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Cognitive Effect of the Sub-Chronic Exposure to Mosquito Coil Smoke in Mice

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Abstract : *The aim of this experiment is to investigate the cognitive effect of the sub-chronic exposure to mosquito coil smoke in mice. A total of 28 adult mice were used. The mice were divided into four groups. Group 1 served as a control group and got exposed to environment air, while group 2, 3 and 4 were exposed to 1 hour, 2 hours and 3 hours of mosquito coil smoke respectively for 6 weeks. Learning and memory test were conducted after 1 week, 3 weeks and 6 weeks of exposure. The brains were harvested, homogenized and samples analysed for Malondialdehyde (MDA) and Acetylcholinestrase (AChE). The result showed that mosquito coil smoke significantly (P<0.05) decrease Learning and Memory. Oxidative stress might have played role in the decreased Learning and Memory. This study indicates the toxic effect of mosquito coil smoke.*
Keywords: *Learning, Lipid peroxidation, Memory, Mosquito coil smoke, Pyrethroids*

I. Introduction

Mosquito coil is a mosquito repelling incense usually shaped into a spiral and made from a dried paste of Pyrethrum powder [1]. The active ingredients found in mosquito coils can be any or some of the following; Pyrethrum, Pyrethrin and allethrin. Other ingredients include Dibutylhydroxyl toluene (BHT), Piperonyl butoxide (PBO), aromatic and aliphatic hydrocarbons [2].

With malaria and other mosquito-borne diseases serving as a major health problems in tropical areas, mosquito coil usage has increased in recent decades and the annual world consumption was estimated to be 32 billion coils by the year 2000 [3]. General abuse and wanton overuse of these mosquito coils and insecticidal sprays in the control of mosquitoes pose a serious public health challenge, especially innocuous and chronic inhalation of the fumes and consumption of produce that may have been laced inadvertently by chemical constituent of the insecticide [4].

Epidemiologic studies have shown that long-term exposure to mosquito coil smoke was associated with asthma and persistent wheeze in children [5,6]. Toxicological effects of mosquito coil smoke on rats include focal deciliation of the tracheal epithelium metaplasia cells and morphological alteration of the alveolar macrophages [7]. Biochemical analysis of serum obtained from rats exposed to mosquito coil smoke showed significant increase in serum levels of urea and creatinine, indicating functional damage to the kidney [8]. Urea level can be increased by many other factors such as dehydration, antidiuretic drugs and diet while creatinine is more specific to the kidney since kidney damage is the only significant factor that increases serum creatinine level [9].

Exposure to mosquito coil smoke decrease the protein biosynthetic activity of the liver. This could affect capacity of serum protein-mediated transport of various substance [10]. Mosquito coil smoke exposure challenge the immune system in experimental rat leading to decreased in neutrophil and lymphocyte count as well as mean body weight [11]. Learning and memory tasks that are dependent upon motor or sensorimotor function may also be distrupted by pyrethroids at levels well below those that induce severe neurotoxicity [12]. Not only pyrethroid based but other mosquito repellents have been shown to cause neuronal degeneration in the brain leading to impairment of learning and memory [13,14]

Oxidative stress is the imbalance between the production and manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage [15]. Increase in erythrocyte lipid peroxidation is correlated with the inhibition in erythrocyte AChE activity and so erythrocyte AChE can be a marker enzyme in pyrethroid toxicity [16]. Despite the fact that mosquito coil smoke may have many potential adverse health effects, large populations in developing countries still use mosquito coils in their daily lives [17]. With paucity of information on the toxicological effects of mosquito coil smoke on learning and memory, research in this area is pertinent.

II. Materials and Methods

2.1 MATERIALS

Materials used were: Goldeer mosquito coil containing 0.03% Transfluthrin manufactured in Kano, Nigeria; Elevated plus maze; Ultrospec Plus Spectrophotometer; partially ventilated chamber (2.00 x 0.98 x 1.55m); 28 adult male mice (20-38gm), grower mash and water

2.2 Methods

A total of 28 adult mice were obtained from the National Institute of Trypanosomiasis and Oncocerciasis Research (NITOR), Kaduna, Nigeria and allowed to acclimatized for two weeks. They were fed mainly with grower mash and water ad Libitum. The mice were divided into four groups of seven (7) mice each. The mice in group 2-4 were exposed to mosquito coil smoke for 6 weeks in the partially ventilated chamber (2.00 x 0.98 x 1.55m)

