Effects of *Rambo Insect Powder* on Glutathione S-Transferase (GST) and Superoxide Dismutase (SOD) Activity in Rats

Otitoju, O. and Onwurah, I. N. E
Pollution Control & Biotechnology Unit, Department of Biochemistry University of Nigeria, Nsukka, Enugu State, Nigeria

Corresponding Author: I. N. E. Onwurah, Pollution Control and Biotechnology Unit, Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria: E-mail: iyknuelo@yahoo.com

Abstract

This study was undertaken to investigate the toxic effect of a locally produced insect powder, Rambo, which contains 0.6% Permethrin on non-target organisms exemplified with albino rats. The rats were divided into three groups of five rats per cage; the newly-weaned rats (NWR), aged between 2 – 4 weeks and weighing 150-180g, middle-aged rats (MAR) aged between 7-12 weeks and weighing 290-335g, and the aged-rats (AR), 13-16 weeks old and weighing 570 –642g. Each group was sub-divided to receive a feeding regime of 1%, 5% or 10% (w/w) of insecticide-contaminated diet. The controls for each group were fed with normal rats diet. Analysis of total plasma protein and lipid peroxidation products did not reveal significantly different results ($p>0.05$) when compared with parallel controls especially for the NWR. The effect of the insecticide at various concentrations on superoxide dismutase (SOD) activity in the blood plasma was not significantly different ($p > 0.05$) in the NWR groups. However, in the MAR and AR groups, the results were significantly different ($p<0.05$) against parallel controls. Pair wise comparison between NWR/MAR, NWR/AR and MAR/AR groups fed with 1%, 5% or 10% (w/w) insecticide contaminated diet did not show significantly different results ($p>0.05$) at any of the concentrations of insecticide used. The glutathione S-transferase (GST) activity was found to be significantly increased ($p <0.05$) in the brain and liver homogenates but was not significantly different ($p>0.05$) in the blood plasma at any of the concentrations of the insecticide in the diet. Pair wise comparison of the effect on GST also gave significantly different results ($p<0.05$) within the groups of NWR/MAR, NWR/AR, and MAR/AR. The above results may suggest that metabolism of "Rambo" insecticide could induce superoxide anion-mediated by oxidative stress, and hence toxicity in adults of higher organisms, including man.

Keywords: Insecticide, Toxicity, Superoxide Dismutase, Glutathione S-transferase.

Introduction

The effect and fate of pesticides in the environment will determine their impact on both humans and non-target organisms. The lack of restraints on the application of pesticides in most Developing countries may have a far-reaching effect on the health of humans and other non-target organisms in the environment. It has been reported that bioaccumulation of pesticides in food chains can lead to potentially adverse effects in humans and animals owing to the putative toxic action (Palmeira, 1999).

Toxicity may occur directly as a result of pesticides being converted to free radicals or via the formation of superoxide as a by-product of their metabolism (Bridges *et al.*, 1983). Lipid peroxidation is regarded as one of the basic mechanisms of tissue damage by free radicals and other reactive oxygen species (ROS) (Estarbauer, *et al.*, 1991). The knowledge that lipid peroxidation can be linked to the electron transport chain of drug metabolism and the recognition that the metabolism of carbon tetrachloride (CCl_4) yields alkane free radicals as well as stimulate the peroxidation of liver microsomal lipids, led to the assumption that lipid peroxidation could be a basic mechanism of toxicity for a wide range of chemicals (Wilson, *et al.*, 1993). These chemicals have been implicated in causing toxic injury and or oxidative stress on membranes via hydrogen peroxide or activated metabolites, which react with some cellular components such as membrane lipids, and produce lipid peroxidation products (Anozie and Onwurah, 2001). Activated metabolites may also react with enzymes and cause inactivation through protein oxidation (Korge and Campbell, 1993), or/and DNA strand breakage (Schnider *et al.*, 1989; Johnson *et al.*, 1981). There is considerable interest on free radical–mediated modifications of polyenoic fatty acid-containing lipids and lipoprotein systems of cells and tissues because of its potential implications in physiological and pathological processes (Wallin *et al.*, 1993).

