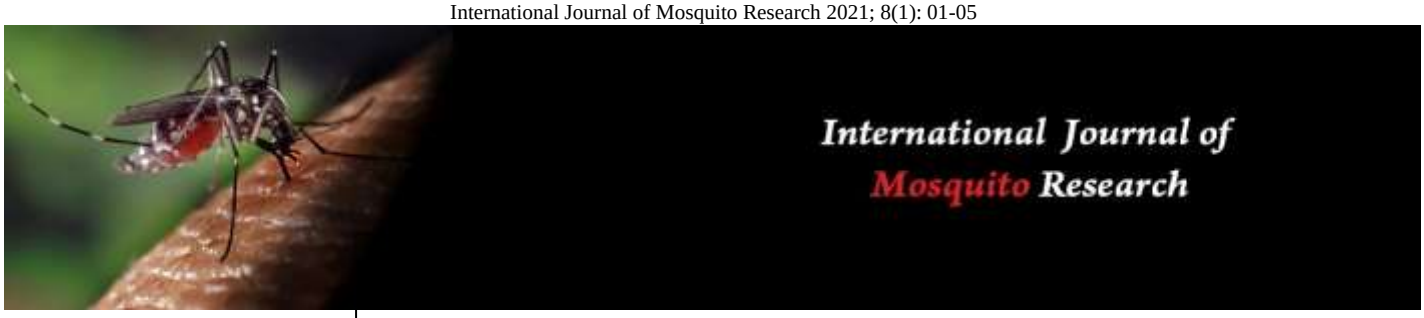

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Experimental validation of vitamin C's defensive function against the biochemical changes triggered by a pyrethroid-based mosquito coil (PBMC) on testis of albino rats

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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 mansonia, 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].

