SUPEROXIDE DISMUTASE (SOD) ACTIVITY AND SERUM CALCIUM LEVEL IN RATS EXPOSED TO A LOCALLY PRODUCED INSECTICIDE *"RAMBO INSECT POWDER"*

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ABSTRACT

Studies of superoxide dismutase (SOD) induction in rats exposed to locally produced insecticide; "Rambo" of which the active chemical compound is permethrin (0.6% w/w) was performed. The calcium levels in the blood plasma of the exposed rats were also evaluated. The rats were divided into three groups of five rats per cage. Each group of rats was fed with 1 %, 5 % or 10 % of the insecticide in their diets. The control group was fed normal diet. The effect of insecticide at various concentrations on superoxide dismutase (SOD) activity in the blood plasma was not significantly different (P > 0.05) in the newly weaned rats (NWR). However, in the middle-aged rats (MAR) and aged rats (AR) groups, the results were significantly different (P < 0.05) against the parallel controls. Comparison of the effect of the insecticide on SOD induction at various concentrations among the groups based on age difference showed significantly different result (P < 0.05), especially among the groups fed with 10 % (w/w) of the insecticide in the diet. Serum Ca^{2+} level (0.51 ± 0.22mg/ml) increased from newly weaned rats groups to (0.66 ± 0.24mg/ml) in the middle-aged rats and (0.63 ± 0.04mg/ml) for the aged rats. The observed Ca^{2+} increase was significantly high for rats fed 10 % (w/w) concentration of the insecticide in the diet (P < 0.05). This increase tends to suggest a concentration- dependent effect. The no-observed-effect-concentration (NOEC) was found to be 0.006 g permethrin per 100 g of the diet, which is equivalent to 1% of the "Rambo" insecticide per 100g of the feed. Results of this study show that in non-target organisms "Rambo" insect powder may induce superoxide dismutase activity, thus, suggesting, oxidative-stress related toxicity. The observed increase in calcium ion especially at 10 % (w/w) of the insecticide showed that permethrin may induce toxic effects associated with cell death via mitochondria uncoupling and loss in ATP metabolism.

Keywords: Superoxide dismutase, Calcium ion, Permethrin insecticide, Rambo insect powder

INTRODUCTION

In our enthusiasm for new successes, we have often overlooked the fact that we have polluted our environment and purposely or inadvertently have exposed ourselves and other life forms to hazardous chemicals (e.g. pesticides). Most of these chemicals have been implicated as causing cell injury and death especially in non-target organisms. (Sotherton, 1991 and Moreby *et al*., 2001). Toxicity may occur directly as a result of a chemical compound being converted to free–radicals or via superoxide anion formation (Bridges *et al*., 1983). Free radicals have been implicated in several human diseases such as cancer and heart diseases (Gutheridge, 1994). More so, they cause membrane damage (Onwurah and Eze, 2000), due to generation of lipid peroxidation product. Detoxification of reactive oxygen species is one of the prerequisites of aerobic life, and the multiple line of defence system. The repertoire to counteract the potentially hazardous reactions initiated by oxygen metabolites includes all levels of protection, prevention, interception and repairs. It comprises non-enzymatic and enzymatic systems. The enzymes involved in antioxidation are the superoxide dismutase, glutathione peroxides and catalases (Ledig and Doffoel, 1988). Superoxide dismutase (SOD) catalyses the destruction (dismutation) of superoxide free – radical ions. These ions are believed to be responsible for lipid peroxidation and peroxidative haemolysis of erythrocytes. The action of SOD therefore results in the protection of the biological integrity of cells and tissues against the harmful effects of superoxide free radicals (Olusi, 2000). To ameliorate the damage caused by the hydroxyl radical formed from superoxide radicals and

hydrogen peroxide, organisms have evolved mechanisms to regulate the concentrations of the two reactants. SOD is an important isoenzyme functioning as superoxide radicals' scavengers in the living organisms. Its activity is also induced by diverse stresses (Bowler *et al*., 1992), presumably, because of the increase in the concentration of superoxide radicals in cells. SOD is an important enzyme family in living cells for maintaining normal physiological conditions and coping with stress.