Detoxification of reactive oxygen species is one of the prerequisites of aerobic life, and the multiple line of defense system. The repertoire to counteract the hazardous reactions initiated by oxygen metabolites includes all levels of protection, prevention, interception and repairs by certain antioxidant enzymes (Rosa *et al.*, 2003). The antioxidant enzymes involved are the superoxide dismutase enzymes, glutathione S-transferases, and catalases (Ledig *et al.*, 1988). Superoxide dismutase (SOD) plays a unique role in the survival of aerobic organisms, especially during the oxidation of chemicals, which exert their toxic effect via superoxide free radicals, directly or indirectly (Heikkila and Cohen, 1973). The action of SOD therefore protects biological integrity of cells and tissues against the harmful effects of superoxide free radicals (Olusi, 2002). It is an important isoenzyme functioning as superoxide radicals' scavengers in the living organisms (Chen and Pan, 1996). Its activity is also induced by divers stresses

(Bowler *et al.*, 1992), presumably in superoxide radicals in cells. Also SOD is an important enzyme family in living cells for maintaining normal physiological conditions and coping with stress.

Another important endogenous compound that protects the cells of living organisms against some highly deleterious compounds is the tripeptide glutathione (GSH), which is the major non-protein thiol in plants and animals (Reed and Fariss, 1984). Glutathione S-transferase is a family of detoxicating enzymes that catalyse the conjugation of reduced glutathione with a group of compounds having electrophilic centers. These include nitrocompounds (Usui *et al.*, 1977), organophosphates (Motoyama and Deuterman, 1977) and organochlorides (Clerk *et al.*, 1986). The reduced glutathione conjugation products become less toxic and more water-soluble so that they can be easily excreted from cells after further metabolism. High levels of GST activity have been associated with pesticides resistance in numerous insect species (Wang *et al.*, 1991). The activity of GSTs has been shown to increase in organisms as a function of xenobiotic concentration in the medium (Sies, 1997). Certain authors have reported that GSTs are biomarkers of toxicity, i.e. GST activity is often elevated in animals in which carcinogenesis has already occurred (Satoh *et al.*, 1985).

Since GSH is essential to the cellular detoxification of many toxic xenobiotics (Deleve and Kaplowits, 1991), monitoring this endogenous thiol during pesticide exposure is very important. Depletion of intracellular GSH is known to be one of the most detrimental effects of toxic injury, since loss of glutathione protection against reactive intermediates leads to failure of vital cell processes and cell death (Orrenius, 1985).

The present study was undertaken to investigate the toxic effect of ingesting *"Rambo" insect powder*-contaminated diets on non-target organisms using albino rats. The results obtained from this study may aid in establishing pathway of injury in man as a result of the consumption of the insecticide via either inhalation, consumption of contaminated diet as a result of the bioaccumulation in food chain or even direct exposure to domestic animals and pets. This study may also be useful for regulatory purpose.

Materials and Methods

Test sample: The test sample for the study was a locally produced insecticide, "Rambo" which is labeled to contain 0.60% permethrin. Rambo insect powder is the product of Gongoni Co. Limited, 89A Sharade Industrial Estate, Phase III, Kano – Nigeria.

Formulation of contaminated diets: Commercial animal feed was contaminated with the insecticide by weighing out a definite amount of the feed and then mixed with the *"Rambo" insect powder* to give 1%, 5%, or 10% (w/w) contamination (Table 1). The feed for control contains no "Rambo" insect powder.