Group 1: Served as control group exposed to room air
Group 2: Exposed to mosquito coil smoke, 1 hour daily for 6 weeks
Group 3: Exposed to mosquito coil smoke, 2 hours daily for 6 weeks
Group 4: Exposed to mosquito coil smoke, 3 hours daily for 6 weeks

2.3 Memory and Learning Test

The mice were passed through the memory and learning test after the first, third and sixth week of exposure. The Elevated Plus Maze for mice as described by [18] but modified to study Learning and Memory as described by [19] was used. The Elevated Plus Maze consisted of two perpendicular open arms (30 x 5 x 25cm) crossed at the central platform (5 x 5cm) to form a plus sign. The maze was raised 45cm above the floor. On the first day of the test (Learning), a mouse was placed at the end of one open arm, facing away from the central platform. The latency for the mouse to move from the open arm to one of the enclosed arms are recorded in seconds. Following entry into the arm, the animals were allowed to explore the apparatus for 30 seconds. The mice were again exposed to the smoke after the learning test. Twenty four hours later, the second trials (Retention test) was performed, the procedure is similar with that of the Learning. Prolong latency in an animal during Retention test as compared to learning test suggest decrease in retention ability.

2.4 Evaluation of Lipoperoxidation

The level of thiobarbituric acid reactive substance, Malondialdehyde (MDA) as an index of lipid peroxidation was evaluated on the brain sample using the method of [20] as mosified by [21]. The principle of the method was based on spectrophotometric measurement of the colour developed during reaction of thiobarbituric acid (TBA) with MDA. The MDA concentration in each sample was calculated by the absorbance coefficient of MDA-TBA complex 1.56×10^5 $cm^{-1}M^{-1}$ and expressed as nmol mg^{-1}

2.5 Evaluation of Acetylcholinestrase Activity

Acetylcholinestrase activity was evaluated on brain sample using the method of [22] with acetylthiocholine iodide as a substrate. Briefly, the whole brain sample of each animal was homogenized in a cold 0.1M phosphate buffer (Ph 7.4). Thiocholine was released because of the cleavage of acetylthiocholine iodide by acetylcholinestrase was allowed to react with the –SH reagent 5,5'dithiobis-(2-nitrobenzoic acid) (DTNB), which was reduced to thionitrobenzoic acid detected using a UV Spectrophotometer was then taken as a direct estimate of acetylcholinesterase activity.

2.6 Data analysis

Data obtained from the study were expressed as Mean ± SEM. The differences between the groups were analysed by one way analysis of variance (ANOVA) followed by post hoc multiple comparison test of Tukey using SPSS statistical tool. Values of $P < 0.05$ was taken to imply statistical significance.

III. Result

Effect of Mosquito coil smoke inhalation on Transfer latency (Learning) in mice

GROUPS	Week 1 (Sec)	Week 3 (sec)	Week 6 (sec)
Control	50.80 ± 16.0	13.80 ± 3.77	26.80 ± 5.70
1 hour	44.20 ± 8.52[b]	44.40 ± 18.9[aa]	57.00 ± 13.4[aa]
2 hours	43.20 ± 12.9[b]	39.00 ± 9.71[aa]	32.40 ± 7.91
3 hours	58.40 ± 13.0[a]	50.40 ± 14.8[aa]	44.40 ± 18.8[aa]

Results are presented as Mean ± SEM N = 5; $P < 0.05$, b = significant decrease, a= significant increase, aa= very significant increase

Effect of Mosquito coil smoke inhalation on Transfer latency (memory) in Mice:

GROUPS	Week 1(sec)	Week 3 (sec)	Week 6 (sec)
Control	13.60 ± 2.80	22.60 ± 6.70	37.00 ± 15.0
1 hour	26.80 ± 16.0[a]	61.60 ± 13.4[aa]	46.20 ± 18.0[a]
2 hours	16.60 ± 3.20	65.20 ± 15.6[aa]	51.20 ± 12.2[aa]
3 hours	56.00 ± 11.5[aa]	58.60 ± 14.4[aa]	79.20 ± 6.60[aa]

