Experimental validation of vitamin C's defensive function against the biochemical changes triggered by a pyrethroid-based mosquito coil (PBMC) on testis of albino rats

Dr. Heena Singh, Dr. RK Diwan, Dr. Ravhendra Singh, Dr. Punita Manik, Dr. Archana Rani and Dr. Sharique Ahmad

DOI: https://doi.org/10.22271/23487941.2021.v8.i1a.492

Abstract
Allethrin is a widely used pyrethroid in mosquito coil and has a potential toxic effect. The purpose of the current research was to explore the effects triggered by allethrin-based mosquito coil smoke using an albino wistar rat model on certain biochemical parameters to ascertain the oxidative stress to be the mechanism of insult to testicular tissue. The animals in our study were clustered among the four groups. Group 1st, with 12 rats represented as control, while group 2nd having 12 rats, mosquito coil smoke was given to them 8 hours per day, seven days per week for twelve weeks. Group 3rd with 8 rats having the same exposure as in group 2nd and then kept for further 8 weeks without any exposure served as the withdrawal group. Group 4th, a maximum of ten rats with similar treatment as group 2nd, together with administration of vitamin C. Group 2nd rats showed derangement in oxidative markers compared to group 1st, 3rd, and 4th as there was a substantial amount of (P<0.001) reduction in glutathione peroxidase (GPX), superoxide dismutase(SOD), catalase(CAT) and reduced glutathione (GSH) activities with a significant rise (P<0.001) in activities of malondialdehyde (MDA). The results of this analysis indicate that vitamin C supplementation is a more effective treatment modality than exposure withdrawal against the oxidative stress triggered by PBMC’s smoke in testis of rat.

Keywords: Pyrethroid based mosquito coil (PBMC), oxidative stress, oxidative markers, antioxidant, Vitamin C

Introduction
Malaria and other mosquito-borne disease have become a nuisance to public health. Mosquito coil is commonly used for controlling mosquitoes across residential areas. These are sluggish-burning and emit insecticide-containing smoke and are being employed in the vicinity for mosquito control to avoid malaria [1]. The pyrethroid based mosquito coil (PBMC) contains various pyrethroid like allethrin, transfluthrin, deltamethrin, etc. To date, large numbers of pyrethroid have been developed. But nowadays the most commonly used pyrethroid is allethrin that is efficient towards various forms of mosquitoes namely mansonria, aedes, and anopheles [2]. A flaming mosquito coil emitting smoke produces submicron particles (< 1) mixed with a large number of heavy metals, allethrin, as well as a strong vapors range like phenol O-cresol [3]. Mosquito coils are typically used during the night in bedrooms, where the elevated exposure to smoke particles can lead to serious damage to various organs according to their susceptibility. These toxic substances mostly act on three major systems of our body which include the nervous system, the reproductive system as well as the immune system. Various studies indicate oxidative stress and compromised germ cell productions are associated with the inadvertent use of these PBMCs [4, 5]. These pyrethroids generate free radicals causing oxidative stress. Oxidative stress induces reactive oxygen species including oxygen ions & peroxides [6]. The aberrant development of such free radicals results in, nucleic acid, lipid, and protein damage. The radicals are likely to take part in chemical reactions, snatching electrons from imperative organs and structures eventually causing damage. Gonad is the primary focus organ for these toxic substances because of abundant Polyunsaturated Fatty acids in its membrane [7].
These Polyunsaturated Fatty acids are prone to peroxidation activity under oxidative stress and thus resulting into membrane disruption. Antioxidants prevent the generation of reactive oxygen species and cell has different antioxidant enzymes like superoxide dismutase (SOD), glutathione reductase (GSH), glutathione-s-transferase (GST), catalase (CAT), & reduced glutathione (GSH) to combat the oxidative damage. Vitamin C is one of the major components of the body antioxidant system [8]. It has an excellent antioxidant characteristic that protects cells and tissues from lipid peroxidation induced by free radicals. So, the analysis was conducted to determine the impact of PBMC smoke on the albino rats’ testes and biochemical markers of oxidative stress on the testes of albino rats

Materials and methods
Current analysis has been performed in the Department of Anatomy in the conjunction with the Department of Biochemistry in King George’s Medical University, Lucknow. A total of 42 healthy male albino wistar rats of the age between 2-3 months and of weight between 275±25 grams, were collected from the animal house of CSIR-IITR, Lucknow. They were kept in polyethylene cages of size 15x12x8 inches in groups with not more than 4 rats in one cage. They were fed freely on a standard pellet diet 5gm/rat/day along with water in ad libitum supply.

