DOI: 10.7860/JCDR/2022/50899.15988

Original Article

Anatomy Section

Protective Role of Vitamin C against Biochemical Enzymes Alterations Induced by Exposure of Allethrin-based Mosquito Coil Smoke on Cerebellum of Male Albino Rats- An Experimental Study

RANJANA SACHAN[1], ARVIND KUMAR PANKAJ[2], ANITA RANI[3], SWATI YADAV[4]

(cc) BY-NC-ND

ABSTRACT

Introduction: Among synthetic pyrethroids, allethrin is widely used as active ingredient in mosquito coils. Many experimental studies have proved that pyrethroids cause neurotoxicity and results into derangements of biochemical enzymes including oxidative stress markers and antioxidant levels in brain.

Aim: To study alterations in biochemical markers (oxidative and antioxidant enzymes) in cerebellum of male albino Wistar rats induced by the exposure of allethrin-based mosquito coil smoke along with protective role of withdrawal and exogenously administered vitamin C on these markers.

Materials and Methods: This experimental study was conducted in the Department of Anatomy, King George's Medical University, Lucknow, Uttar Pradesh, India, during October 2014-September 2015. Total 42 male albino Wistar rats were divided into four groups. The first group contained 12 rats was used as control, group II with 12 rats was given 8 hours daily exposure of allethrin-based mosquito coil smoke via whole body inhalation for 6 days in a week for a total of 12 weeks. Group III contained eight rats and was given exposure same as group II and were further kept for 8 weeks to see the withdrawal effect. Group IV with 10 rats was given same exposure as group II, along with oral administration of 20 mg/kg body weight vitamin C. After 12 weeks of exposure, cerebellum was dissected and then sent for biochemical enzyme estimation. Rats in group III were dissected after 8 weeks post 12 weeks of exposure. Comparisons were made between groups by using one way Analysis of Variance (ANOVA).

Results: The exposure Group II showed significant rise ($p<0.001$) in level of oxidative stress marker enzyme Malondialdehyde (MDA) and significant ($p<0.001$) reduction in activities of antioxidants Glutathione Peroxidase (GPO), reduced Glutathione (GSH), Superoxide Dismutase (SOD) and Catalase (CAT) in comparison to Group I, III and IV. In vitamin C administered group IV the above parameters were found to be less affected. Intergroup comparison of oxidative stress markers level showed significant intergroup difference between group I, II, group II, III and group II, IV.

Conclusion: Allethrin-based mosquito coil smoke causes oxidative stress in cerebellum of albino Wistar rats and vitamin C has ameliorative effect on biochemical alterations induced by sub-chronic whole body exposure of mosquito coil smoke.

Keywords: Antioxidants, Ascorbic acid, Neurotoxicity, Oxidative stress, Pyrethroids, Subchronic

INTRODUCTION

Mosquito coils are effective against genera of mosquitoes including Aedes, Anopheles and Mansonia [1]. Mosquito coils are burned indoors and outdoors mostly in East Asia, uses are limited to some extent in other parts of world. In tropical countries, the use is widespread and the population is exposed during night hours throughout the year along with children [2]. Synthetic derivatives of pyrethrum are pyrethroids and are most widely used class of insecticides worldwide. Most common active ingredient in mosquito coil are various pyrethroids among which first discovered synthetic pyrethroid named allethrin and esbiothrin are used most frequently [3] . Most mosquito coils sold in India contain allethrin concentration 0.05-0.1% w/w is reported to be a weak to moderately toxic pyrethroid with inhalation LC50 >1500 mg/m^3 in case of rat and mice [4].

Synthetic pyrethroids are reported to increase lipid and protein oxidation and induce GSH depletion in cerebellum of adult rats [5]. Oxidative stress is initiated by formation of free radicals and reactive oxygen species which attack glial cells and neurons leading to damage [6]. It is well known fact, that, some organs are more sensitive to adverse conditions than others. Incidence of pyrethroid induced neurotoxicity has increased as brain is primary target of synthetic pyrethroids [7]. Pyrethroids are relatively more hydrophobic as compared to other classes of insecticides and biological membranes are vulnerable target for them [8,9]. Pyrethroids have propensity to cause significant damage in rat brain, associated with marked perturbations in oxidative defence system [10].