Table 1: Composition of Experimental Diet (g)

Concentration	Pesticide conc. By weight (g)	Diet conc. By weight (g)	Total weight (g)
1%	1	99	100
5%	5	95	100
10%	10	90	100

Experimental animals: Wilstar albino rats weighing between 120 – 720g were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka (UNN), and were maintained on a commercial feed (grower's marsh) for about one week in the laboratory before commencing the experiment. The animals were divided into three groups based on their ages as newly weaned rats (NWR), 2-4 weeks old and weighing 150 –185g, middle–aged rats (MAR) 7-12 weeks old and weighing between 570 -- 642g and aged rats (AR) 13-16 weeks old weighing 290 – 355g, of five rats per cage. The different diet compositions were hand – mixed to give a uniform mixture. The animals were fed *ad libitum* for twenty-eight days with the insecticide contaminated diets, except the controls for each group. Each group was fed with different concentrations of the Rambo contaminated diet (1 %, 5 % and 10 % w/w); the control groups were fed with the normal diet. The animals were provided with adequate water supply daily.

Total protein determination: Total protein in blood plasma was assayed by a modified Lowry method with Folin Ciocalteau reagent as described by Cunha-Bastos *et al.*, (1991). To 0.1 ml of the test sample was added 2.5 ml of reagent C (Reagent C is a mixture of 2% Na_2CO_3 in 100 ml of 0.1N NaOH, 0.5% $CuSO_4$ and 1% Na/K tartrate) and 0.5 ml of Folin Ciocalteau reagent. Bovine serum albumin (BSA) was used as the standard.The absorbance was taking at 750 nm against the blank using Sp 500 spectrophotometer.

Lipid Peroxidation: Lipid peroxidation was assayed as thiobabituric acid reacting substances (TBARS) using the method described by Wallin *et al.*, (1993). 1ml each of the samples was transferred to clean test tubes. To each of the tube was added 0.05 ml of 50% (w/v) TCA and 0.075 ml of 1.3% TBA (w/v) dissolved in 0.3% NaOH. The mixture was incubated between 90 - 95 ^{o}C in a water bath for 40 minutes and then allowed to cool on iced-water, and 0.01 ml of 20% (w/v) sodium dodecyl sulphate (SDS) was added to each test-tube to reduce turbidity. The absorbance at 350 nm minus the absorbance at 600 nm was read in Sp 500 spectrophotometers against the blank. Results were expressed in mol MDA/mg protein.

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 (Misra and Fridovich, 1971). Auto-oxidation of epinephrine was initiated by adding 1 ml of Fenton reagent (prepared by the addition of 0.1 Mol/L of freshly prepared $FeCl_3$. $6H_2O$ to 0.5 mol/L of H_2O_2 in the ratio 1:2) to 4 ml of auto-

oxidation mixture (Epinephrine (3 x10^{-4} M), Na_2CO_3 (10^{-3}M), EDTA (10^{-4}M)), and 1.0 ml of deionised water at a final volume of 6 ml (Onwurah, 1999). The auto-oxidation was monitored in a spectrophotometer (Sp 500) at 480 nm every 30 secs 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 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 (Jewett and Rocklin, 1993). Superoxide dismutase activity was subsequently calculated for each sample.

Glutathione S-transferase assay: The glutathione S-transferase (GST) activity was monitored according to the method of Habig *et al.*, (1974). 0.1 ml of the sample was added to a reaction mixture containing 2.3 ml of 0.1M potassium phosphate buffer (pH 6.5) and 0.3 ml (1 mM) of reduced glutathione (GSH). Then, 0.3 ml of 1 – Chloro-2, 4-dinitrobenzene was added to the mixture to start the reaction. The contents were quickly shaken and the increase in absorbance measured at 340 nm for 4 minutes in an Sp 500 spectrophotometer.

Statistical analysis: Values are means ± standard deviations of quadruplicate (n = 4) determinations. Data were also analyzed by one-way analysis of variance (ANOVA), and means were compared by Duncan's (1955) multiple range test. Significance was accepted at a P – value of 0.05.

