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Full Length Research Paper

Glutathione S-transferase (GST) activity as a biomarker in ecological risk assessment of pesticide contaminated environment

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The behaviour and fate of pesticides in the environment will determine their impact on both humans and non-target organisms. Biochemical biomarkers are increasingly used in ecological risk assessment to identify the incidence of exposure to and effects caused by xenobiotics. This study was undertaken to investigate the potential toxic effect of a locally produced insect powder called "Rambo" (which contain 0.6% permethrin) on non-target organisms exemplified with albino rats. The results obtained showed that glutathione S-transferase (GST) activity in the newly-weaned rats (NWR) and middle-aged rats (MAR) groups were found to increase significantly ($p<0.05$) in the liver homogenates at the concentrations used (1, 5 and 10%) compared with their parallel controls. In the plasma and brain homogenates, a decrease in GST activity was observed, this decrease was significant ($p<0.05$) in the brain homogenates, but in the blood plasma the decrease in GST activity was not significant ($p>0.05$). However, the highest GST activity (398.44 ± 23.44) U/L was recorded in the liver homogenates while the least activity (9.07 ± 3.44) U/L was obtained in the plasma sample. The significance of such a decrease in intracellular GST is that, protection against reactive intermediates may be lost and thus affect vital metabolic processes that may result to death. This shows that GST can be used as a biomarker in ecological risk assessment of pesticide contaminated environment.

Key words: Glutathione S-transferase, biomarkers, pesticides, risk assessment.

INTRODUCTION

The application of man-made chemicals to the environment has resulted in the need for development of methods to assess, monitor and mitigate their impact. Biochemical biomarkers are increasingly used in ecological risk assessments of aquatic and terrestrial ecosystems to identify the incidence of exposure to and effects caused by xenobiotics. Until recently, the most common end point measured when evaluating toxicity of chemicals were mortality values. This, according to Neuhauser et al. (1984) can only provide a measure of short-term acute toxicity and are not always useful for predicting the ecological consequences of exposure to a particular chemical, e.g., as seen with reproduction, where effects are observed at concentrations well below the lethal concentration (LC_{50}) value. Biomarkers can provide information on the potential adverse impacts of contaminants and can act as early warning signals of impending environmental damage.

Pesticide ingestion either by direct or indirect exposure may lead to generation of reactive oxygen species (ROS), which are detrimental to the health of humans and non-target organisms such as domestic fowls and pets. It has been reported that bioaccumulation of pesticides in the food chain can lead to potentially adverse effect in humans and useful animals owning to their 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 by-product of their metabolism.

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The repertoire to counteract the potentially hazardous reactions initiated by oxygen metabolites include all levels of protection, prevention, interception and repairs by certain antioxidant enzymes or biomarkers, such as glutathione S-transferase (GST), and superoxide dismutase (SOD). Glutathione S-tranferase is a family of detoxifying enzymes that catalyzed the conjugation of reduced glutathione with a group of compounds having electrophilic centers e.g., nitrocompounds, organophosphates and organochlorides (Clark et al., 1986). Since glutathione (GSH) is essential to cellular detoxification of many toxic xenobiotics, monitoring this endogenous thiol during pesticide exposure is very important.

The present study was undertaken to investigate the potential toxic effect of "Rambo" insect powder on non-target organisms and the possible utilization of GST as a biomarker for ecological risk assessment of pesticide contaminated environment .The result obtained from this study may aid in formulating pathway of injury in man or non-target organisms as a result of consumption of the insecticide via either inhalation or 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 libeled to contain 0.60% permethrin. Rambo insect powder is the product of Gongoni Co. Limited, 89A Sharade Industrial Estate, Phase III, Kano – Nigeria.

Formulating 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. The feed for control contains no "Rambo" insect powder.

Experimental animals

Wilstar albino rats weighing between 120 – 720 g 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), middle–aged rats (MAR) and aged rats (AR), of five rats per cage. The animals were fed *ad libitum* for about two months with the insecticide contaminated diets, except the controls for each group. 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-Ciocaltou reagent as described by Cunha-Bastos et al. (1991). Bovine serum albumen (B.S.A) was used as standard protein.

Lipid peroxidation

Lipid peroxidation was assayed as thiobabituric acid reacting substances (TBARS), using the method described by Wallin et al. (1993).

Glutathione S-transferase assay

Glutathione S-transferase activity of the blood plasma of rats fed with contaminated-diet was measured spectrophotometrically at 480 nm and 37°C by following conjugation of the acceptor substrate 1-chloro-2, 4-dinitrobenzen as described by Herbig et al. (1974).

Statistical analysis

Mean values (±SD) of duplicate experiment with duplicate sampling (N = 4) were taken for each analysis. A significantly different result was established by one-way ANOVA and differences between groups by Duncan multiple range test. The accepted level of significance was $p < 0.05$.

RESULTS

Table 1 shows the levels of lipid peroxidation products and total protein in blood plasma of rats exposed to different concentrations of insecticide-contaminated diet. The values of lipid peroxidation products determined as MDA equivalent (mol MDA/mg protein) were not significantly different against the values obtained for the controls ($p>0.05$). The highest lipid peroxidation products (9.38 molMDA/mg protein) were found in the plasma of NWR group fed with the 10% insecticide contaminated feed.

The plasma GST activity for each group of exposed rats (Table 2) 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 ($p <0.05$) as shown in Table 3.

DISCUSSION

Biochemical biomarkers are increasingly used in ecological risk assessment 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

Table 1. Total plasma protein (mg/ml) and plasma lipid peroxidation (molMDA/mg protein).

	Newly weaned rats (NWR)		Middle aged rats (MAR)		Aged rats (AR)	
Group	**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**
Normalfeed (control)	0.34±0.02	0.64±0.15	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

Table 2: glutathione levels in the plasma, brain andliver 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 3: Duncan multiple range test of one-way anova for pairwise comparison of the ages of rats exposed to varying concentrations of insecticide contaminated diet with respect to gst activity.

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

Values are Means ± Standard deviations (N = 4)

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 rats (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 organisms also. Estarbaner et al. (1994) reported that lipid peroxidation is regarded as one of the basic mechanisms of tissue damage.

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 GST 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.

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 such as silver, mercury and crude oil. 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.

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. Hence reduction in the percentage activity of GST may serve as a marker for environmental toxicants. In the NWR and AR groups, activity levels of GST were higher at lower concentration of 1% tested. This may be due to the 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 activity 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 (permethrin) from Rambo insect powder on the GST activity. Prolonged exposure to Rambo insect powder may lead to the depletion 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 5% (w/w) contamination of Rambo insect powder. This induction may be as a result of the lipophilicity of the active ingredient (permethrin), 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 insecticide 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 (Result not shown).

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. In conclusion, the reduction in GST activity as observed in this study shows that this biomarker is important in ecological risk assessment of pesticide-contaminated environment.

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