Antioxidants are produced either endogenously or received from exogenous sources and are further supported by enzymes like SOD, CAT, GPO/GPX and reduced GSH and all these act synergistically to remove free radicals produced during oxidative stress [11]. Lamda cyhalothrin (a synthetic pyrethroid) induced cerebellum damage in adult rats by oxidative stress and vitamin C played a protective role as antioxidant [5].

Inhalation of fumes of 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 [12]. The mosquito repellent inhalation in rats during early prenatal/postnatal and perinatal life have many adverse effects, leading to central nervous system abnormalities and if a similar mechanism operates in humans they are also vulnerable target [13]. Despite of known neurotoxic role of allethrin, studies specifically focusing on biochemical enzymes alteration in cerebellum, withdrawal effect and role of exogenously

administered antioxidant are scarce. Most of the previous reports are based on experiments on immature animals and other routes of exposure and not via respiratory route, though it is the common. With all these known facts, the present experimental study aims to analyse the protective role of vitamin C and role of withdrawal on the pyrethroid induced toxicity in rat cerebellum. Vitamin C is most widely used antioxidant in general population mainly obtained via citrus fruits. Water soluble tablets are easily available and administrable to experimental rats.

MATERIALS AND METHODS

The present experimental study was conducted in the Department of Anatomy, King George's Medical University, Uttar Pradesh, India in collaboration with Department of Biochemistry during the period October 2014 to September 2015. Approval of Institutional Animal Ethical Committee (Project no. 58/IAEC/2014, Ref.no.66/IAH/Pharma-14) was taken prior to start of the present study.

Inclusion criteria: A total of 42 healthy male albino Wistar rats aged 2-3 months, weighing approximately 250 to 300 grams were used for study and randomly divided into four groups.

Exclusion criteria: Female rats were excluded because of their cyclic hormonal variations.

Study Procedure

Male albino Wistar rats were obtained from Industrial Toxicological Research Centre (ITRC), Lucknow. Total 42 rats were taken for the study and they were acclimatised for two weeks and then on the basis of mean body weight, they were randomly divided into four groups. No prior sample size calculation was done. It was done on the basis of general rule of thumb for experimental study with minimum sample size (n≥5) in each group. They were kept in polyethylene cages of size 15×12×8 inches in groups of four rats in each cage. Animals were fed on standard pellet diet 5 gm/rat/day and water was given ad-libitum.

Distribution in different groups: Animals were divided into four groups:

1) Group I- contained 12 rats with no exposure. It was control group.
2) Group II- contained 12 rats and were given 8 hours daily exposure via nose and whole body inhalation for six days in a week for total 12 weeks.
3) Group III- contained eight rats and was given same exposure as group II and this group was further kept for 8 weeks without any exposure to see the withdrawal changes.
4) Group IV- contained 10 rats and were given exposure same as group II along with oral administration of vitamin C in a dose 20 mg/kg body weight once daily for 12 weeks.

For identification of individual rat common picric acid solution was applied on different sites of body of individual rat.

The present study has followed standard criteria for realistic room condition for exposure considering the information provided by Achmadi UF and Jpauluhn J [14].

Mosquito coil: Mortien Power Guard was the coil brand used in our study as it is commonly used. Length and weight of d-trans allethrin-based coil was measured to be 15 cm and 12 grams and as per product details each coil is supposed to burn for 8 hours continuously. The chemical composition of mosquito coil which is mentioned on the product in terms of w/w is as follows, 0.1% w/w d-trans allethrin with some other major constituents like coconut shell powder 40% w/w, wood binder, starch binder 10.0%, Genopol LO 88 emulsifier 0.1% w/w, red dye 0.1% w/w, fragrance 0.5% w/w, sodium benzoate 0.3% w/w, potassium nitrate 0.1%, and jiggat (joss) 6.0%.

