Neurotoxicol Teratol,* 28(4), 472-81. DOI: 10.1016/j.ntt.2006.03.005

Soderlund, D. M. (2010). State-Dependent Modification of Voltage-Gated Sodium Channels by Pyrethroids. *Pestic Biochem Physiol,* 97(2), 78–86. DOI: 10.1016/j.pestbp.2009.06.010.

Soderlund, D. M. (2012). Molecular Mechanisms of Pyrethroid Insecticide Neurotoxicity: Recent Advances. *Arch Toxicol,* 86, 165–181. DOI: 10.1007/s00204-011-0726-x.

Srivastava, A. A., Srivastava, M. K., Raizada, R. B. (2006). Ninety-Day Toxicity and One-Generation Reproduction Study in Rats Exposed to Allethrin-Based Liquid Mosquito Repellent. *J Toxicol Sci*, 31(1), 1-7. DOI: 10.2131/jts.31.1.

Susilowati, R. P. (2013). Efektivitas Ekstrak Daun Permot (*Passiflora foetida*) Terhadap Mortalitas Larva Nyamuk *Aedes aegypti*. Laporan Penelitian. Fakultas Kedokteran, Univrsitas Kristen Krida Wacana: Jakarta.

Susilowati, R. P. (2016). Uji Toksisitas Obat Nyamuk Bakar Berbahan Ekstrak Daun Permot (*Passiflora foetida*): Kajian Histopatologis Hati dan Ginjal Mencit. Prosiding Seminar Nasional Pendidikan Biologi dan Saintek: Isu-Isu Kontemporer Sains, Lingkungan, dan Inovasi Pembelajarannya. Universitas Muhammadiyah Surakarta, 21 Mei 2016. PP. 214-218.

Susilowati, R.P. (2017). Efektivitas Daun Permot (Passiflora foetida) Sebagai Obat Nyamuk dan Pengaruhnya pada Sel Darah Mencit. *J Kedokt Meditek,* 23(62), 1-10. DOI: 10.36452/jkdoktmeditek.v23i62.1544

Syed, F., Chandravanshi, L., Khanna, V. K., Soni, I. (2016). Beta-Cyfluthrin Induced

Neurobehavioral Impairments in Adult Rats. *Chemico-Biological Interactions,* 243. DOI: 10.1016/j.cbi.2015.11.015

Venegas, G. F., Donoso, C., Arriaza, C., Espinoza-Navarro, O., Castro, M. E., Torres, C., Felipe Lillo, F., Rodríguez, H. B. (2014). Evaluation of the Cerebellar Cortex of CF-1 Mice Exposed to a Single Dose of Cypermethrin. *Interciencia,* 39(11), 816-820.

Xu, M.Y., Wang, P., Sun, Y.J., Wang, H.P., Liang, Y.J., Zhu, L., Wu, Y.J. (2015). Redox Status in Liver of Rats Following Subchronic Exposure to the Combination of Low Dose Dichlorvos and Deltamethrin. Pesticide Biochemistry and Physiology, 124, 60-65. DOI: 10.1016/j.pestbp.2015.04.005

Yadav, S., Rani, A., Dewan, R,K. (2021). Effect of Inhalation of Pyrethroid Based Mosquito Vaporisers Fumes on the Body Weight of Male Albino Wistar Rats-An Experimental Study. *Journal of Cliinical and Diagnostic Research,* 15(4), 1-3. DOI:10.7860/JCDR/2021/47261.14686

Yuan, Y., Ding, Z., Qian, J., Zhang, J., Xu, J., Dong, X., Han, T., Ge, S., Luo, Y., Wang, Y., Zhong, K., Liang, G. (2016). Casp3/7-Instructed Intracellular Aggregation of Fe_3O_4 Nanoparticles Enhances T_2 MR Imaging of Tumor Apoptosis. *Nano Lett,* 16(4), 2686–2691. https:// DOI: 10.1021/acs.nanolett.6b00331.