Results

Table 2 shows the mean daily consumption of the contaminated feed by the various groups of rats. There was no significant difference in food intake during the period (p>0.05) for the groups (NWR, MAR and AR).

The mean body weights of rats at various days of exposure to contaminated diet are shown in Table 3. The mean body weights of the rats in all the groups (NWR, MAR and AR) after acclimatization were 180.0±19.1g, 323.1±22.7g and 567 ± 78.1g respectively. At the 7th day of feeding there was an increase of 15.0g, 77.0g and 93.3g in the body weights of the control groups of the NWR, MAR and AR respectively while the rats fed with contaminated diets also showed increase in body weights for all the groups. However, for the rats fed with 10% contaminated diet, a marked decrease in the body weights of 22.5g, 25.0g and 182.5g for NWR, MAR and AR respectively were observed. The loss in weights was gradual over the periods of 14, 21 and 28 days of exposure.

The levels of lipid peroxidation products and total protein in blood plasma of rats exposed to different concentrations of insecticide-contaminated diet are shown in Table 4. The values of lipid peroxidation products determined as MDA equivalent (mol MDA/mg protein) were not significantly different from the values obtained for the controls (p>0.05). The highest lipid peroxidation products (9.38 molMDA/mg protein) were found in NWR group fed with the 10% insecticide contaminated feed.

Table 5 shows the results of inhibition studies on the auto-oxidation of epinephrine at pH 10.2 by blood plasma protein of rats exposed to 1%, 5% and 10% (w/w) "Rambo" insecticide-contaminated diet. The concentrations of plasma protein (mg/ml) in the blood and SOD activity are shown in Table 6. The result showed that plasma protein levels were not significantly different (p>0.05) within the groups (NWR, MAR and AR) of experimental rats relative to their controls.

The specific activity of SOD (units/mg protein) calculated from various enzyme units was not significantly different (p >0.05) within NWR groups at various concentrations of 1%, 5% and 10% (w/w) of the insecticide. On the other hand, MAR and AR groups showed significantly different results in the activity of SOD (p <0.05) relative to their controls. Pair wise comparison of SOD between NWR/MWR, NWR/AR and MAR/AR groups fed with 1%, 5% and 10% (w/w) insecticide-contaminated diet between 7-21 days of exposure did not show significantly different results (p >0.05) as shown in Table 7.

The plasma GST activity for each group of exposed rats (Table 8) was not significantly different (p >0.05) at any of the concentrations within the groups NWR, MAR and AR. Pair wise comparison of plasma GST activities in the exposed rats based on age differences at concentrations of (1%, 5% or 10% w/w) of the insecticide in the diet showed no significant results (p>0.05). GST activities in the brain and liver homogenates showed significantly different results (p <0.05) at any of the given concentrations but pair wise comparison based on age grouping (NWR/MAR, NWR/AR, and MAR/AR) showed significant different result (p <0.05).

Discussion

Biochemical biomarkers are increasingly used in ecological risk assessments of the ecosystem to identify the incidence and effects of xenobiotics. This is because of their potential as rapid early warning signal against potentially damaging effects caused by stressor. Ideally, biochemical biomarkers will identify effects at a sub-cellular level before they are apparent at higher levels of biological organization (Olsen *et al.*, 2001). This study has shown that rats can be used to measure successfully the harmful effect of insecticide on non-target organisms. The ingestion of pesticide-contaminated diet has been known to elicit toxic cell damage mediated by xenobiotic metabolism, free radical formation, and lipid peroxidation (Dargel, 1992). The highest level of lipid peroxidation product (9.38±3.00 molMDA/mg protein) was obtained in the newly weaned rat (NWR) groups at 10 %(w/w) of insecticide exposure though not significantly higher than in the control. This high level of lipid peroxidation products is an index of oxidative stress and tissue damage. Hence, Rambo insecticide may be deleterious to tissues of higher

Table 2: Food intake of rats/ day (g)