Calcium ions are important and are required for many physiological functions. Although, the role of Ca^{2+} as a mediator of toxicant induced cell death has been a subject of interest, intracellular Ca^{2+} homeostasis is of importance to cell viability (Palmeira, 1999). The critically important cellular calcium pool for regulation of intracellular events is the cytosolic Ca^{2+}, and this is control by hormones and growth factors (Thomas *et al.,* 1984). Loss of the ability to respond to such hormones and growth factors may result in cell death (Orrenius *et al*., 1989). The mechanisms by which Ca^{2+}-mobilising hormones, like vasopressin, induce intracellular Ca^{2+} transients have been extensively studied (Kawanishi *et al*., 1989 and Glennon *et al*., 1992). Normally, intracellular Ca^{2+} homeostasis is maintained by the concerted operation of cellular transport and compartmentation systems (Carafoli, 1987). Damages caused by free radicals or superoxide radical on the plasma membrane will lead to a rise in cytosolic Ca^{2+} concentration, which may cause cell injury and finally cell death (Comporti, 1993).

Most cells incorporate a variety of very active defense and repair systems when exposed to environmental toxicants, and these defense systems may be overwhelmed during prolonged exposure. The imposed damage may be qualitative or quantitative in nature. In this study, the effects of a locally produced insecticide *Rambo insect powder* on serum Ca^{2+} level and induction of SOD were investigated.

MATERIALS AND METHODS

Test Sample: The test sample for the experiment was a locally produced insecticide, Rambo insect powder that contains 0.60 % (w/w) Permethrin as the active ingredient. *Rambo insect powder* is produced by Gongoni Co. Limited, 89A Sharada Industrial Estate, Phase 111, Kano, Nigeria.

Formulation of Treatment: Different concentrations of the insecticide powder in the diet were prepared by weighing-out a definite amount of growers' mash (feed) and then mixed with the "Rambo" insect powder. The concentrations of the active ingredient of the "Rambo" insecticide (permethrin) in the feed were 0.006 g, 0.03 g and 0.06 g. This produced either 1 %, 5 % or 10 % (w/w) of the "Rambo" insect powder in the feed. The feed for control contains no "Rambo" powder. All the animals were given sufficient quantity of water daily.

Procurement and Management of Experimental Animal: Wilster albino rats weighing between 120 – 720 g were obtained from the Faculty of Veterinary Medicine, University of Nigeria Nsukka (UNN) and maintained on a commercial feed (growers' mash) for five days in the animal house of the Department of Biochemistry, UNN, before the commencement of the experiment. The animals were grouped into three; newly weaned rats (NWR; 2 – 4 weeks, weighing 150 – 185 g), middle aged rats (MAR; 7 – 12 weeks, weighing 290 – 335 g), and aged rats (AR; 13 – 16 weeks, weighing 570 – 642 g). Each group was fed with different concentrations of the Rambo contaminated diets (1 %, 5 % and 10 % w/w), the control groups were fed with the normal diet.

Protein Determination in the Plasma: Total protein concentrations (mg/ml) in the plasma were analysed with Follin-Ciocalteau reagent as described by Cunha-Bastos *et al*. (1999). Bovine Serum Albumin (BSA) was used as standard protein.

Superoxide Dismutase Assay: An indirect method of inhibiting auto-oxidation of epinephrine to its adrenochrome was used to assay SOD activities in blood plasma (Misra and Fridovich, 1971). Auto-oxidation of epinephrine was initiated by adding 1ml of Fenton reagent prepared as described by Onwurah, (1999) to a mixture of epinephrine (3×10^{-4} M), Na_2CO_3 (10^{-3}M), EDTA (10^{-4}M), and 1.0ml of deionized water at a final volume of 6 ml. The auto-oxidation was read in a spectrophotometer at 480 nm every 30 sec for 5 min. The experiment was repeated with 1.0 ml of the blood plasma from different blood samples collected from different groups of animals. A graph of absorbance against time was plotted for each, 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 (Jewett and Rockling, 1993). Superoxide dismutase activity was subsequently calculated for each sample.

Serum Calcium Assay: This was based on the method of precipitation by chloranilic acid (Cerioti, 1974). To 0.5 ml of the serum in a centrifuge tube was added 0.5 ml of chloranilic acid. This was mixed thoroughly and centrifuged. The precipitate was washed in 3 ml of 50 % prapanol–water mixture, and later dissolved in 3.5 ml of citrate

buffer (0.2 mol/l). The mixture was shaken on a "cyclomixer", and the absorbance read at 530 nm against water as blank.