Mode of exposure of mosquito coil smoke: The dimensions of the room used for exposure were 9.5×9.0×9.0 feet. The room was properly ventilated. Group II, III and group IV were kept in this room and were given 8 hours daily whole body inhalation of mosquito coil smoke for 6 days in a week for a total of 12 weeks. The mosquito coils were burnt from 9 am to 5 pm to mimic the human exposure of 8 hours daily.

Vitamin C: A 500 mg vitamin C chewable tablet Limcee with ascorbic acid IP 100 mg and Sodium Ascorbate- IP 450 mg (equivalent to 400 mg of ascorbic acid) were used.

Dose calculation and administration of vitamin C: Prior to administration of recommended dose of vitamin C, weight of each rat of group IV was recorded on electronic weighing machine. A freshly prepared aqueous solution of vitamin C was orally administered in a dose of 20 mg/kg body weight (Recommended dose for human is 10 mg/kg body weight). For dose calculation, aqueous solution of 500 mg vitamin C tablet was prepared, in 10 mL of water, so 1 mL of this solution contained 50 mg of vitamin C. A rat approximately weighing 250-300 mg required 5-6 mg of vitamin C which was present in approximately 0.1-0.12 mL of solution [15]. Required solution was repeatedly filled in 1 mL syringe and was orally administered to each rat of group IV with help of rat feeding cannula attached to syringe.

Sample procurement for biochemical assay: After completion of 12 weeks control group and exposure group II and group IV were euthanized by cervical dislocation as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines [16]. Group III rats were dissected after 8 weeks of withdrawal. After giving midline incision in head and neck, the skull cap was removed with the help of bone nibbler, toothed forceps, scissors and cerebellum was separated from brainstem and quickly removed then taken to Biochemistry Department in pre-cooled normal saline containing glass bottle where it was kept in deep freezer at -80°C for further biochemical enzymes assay.

Biochemical enzyme assessment: About 10% (w/v) homogenate of cerebellum was prepared with the aid of York's homogeniser fitted with Teflon plunger in 0.1 M Phosphate buffer (pH 7.1) as per requirement. The whole homogenate was first centrifuged at 2500 rpm for 10 minutes, nuclear and cellular debris at bottom were discarded and the supernatant was centrifuged at 11,000 rpm for 15 minutes, the obtained clear supernatant was further centrifuged at 105,000 rpm for 90 minutes, the final resultant supernatant was used for enzyme estimation. Lipid Peroxidase (LPO) level estimation (N mol MDA/mg of protein) was done by method given by Ohkawa H et al., [17]. The CAT estimation (unit/mg protein) was done according to Aebi method [18]. SOD estimation was done according to the process defined by McCord JM and Fridovich I. For estimation of reduced GSH and GPO, Ellman GC, Paglia and Valentine methods were used, respectively [19-21].

STATISTICAL ANALYSIS

The statistical analysis was done by using Statistical Package of Social Sciences (SPSS) version 16.0. For comparison between groups, ANOVA and Turkey HSD tests were employed. Turkey Honest Significant Test is post HOC test that ensure low type 2 error and the sample size was small, hence we required a comparative test that could show only Honestly Significant Difference (HSD). The values were represented in frequency and percentage and Mean±SD. p-value <0.05 was considered statistically significant.

RESULTS

During the study, the expiry of experimental rats was observed, in group I two rats, group II four, group III two and in group IV only one rat was expired. So, the survival rate in groups ranged from 66.7% in group II to 90% in group IV. In [Table/Fig-1], mean LPO/MDA levels ranged from 1.87±0.90 in vitamin C administered (group IV) to 4.85±1.18 in exposure (group II), thereby showing statistically significant intergroup differences (p<0.001). Mean LPO/MDA ranged from 2.01±0.07 in withdrawal (group III). Mean GPO/GPX levels ranged from 24.79±5.17 in exposure (group II) to