Feeding regime	Newly-weaned rats (NWR) body weight (g)				Middle-age rats (MAR) Body weight				Aged-rats (AR) Body weight (g)			
	7 days	14 days	21 days	28 days	7 days	14 days	21 days	28 days	7 days	14 days	21 days	28 days
Normal feed (control)	14.47 ± 7.9	26.8 ± 2.3	28.1 ± 2.3	28.7 ± 29	50.8 ± 4.2	46.8 ± 11.5	48.1 ± 0.3	46.1 ± 0.8	53.5 ± 2.6	54.9 ± 3.3	53.4 ± 5.9	47.9 ± 0.5
1% contamination	26.1 ± 3.2	24.3 ± 1.1	26.8 ± 1.5	29.0 ± 2.4	47.7 ± 0.9	47.3 ± 1.3	47.7 ± 0.9	48.6 ± 0.1	49.9 ± 1.8	51.5 ± 0.7	50.1 ± 5.5	44.6 ± 2.7
5% contamination	21.7 ± 4.2	22.8 ± 1.6	23.2 ± 8.3	25.1 ± 0.8	44.6 ± 1.8	44.0 ± 0.7	44.6 ± 3.3	45.9 ± 1.6	48.4 ± 2.0	48.8 ± 0.6	48.2 ± 4.4	44.7 ± 0.1
10% contamination	21.8 ± 2.7	21.9 ± 1.8	23.5 ± 3.3	28.0 ± 1.0	34.0 ± 6.2	42.1 ± 1.1	42.5 ± 1.7	42.2 ± 1.4	48.2 ± 1.6	46.0 ± 1.5	47.8 ± 3.1	42.8 ± 2.3

Values are Means ± Standard deviations (N = 4)

Table 3: Mean body weight of rats at various days of exposure (g)

Feeding regime	Newly-weaned rats (NWR) body weight [g]				Middle-age rats (MAR) Body weight [g]				Aged-rats (AR) Body weight [g]			
	7 days	14 days	21 days	28 days	7 days	14 days	21 days	28 days	7 days	14 days	21 days	28 days
Normal feed (control)	195.2 ± 45.0	230.6 ± 40.2	241.0 ± 40.0	261 ± 42.5	305.0 ± 18.4	320.5 ± 14.1	356.7 ± 37.4	366.0 ± 21.6	530.2 ± 21.2	546.3 ± 21.3	583.7 ± 22.5	590.3 ± 13.0
1% contamination	185.0 ± 41.5	202.5 ± 32.7	217.5 ± 37.0	225.0 ± 38.8	345.3 ± 16.5	375.0 ± 15.6	375.0 ± 15.0	362.0 ± 11.4	505.0 ± 15.0	507.5 ± 29.4	470.5 ± 27.6	447.1 ± 14.9
5% contamination	172.5 ± 32.7	192.5 ± 30.3	202.5 ± 32.7	216.8 ± 11.0	307.5 ± 25.5	352.5 ± 32.7	347.5 ± 27.7	339.5± 14.5	540.5 ± 26.5	512.1 ± 24.0	475.0 ± 18.0	467.8 ± 8.7
10% contamination	147.5 ± 43.3	173.3 ± 4.70	156.7 ± 4.7	150.3 ± 6.1	285.2 ± 18.2	292.0 ± 15.0	305.0 ± 213.5	295.0 ± 17.4	462.5 ± 16.0	440.1 ± 45.8	417.9 ± 12.8	397.0 ± 15.8

Values are Means ± Standard deviations (N = 4)

Table 4: Total plasma protein (mg/ml) and plasma lipid peroxidation (molmda/mg protein)

