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Evaluation of Oxidative Stress Biomarkers and Atherogenic Indices in Adult Wistar Rats Exposed to Pyrethroid Insecticides

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Abstract

Indiscriminate use of pesticides appears to increase the production of free radicals in organisms such as humans and other animals. Thus, the aim of this study was to investigate the effect of pyrethroid insecticides on oxidative stress biomarkers and atherogenic indices in exposed adult Wistar rats. A total number of apparently healthy 60 Wistar rats were randomly selected for this study and divided into two groups. Thirty six (36) were exposed to 1.2 % w/v pyrethroid insecticides while the remaining twenty four (24) were grouped as non-exposed. The animals in each group were anesthetized and blood sample was collected from the inferior vena cava after days of exposure. Plasma levels of total antioxidant status (TAS), malondialdehyde (MDA), glutathione (GSH), hydrogen peroxide (H_2O_2), Nitric Oxide (NO) and lipids profiles were determined using standard spectrophotometric techniques. Plasma activities of superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) were determined using standard spectrophotometric techniques. Statistical analysis of data was obtained and $P<0.05$ was considered significant. The mean TChol and HDL were significantly higher in 7days than 41days exposure, while plasma mean TChol and HDL were significantly lower in 41days than 21days exposure. The plasma mean levels of SOD, GPx, CAT, GSH and TAS were significantly decreasing while plasma mean levels of MDA, H_2O_2 and NO were significantly increasing from 7 days through 41 days exposure in exposed groups irrespective of the gender. In conclusion, this study observed that there is oxidative stress in Wistar rats exposed to pyrethroids based insecticides and lowered antioxidant defenses also observed in the studied population. Also, there was increased in weight differences with possibility of becoming obese. Thus, antioxidant supplements are advised as a prophylactic supportive therapy for adequate measures in preventing development of oxidative stress-associated complications among exposed individuals.

Keywords: Organic pollutants, pyrethroid insecticides, free radicals, antioxidants, atherogenic indices

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1. Introduction

Persistent organic pollutants (POPs) are a group of diverse substances, including polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), that are resistant to biodegradation and ubiquitously present in our environment. Humans are predominantly exposed through the consumption of contaminated food, mainly meat, fish, and dairy products [1], in agriculture for crop protection, in homes for controlling any pest and gardens for destroying unwanted species of plants [2]. Because of their ability to resist environmental degradation, these substances are omnipresent in food products, and found all around the world, even in areas where they have never been used like Antarctica [3, 4]. POPs are lipophilic chemicals that can pass through biological phospholipid membranes and accumulate in fatty rich tissues of humans [4]. This affinity for the adipose tissues means that POPs are likely to accumulate, persist and could eventually; achieve toxicologically relevant concentrations, even though exposure episodes may appear limited [5]. POPs are grouped according to their use and origin into pesticides which include aldrin, chlordane, dichlorodiphenyl trichloroethane (DDT) etc and industrial chemicals which include polychlorinated biphenyls and hexachlorocyclohexane [5]. Among the classes of pesticides, insecticides have been implicated as having the most effects on human health because they are designed to act on insect receptors, ion channels, and enzymes which are very similar to those found in humans [6, 7]. Specifically, exposure to persistent organic pollutants (POPs) has been shown to have a strong positive association with type 2 diabetes and related metabolic conditions [8, 9].

In a healthy body, reactive oxygen species (ROS) and antioxidants remain balance. Oxidative stress occurs when the generation of reactive oxygen species and other radical species exceeds the scavenging capacity by antioxidants of oxidative agents in organism or it is due to excessive production of reactive oxygen species and/or inadequate intake or increased utilization of antioxidants [10-12]. Most ROS are formed either by endogenous source such as operation of electron transport chains in mitochondria, endoplasmic reticulum, plasmatic and nuclear membranes or by exogenous exposures which include alcohol, smoking and environmental pollutants [11]. It has been suggestive that exposure to pesticides has an effect on the increase of oxidative biomarkers, because it is one of exogenous source of free radicals production. This may eventually lead to atherogenic indices alteration in the body if it is not been controlled on time [13, 14]. Elimination of reactive oxygen species is catalyzed by certain enzymes such as superoxide dismutase (SOD), catalases and peroxidases. Antioxidants (including vitamins C and E) and antioxidant cofactors (such as selenium, zinc, and copper) are capable to dispose, scavenge, or suppress ROS formation [15].

