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Oxidative stress and superoxide dismutase activity in brain of rats fed with diet containing permethrin

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Abstract

Many pesticides have been reported to cause a lot of health problems in workers, users and non-target organisms in the environment. Permethrin containing insect pesticide has been reported to be toxic to non-target organisms. However, the underlying mechanism involved in the toxicity is not well understood. The present study was envisaged to investigate the possible role of oxidative stress in permethrin neurotoxicity and to evaluate the protective effect of superoxide dismutase (SOD) activity in brain homogenates of Wistar rats. Oxidative stress measured as thiobarbituric acid reacting substances (TBARS) was found to significantly increase ($p < 0.05$) in all the experimental groups compared with their parallel controls. Concomitantly, the activity of SOD was found to decrease or increase significantly ($p < 0.05$) in the experimental groups compared with their controls. Our result also showed that activity of SOD was aged and concentration dependent. Hence, the newly weaned rats appear to be more susceptible to the pesticide contaminated diet because the SOD activity decreased more in the brain homogenates compared with the middle aged rats or aged rats. Observed aggressive behaviour was noticed in the pesticide exposed rats, hence a possible neurobehavioral effect. The result demonstrated that the pyrethroid insect powder exerts its toxic effect by promoting oxidative stress in the brain and this may affect normal brain functioning and growth.

Keywords: pesticides, reactive oxygen species, superoxide dismutase, lipid peroxidation

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INTRODUCTION

Pesticide use in developing nations like Africa has increased dramatically in recent times the adverse effects in humans and other non-target organisms[1]. Although relative to global utilization of pesticides, African pesticides' use may be considered as low, but the ways in which they are being used are causing serious environmental and health problems. In most developing nations safety equipment is rarely used and in some cases completely lacking, storage methods are unsafe and the instructions for pesticide use are not always understood since most farmers or pesticide users are uneducated hence, increased risk of exposure.

The adverse effect of some pesticides has necessitated legislation in most developed countries against potentially damaging ones[2]. However, in most developing nations, regulatory laws governing pesticide production and utilization are almost completely lacking or where available these laws are poorly implemented thereby having a far-reaching effect on the ecosystem[3].

The use of pesticides has been widely expanded as a consequence of increased availability and improved prices[4]. However, the benefits of pesticides use must be balanced in regards to environmental degradation, worker safety and public health. Environmental contamination of pesticide is a threat to life and the hazard goes beyond the farming community because pesticide residues are found in the food chain, soil and water supplies.

Numerous pesticides such as paraquat, DDT, PCB, Arochlor® etc have been used as model factors inducing oxidative stress both *in-vivo* and *in vitro*[5,6]. The conversion of pesticides to free radicals or via the formation of superoxide radical is a by-product of their metabolism and this is regarded as one of the basic mechanisms of tissue damage. Several studies have pointed out the risk of carcinogenic effect, neurological actions and brain damage in living organisms exposed to various concentrations of xenobiotics in the environment[4]. In other to ameliorate these damages, organisms have evolved mechanisms to control the amount of hydroxyl and superoxide radicals generated. These fragments are quickly scavenged by natural protective molecules in the cells called antioxidants[7]. Antioxidants are intimately involved in the prevention of cellular damage – the common pathway for cancer, aging and a variety of diseases[4]. They safely interact with free radicals and terminate the chain reactions before vital molecules are damaged, antioxidants could be enzymatic or non-enzymatic, the latter include glutathion reductase, glutathion-S-transferase, glutathion peroxidase, superoxide dismutase (SOD) and catalase[8].

SOD is an important enzyme family in living cells for maintaining normal physiological conditions and for coping with stress. The action of SOD therefore is to protect the biological integrity of the cells and tissues against harmful effects of superoxide free radical[9]. Humans and other non-target organisms are sensitive to a great number of pollutants in the environment such as pesticides and heavy metal. Bioaccumulation of these pollutants in the biological system may pose a serious challenge to public health, hence the aim of this study.

MATERIALS AND METHODS

Test samples:

The pesticide used for this experiment was permethrin, formulated as Rambo insect powder. It is a product of Gogoni Co. limited Nigeria.

Treatment formulation

Commercial animal feed was contaminated with the pesticide to give the formulation of 1%, 5% or 10% (w\w) contamination (Table 1). The control groups were not given contaminated diet.

Table 1: Formulation of experimental diet

Formulation control	Pesticide qty (g)	Weight of feed (g)	Total (g)
1%	1	99	100
5%	5	95	100
10%	10	90	100

Lipid Peroxidation

Lipid peroxidation was assayed as thiobabituric acid reacting substances (TBARS) using the method described by

Wallin *et al*[10]. The differences in absorbance at 350 nm and 600 nm were read in Sp 500 spectrophotometer against the blank.