Statistical Analysis: Mean values (± SD) of duplicate experiment with duplicate sampling (N = 4) were taken for each analysis. Significantly different results were established by one – way ANOVA and differences between groups, age, and concentrations were determined by DUNCAN multiple range test. The accepted value of significance was $p<0.05$ (Duncan, 1955).

RESULTS

The inhibition of the initial rate of auto-oxidation of epinephrine brought about by SOD has been used in a rapid, sensitive, and convenient method of assessing the presence of this enzyme in protein extracts of cell homogenates (Misra and Fridovich, 1971). The corollary also holds: The activity of SOD was measured due to the presence of superoxide anion (McCord and Fridovich, 1970). Table 1 showed the results of inhibition studies on the auto-oxidation of epinephrine (pH 10.2) by blood plasma protein of rats exposed to "Rambo" insecticide contaminated diet at various concentrations of 1 %, 5 % or 10 % (w/w). The result showed that a plasma protein level was not significantly different ($P > 0.05$) within the groups (NWR, MAR and AR) of experimental rats and the controls.

The specific activity of SOD did not significantly increased in the NWR groups fed with 1 %, 5 % or 10 % (w/w) – contaminated diet relative to control ($P > 0.05$). In the contrary, the MAR groups and AR groups fed with 1 %, 5 % or 10 % - contaminated diet showed significant increase in the specific activity of SOD ($P < 0.05$) relative to their controls. Pair wise comparison between NWR/MAR, NWR/AR and MAR/AR groups, fed with 1 %, 5 % or 10 % (w/w) insecticide – contaminated diet between 7 – 21 days of exposure showed significantly different results ($P < 0.05$) on SOD only at 10 % (w/w) insecticide – contaminated diet; but the 1 % and 5 % (w/w) insecticide – contaminated showed non significant difference ($P > 0.05$) (Table 3).

Serum Ca^{2+} levels is shown in Figure 1. The results were not significantly different within the groups fed with 1 % and 5 % of the insecticide – contaminated diet ($P > 0.05$). The results were however significantly different within the groups NWR, MAR and AR fed with 10 % of the insecticide – contaminated diet ($P < 0.05$). Comparison of the effect of the insecticide on Ca^{2+} levels between the pairs of NWR/MAR, NWR/AR and MAR/AR groups showed significantly different results ($P < 0.05$) at 10 % concentration of insecticide-contaminated diet (Table 3).

DISCUSSION

The effect of pesticides on non-target organisms is well documented (Moreby and Southway, 1999). The present study reports the effect of permethrin (formulated as "Rambo" insect powder) on non-target organisms. Our results demonstrated that SOD activity decreased in the middle-aged rats (MAR) and aged–rats (AR) groups. The newly – weaned rats (NWR) groups showed a marked increase in the SOD activity when compared with the control. These differences in plasma SOD levels may be due to several factors, such as age, concentration of toxicants, sex, diet etc. The low levels of SOD in the plasma of MAR and AR rats fed with insecticide-contaminated diet may be due to the overwhelming influence of superoxide radicals or activated metabolites generated by the insecticide exposure on the cell membrane of the exposed rats. Determination of SOD in plasma protein samples is based on the ability of the enzyme to inhibit superoxide anion- dependent reactions (Marklund and Marklund, 1974).

The increase in SOD activity in the NWR groups may be due to an induction of the enzyme protein in the presence of reactive metabolites of permethrin (Ledig and Doffoel, 1988). This is obvious from the results in Table 2 where the plasma protein level for NWR fed with varying concentration of the insecticide in the diet were significantly high than that of the control. Similarly, Deuterman (1980) showed that at birth and at the earlier stage of life, there was a marked increase in the activity of many enzymes in the body system of rats. These enzymes are involved in many reactions relating to xenobiotic metabolism and more so, a number of them are age-dependent. The increase in enzyme activity at an earlier stage in life may suggest that NWR groups with increase level of SOD activity could metabolize the permethrin such that its putative toxic effect was not overwhelming to subjugate the mechanism of action of SOD. SOD is an extremely potent antioxidative enzyme that fights cellular damage arising from free radical induction of reactive metabolites from oxidation of hydrocarbon compounds (Onwurah and Eze, 2000). Hence, induction of SOD activity in rats' blood plasma when exposed to environmental toxicants such as "Rambo" insecticide may be an adaptive mechanism for its survival. The rate at which individual and/or groups of rats metabolized the toxicant is age-dependent. This is justified by the mortality ratio (1:4) of rats in favour of newly weaned rats when compared with aged rats. SOD activity is also induced by diverse stresses (Bowler *et al.*, 1992) which may include exposure to hydrocarbon compound, copper, ultra-violet radiation, thermal pollution, disease etc.