Oxidative stress has been established to be harmful because oxygen free radicals attack biological molecules such as lipids, proteins, and DNA [16]. The biomarkers that can be used to assess oxidative stress in-vivo have been attracting interest because the accurate measurement of such stress is necessary for investigation of its role in lifestyle diseases as well as to evaluate the efficacy of treatment. The involvements of free radicals in several disease states are becoming well established. These include atherosclerosis, some forms of cancer, cataract formation and other disorders of eye, the diseases which involve the inflammatory response such as rheumatoid arthritis, ulcerative colitis and crohn's disease [13]. Obesity epidemic roughly correlates with a marked increase in the use of chemicals (pesticides). It is known that chemical toxins like pyrethroids can act as obesogens [17]. Obesogens are functionally defined as chemicals that inappropriately alter lipid homeostasis and fat storage, alter metabolic set points, disrupt energy balance or modify the regulation of appetite and satiety to promote fat accumulation and obesity [18]. The resistant nature of malaria Plasmodium falciparum to anti-malaria drugs and the nuisance caused by other household pests have led to increase in the use of pesticide to control these pests. It has been suggested that exposure to pesticides has an effect on the increase of oxidative biomarkers and atherogenic indices in the body. Therefore, this present study was aimed at exposing adult Wistar rats to pyrethroid pesticides

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to assess its effects on the oxidative stress biomarkers and atherogenic indices pattern in their body system.

2. Materials and Methods

2.1 Study Design

This is Experimental and observational study. Sixty (60) apparently healthy adult male and female Wistar rats weighing between 120g-250g were be obtained from the laboratory animal house of the Department of Anatomy, University of Benin and divided into two groups. The animals were housed within the facility and maintained on standard rodent pellets and water ad libitum. On transfer to the work area, the animals were allowed 14 days for acclimatization. Animals were housed under standard conditions of temperature and relative humidity with both light and dark cycle. Animal handling was performed according to Good Laboratory Practice (GLP). All animal experiments were in accordance with the National Institute of Health Guide for care and Use of Laboratory Animals.

2.2 Ethical Consideration

Approval for this study was obtained from the biomedical research ethics committee of Ministry of Agriculture and Natural resources, Benin City (With ethical clearance registration number: V.1041/15) and was carried out in strict compliance with the guidelines for the care and use of animals for research committee which is in line with that set by World Health Organization.

2.3 Choice of Pesticides

Pyrethroid pesticides with trade name 'Raid' registered by National Administration of Food and Drug Agency Control (NAFDAC) with registration number; A5-0417 was used for the experiment. This pyrethroid pesticide was chosen for the study because it is registered by NAFDAC for use in homes and it is multi-purpose insects' killer with active ingredients like D-allethrin, tetramethrin and deltamethrin.

2.4 Description of Method Used

The Wistar rats were randomly divided into two (2) groups which include expose and non-expose groups and each of the groups was further subdivided into three groups based on the period of exposure to pyrethroid based insecticides. Thus, identification tag was given to the rats in each of the groups.

Group 1 (36 rats); TG (TGM & TGF),

Group 2 (24 rats); CG (CGM & CGF); normal control

Key: TG = treated group, TGM= treated male group, TGF=treated female group, CG=Control group, CGM = control or non-expose male group, CGF=control or non-expose female group

All the experimental rats were housed in small iron cages (36cm x 22cm x 14cm) with many holes, where temperature was maintained at room temperature. The male and female rats were kept in different compartment of the cages to avoid mating. They were exposed to pyrethroid vapours inside a closed room (180cm x 240cm) according to method described by Hasan et al. [19]. The animals were exposed to 1.2% w/v pyrethroid vapours for 8 hours daily for a period of 7, 21 and 41 days respectively. Prior to the experiment the weight of the rats in each of the groups was recorded (as their initial weight) and their behaviours noted. The control animals were kept under identical conditions without exposure to the named insecticides. The description will be fully represented in Table 1.