Group	Newly weaned rats (NWR)		Middle aged rats (MAR)		Aged rats (AR)	
	Plasma protein (mg/ml)	Plasma lipid peroxidation molMDA mg protein	Plasma protein (mg/ml	Plasma lipid peroxidation molMDA mg protein	Plasma protein (mg/ml)	Plasma lipid peroxidation molMDA/mg protein
Normal feed (control)	0.34±0.02	0.64±0.i5	0.67±0.19	1.12±0.25	0.56±0.07	0.67±0.24
1% contamination	0.66±0.14	1.14±0.25	0.56±0.11	2.46±0.38	0.52±0.08	1.68±0.63
5% contamination	0.64±1.7	2.54±1.38	0.68±0.22	1.84±0.08	0.47±0.05	1.86±0.63
10% contamination	0.48±0.04	9.38±3.00	0.65±0.18	1.15±0.25	0.41±0.01	2.44±0.50

Values are Means ± Standard deviations (N = 4)

Table 5: 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 + 1.0ml Distilled H_2O	0.078±0.003	-
Am + 1.0ml plasma NWR 1%*	0.032 ±0.018	66.67 ±0.047
Am + 1.0ml plasma NWR 5%	0.053 ±0.020	41. 03±0.047
Am + 1.0ml plasma NWR 10%	0.052 ±0.021	6.41±0.047
Am + 1.0ml plasma NWR control	0.013 ±0.000	52.56±0.013
Am + 1.0ml plasma MAR 1%	0.015 ±0.002	10.26±0.030
Am + 1.0ml plasma MAR 5%	0.021 ±0.001	15.38±0.044
Am + 1.0ml plasma MAR 10%	0.025 ±0.002	39.74 ±0.003
Am + 1.0ml plasma MAR control	0.011 ±0.001	15.38±0.044
Am + 1.0ml plasma AR 1%	0.021 ±0.001	57.69±0.003
Am + 1.0ml plasma AR 5%	0.019 ±0.002	46.15±0.019
Am + 1.0ml plasma AR 10%	0.053 ±0.005	38.46±0.023
Am + 1.0ml plasma AR control	0.009 ±0.002	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 6: SOD activity in rats exposed to insecticide contaminated diets

GROUP	Plasma total protein (mg/ml)	Superoxide dismutase (SOD)	
		Activity (units)[a]/ml	*Specific activity. Unit/mg protein
NWR 1%	0.66 ±0.14	0.82 ±0.46	1.25 ±0.70
NWR 5%	0.64 ±0.17	1.35 ±0.50	2.81 ±1.04
NWR 10%	0.48 ±0.04	1.34 ±0.54	3.11 ±1.25
NWR control	0.43 ±0.02	0.33 ±0.00	0.52 ±0.00
MAR 1%	0.56 ±0.11	0.39 ±0.06	0.69 ±0.10*
MAR 5%	0.68 ±0.22	0.55 ±0.02	0.80 ±0.02*
MAR 10%	0.65 ±0.18	0.64 ±0.05	0.99 ±0.08*
MAR control	0.67 ±0.19	0.29 ±0.03	0.43 ±0.04
AR 1%	0.52 ±0.08	0.54 ±0.26	1.04 ±0.50*
AR 5%	0.47 ±0.05	0.50 ±0.01	1.05 ±0.04*
AR 10%	0.41 ±0.01	1.36 ±0.13	3.32 ±0.32*
AR control	0.56 ±0.07	0.22 ±0.04	0.39 ±0.07

**Specific activities for the SOD in all the experimental groups are 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 7: Glutathione levels in the plasma, brain and liver homogenates of rats exposed to "Rambo" contaminated diet