TABLE 1: Rate of auto-oxidation of epinephrine in rats exposed to insecticide-contaminated diet

Auto-oxidation mixtures (Am)	Auto-oxidation rate (Units/min)	Percent inhibition (%)
Am + 1.0 ml Distilled H_2O	0.078 ± 0.003	--
Am + 1.0 ml plasma NWR 1 %*	0.026 ± 0.014	66.67 ± 0.047
Am + 1.0 ml plasma NWR 5 %	0.073 ± 0.047	6.41 ± 0.047
Am + 1.0 ml plasma NWR 10 %	0.037 ± 0.013	52.56 ± 0.013
Am + 1.0 ml plasma NWR control	0.070 ± 0.030	10.26 ± 0.044
Am + 1.0 ml plasma MAR 1 %	0.066 ± 0.044	15.38 ± 0.044
Am + 1.0 ml plasma MAR 5 %	0.047 ± 0.003	57.69 ± 0.003
Am + 1.0 ml plasma MAR 10 %	0.066 ± 0.044	15.38 ± 0.044
Am + 1.0 ml plasma MAR control	0.033 ± 0.003	57.38 ± 0.003
Am + 1.0 ml plasma AR 1 %	0..021± 0.010	26.92 ± 0.032
Am + 1.0 ml plasma AR 5 %	0.042 ± 0.019	46.15 ± 0.019
Am + 1.0 ml plasma AR 10 %	0.048 ± 0.023	38.46 ± 0.023
Am + 1.0 ml plasma AR control	0.030 ± 0.010	61.54 ± 0.010

** Plasma taken from different groups of rats eg. Newly weaned rats (NWR) fed with 1% (w/w) contaminated diet. For details see materials and method.*

TABLE 2: SOD activity and total plasma protein levels in rats exposed to insecticide-contaminated diets

Group	Plasma Total Protein (mg/ml)	Superoxide Dismutse (SOD)	
		Activity (units[a] / ml)	*Specific activity Unit/mg protein
NWR 1 %	0.66 ± 0.14	1.33 ± 0.0003	2.02 ± 0.34
	0.64 ± 0.17	0.13 ± 0.0009	0.20 ± 0.26
NWR 10%	0.48 ± 0.04	1.05 ± 0.0003	2.19 ± 0.29
NWR control	0.43 ± 0.02	0.21 ± 0.0006	0.49 ± 0.11
	0.56 ± 0.11	0.31 ± 0.0009	0.55 ± 0.13
	0.68 ± 0.22	0.80 ± 0.0006	0.18 ± 0.10
MAR 10%	0.65 ± 0.18	0.31 ± 0.0009	0.48 ± 0.17
MAR control	0.67 ± 0.19	1.15 ± 0.0006	1.72 ± 0.24
AR 1 %	0.52 ± 0.08	0.23 ± 0.0008	0.44 ± 0.15
AR 5%	0.47 ± 0.05	0.92 ± 0.0004	1.96 ± 0.23
AR 10%	0.41 ± 0.01	0.77 ± 0.0005	1.88 ± 0.18
AR control	0.56 ± 0.07	1.23 ± 0.0002	2.20 ± 0.45

**Specific activity for the SOD in all the groups is not significantly different (P < 0.05) [a]One unit (of activity) of Sod is generally define as the amount of the enzyme that inhibits the autoxidation of epinephrine by 50 %.*

Table 3: Duncan multiple range test of one-way ANOVA for comparing the ages of rats exposed to varying concentrations of insecticide-contaminated diet on SOD activity and serum calcium levels

Combinations	Sod in Plasma		Serum Ca^{2+} Level	
	Differences	LSR	Differences	LSR
NWR 1% / MAR 1%	0.043	0.162	0.12	0.32
NWR 1% / AR 1%	0.003	0.162	0.23	0.32
AR1% / MAR 1%	0.043	0.162	0.11	0.32
NWR 5% / MAR 5%	0.026	0.130	0.23	0.36
NWR 5% / AR 5%	0.005	0.130	0.23	0.36
AR 5% / MAR 5%	0.031	0.130	0.00	0.35
NWR 10% / MAR 10%	0.037	- 0.049*	0.17	0.09*
NWR 10% / AR 10%	0.000	- 0.049*	0.16	0.09*
AR 10% / MAR 10%	0.037	- 0.049*	0.23	0.09*

**Significantly different results (P < 0.05)*

SOD is an important enzyme in living cells for maintaining normal physiological conditions and coping with oxidative stress.