2.5 Sacrifice of the animals and blood collection

After 7, 21, and 41 days exposure to insecticide, the rats in each group was reweighed to determine their final weight and the animals in each group were anesthetized respectively. Blood sample was collected from the inferior vena cava by the use of five (5) ml syringe and was dispensed into sterile vacutainer bottle containing lithium heparin anticoagulant and gently mixed by inverting the container severally for the determination of plasma lipids profile and oxidative stress markers. Plasma was separated from the blood by centrifugation for 5 minutes at 4000rpm, into plain bottles and stored at -20°C until time of analysis.

2.6 Analytical Methods and Procedures

Plasma levels of total antioxidant status (TAS) were determined using DPPH Method (1, 1 diphenyl 2, picryl hydrazyl) [20], Lipid peroxidation was determined by measuring the

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thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation [21], GSH was measured by the method of Beutler et al. [22], Hydrogen peroxide (H_2O_2) was estimated spectrophotometrically using Wolff's method [23] and Nitric oxide (NO) generated from the reaction of N‑1‑napthylethylenediamine dihydrochloride (NED) and sulfanilamide (Griess reagent) was measured colorimetrically [24]. Lipids profiles were determined appropriately using standard spectrophotometric techniques [25]. Plasma activities of superoxide dismutase (SOD) were determined using the method of Misra and Fridovich, [26] and measured at 480nm. Glutathione peroxidase (GPX) was assayed by the method proposed by Reddy et al. [27]. In the presence of the hydrogen donor pyrogallol or dianisidine, peroxidase converts H_2O_2 to H_2O and O_2. The coloured product formed was measured colorimetrically at 430nm. Catalase (CAT) was determined using direct colorimetric method of Sinha [28]. The method is based on the fact that dichromate is reduced to chromic acetate when heated in the presence of H_2O_2. The chromic acetate produced is measured colorimetrically at 570nm.

2.7 Statistical analysis

A statistical package for social sciences (SPSS) 19.0 versions was used for the analysis of the data appropriately. One way analysis of variance (ANOVA) was used for comparison within the groups. Spearman correlation was used to test the association between variables. Data were presented using mean ± standard deviation (mean ± SD) for all quantitative values. The level of significance was taken at 95% confidence interval and $P<0.05$ was considered significant.

Table 1. Grouping of Animals and Sex distribution of the rats' population in percentage (%)

Groups	Group A (7 days exposure)		Group B (21 days exposure)		Group C (41 days exposure)		Total
	Male	Female	Male	Female	Male	Female	
Exposed (Group 1)	6 (10)	6 (10)	7 (11.3)	5 (8.3)	6 (10)	6 (10)	36 (60)
Non-exposed (Group 2)	4 (6.67)	4 (6.67)	4 (6.67)	4 (6.67)	4 (6.67)	4 (6.67)	24 (40)
Total	10 (16.67)	10 (16.67)	10 (18.33)	10 (16.67)	10 (16.67)	10 (16.67)	60 (100)

3. Results

A total number of apparently healthy sixty (60) Wistar rats were randomly recruited for this study and divided into two groups. Thirty six (36) Wistar rats were exposed to pyrethroid insecticides while the remaining twenty four (24) were grouped as non-expose. Table 1 shows the grouping and sex distribution of all experimental animals used in percentage (%). Females constituted 48.34% while males constituted 51.67% in overall.

Figure 1 shows comparisons of mean atherogenic indices among exposed groups and control. The mean TChol and HDL were significantly higher in 7days than 41days exposure to insecticides, while plasma mean TChol and HDL were significantly lower in 41days than 21days exposure to insecticides. In Table 2, plasma mean levels of SOD, GPx, CAT, GSH and TAS were significantly decreasing from 7 days through 41 days exposure in exposed groups, while plasma mean levels of MDA, H_2O_2 and NO were significantly increasing from 7 days through 41 days exposure in exposed groups irrespective of the gender.