Results are presented as Mean ± SEM N = 5; P < 0.05, a= significant increase, aa= very significant increase

Effect of Mosquito Coil Smoke Inhalation on MDA Concentration in Mice

GROUPS	MDA
Control	1.5 ± 0.13
1 hour	1.7 ± 0.15
2 hour	1.6 ±0.16
3 hour	2.0 ± 0.09[a]

Results are presented as Mean ± SEM N= 6; P < 0.05, a=significant Increase

Effect of Mosquito coil Smoke Inhalation on AChE activity in Mice

GROUPS	AChE
Control	20.00 ± 1.16
1 hour	21.67 ± 1.20
2 hour	22.83 ±1.17
3 hour	26.17 ± 1.72[a]

Results are presented as Mean ± SEM N= 6; P < 0.05, a=significant Increase,

IV. Discussion

The result obtained for learning showed very significant increase in latency, implying very significant (P<0.05) decrease in learning; (44.40 ± 18.90; 39.00 ± 9.71; 50.40 ± 14.80) for week three and (57.00 ± 13.40; 44.40±18.86) for week six as compared to the control groups: (13.80±3.77) and (26.80±5.70) respectively. There was a significant increase(P<0.05) in learning in 1 hour (44.20±18.52) and 2 hours (43.20±12.9) groups as compared to control group (50.80±16.0) after one week of exposure. While the result for memory showed very significant decrease (P<0.05) in retention ability of mice with increase duration and period of exposure: (56.00±11.5) for week one, (61.60±13.4, 65.20±15.6 and 58.60±14.4) for week three and (46.20±18.0, 51.20±18.0 and 79.20±6.60) for week six as compared to the control groups (13.60±2.80), (22.60±6.70) and (37.00±15.00) respectively .The decreased in memory could be due to the fact that both type I and type II pyrethoids produced dose – dependent decrease in the responsiveness of granule cells of the hippocampus [23]. Not only pyrethoids based, but other mosquito repellent, for example formulations having N–N – diethyl – m – toluamide (DEET) have also been shown to cause neuronal degeneration in the brain leading to impairment of learning and memory [13, 14]. Also the exposure protocol during the experiment was a stressful one and could have been responsible for the decrease in memory which agreed with the finding of [24] that exposure to stress of an unfamiliar environment resulted in deficits of working memory indicative of hippocampal dysfunction.

Since mosquito coil releases carbon monoxides, studies have shown that chronic exposure to low levels of carbon monoxides can lead to depression, confusion and memory loss [25, 26]. The significant (P < 0.05) increased Malondialdehyde (MDA) concentration in the mosquito coil smoke inhaled group after 3 hours exposures showed that oxidative stress could have been partly involved in the molecular mechanism of neurobehavioural deficits observed in the present study. The brain, due to its biochemical and physiological properties is especially sensitive to free radicals which destroys its function and structures [27, 28]. MDA is the end – product of lipid peroxidation, which is a process where reactive oxygen species degrade polyunsaturated fatty acids [29]. Carbon monoxides causes endothelial cell and platelet release of nitric oxide, and the formation of oxygen free radicals including peroxynitrite [30]. The result of these effects is lipid peroxidation, which causes delayed reversible demyelinization of white matter in the central nervous system known as Grinker Myelinopathy, which leads to edema and necrosis within the brain [31]. This may result in cognitive defects, especially affecting memory and movement disorder, typically related to damage to the cerebral white matter and basal ganglia [32, 33]

Significant increase (P<0.05) in Acetylcholinestrase (AChE) activity as shown in the study , indicated that the coil components, such as transfluthrin also work like other pyrethoid such as Deltamethrin which increase the release of hippocampal acetylcholine [34], which could in turn induce a regulatory overcompensation by increasing AChE [35]. However, it is well established that there is linear relationship between increase in lipid peroxidation and the decreased in hippocampal AChE activity. Reduced enzyme activity results in reduced breakdown of release acetylcholine (Ach) [36]. Acetylcholine is one of the neurotransmitter involved in learning and memory. Increase in AChE will result in decrease in Ach, which may in turn impair learning and memory, because regulation of cortical Ach could influence cognitive processes and behaviour [37]. This could have contributed in the decrease in memory found in the course of the study.

V. Conclusion

Chronic exposure to mosquito coil smoke can lead to learning and memory impairment.

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