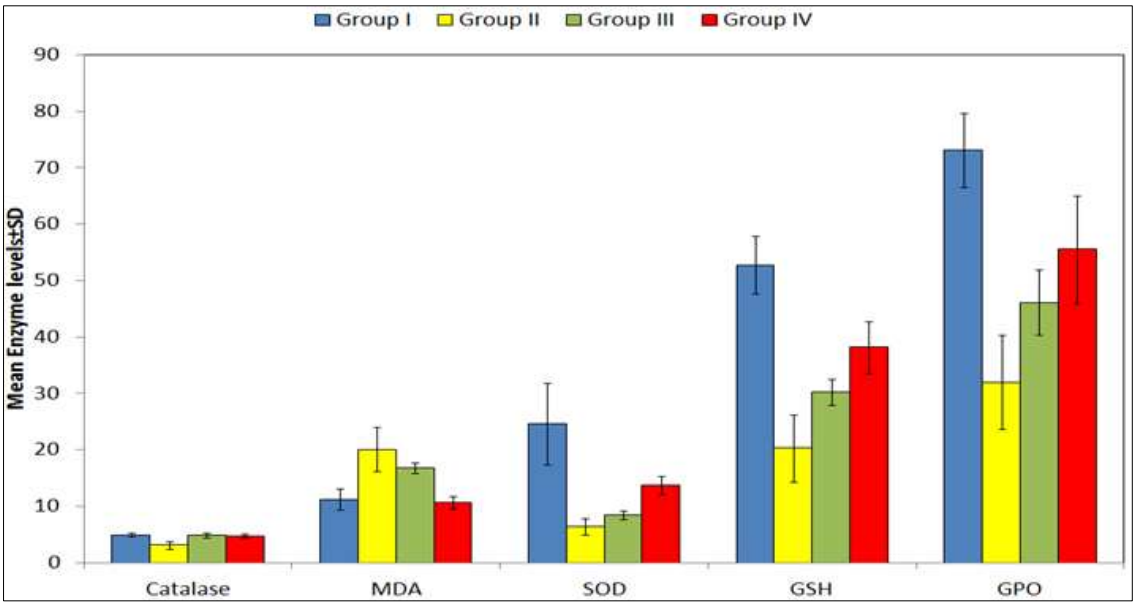
Statistical evaluation

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 2nd) (31.98±8.30 units) and maximum in control (Group 1st) (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 2nd), control (Group 1st) had significantly higher catalase, SOD, GSH, and GPO levels and significantly lower MDA levels (P<0.001) (Table.2). No important distinction between control (Group 1st) & PBMC + withdrawal group (Group 3rd) was observed for Catalase levels. Mean MDA levels in control (Group 1st) were significantly lower as compared to that in PBMC + withdrawal group (Group 3rd) (P<0.001) and mean SOD, GPO & GSH levels were higher significantly in control (Group 1st) as compared to those in PBMC + withdrawal group (Group 3rd) (P<0.001) (table2). No important distinction between the control (Groups 1st) and PBMC + VIT. C treated group (Group 4th) was observed for Catalase and MDA levels. For SOD, GSH and GPO rates were substantially higher in the control group 1st than those in PBMC + VIT C treated group (Group 4th) (P<0.001) (table 2). PBMC exposed (Group 2nd) had significantly lower mean Catalase, SOD, and GPO levels as compared to PBMC + withdrawal group (Group 3rd) and significantly higher MDA levels as compared to PBMC + withdrawal group (Group 3rd). No significant difference between these two groups was seen concerning mean SOD levels (table2). PBMC exposed (Group 2nd) had significantly lower catalase, SOD, GSH, and LPO levels and significantly higher MDA levels as compared to PBMC + VIT C treated group (Group IV) (table2). PBMC + withdrawal group (Group 3rd) had substantially higher MDA & significantly lower GSH rates as compared to PBMC + VIT C treated group (Group 4th). For other enzymes, no statistically significant changes were observed between the two groups (Table.2).



Enzymatic activities of Catalase (unit/mg of protein), SOD (unit/mg of protein), GSH (nmol/mg of protein), and GPX (moles of NADPH oxidized per minute/mg of protein) are improving more with vitamin C treated group in comparison to withdrawal group. Whereas MDA (nmol/mg of protein) activity is found to be minimum in the vitamin C treated group in comparison to group 2nd and Group 3rd.

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

Table 1: Intergroup comparison of Mean Enzyme Levels

Parameter	Control (Group 1st)		Exposure (Group 2nd)		Withdrawal (Group 3rd)		VIT C exposure (Group 4th)		Statistical analysis	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F	p
Catalase	4.93	0.39^{2nd}	3.10	$0.68^{2st,3rd,4th}$	4.8	0.44^{2nd}	4.75	0.38^{2nd}	26.412	<0.001
MDA	11.22	$1.81^{2nd,3rd}$	20.07	$3.86^{1st,4th}$	16.72	$0.94^{1st,4th}$	10.64	$1.03^{2nd,3rd}$	34.101	<0.001
SOD	24.62	$7.26^{2nd,3rd,4th}$	6.38	1.45^{1st}	8.40	0.79^{1st}	13.72	1.61^{st}	33.513	<0.001
GSH	52.73	$5.09^{2nd,3rd,4th}$	20.28	$5.90^{1st,4th}$	30.25	2.35^{1st}	38.16	$4.65^{1st,2nd}$	71.287	<0.001
GPX	73.13	$6.55^{2nd,3rd,4th}$	31.98	$8.30^{1st,4th}$	46.13	5.84^{1st}	55.56	$9.55^{1st,2nd}$	43.344	<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.

Table 2: Between Group Comparison (Tukey HSD test) of Enzymes Levels

Test No.	1st vs. 2nd			1st vs. 3rd			1st vs. 4th			2nd vs. 3rd			2nd vs. 4th			3rd vs. 4th		
	MD	SE	p	MD	SE	p	MD	SE	p	MD	SE	P	MD	SE	p	MD	SE	P
Catalase	1.83	0.23	<0.001	-0.17	0.25	0.895	0.17	0.22	0.857	-2.00	0.26	<0.001	-1.65	0.23	<0.001	0.35	0.25	0.521
MDA	-8.85	1.07	<0.001	-5.51	1.16	<0.001	0.58	1.03	0.943	3.35	1.21	0.047	9.43	1.09	<0.001	6.09	1.18	<0.001
SOD	18.25	2.00	<0.001	16.22	2.17	<0.001	10.91	1.93	<0.001	-2.03	2.27	0.809	-7.34	2.04	0.006	-5.32	2.22	0.100
GSH	32.46	2.29	<0.001	22.48	2.50	<0.001	14.58	2.22	<0.001	-9.98	2.61	0.003	-17.88	2.35	<0.001	-7.91	2.55	0.021
GPX	41.16	3.70	<0.001	27.01	4.03	<0.001	17.58	3.59	<0.001	-14.15	4.22	0.011	-23.58	3.79	<0.001	-9.43	4.11	0.123

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)

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 spermatotoxin', 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 2nd) 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 its 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 H_2O_2 then CAT metabolizes H_2O_2 to water. When they get saturated GPX is activated that to detoxifies H_2O_2. 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 defence 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 oxidant (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.

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Conflict of interest

The authors declare that there is no conflict with any agencies on any term.

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