Groupings of the animals
The rats were split into four groups.
1) Group 1st -12 rats with no exposure served as control group
2) Group 2nd - total of 12 rats, with 8 hours exposure to smoke of the mosquito coil, per day for 12 weeks.
3) Group 3rd - 8 rats with 8 hours exposure to mosquito coil smoke, every day for 12 weeks, and then kept for 8 weeks without exposure.
4) Group 4th - 10 rats with 8 hours exposure to smoke of the mosquito coil, every day for 12 weeks along with oral supplementation of ascorbic acid (VIT. C) at a 20 mg/kg body weight dosage once daily for the exposure duration

Product detail
A mosquito coil
We used commonly available Mosquito Coils in our region. The composition of the mosquito coil (in terms of w/w) is as follow 0.1% w/w d-trans allethrin with some other major components like wood binder, 10% w/w Starch, coconut shell powder 40% w/w, Genopol LO 88 emulsifier 0.1%w/w, 0.5%w/w Fragrance, 0.1%w/w Red dye, 0.1%w/w/Potassium Nitrate, 0.3%w/w Sodium, & 6% w/w Jiggat (joss). As per product details, it is anticipated that each coil will burn for approx. 8 hours.

Vitamin C
VIT. C tablets of weight 500mg containing ascorbic acid IP-100mg and Sodium ascorbate-450mg (equivalent to 400 mg of ascorbic acid) was used.

Mode of treatment
Mosquito coil exposure
Animals from experimental groups 2nd, 3rd, and 4th were kept in a room of dimension 9.5 feet × 9 feet × 9 feet having proper cross ventilation. Rats were subjected to whole-body PBMC smoke inhalation by burning it from 9 PM to 5 AM for 8 hours at night to mimic human exposure. All the cages were kept in a circle of radius 1 feet and the mosquito coil was kept at the centre of the circle so that each rat is equally exposed to smoke.

The vitamin C therapy
A fresh aqueous solution of VIT C was prepared by dissolving one VIT C tablet of 500 mg (lime, Abbott healthcare) in 10 ml of water. The freshly prepared solution was orally administered with the help of feeding canula to rats of experimental group IV at a dose of 20mg/kg. (Twice the recommended human dosage of 10 mg/kg of body weight) as documented that double the human therapeutic dose is very efficient as just an antimutagen, accompanied by doses of 40 mg and 10 mg, [9].

Sample collection and Sacrifice
The rats were first weighed and then anesthetized at the time of sacrifice by putting them in a sealed jar including chloroform-soaked cotton wool. The rats were exposed to reveal the reproductive organs with a midline abdominal laparotomy. The testicles were then removed. Testes were kept at -80 ° C of each animal and biochemical evaluation was carried out at room temperature.

Biochemical assessment
With the assistance of York’s homogenizer equipped with Teflon plunger, 10 % ( w / v) testis homogenate was prepared in KCl (0.15 M) for MDA & GSH, while 0.1 M phosphate buffer (pH 7.1) employed for estimation of CAT, SOD, and GPX as per requirement. The whole homogenate was centrifuged at three steps and the resultant supernatant was used for enzyme activities. The measurement of proteins was achieved by the process defined by Lowry et al. [10]. Lipid peroxide levels (Nmol MDA/mg of protein) were done by the method elaborated by Ohkawa et al. [11] Catalase activity (CAT in unit/mg of protein) was detected using the process of Aebi in [12]. Superoxide dismutase (SOD in unit/mg of protein) detection was done by the process described by McCord & Fridovich [13]. Estimation of Glutathione peroxidase (GPX in moles of NADPH oxidized/minute/mg of protein) was done by the method mentioned by Paglia & Valentine [14] while estimation of reduced Glutathione (GSH in nmol/mg of protein) was done by the method of Ellman [15].

Statistical evaluation
The statistical assessment was done with SPSS Version 15.0 SAS (statistical analysis software). ANOVA and Tukey HSD test was employed for comparison between groups. The values were represented in Mean ± SD.