Lastly, Table 3 shows correlation between oxidative stress parameters and weight with atherogenic indices (TChol, TAG, HDL and LDL) in exposed groups. TChol and LDL showed statistical positive significantly correlation with GSH and (GPx and GSH) respectively, while both of them had significant inverse correlation with final weight (wt2). Figure 2 shows comparison between initial weight (Wt1) and final weight (Wt2) among exposed groups and control.

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Table 2. Comparison of mean oxidative stress indices among exposed groups and control

Parameters		7 days Exposure (n=12)	21 days Exposure (n=12)	41 days Exposure (n=12)	Control (n=24)	P value
SOD (U/ml)	**M**	3.27 ± 0.88 [a, b, c]	1.99 ± 0.56	2.21 ± 0.55	2.30 ± 0.52	0.005*
	F	2.98 ± 0.51 [a, b, c]	2.04 ± 0.51	1.81 ± 0.29	1.87 ± 0.33	0.000*
	C	3.12 ± 0.70 [a, b, c]	2.01 ± 0.44	2.01 ± 0.47	2.08 ± 0.48	0.000*
MDA (µmol/l)	**M**	1.79 ± 0.25 [b]	2.42 ± 0.66	2.84 ± 0.38	2.36 ± 1.05	0.142
	F	2.12 ± 0.38 [b]	2.22 ± 0.48 [b]	2.94 ± 0.58 [a, c]	1.95 ± 0.32	0.001*
	C	1.95 ± 0.35 [b]	2.33 ± 0.55 [b]	2.89 ± 0.47 [a, c]	2.15 ± 0.79	0.002*
GPx (U/ml)	**M**	3.43 ± 0.28 [a, b, c]	2.04 ± 0.52 [a,]	2.13 ± 0.49	2.74 ± 0.89	0.003*
	F	3.31 ± 0.23 [a, b, c]	2.33 ± 0.26 [b]	1.73 ± 0.27 [a, c]	2.40 ± 0.28	0.000*
	C	3.30 ± 0.28 [a, b, c]	2.16 ± 0.44 [a]	1.94 ± 0.43 [a]	2.57 ± 0.67	0.000*
CAT (U/L)	**M**	34.14 ± 4.71 [a, b, c]	24.06 ± 2.06	22.11 ± 3.27	21.62 ± 3.55	0.000*
	F	27.03 ± 3.77 [a, b]	24.77 ± 3.33 [b]	20.65 ± 1.34 [c]	23.09 ± 1.71	0.001*
	C	30.58 ± 5.51 [a, b, c]	24.36 ± 2.55 [b]	21.38 ± 2.50 [c]	22.35 ± 2.82	0.000*
H_2O_2 (µmol/l)	**M**	3.60 ± 1.06 [c]	4.48 ± 0.51 [a]	4.15 ± 0.64 [a]	3.11 ± 0.15	0.000*
	F	3.27 ± 0.61 [b]	4.18 ± 0.63 [b]	6.93 ± 1.90 [a, c]	3.38 ± 0.34	0.000*
	C	3.44 ± 0.84 [b, c]	4.36 ± 0.56 [a, b]	5.54 ± 1.98 [a, c]	3.25 ± 0.29	0.000*
GSH (µmol/l)	**M**	11.54 ± 1.07 [a, b, c]	7.98 ± 0.97	6.05 ± 2.13	7.24 ± 3.87	0.009*
	F	10.59 ± 0.87 [a, b, c]	8.92 ± 1.09 [a, b]	4.70 ± 0.63 [a, c]	7.18 ± 1.19	0.000*
	C	11.06 ± 1.06 [a, b, c]	8.38 ± 1.09 [b]	5.38 ± 1.65 [a, c]	7.21 ± 2.80	0.000*
NO (µmols/L)	**M**	24.92 ± 3.94 [a, b, c]	33.02 ± 8.07	38.51 ± 4.60	33.50 ± 5.72	0.004*
	F	24.81 ± 3.85 [a, b]	28.38 ± 2.01 [b]	37.92 ± 2.31 [a, c]	30.10 ± 3.14	0.000*
	C	24.86 ± 3.72 [a, b, c]	31.08 ± 6.54 [b]	38.21 ± 3.48 [a, c]	31.80 ± 4.84	0.000*
TAS (U/L)	**M**	5.13 ± 0.76 [a, b, c]	2.26 ± 0.52 [a]	2.28 ± 0.59 [a]	3.07 ± 0.47	0.000*
	F	3.88 ± 0.33 [b, c]	2.71 ± 0.57 [a, b]	1.93 ± 0.13 [a, c]	3.87 ± 0.76	0.000*
	C	4.50 ± 0.86 [a, b, c]	2.45 ± 0.57 [a]	2.11 ± 0.45 [a]	3.47 ± 0.74	0.000*

