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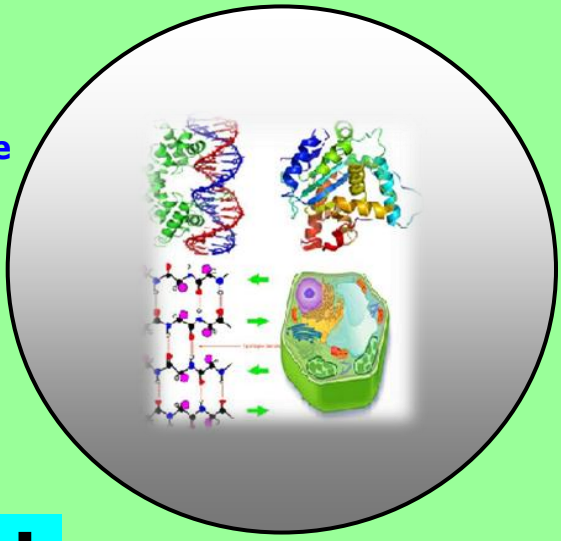

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RESEARCH PAPER

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Biochemical Alterations in the Cerebellum of Male Albino Rats Following Exposure to Allethrin Based Mosquito Coil Smoke

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ABSTRACT

Synthetic pyrethroid classes of insecticides are well known for their neurotoxic role and propensity to cause oxidative damage in the body. The most commonly used active ingredient in mosquito coil is allethrin which is the first derived synthetic pyrethroid. Present study was carried out to investigate the effect of sub-chronic whole body inhalation of mosquito coil smoke on antioxidant levels in cerebellar tissue.

Total twenty four male albino rats were used for the study in Department of Anatomy, King George Medical University U.P. Lucknow (UP), India. Rats were randomly divided into control (I) and experimental (II) group, each containing 12 rats. Group II animals were exposed to mosquito coil smoke for eight hours daily for 90 days. Rats were sacrificed and cerebellum from animals of control and exposed groups was used to prepare tissue homogenate for biochemical marker study.

Cerebellar tissue antioxidant levels (superoxide dismutase, catalase, reduced glutathione and glutathione peroxidase) were found to be significantly lower in exposed rats as compared to control group.

These assessments suggest that inhaling mosquito coil smoke may cause oxidative stress leading to decline in antioxidant enzyme levels.

Keywords: Allethrin, Mosquito Coil, Cerebellum, Neurotoxic and Antioxidants.

INTRODUCTION

Mosquito coil is slow burning incense and typically made from dried paste of pyrethrum powder (McKean, 2005). Synthetic derivatives of pyrethrum are pyrethroids and are most widely used class of insecticides worldwide (Elliot, 1976). Commonest active ingredient in mosquito coils are various pyrethroids among which first discovered synthetic pyrethroids

named allethrin is used most frequently (Krieger et al., 2003). Most mosquito coils sold in India contain allethrin concentration 0.05-0.1% w/w and is reported to be a weak to moderately toxic pyrethroid with inhalation LC_{50} >1500mg/m^3 in mouse and rat (Tomlin, 1994).

Owing to its easy affordability low income communities in Asia, Africa, South America are also adapting the mosquito repelling coils essentially made up of pyrethrum (Mulla et al., 2001). Inhalation of fumes of the mosquito repellent like liquid vaporizers may get entry into the brain by breaching the developing blood-brain barrier, hence deleterious to developing nervous system and can lead to long-term functional deficits (Sinha et al., 2006). Extensive electrophysiological and pharmacological studies have collectively revealed that pyrethroid inhibit sodium channel deactivation thus primarily stabilizing open configuration of activated channels in neurons (Vijverberg et al., 1982). All studies mentioned above are indicative of well known neurotoxicity of pyrethroids and it is reasonable to assume therefore that they will also have similar toxic effects in human also.

Cells exposed to environment are continuously producing oxygen free radicals. These free radicals and reactive oxygen species are chiefly produced by aerobic metabolism. Superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase are antioxidative enzymes generated to eliminate free radicals (Poulson et al. 1998; Uday et al., 1990). Increased oxidative stress in brain is associated with learning and memory declination, chronic application of SOD and CAT at low doses had reversed the behavioral and biochemical changes in mice (Liu et al., 2003).With this background the present study was carried out to investigate the effect of sub-chronic whole body inhalation of mosquito coil smoke on antioxidant enzyme levels in cerebellar tissue using an experimental randomized controlled design.