Results
Table 1 showed that mean catalase levels ranged from 4.93±0.39 units in control (Group 1st) to 3.10±0.68 units in PBMC exposed (Group 2nd). The intergroup difference was also statically significant (P<0.001). Similarly, mean SOD levels ranged from 24.62±7.26 units in control group 1st to 6.38±1.45 units in PBMC exposed (Group 3rd). Statistically, the intergroup difference was significant (P<0.001). Mean GSH levels was also maximum in control (Group 1st) (52.73±5.09 units) and minimum in PBMC exposed (Group 2nd) (20.28±5.90 units). Statistically, there was a significant
difference among groups. Mean GPO levels were minimum in PBMC exposed (Group 2\textsuperscript{nd}) (31.98±8.30 units) and maximum in control (Group 1\textsuperscript{st}) (73.13±6.55 units). Statistically, this difference was significant. The value for MDA was reversed. Mean MDA levels ranged from 10.64±1.03 units (PBMC + VIT C treated) to 20.07±3.86 units (PBMC exposed group). Statistically, there was a significant difference among groups (P<0.001) (Fig.1). As compared to PBMC exposed (Group 2\textsuperscript{nd}), control (Group 1\textsuperscript{st}) had significantly higher catalase, SOD, GSH, and GPO levels and significantly lower MDA levels (P<0.001) (Table.2). No important distinction between control (Groups 1\textsuperscript{st}) and PBMC + VIT C treated group (Group 4\textsuperscript{th}) was observed for Catalase and MDA levels. For SOD, GSH and GPO rates were substantially higher in the control group 1\textsuperscript{st} than those in PBMC + VIT C treated group (Group 4\textsuperscript{th}) (P<0.001) (table 2). PBMC exposed (Group 2\textsuperscript{nd}) had significantly lower mean Catalase, SOD, and GPO levels as compared to PBMC + withdrawal group (Group 3\textsuperscript{rd}) and significantly higher MDA levels as compared to PBMC + withdrawal group (Group 3\textsuperscript{rd}). No significant difference between these two groups was seen concerning mean SOD levels (table2). PBMC exposed (Group 2\textsuperscript{nd}) had significantly higher catalase, SOD, GSH, and GPO levels as compared to PBMC + withdrawal group (Group 3\textsuperscript{rd}) (table2). No statistically significant changes were observed between these two groups concerning mean SOD levels (Table.2).

![Enzymatic activities of Catalase (unit/mg of protein), MDA (nmoles/mg of protein), SOD (unit/mg of protein), GSH (nmoles/mg of protein), and GPX (moles of NADPH oxidized per minute/mg of protein)](image)

**Fig 1:** Intergroup and between-group comparison of Mean Enzyme Levels

| Parameter | Control (Group 1\textsuperscript{st}) | Exposure (Group 2\textsuperscript{nd}) | Withdrawal (Group 3\textsuperscript{rd}) | VIT C exposure (Group 4\textsuperscript{th}) | Statistical analysis |
|-----------|-------------------------------------|--------------------------------------|---------------------------------|---------------------------------|---------------------|
| Catalase  | Mean 4.93, SD 0.39\textsuperscript{2nd} | Mean 3.10, SD 0.69\textsuperscript{3rd,4th} | Mean 4.8, SD 0.44\textsuperscript{2nd} | Mean 4.75, SD 0.38\textsuperscript{2nd} | F 26.412, p < 0.001 |
| MDA       | Mean 11.22, SD 1.81\textsuperscript{2nd,3rd} | Mean 20.07, SD 3.86\textsuperscript{1st,4th} | Mean 16.72, SD 0.94\textsuperscript{1st,4th} | Mean 10.64, SD 1.03\textsuperscript{2nd,3rd} | F 34.101, p < 0.001 |
| SOD       | Mean 24.62, SD 7.26\textsuperscript{2nd,3rd} | Mean 6.38, SD 1.45\textsuperscript{1st} | Mean 8.40, SD 0.79\textsuperscript{1st} | Mean 13.72, SD 1.61\textsuperscript{1st} | F 33.513, p < 0.001 |
| GSH       | Mean 52.73, SD 5.09\textsuperscript{2nd,3rd} | Mean 20.28, SD 5.90\textsuperscript{1st,4th} | Mean 30.25, SD 2.35\textsuperscript{1st} | Mean 38.16, SD 4.65\textsuperscript{1st,4th} | F 71.287, p < 0.001 |
| GPX       | Mean 73.13, SD 6.55\textsuperscript{2nd,3rd} | Mean 31.98, SD 8.30\textsuperscript{1st,4th} | Mean 46.13, SD 5.84\textsuperscript{1st} | Mean 55.56, SD 9.55\textsuperscript{1st,2nd} | F 43.344, p < 0.001 |

Catalase (unit/mg of protein), MDA (nmoles/mg of protein), SOD (unit/mg of protein), GSH (nmoles/mg of protein) and GPX (moles of NADPH oxidised per minute/mg of protein)