*Significant at P <0.001

a = significantly different from control

b = significantly different from 41 days exposure

c = significantly different from 21days exposure

Key: SOD = Superoxide dismutase, MDA= Malondialdehyde, GP_X = Glutathione peroxidase, CAT= Catalase, H_2O_2 =Hydrogen peroxide, GSH= Glutathione, NO= Nitric oxide, TAS= Total antioxidant status, M=Male, F=Female, C=Combined of both male and female

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Table 3. Correlation between oxidative stress parameters and weight with TChol, TAG, HDL and LDL in exposed groups

	TChol		TAG		HDL		LDL	
	r-value	**p-value**	**r-value**	**p-value**	**r-value**	**p-value**	**r-value**	**p-value**
SOD (U/ml)	0.182	0.288	0.046	0.788	0.053	0.761	0.304	0.072
MDA (µmol/l)	-0.143	0.407	0.026	0.879	-0.128	0.458	-0.181	0.291
GPx (U/ml)	0.325	0.053	-0.074	0.669	0.124	0.470	0.530	0.001*
CAT (U/L)	0.265	0.119	-0.024	0.887	0.238	0.163	0.290	0.086
H_2O_2 (µmol/l)	-0.175	0.307	0.203	0.236	-0.166	0.333	-0.374	0.024
GSH (µmol/l)	0.501	0.002*	0.130	0.449	0.341	0.042	0.487	0.003*
NO (µmols/L)	-0.225	0.187	0.188	0.273	-0.181	0.291	-0.272	0.108
Wt1 (gm)	-0.074	0.669	0.126	0.463	-0.097	0.574	-0.119	0.488
Wt2 (gm)	-0.461	0.005*	-0.027	0.875	-0.304	0.071	-0.439	0.007*

* Correlation is significant at the 0.05 level (2-tailed)