MATERIAL AND METHODS

Present study was done in the Department of Anatomy, King George Medical University U.P. Lucknow (UP), India. Total 24 healthy male albino Wistar rats were obtained from Indian Institute of Toxicology Research (IITR), Lucknow. Approval of Institutional Animal Ethical Committee (IAEC) was taken prior to start of study. Each rat was approximately weighing 180 to 200 grams. After being transferred from their colonies animals were acclimatized for 2 weeks under favorable climatic condition. After acclimatization these rats were randomly allocated to one of the two groups – a total of 12 animals were allocated to the Group I and did not undergo mosquito coil smoke exposure (Control Group) whereas remaining 12 rats were allocated to Group II and underwent mosquito coil smoke exposure (Experimental Group). The animals were housed in separate aluminium cages. Light and dark cycle of 12 hours and temperature was maintained. Animals were fed on standard pellet diet (5gms/rat) and water was given ad-libitum according to CPCSEA guidelines (**CPCSEA Guidelines, 2003**).

EXPERIMENTS

Product Details: Mosquito repellent coil brand Mortien Powerguard manufactured by Lotus Household Products Pvt. Ltd**.** containing 0.1% w/w of d–trans allethrin were purchased from a local store in Lucknow and used for the experiment. Length and weight of d trans- allethrin based coil was measured to be approximately 15 cms and 12 grams respectively.

Group II rats were exposed to mosquito coil smoke via whole body inhalation route according to the guidelines set by **Achmadi and Pauluhn (1998).** The coil was lit for 8 hours daily and 6 days in a week for a period of 12 weeks.

Sample collection-At the end of exposure period the animals from group I and II were euthanized by cervical dislocation as per CPCSEA guidelines. The brains were harvested and kept in a clean petridish, cerebellum was separated from brain stem and cerebrum and was quickly removed and taken to Biochemistry department in pre-cooled normal saline containing glass bottle, where it was kept in deep freezer at -80ºC for biochemical assay. Estimation of cerebellar tissue lipid peroxidase(LPO/MDA) (Okhawa et al,1979) ,Glutathione peroxidase (GPO/GPX) (Paglia and Valentine,1967), reduced Glutathione (GSH)(Ellman G,1959), Catalase (Aebi,1974)and Superoxide dismutase (SOD)(McCord and Fridovich,1969) levels were assessed using standardized laboratory procedures.

Statistical Analysis: Data was analyzed using Statistical Package for Social Sciences version 15.0. Mean antioxidant enzyme levels were compared between control and experimental groups using Independent Samples 't' test.

RESULTS

Mean LPO/MDA levels in Experimental group II (4.85±1.18) were significantly higher as compared to that of Control Group (2.14±0.37) ($p<0.001$) whereas mean GPO/GPX, GSH, Catalase and SOD levels of control group were significantly higher as compared to that of experimental group ($p<0.001$) (Table 1, Fig. 1). The findings of present study showed that allethrin based mosquito repellent coil smoke exposure induces oxidative stress and are potential source of tissue damage in the cerebellum of animals. Significant decrease in all antioxidant enzymes such as SOD, GPO/GPX, CAT, GSH in cerebellar tissue of exposure group favours the fact that cooperation of all intracelleular antioxidant is needed to detoxify the reactive oxygen species in nervous tissue. The increased oxidative stressed might be responsible for degenerative changes and can have an impact on neurobehavioral aspect for this further investigations are recommended.

DISCUSSION

In the present study cerebellum of allethrin exposed animals showed significant increase in lipid peroxidase (LPO) concentration whereas antioxidants like GPO/GPX, GSH, Catalase and SOD were lowered. LPO is a marker of oxidative stress and is presumed to be involved in molecular mechanism of neurobehavioral deficits (Mshelia, et al., 2013)**.** It is documented that central nervous system is the principal target of synthetic pyrethroids (Ansari *et al*., 2012). Low level of antioxidant defense and increased oxygen consumption enhances the risk of oxidative damage to brain (Ali, 2012)**.** A result of the present study indicates that the oxidative stress generated by exposure to repellent smoke had an inhibiting effect on the antioxidant levels. Similar to results of present study Gupta *et al.* (1999) also observed a decrease in brain glutathione (GSH) levels of rats exposed to pyrethroid-based liquid mosquito repellent. In another study, Sinha *et al.* (2006) observed significant oxidative stress, where an increase in lipid peroxidation and a decrease in antioxidants, glutathione, superoxide dismutase and catalase in various brain areas (cerebellum, corpus striatum, frontal cortex and hippocampus) were evident at all the exposure schedules.