**Denotation:** superscript denotes statistically significant difference with the groups.
Discussion

PBMCs are the most commonly used mosquito repellents due to their effectiveness and low cost. Numerous studies have shown in recent years that sensitivity to environmental toxicants has impaired reproductive functions [16]. These environmental toxicants may act through various pathways like direct spermatozoa, alteration of hormonal pathway, and by causing oxidative stress, etc [17]. Pyrethroids have been reported to cause oxidative damage, evidenced by deranged markers in studies conducted in various animals by using various dosages at various periods [18]. Although the antioxidant mechanism of our body is capable to counter these oxidative damages to some extent, a higher exposition to these compounds leads to severe oxidative stress and toxicity. In present analysis, there was a considerable rise in MDA content in PBMC exposed (Group 2th) in contrast to regulation, (Group 1st) implying that allethrin triggered free radicle development by lipid peroxidation in testicular tissue. Pyrethroids having lipophilic property, penetrate and accumulate in these biological membranes. This leads to increased production of ROS, eventually causing increased MDA levels. Testis is the more susceptible organ as they have high lipid content in their membrane. These findings verify with the prior studies that presented a substantial increase in the level of MDA signifying high peroxidation of lipid & testicular toxicity [19, 20]. Issam et al also reported increased MDA levels in rats on treating them with subcutaneous deltamethrin [18]. This increase in MDA level is partially reversible in PBMC + withdrawal group (Group III), and group IV (Exposure + VIT. C). VIT. C prevents oxidation of lipids through its chain-breaking antioxidant activity [21]. Other studies also demonstrated that VIT. C prevents biochemical alterations in serum and renal tissue against deltamethrin-induced toxicity [22]. The primary antioxidant enzymes (SOD, Catalase (CAT) and Glutathione peroxidase), which inactivate the ROS into intermediates molecules. SOD converts superoxide radicals to H2O2 then CAT metabolizes H2O2 to water. When they get saturated GPX is activated that to detoxifies H2O2. Similar to other studies, oxidative stress is increased with a decreased level of GPX, SOD & CAT activities in group II [21, 23]. In PBMC + withdrawal group (Group III) partial recovery of these enzymes was evident in our study. VIT. C protect testis from the deterrent effects of pyrethroids through inhibition of lipid peroxidation and activation of endogenous antioxidative defense system. With supplementation of VIT. C, activities of GPX, SOD and CAT in PBMC + VIT C treated group (Group IV) are significantly restored probably because of increased bioavailability of these enzymes. Similar to other study, there was a significant rise in the activities of these enzymes in rats treated with PBMC plus oral administration of vitamin C [24].

Secondary antioxidant enzymes like Glutathione reductase (GSH), work directly to detoxify ROS by decreasing the peroxide level. In our investigation, GSH level was found to be decreased significantly in PBMC exposed (Group II) against the control (Group I), shows its consumption for detoxification of free radicals and therefore increased susceptibility of testis to free radicals. Similar effects have been observed by other authors across the globe in vitro and in vivo [20, 23, 25]. However, VIT. C administration in PBMC + VIT C treated group (Group IV) has supplemented the antioxidant enzyme activities, probably because of its ability to reduce the accumulation of free radicals and regenerates the GSH. Hence in our study biochemical assessment revealed an increased level of lipid peroxidation product i.e. MDA, which is the marker of oxidative injury in PBMC exposed (Group II) in comparison to control (Group I), PBMC + withdrawal group (Group III), and PBMC + VIT C treated group (Group IV). All the antioxidant enzymes (GSH, SOD, CAT, GPO) decreased significantly in PBMC exposed (Group II) in comparison to the rest of the groups. The antioxidant (MDA) and antioxidant enzyme (GSH, SOD, CAT, GPX) level improved in PBMC + withdrawal group (Group III) but again the improvement was more marked in PBMC + VIT C treated group (Group IV).

Limitation

Rats were kept in a room of dimension 9.5 feet × 9 feet × 9 feet having proper cross ventilation to simulate human settings but a closed inhalation chamber would be better for accurate monitoring of allethrin exposure.

Conclusion

It is concluded that PBMCs cause an increased level of lipid peroxidation product i.e. MDA while it decreased level of GSH and other antioxidant enzymes i.e. GPX, CAT and SOD due to oxidative injury to testicular tissue. This oxidative injury may further damage germ cell lines, which needs to be studied further. The values of these enzymes are partially reversible with withdrawal from the PBMC. But our study indicates the level of these enzymes can be markedly improved by supplementation of vitamin C in double the human therapeutic dose in comparison to exposure withdrawal. So vitamin C supplementation can be a good treatment modality for people who are exposed to PBMC.

Ethical and Legal aspects

Ethical clearance was obtained from the Animal Institutional Ethical Committee, King George’s Medical University via ref. no. 66/IAH/Pharma-14.