Superoxide Dismutase Assay

An indirect method of inhibiting auto-oxidation of epinephrine to its adrenochrome was used to assay SOD activity in the blood plasma[11]. The auto-oxidation was monitored in a Spectrophotometer (Sp 500) at 480nm every 30 secs for 5min. A graph of absorbance against time was plotted for each absorbance, and the initial rate of auto-oxidation calculated. One unit of SOD activity was defined as the concentration of the enzyme (mg protein/ml) in the plasma that caused 50% reduction in the auto-oxidation of epinephrine[12].

Statistical analysis

Mean values (±SD) of replicate experiment with quadruplet sampling (N=2x4) were taken for each analysis. Significantly different results were established by one-way ANOVA and differences between groups, and concentrations were determined by DUNCAN multiple range tests[21]. The accepted level of significance was $p<0.05$.

RESULTS

Inhibition study on the autoxidation of epinephrine on brain homogenates of rats exposed to various concentrations of pesticides is shown in Table 2. The result showed that percent inhibition of SOD in the brain was significantly different ($p<0.05$) within the exposed groups when compound with their parallel control groups. The trend was such that the newly weaned rat (NWR) groups showed marked decrease in percent inhibition while the middle aged rats (MAR) and aged rat (AR) groups showed significant increase when compared with their control groups.

SOD activity result (Table 3) showed that in the NWR group, there was a significant decrease ($p<0.05$) in activity but MAR and AR groups showed significant induction in SOD activity compared with their respective controls.

Lipid peroxidation value was determined as thiobarbituric acid reacting substances (TBARS). The result showed that the levels of liquid peroxidation increased significantly ($p<0.05$) in all the pesticide exposed groups when compared with their controls (Table 2).

Table 2: Rate of Autoxidation of Epinephrine in Rats Exposed To Insecticide-Contaminated Diet

Auto-oxidation mixture	Autoxidation rate (Unit/min)	% inhibition
AM + 1.0ml distilled water	0.0035 ± 0.00005	-
AM + 1.0ml BH NWR 1 %	0.0073 ± 0.00001	212.08 ± 3.51
AM + 1.0ml BH NWR 5 %	0.0108 ± 0.00005	311.64 ± 3.07
AM + 1.0ml BH NWR 10 %	0.0121 ± 0.00000	349.37 ± 6.51
AM + 1.0ml BH NWR Control	0.0224 ± 0.00020	646.64 ± 18.07
AM + 1.0ml BH MAR 1 %	0.0122 ± 0.00060	352.02 ± 10.84
AM + 1.0ml BH MAR 5 %	0.0164 ± 0.00230	474.50 ± 59.79
AM + 1.0ml BH MAR 10 %	0.0150 ± 0.00010	434.92 ± 9.21
AM + 1.0ml BH MAR Control	0.0036 ± 0.00005	103.15 ± 1.56
AM + 1.0ml BH AR 1 %	0.0039 ± 0.00005	110.00 ± 1.43
AM + 1.0ml BH AR 5 %	0.0236 ± 0.00030	684.33 ± 18.62
AM + 1.0ml BH AR 10 %	0.0265 ± 0.00025	766.93 ± 18.36
AM + 1.0ml BH AR Control	0.0125 ± 0.00005	360.97 ± 6.67

Table 3: SOD activity and lipid peroxidation products in brain homogenates of rats exposed o insecticide-contaminated diet

GROUP	SOD activity (Unit / ml)	Lipid peroxidation (nMol / ml)
AM + 1.0ml BH NWR 1 %	4.24 ± 0.07	2.85 ± 0.15
AM + 1.0ml BH NWR 5 %	6.23 ± 0.06	5.13 ± 0.13
AM + 1.0ml BH NWR 10 %	6.99 ± 0.13	6.10 ± 0.10
AM + 1.0ml BH NWR Control	12.93 ± 0.36	0.53 ± 0.03
AM + 1.0ml BH MAR 1 %	7.04 ± 0.22	5.60 ± o.60
AM + 1.0ml BH MAR 5 %	9.49 ± 1.20	5.59 ± 0.09
AM + 1.0ml BH MAR 10 %	8.69 ± 0.19	5.43 ± 0.58
AM + 1.0ml BH MAR Control	2.06 ± 0.03	1.25 ± 0.25
AM + 1.0ml BH AR 1 %	2.20 ± 0.03	2.43 ± 0.43
AM + 1.0ml BH AR 5 %	13.69 ± 0.38	11.83 ± 0.18
AM + 1.0ml BH AR 10 %	15.34 ± 0.37	9.25 ± 0.25
AM + 1.0ml BH AR Control	7.22 ± 0.13	1.38 ± 0.38

DISCUSSION

The use of pesticides in field and domestic pest control programs seems to have produced many physiological biochemical and behavioural changes in man and other non-target organism by influencing both the activities of many enzymes and other cellular processes. All pesticides must be toxic or poisonous to be effective against the pest they are intended to control they are also potentially hazardous to humans and pets. During application they may contaminate food or bioaccumulate in crops or in food chain causing liver damage or become carcinogenic after uptake[1, 13].