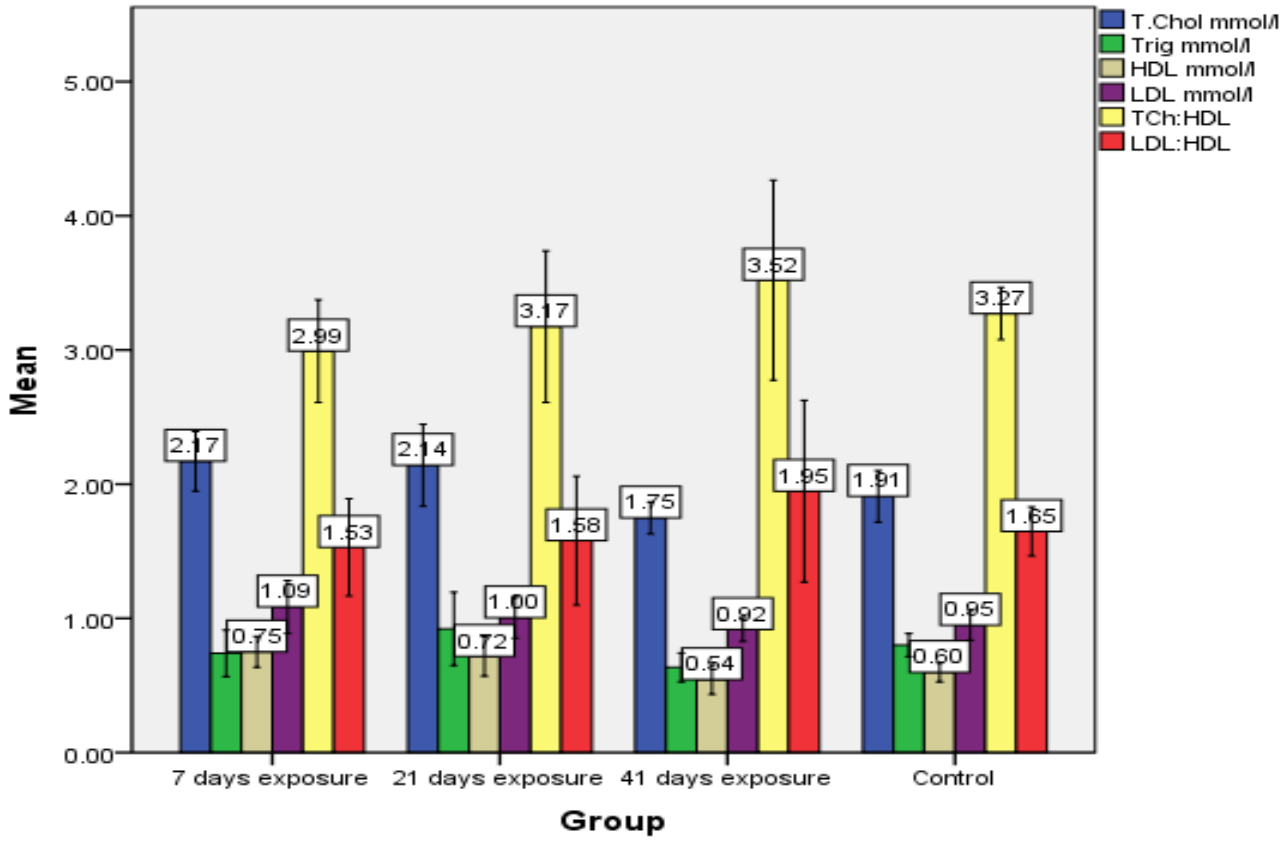

Figure 1. Comparison of mean atherogenic indices among exposed groups and control
Key: TChl = Total cholesterol, TAG = triglycerides, HDL = High density lipoprotein, LDL = Low density lipoprotein

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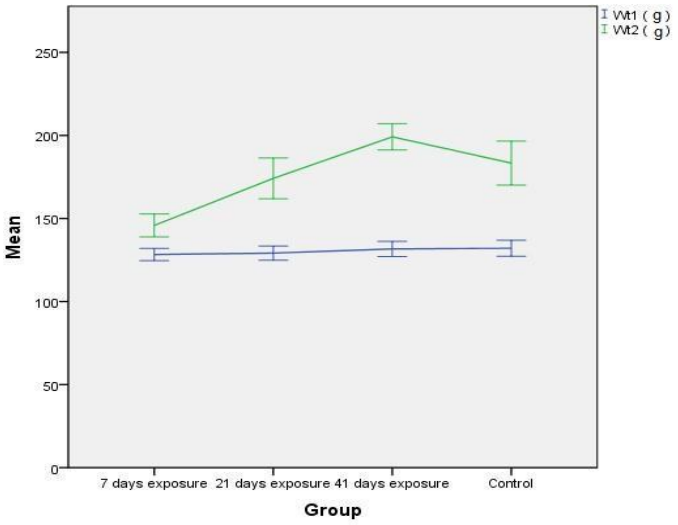

Figure 2. Comparison between initial weight (Wt1) and final weight (Wt2) among exposed groups and control

4. Discussion

It has been suggested that exposure to pesticides has an effect on the increase of oxidative biomarkers, because it is one of exogenous source of free radicals production. This may eventually lead to atherogenic indices alteration in the body if it is not been controlled on time [13]. Oxidative stress has been defined as harmful because oxygen free radicals attack biological molecules such as lipids, proteins, and DNA [14, 29]. Oxidative stress is well known to be involved in the pathogenesis of lifestyle-related diseases, including atherosclerosis, hypertension, diabetes mellitus, ischemic diseases, and malignancies [30].