Parameters	Control (Group I) (n=10)		Exposure (Group II) (n=8)		Withdrawal (Group III) (n=6)		Vitamin C exposure (Group IV) (n=9)		Statistical analysis	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F	p-value
LPO/MDA	2.14	0.37	4.85	1.18	2.01	0.07	1.87	0.90	27.06	<0.001
GPO/GPX	43.09	3.60	24.79	5.17	37.90	5.14	37.52	5.90	20.97	<0.001
GSH	43.92	4.03	28.07	13.34	33.04	5.50	35.15	7.38	5.79	0.003
Catalase	2.91	0.54	1.87	0.49	2.51	0.41	2.36	0.43	7.20	0.001
SOD	2.86	0.38	1.51	0.33	2.60	0.37	2.54	0.41	18.77	<0.001

[Table/Fig-1]: Intergroup comparison of Oxidative Stress Markers and antioxidant enzyme levels between control, exposure, withdrawal and vitamin C administered groups. For analysis, N=33, p-value <0.05 was considered statistically significant

Parameters	I vs II			I vs III			I vs IV			II vs III			II vs IV			III vs IV		
	Mean	SE	p-value	Mean	SE	p-value	Mean	SE	p-value	Mean	SE	p-value	Mean	SE	p-value	Mean	SE	p-value
LPO	2.71	0.37	<0.001	0.13	0.40	0.988	0.26	0.36	0.880	2.84	0.42	<0.001	2.97	0.38	<0.001	0.13	0.41	0.988
GPO/GPX	18.30	2.35	<0.001	5.18	2.56	0.203	5.57	2.28	0.091	-13.11	2.68	<0.001	-12.73	2.41	<0.001	0.38	2.62	0.999
GSH	15.85	3.92	0.002	10.87	4.27	0.073	8.76	3.80	0.119	-4.97	4.46	0.683	-7.08	4.01	0.310	-2.11	4.35	0.962
Catalase	1.04	0.23	<0.001	0.40	0.25	0.380	0.55	0.22	0.079	-0.64	0.26	0.083	-0.49	0.23	0.170	0.15	0.25	0.933
SOD	1.25	0.18	<0.001	0.16	0.19	0.851	0.22	0.17	0.591	-1.09	0.20	<0.001	-1.03	0.18	<0.001	0.06	0.20	0.989

[Table/Fig-2]: Intergroup comparison (Tukey HSD test). p-value <0.05 was considered statistically significant

43.09±3.60 units in control (group I), thus showing statistically significant intergroup difference (p<0.001). Mean GSH levels ranged from 28.07±13.34 units in exposure (group II) to 43.92±4.03 units in control (group I). Statistically, the intergroup difference was significant (p=0.003). Mean CAT levels ranged from 1.87±0.49 unit in exposure (group II) to 2.91±0.54 units in control (group I). Statistically, the intergroup differences were significant (p=0.001). Mean SOD level ranged from 1.51±0.33 in exposure (group II) to 2.86±0.38 units in control (group I).

In [Table/Fig-2] between exposure group II and withdrawal group III a significant difference was observed only for LPO/MDA, GPO/GPX and SOD. For LPO mean value in exposure group II were higher as compared to that in withdrawal group III, for other parameters mean value in withdrawal group III was higher as compared to exposure group II (p<0.05). Between exposure group II and vitamin C administered group IV a statistically significant difference was observed for all the parameters except GSH and CAT. For GPO/GPX and SOD mean value in exposure group II was lower as compared to vitamin C administered group IV. For LPO mean value in exposure group II was higher as compared to vitamin C administered group IV (p<0.001).

DISCUSSION

In the present study, LPO/MDA, a marker of lipid peroxidation was found to be significantly higher in cerebellar homogenate of exposure group II in comparison to withdrawal group III and vitamin C administered group IV. The LPO is the end product of lipid peroxidation which produces reactive oxygen species by degrading Polyunsaturated Fatty Acid (PUFA) in brain [22]. The free radicals produced during oxidative stress can destroy protein, lipids and DNA by attacking on cell membrane by increasing its fluidity [23]. Inhaling mosquito coil smoke causes significant histological damage in rats's lung and kidney but least effect on their heart [24]. Mammalian neurotoxicity of pyrethroids has been well-documented and review regarding toxicity, metabolism and actions are available [25]. The probable cause behind neurotoxicity may be presence of high levels of PUFA in brain owing to increase sensitivity of it to toxic insults [26].