	PLASMA GST (mg/ml)				BRAIN GST (U/liter)				Liver GST (U/Litre)			
	1%	5%	10%	(0%) control	1%	5%	10%	(0%) control	1%	5%	10%	(0%) control
NWR	21.88] ± 12.5	9.53 ± 3.28	12.82 ± 3.13	13.29 ± 2.35	15.63 ± 0.00	21.25 ± 0.31	30.32 ± 3.13	19.82 ± 1.72	107.82 ± 4.60	145.32 ± 1.57	79.69 ± 1.56	53.59 ± 12.03
MAR	9.38 ± 0.32	9.07 ± 3.44	9.38 ± 4.07	13.28 ± 5.47	24.07 ± 0.32	17.5 ± 1.25	17.66 ± 0.47	19.85 ± 1.10	114.07 ± 10.94	159.38 ± 3.1	68.75 ± 6.25	118.75 ± 0.00
AR	15.32 ± 4.07	8.13 ± 0.00	16.25 ± 6.56	17.50 ± 1.25	15.16 ± 0.16	28.44 ± 0.63	12.50 ± 2.19	15.32 ± 1.57	175.0 ± 0.00	233.28 ± 20.78	398.44 ± 23.44	453.13 ± 9.38

Values are Means ±Standard deviations (N = 4)

Table 8: Duncan multiple range test of one-way ANOVA for pair wise comparison of the ages of rats exposed to varying concentrations of insecticide contaminated diet with respect to sod and plasma GST activity

COMBINATION	PLASMA GST (mg/ml)		BRAIN GST (U/liter)		LIVER GST (U/liter)		SOD IN PLASMA	
	differences	L.S.R.	differences	L.S.R.	differences	L.S.R.	differences	L.S.R.
NWR1%/MAR1%	12.50	34.16	8.74	0.90*	6.25	32.49.	0.043	0.162
NWR1%/AR1%	6.56	34.16	0.47	0.90	67.18	32.49*	0.003	0.162
MAR 1%/AR1%	5.92	34.16	8.91	0.90*	60.93	32.49*	0.043	0.162
NWR5%/MAR5%	0.46	12.34	3.75	3.72*	14.06	54.69	0.026	0.130
NWR5%/AR5%	1.40	12.34	7.19	3.72*	87.96	54.69*	0.005	0.130
MAR5%/AR5%	0.94	12.34	10.94	3.72*	73.90	54.69*	0.031	0.130
NWR10%/MAR10%	3.47	21.63	12.66	9.81*	10.94	70.10	0.037	-0.049*
NWR10%/AR10%	3.40	21.63	17.82	9.81*	318.75	70.10	0.000	-0.049*
MAR10%/AR10%	6.87	21.63	5.16	9.81	329.69	70.10*	0.037	-0.049*

Values are Means ±Standard deviations (N = 4)

organisms. Esterbauer *et al.*, (1991), reported that lipid peroxidation is regarded as one of the basic mechanisms of tissue damage. The reduction in the body weight of aged rats (AR) and middle-aged rats (MAR) even at the lowest contamination with 1% (w/w) insecticide may be attributed to oxidative stress arising from the insecticide in the feed. This can be linked with the result of lipid peroxidation and protein oxidation. This agreed with the work of Koshio *et al.*, (1994), who showed that the loss in weight by albino rats was as a result of their exposure to contaminated diet. Weight loss is also an indication of ill health. Bruckner *et al.* (1974), also observed that experimental animals fed with poly-chlorinated biphenyl (PCB) mixtures such as Arochlor 1242® retarded body weight in experimental animals. However, our observation showed that there was gradual gain in body weight in the NWR and MAR groups, contrary to that for the AR group. The difference in the body weight

loss or gain may be due to several factors. Deuterman (1980) showed that at birth there is a marked increase in the activity of many enzymes located in the mammalian liver. These enzymes are involved in many reactions relating to xenobiotic metabolism and a number of them are age-dependent. The increase in enzyme activity at an earlier stage in life may suggest that NWR and MAR groups could metabolize the toxicant such that its putative toxic effect was not overwhelming to subjugate the mechanism of growth. Exposure to a pollutant can impact on the growth of an organism by direct toxic effects or indirectly by impacting on the energy of the organism as it attempts to detoxify the contaminant (Spurgeom and Hopkin, 1996).