The plasma mean of TChol and HDL were significantly decreasing from 7 days through 41 days exposure among exposed groups in this study. The reasons might be due to loss of appetite observed among exposed groups in comparing to controls. The result of this study is in agreement with Igho and Afoke [7] which reported that insecticides were associated with extensive cerebellar and cerebral damage with impaired behavioral changes and feeding habit. It is believed that the adverse effects of an insecticide are dependent on a number of factors as reported by WHO [5]. The most vital factor is the dose-time relationship which is how much of the substance is involved and how often the exposure to the substance occurs, thus giving rise to different types of toxicity in humans or animals. Pyrethroids are not easily absorbed through the skin, but are absorbed through the gut and pulmonary membrane. However systemic toxicity by inhalation and dermal absorption is low as reported by Reigart and Roberts [31] and this might take a longer time to be achieved.

In this study, we observed a significantly decrease in the plasma activity of SOD, GPX and CAT, and plasma levels of GSH and TAS in the exposed groups when compared to the controls, while plasma mean levels of MDA, H_2O_2 and NO were significantly increasing from 7 days through 41 days exposure in exposed groups irrespective of the gender. This is similar to what Aly et al. [32], Surajudeen et al. [33] reported when mice and farmers were exposed to pesticides respectively.

Oxidative stress results when there is increased production of free radicals or decreased activity of counter-actors, antioxidants or both in a combination [29]. Antioxidant which can either be enzymatic (SOD, CAT, GPx) or non-enzymatic (GSH, TAS, Vitamin C, A etc) protects against effect(s) of free radicals in order to maintain homeostatic balance of reactive oxygen species [15, 34, 42]. SOD plays a major role as first line of the antioxidant defense system by catalyzing the dismutation of superoxide radical to form hydrogen peroxide (an oxidant) and molecular oxygen [15, 35]. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS) [36]. GPx is a selenium dependent enzyme with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water [37]. Significant reduction in SOD, CAT and GPx activity might be an indication of accumulation of H_2O_2 that required to mump up these reactive species. Our observation possibly depicts increased formation of free radicals that could lead to oxidative damage [33, 35] as a result of overwhelming antioxidant activities of all these enzymes. In other way round, GSH and TAS were

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significantly decreasing from 7 days through 41 days among exposed groups in this study. Glutathione had been reported to play a key role in maintaining proper function and preventing oxidative stress in human cells. Reduced glutathione reduces the oxidized form of the enzyme glutathione peroxidase, which in turn reduces hydrogen peroxide (H_2O_2) dangerously reactive species within the cell [37]. Significant reduction in plasma levels of reduced glutathione (GSH) and TAS are thus a result of overwhelming antioxidant effects to reduce free radicals generated.

This finding suggests increase in oxidative stress, resulting in increased lipid peroxidation and this is corroborated by Pasupathi *et al*. [39] report. This study shows inverse correlation plasma levels of MDA with TChol, HDL & LDL, even though it is not statistical significant. The disparities might be due to route of exposure (Inhalation), which has low systemic toxicity [31] and perhaps exposure to pyrethroid pesticides could affect lipid metabolism, and it also indicates that lipid peroxidation is occurring even though the effect might be low. Total cholesterol is a major constituent of cell membranes, steroids and signaling molecules and these molecules are easily damaged by free radicals produced due to exposure to pyrethroid pesticides [40]. The decreased in plasma SOD and CAT activity might be due to increased H_2O_2 production in exposed groups. When initial weight (Wt1) was compared with final weight (Wt2) among exposed groups and control in this study, there was increased in weight difference despite the fact that feeding habit and cholesterol levels decreasing from 7 days to 41 days exposure to insecticides. This might be due to insulin resistant owing to the action of oxidative stress effects on insulin receptors leading to obesity as reported by previous work [41].

5. Conclusion

In conclusion, this study observed that there is oxidative stress in Wistar rats exposed to pyrethroids insecticides and lowered antioxidant defenses also observed in the studied population. Antioxidant supplements are thereby advised as a prophylactic supportive therapy for adequate preventing development of oxidative stress-associated complications among exposed individuals.

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