Significantly, higher brain markers of lipid peroxidation and protein oxidation were observed in lambda-cyhalothrin (LTC) (a synthetic pyrethroid) intoxicated rats as compared to control rats [27].

In present study, also decreased level of antioxidative enzymes (GPO, GSH, SOD and CAT) in exposed group II could be due to high utilisation of these enzymes for conjugation or its involvement in neutralising increased free radical species. Pyrethroids have propensity to cause significant oxidative damage in rat brain and is associated with marked pertubations in antioxidant defense system [10]. The second line of antioxidant such as GPO is activated when SOD, CAT are saturated [28]. This decrease in antioxidant enzyme levels also reflects antioxidant mechanism failure to overcome influx of ROS induced by toxins exposure as LTC exposure in rat brain tissue [29]. Pyrethroid are known to increase lipid and protein oxidation by inducing GSH depletion in cerebellum of adult rats and these effects can be neutralised by antioxidant like vitamin C [5]. Non enzymatic antioxidant like vitamin A, C, E, GSH and trace elements like zinc and selenium are shown to scavenge free radicals and reactive oxygen species [30]. The protective effect of ascorbic acid (AA/VitC) and alpha tocopherol (E-307/Vit E) at 100 mg/kg oral doses administered daily for entire period of toxicant exposure of 3 weeks to experimental mice of 3-4 months and weight=30 gm ameliorated the tissue damage as observed through histopathological examination [31]. In present study, even lower dose (20 mg/kg body weight) of vitamin C has shown protective effect on biochemical markers.

In present study, in comparison to exposure group II, significantly decreased LPO/MDA levels were found in vitamin C administered group IV. Partial recovery of antioxidant levels was seen in group III after withdrawal of 8 weeks, these levels were significantly increased in vitamin C exposed group IV. Both the results indicate recovery by exogenous administration of vitamin C and also due to withdrawal. In the present study, the biochemical estimation revealed increased level of LPO (a marker of oxidative stress) in exposed group II, in comparison to control group I. In comparison to control group I, LPO level was also increased in withdrawal group III but this increase was lower than exposure group II. In vitamin C administered group IV this increase in LPO level was less in comparison to only exposure and withdrawal groups as well. This result clearly indicates importance of role of vitamin C as antioxidant against oxidative stress. The levels of different antioxidants GPO, GSH, SOD, CAT were found to be significantly decreased in group II in comparison to control group. In exposure plus vitamin C administered group, levels were similar to control group. There is defensive function of vitamin C against biochemical changes triggered by pyrethroid-based mosquito coil smoke on testis of albino rats [32].

Present study is different as it has also included the effect of withdrawal from exposure and role of vitamin C on cerebellum (to evaluate neurotoxic role) of albino rats. Withdrawal group also showed recovery of antioxidants levels. So in present study,

biochemical estimation provides strong evidence that oxidative stress is involved in pathogenesis of neurotoxicity of allethrin-based coil smoke and vitamin C administration has ameliorative role in recovery from oxidative stress.

Limitation(s)

Due to complex nature of exposure atmosphere generated by mosquito coil smoke, it is scientifically challenging to characterise accurate inhalation rate of main chemical constituent in realistic exposure conditions. The present study has one limitation with regards to calculation of proper inhalation rate of main chemical constituent as authors had not used inhalational chamber for only exposure of smoke via nose.

CONCLUSION(S)

Inhalational exposure of allethrin-based mosquito coil smoke causes significant increase in LPO activity in cerebellum of rats suggestive of severe oxidative stress. Simultaneous vitamin C administration results in increased levels of antioxidant SOD, GPO, CAT and GSH. Withdrawal after exposure also causes increase in level of antioxidant enzymes. So, by the present study, it can be concluded that vitamin C intake can provide protection from harmful effects caused by exposure of allethrin-based mosquito coil smoke.