The role of calcium ion (Ca^{2+}) as a mediator of toxicant-induced cell death is very important to this study because intracellular Ca^{2+} homeostasis is very important to cell viability. Our results demonstrated that there is an increase in the serum Ca^{2+} level from NWR to MAR and ARs. This agrees with the work of Thomas *et al.*, (1984). Similarly, there seems to be an inverse correlation between the SOD and serum Ca^{2+} level.

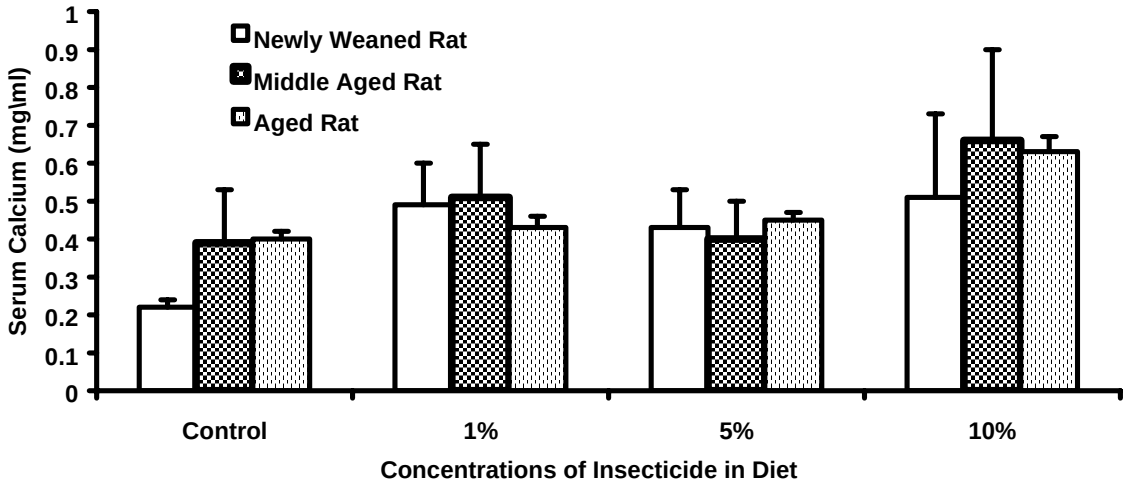

Figure 1: Serum calcium levels (mg\ml) of rats exposed to insecticide contaminated diets

The group with high SOD activity possessed very low level of serum Ca^{2+}. This may be as a result of the scavenging ability of SOD on the superoxide radicals generated by the increase in cytosolic Ca^{2+} level. The elevated Ca^{2+} level can cause several tissue injuries and subsequently affect membrane potential and mitochondrial uncoupling (Marklund and Marklund, 1974). Several studies with xenobiotics demonstrated mitochondrial energy uncouplers (Deuterman, 1980), which suggest disruption of energy supply as a common principal cause of cellular cytotoxicity.

Acute pesticide poisoning, particularly in developing countries, is frequent and thus of great importance in public health. The magnitude of the problems depends on a number of contributing factors, such as types of pesticide regulations, awareness of the degree of danger, training to minimized exposure and availability of medical treatment facilities. The use of pesticide in developing countries is often characterized by lack of vital knowledge of its toxicity and procedures for safe use. This plays a critical role in obtaining direct exposure by both target and non-target organisms.

The action of "Rambo" insecticide on non-target groups may vary widely in comparison to the other insecticides like paraquat (Palmeira, 1999); deltamethrin, zeta–cypermethrin and dimethoate (Moreby *et al.*, 2001). It has become apparent that both SOD and Ca^{2+} are important to both toxicological and physiological processes. The relative importance of the various Ca^{2+} dependent processes in cells needs to be further clarified and the toxicity of "Rambo" insecticide with other pyrethroid could be further compared using biochemical markers.

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