Acknowledgement

The authors have this opportunity to thanks Department of Biochemistry & Pharmacology, King George’s Medical University, India to carry out present study.
Conflict of interest
The authors declare that there is no conflict with any agencies on any term.

References
1. Garba SH, Shehu MM, Adelaiye AB. Toxicological effects of inhaled mosquito coil smoke on rat’s spleen: A hematological and histological study. J Med. Sci 2007;(1):94-99.
2. Krieger RL, Dinoff M, Zhang X. Octachlorodiphenyl ether. Mosquito coils are inadequately studied for residential use in Asia and illegal in the United States. Environ. Health Perspect. 2003;3:12-15.
3. Liu W, Zhang J, Hashim JH, Jalaludin J, Hashim Z, Goldstein BD. Mosquito coil emissions and health implications. Environ. Health Perspect. 2003;111(12):1454-1460.
4. Perry MJ. Effects of environmental and occupational pesticide exposure on human sperm: a systematic review. Hum Reprod Update 2008;14:233-242.
5. Madhubabu G, Yenugu S. Effect of continuous inhalation of allethrin-based mosquito coil smoke in the male reproductive tract of rats. Inhal Toxicol 2012;24(3):143-152.
6. Li HY, Wu SY, Ma Q, Shi N. The pesticide deltamethrin increases free radical production and promotes nuclear translocation of the stress response transcription factor Nrf2 in rat brain. Toxicol Ind Health 2011;27(7):579-590.
7. Wathes DC, Abayasekara DR, Aitken RJ. Polyunsaturated fatty acids in male and female reproduction. Biol Reprod 2007;77(2):190-201.
8. Figueroa-Méndez R, Rivas-Arancibia S. Vitamin C in Health and Disease: It’s Role in the Metabolism of Cells and Redox State in the Brain. Front Physiol 2015;6:397.
9. Kahn PK, Sinha SP. Antimutagenic efficacy of higher dose of Vit c. mutat.res 1993;298(3),157-161
10. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193(1):265-275
11. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95(2):351-358.
12. Aebi HE. Catalase in vitro Methods of Enzymatic Analysis, Verlag Chemie, Weinheim 1983;273-286.
13. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969;244(22):6049-6055.
14. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab. Cm. Med 1967;70:158-169.
15. Ellman GL. Tissue sulphhydryl groups. Archives of Biochemistry and Biophysics 1959;82:70-77.
16. Veras MM, Caldini EG, Dolhnikoff M, Saldiva PH. Air pollution and effects on reproductive-system functions globally with particular emphasis on the Brazilian population. J Toxicol Environ Health B Crit Rev 2010;13(1):1-15.
17. Sinawat S. The environmental impact on male fertility. J Med Asssoc Thai 2000;83(8):880-885.
18. Issam C, Samir H, Zohra H, Monia Z, Hassen BC. Toxic responses to deltamethrin (DM) low doses on gonads, sex hormones and lipoperoxidation in male rats following subcutaneous treatments. J Toxicol Sci 2009;34(6):663-670
19. Brigelius-Flohe R. Tissue-specific functions of individual glutathione peroxidases. Free Radic Biol Med 1999;27:951-965.
20. Akunna G, Saalu LC, Ogunlade B, Ogunmode OS and Akinbade AM. Pyrethroid-Based Insecticide Induces Testicular Toxicity via Oxidative Pathway. Oriental Journal of Scientific Research 2013;2:1.
21. Djeffa A, Messarah M, Boumendjel A, Kadeche L, El-Fek A. Protective effects of VitC and selenium supplementation on methomyl-induced tissue oxidative stress in adult rats. Toxicology and Industrial Health 2012;1-13.
22. Abdel-Daim MM, El-Ghoneimy A. Synergistic protective effects of ceftriaxone and ascorbic acid against subacute deltamethrin-induced nephrotoxicity in rats. Ren Fail 2015;37(2):297-304.
23. Afolabi OK, Aderibigbe FA, Folarin DT, Arinola A, Wusu AD. Oxidative stress and inflammation following sub-oral exposure of cypermethrin in rats: mitigating potential of epicatechin. Heliyon 2019;5(8):02274.
24. Ghorbani Taherdehi F, Nikravesh MR, Jalali M, Fazel A, Gorji Valokola M. Evaluating the Protective Role of Ascorbic Acid in Malathion-induced Testis Tissue Toxicity of Male Rats. Int J Prev Med 2019;10:45.
25. Maran E, Fernandez M, Barbieri P, Font G and Ruiz MJ. Effects of four carbamate compounds on antioxidant parameters. Ecotoxicology and Environmental Safety 2009;72:922-930.