Pyrethroid insecticides are acute neurotoxicants that modulate the function of voltage-gated sodium channels (Vijverberg *et al.*, 1982); specifically, they alter the permeability of excited nerve cells to sodium ions and cause repetitive nerve impulses (Aldridge *et al.*, 1990; Bradbury and Coats, 1989).

Table 1. Comparison of Antioxidant Enzyme levels between Control and Experimental Groups.

Parameters	Control (Group I) (n=12)		Experimental (Group II) (n=12)		Statistical Analysis	
	Mean	SD	Mean	SD	't'	'p'
LPO/MDA	2.14	0.37	4.85	1.18	7.591	<0.001
GPO/GPX	43.09	3.60	24.79	5.17	10.063	<0.001
GSH	43.92	4.03	28.07	13.34	3.940	0.001
Catalase	2.91	0.54	1.87	0.49	4.941	<0.001
SOD	2.76	0.38	1.51	0.33	8.604	<0.001

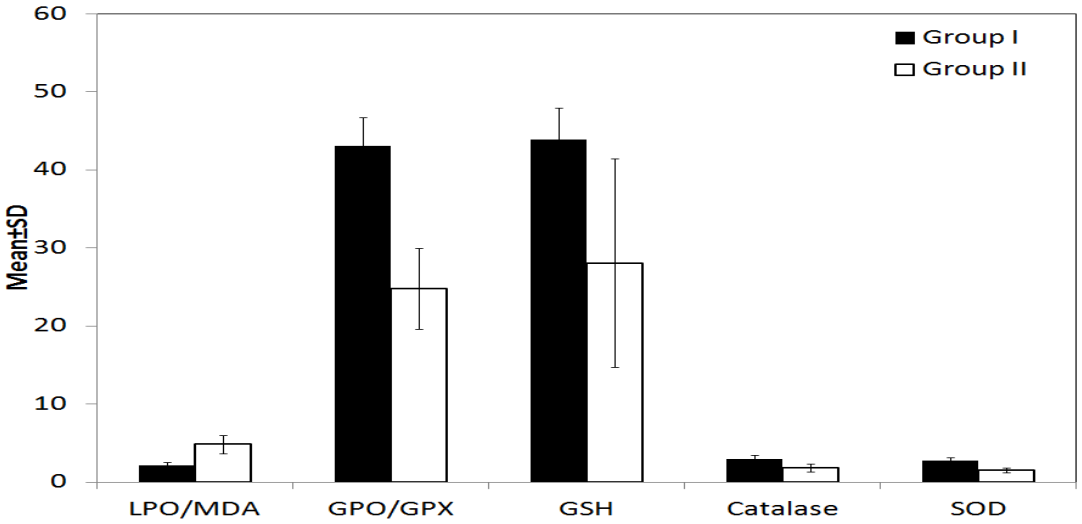

Figure 1. Comparison of Antioxidant Enzyme levels between Control and Experimental Groups.

Pyrethroid repellents are lipophilic in nature (Michelangeli *et al.*, 1990), they easily cross biological membranes and accumulate in biological membranes thereby stimulating the production of reactive oxygen species (ROS) which produces oxidative stress and damage to essential cell components in mammals (El-Demerdash, 2007). Previous research has indicated that these effects could be directly related to pesticide toxicity which may be due to changes in membrane fluidity (Sarkar *et al.*, 1993; Antunes-Madeira *et al.*, 1994), in lipid composition (Perez-Albarsanz *et al.*, 1991), and inhibition of enzyme activities (Jones and Lee, 1986). The findings in present study thus indicated the need for exercise of caution while using these mosquito repellents considering the fact that they induce oxidative stress and are potential source of tissue damage in the vital areas of animals. Further studies are recommended to explore the clinical aspects such as neurobehavioral changes owing to this increased oxidative stress.

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