Acknowledgement

The authors want to thank Department of Anatomy, Biochemistry and Department of Pharmacology of King George's Medical University, Lucknow for allowing to conduct the present study.

Conflict of interest: Author declares that there is no conflict of interest with any particular brand. Mortien power guard was used for research purpose only.

REFERENCES

[1] Yap HH, Lim MP, Chong NL, Lee CY. Efficacy and sublethal effects of mosquito coils on Aedes aegypti and Culex quinquefasciatus (Diptera: Culicidae), 1996. Pp. 177-184. In K.B. Wildey (ed.). Proceedings of the 2nd International Conference on Insect pests in the urban environment. Heriot-Watt University, Edinburgh, Scotland, United Kingdom.
[2] Gupta A, Agarwal R, Shukla GS. Functional impairment of blood brain barrier following pesticides exposure during early development in rats. Hum Exp Toxicol. 1999;18:174-79.
[3] Krieger RI, Dinoff TM, Zhang X. Octachlorodipropyl ether (s-2) mosquito coils are inadequately studied for residential use in Asia and illegal in the United States. Environ Health Perspect. 2003;111(12):1439-42.
[4] Tomlin CA. World Compendium: The pesticide manual. Tenth edition.UK .British Crop Protection Concil. 1994;27-28.
[5] Fetoui H, Gdoura R. Synthetic pyrethroid increases lipid and protein oxidation and induces glutathione depletion in cerebellum of albino rats: Ameliorative effect of vitamin C. Hum Exp Toxicol. 2012;31(11):1151-60.
[6] Gilgun-Sherki Y, Melamed E, Offen D. Oxidative stress induced-neurodegenerative diseases: The need for antioxidants that penetrate the blood brain barrier. Neuropharmacology. 2001;40(8):959-75.
[7] Wolansky MJ, Harrill JA. Neurobehavioral toxicology of pyrethroid insecticides in adult animals: A critical review. Neurotoxicol Teratol. 2008;30(2):55-78.
[8] Michelangeli F, Robson MJ, East JM, Lee AG. The conformation of pyrethroids bound to lipid bilayers. Biochem Biophys Acta. 1990;1028;49-57.
[9] Kale M, Rathore, John N, Bhatnagar SD. Lipid peroxidase damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: A possible involvement of reactive oxygen species. Toxicol Lett. 1999;105:197-205.
[10] Demerdash EFM. Lipid peroxidation, oxidative stress and acetyl cholinesterase in rat brain exposed to organophosphate and pyrethroid insecticides. Food Chem Toxicol. 2011;49(6):1346-52.
[11] Yun-Zhong F, Sheng Y, Guoyao W. Free radicals, antioxidants and nutrition. Nutrition. 2002;18:872-79.
[12] Sinha C, Seth K, Islam F, Chaturvedi RK, Shukla S, Mathur N, et al. Behavioral and neurochemical effect induced by Pyrethroid based mosquito repellent exposure in rat offsprings during prenatal and early postnatal period. Neurotoxicol Teratol. 2006;28(4):472-81.
[13] Sinha C, Agarwal AK, Islam F, Seth K, Chaturvedi RK, Shukla S, Seth PK. Mosquito repellent (Pyrethroid-based) induced dysfunction of blood brain barrier permeability in developing brain. Int J Dev Neurosci. 2004;22(1):31-37.
[14] Achmadi UF, Jpauluhn J. Household insecticides: Evaluation and assessment of inhalation toxicity: A workshop summary. Experimental and Toxicologic Pathology. 1998;50(1):67-72.
[15] Kahn PK, Sinha SP. Antimutagenic efficacy of higher dose of vit C. Mutat Res. 1993;298(3):157-61.
[16] CPCSEA Guidelines for laboratory animal facility. Commitee for the purpose of control and Supervision of experiments on animals. Indian Journal of Pharmacology. 2003;35:254-57.
[17] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95:351-58.
[18] Catalase AH. In: Bergmeyer HU(ed). Methods of Enzymatic Analysis. 1974;673-84.
[19] McCord JM, Fridovich I. Superoxide dismutase: An enzymic function for erythrocuprein (hemocuprein). J Biol Chem. 1969;244:6049-55.