The brain is an important part of the biological system whose function helps to regulate other parts of the body. However, any damage or form of stress experienced in this part of the body may have serious impact on the entire organism.Many studies have shown that the mechanism of pesticide action in animals is associated with the production of reactive oxygen species (ROS)[1]. This study showed that lipid peroxidation activity increased significantly in the brain homogenate of rats. This increase may be associated with possible damage to the brain cells. The increase in lipid peroxidation was found to be concentration dependent. This is in agreement with earlier work by Gangadaharam *et al*.[14], who reported increased lipid peroxidation in incubated goats' sperm cells. A similar result was also obtained by Latchoumycandane *et al*[15], who reported an increase in lipid peroxidation in the testes of rats exposed to different concentration of metoxyclor and showed that the level of lipid peroxidation was age-dependent as NWR group showed decrease activity of SOD compared to their parallel control. Organisms have evolved mechanism to counteract the effect of radicals generated in the biological membrane. This mechanism involves antioxidant system such as glutathione reductase, glutathione peroxidase, superoxide dismutase (SOD) e.t.c. The function of antioxidant systems is to modify the highly reactive oxygen species to form less reactive intermediate which no longer pose a threat to the cell[8].

However, there must be a balance between oxidation and antioxidant's level in the system for healthy biological integrity to be maintained. Oxidants such as superoxide anions ($O^{\cdot}_2$) Hydroxyl radical ($HO^{\cdot}$) may attack the membranes of the brain cells thereby causing oxidative stress our observation has shown that (SOD) activity in brain homogenate of NWR group decreased

significantly compared with parallel control group this decrease may be as a result of imbalance between oxidants and antioxidants level in favour of the oxidants (Rambo pesticides). SOD is considered to be one of the most active enzymes whose activity is sufficient for dismutation of superoxide anions produced during oxidative stress in cells[16]. The MAR and AR groups showed increased SOD activity compared with the control groups this increase in SOD activity may suggest a possible survival mechanism for the organism in order to reduce possible neurobehavioural effects such as aggression and body tremor.

Although, metabolism in the brain has been associated with ROS generation[17], significant Increase compared with the control group may be associated with permethrin containing pesticide poisoning. Oxidative metabolism of xenobiotics or endogenous compounds is the most important source for ROS[18]. The control of ROS by antioxidant defense systems appears to maintain low concentrations rather than complete elimination. Oxidative stress occurs in a cell or tissue when the concentration of ROS generated exceeds the antioxidants capability of that cell[19]. Hence we suggest that NWR group experiences oxidative stress.

Permethrin, a pyrethroid is used in pest control in agriculture and public health programs. This study has shown that permethrin pesticide formulation could have negative health impact on the brain and nervous system of humans as handlers and producers, therefore adequate precaution must be taken especially among the children population. Soderlund *et al*.[6] has reported that neurotoxin effect of permethrin include tremors in coordination, hyperactivity, increase activity like chewing, aggressive behaviours, resistance to been capture and disruption in learning. Similar observations were recorded in this study in the entire group 1% 5% or 10% irrespective of age. Although SOD activity was high in MAR and AR some of these behavioral effects we noticed but not as high as in NWR groups.

However, increase in lipid peroxidation has been reported in several cases of toxicity induced by ethanol, heavy metals and some xenobiotics[20]. The increase in lipid per oxidation products observed in brain homogenates of the exposed rats might be associated with toxicity and tissue damage. This may depend on a lot of factors such as age, sex and concentration. Children exposed to insecticide contaminated diet or environment may be at higher health risk in that their internal organs and tissues are still developing. They also have higher feeding metabolic rate hence, increased the generation of oxygen free radicals which may attack or damage cell membranes. The overall consequence is breakdown in membrane integrity and membrane dependent functions.

However, due to selective vulnerability of neurons, the brain contain in addition to antioxidant defense, the blood brain barriers which controls the entry of many types of solutes from general circulation to the cerebral parenchyma. It is possible that the blood brain barrier of the NWR group is not well forms or may be the Rambo pesticide is lipophilic thereby crossing the blood brain barriers into the brain to elicit peroxidative activity on the polyunsaturated fatty acids of the neuronal membranes. This may be a possible mechanism by which permethrin elicits its neuronal or behavioral effects especially the young ones or neonates.

We therefore suggest that this pesticide be kept out of the reach of children or from pregnant mothers. Although the MAR and AR groups showed significant increase in SOD activity, within the experimental regime possible exposure may be toxic to non-target organisms irrespective of their age or sex differential. Proper disposal method should be adopted in disposing the carcasses of rats, cockroaches and other pests treated with Rambo so that non-target organisms (domestic fowls, cats and dogs) may not be affected as the residues flow through the food chain.

Developing nations must learn to balance pesticide use with environmental degradation as well as health safety especially in humans and other non-target organisms. Indiscriminate short term exposure may not have immediate obvious health effect but in the long-run the health impact if not taken serious by producers, marketers and users most especially may be very heinous.

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