The levels of plasma protein in the rats exposed to different concentrations of the insecticide were low for MAR and AR groups when compared with parallel control groups. Thus, the reduction in total plasma protein in these groups of experimental animals may be due to the formation of high protein carbonyl derivatives as a result of free radical attack on the cell proteins. This agreed with the earlier work reported by Onwurah (1999), who suggested that there was relatively low level of protein in the cells of *Azotobacter vinelandii* exposed to different concentrations of environmental contaminants. Similarly, Schuppe *et al.*, (1992), showed that during lipid peroxidation and glutathione depletion, proteins are exposed to a wide range of free radical species capable of oxidizing protein thiols, thus promoting the formation of disulphide bridges and even induction of protein fragmentation and catabolism.

Since GST and SOD, amongst other enzyme systems are essential to the cellular detoxification of many toxic xenobiotics (Deleve and Kaplowitz, 1991), monitoring these endogenous enzymes (biomarkers) during pesticides exposure is very important. SOD activity increased 2.4 fold at 1% contamination for NWR and about 6 folds at 10% contamination for the AR group. This may be evidence that the insecticide stimulated SOD activity. As reported by Rosa *et al.* (2003), increase in SOD activity is synonymous with an elevated oxidative stress. However, for the MAR/AR, the induction of SOD activity was lower when compared with the NWR groups. This may explain why the NWR survived more than the AR group. Hence, 4 deaths were recorded in the AR at 10% contaminated diet as against 1 in the NWR groups at 10%.

GST activity in the plasma decreased by 0.72 folds at 5% contamination for NWR and about 0.5 fold at 10% contamination for AR. Glutathione is a very important detoxifying agent, enabling the body to get rid of undesirable toxins and pollutants. As protective antioxidant, it plays an important role in detoxification and elimination of xenobiotics (pesticides) as the first step of the mercapturic acid pathway. In the NWR and AR groups, activity levels of GST were higher at lower concentration of 1% tested. This may be due to a toxic effect associated in high doses that could have reduced GST induction by interacting with cell metabolism. The induction of glutathione S-transferase as a major antioxidant produced by the cell, protect it from free radicals. These highly reactive substances, if left unchecked, will damage or destroy key cell components (e.g. membranes, DNA). Generally, depletion of GST in the plasma and liver homogenates at 5% contaminated diet was observed. This depletion may be as a result of the overwhelming influence of the active ingredient (permethrine) from Rambo insect powder on the GST activity. Prolonged exposure to Rambo insect powder may lead to the deficiency of glutathione in the body system thereby, making the whole organism susceptible to other opportunistic infections.

However, GST induction was observed in the brain homogenates of Rats exposed to [illegible] (w/w) contamination of Rambo insect powder. This induction may be as a result of the lipophilicity of the active ingredient (permethrine), which enables it to by-pass the blood-brain barrier of the exposed rats. This may explain why most of the exposed animals displayed unusual aggressive behaviour, agitation and resistance to being captured during handling. A similar report was observed by Soderlund *et al.*, (2002), who observed that the principal effects of pyrethroids as a class of insecticides are various signs of excitatory neurotoxicity.

Delayed reproduction in the exposed rats was observed because no case of littering in any of the groups throughout the experimental period was recorded but three months later, the animals' reproductive ability was reactivated as evident in some cases of littering among the groups NWR and MAR.

Since "Rambo" insect powder contains 0.60% permethrin, (pyrethroid compound) as its active ingredient (according to the manufacturer), its toxicity may follow the same pattern for other pyrethroid insecticides such as deltamethrin, cypermethrin, and zeta-cypermethrin.

Conclusion: Rambo insect powder administered even at sub – lethal concentrations are toxic to rats though the animals' defense mechanisms were not overwhelmed to the point of death at the concentrations applied. This study tends to suggest that ingestion of Rambo insect powder contaminated diets could lead to multiple toxic effects, such as increase in the levels of lipid peroxidation and protein oxidation products, depletion in body antioxidants (SOD and GST activities) and delayed reproductive activity in non-target organisms.

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