[20] Ellman GC. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82:70-77.
[21] Pagelia DE, Valentine WN. Studies on quantitative and qualitative characterisation of erythrocyte glutathione peroxidase. 1967;70(1):158-69.
[22] Oboh G, Ayodele JA, Adebayo OA. Antioxidant and inhibitory effect of red ginger (Zingiber Officianale Val Rubra) and white ginger (Zingiber Officianale Ruscoe) on Fe+ induced lipid peroxidation in rat brain in vitro. Experimental Toxicologic Pathology. 2012;64:31-36.
[23] Piner P, Unerb N. Oxidative and apoptotic effects of lambda-cyhalothrin modulated by piperonyl butoxide in the liver of Oreochromics niloticus. Environmental Toxicology and Pharmacology. 2012;33:414-20.
[24] Singh R, Singh H, Ranjana, Manik P, Diwan RK, Kumari S. Protective role of Vitamin C against toxic indoor pollutants released from pyrethroid base mosquito coil on a rat model: A histopathological study. Era J Med Res. 2020;7(1):01-06.
[25] Soderlund DM, Clark JM, Sheets LP, Mullin LS, Piccirillo VJ, Sargent D, et al. Mechanism of actions of the pyrethroids neurotoxicity: Implications of cumulative risk assessment. Toxicology. 2002;171:03-59.
[26] Siddappa AJ, Rao RB, Wobken JD, Liebold EA, Connor JR, Georgieff MK. Developmental changes in the expression of iron regulatory protein and iron transport proteins in the perinatal rat brain. J Neurosci Res. 2002;68:761-75.
[27] Ali ZY. Neurotoxic effect of Lambda-Cyhalothrin, a synthetic pyrethroid pesticide: Involvement of oxidative stress and protective role of antioxidant mixture. N Y Sci J. 2012;5(9):93-103.
[28] Duntas LH. The evolving role of selenium in the treatment of Graves' disease and ophthalmopathy. J Thyroid Res. 2012;2012:736161.
[29] Hfaiedh N, Murat JC, Elfeki A. A combination of ascorbic acid and alpha tocopherol or a combination of Mg and Zn are both able to reduce the adverse effects of lindane poisoning of rat brain and liver. Journal of Trace Elements in Medicine and Biology. 2012;04:002.
[30] Halliwell B. Oxidative stress and neurodegeneration: Where are we now? Journal of Neurochemistry. 2006;97(6):1634-58.
[31] Al-Omar MS, Naz M, Mohammad SAA, Mansha M, Ansari MN, Rehman NU, et al. Pyrethroid– induced organ toxicity and anti-oxidant-supplemented amelioration of toxicity and organ damage: The protective roles of ascorbic acid and alpha tocopherol. Int J Environ Res Public Health. 2020;(17):6177.
[32] Singh H, Diwan RK, Singh R, Manik P, Rani A, Ahmad S. Experimental validation of vitamin C's defensive function against biochemical changes triggered by pyrethroid-based mosquito coil (PBMC) on testis of albino rats. International Journal of Mosquito Research. 2021;8(1):01-05.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Anatomy, Autonomous State Medical College, Hardoi, Uttar Pradesh, India.
2. Additional Professor, Department of Anatomy, King George's Medical University, Lucknow, Uttar Pradesh, India.
3. Professor, Department of Anatomy, King George's Medical University, Lucknow, Uttar Pradesh, India.
4. Assistant Professor, Department of Anatomy, Hind Institute of Medical Sciences, Mau, Ataria, Sitapur, Uttar Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Swati Yadav,
C-17, Dilkusha Colony, Near Dilkusha Garden, Lucknow-226002, Uttar Pradesh, India.
E-mail: swatiyadav67@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jun 23, 2021
- Manual Googling: Nov 11, 2021
- iThenticate Software: Dec 02, 2021 (20%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jun 15, 2021**
Date of Peer Review: **Aug 16, 2021**
Date of Acceptance: **Dec 03, 2021**
Date of Publishing: **Feb 01, 2022**