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Full Length Research Paper

Effects of dietary exposure to insecticide Raid® on the survival, growth and inhibition of metabolic processes in Wistar rats

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This study was aimed at determining the effects of an insecticide, 'Raid' contained in feed on the survival, growth and activity of alkaline phosphatase (ALP), glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD) activities, as well as the effects on glutathione content (GSH), lipid peroxidation (LPO) and utilization of basal energy in Wistar rats. The results indicate that mortality was observed at higher concentrations of the pesticide. Mean total body weight of rats fed 430 µg/g Raid was not significantly different from controls ($p > 0.05$). Significant effects of insecticide Raid on survival and growth were observed with concentration of 961 µg/g compared to control ($p < 0.05$) after 10 days. Alkaline phosphatase in serum and lipid peroxidation in the liver tissue significantly increased whereas glutathione contents, glutathione reductase, catalase, and superoxide dismutase activities were significantly decreased in the liver tissue, while there was substantial residual glucose level, in both plasma and liver. Increases in ALP and LPO suggest that Raid induced hepatotoxicity and oxidative stress in animals and effect on glucose utilization suggests liver functions may have been impaired in the insecticide-fed rats, resulting in abnormal uptake of glucose.

Key words: Raid®, alkaline phosphatase, lipid peroxidation, antioxidants, glucose utilization.

INTRODUCTION

The main exposure of the general population of Nigerians to insecticides occur in the homes, though, no study in the country has shown the percentage of Nigerian households that use pesticides. The scourge of malaria parasite-carrying mosquitoes necessitates the use of insecticides in homes as a typical resident spends more time in the home than outside (Eddleston, 2000). Other studies have revealed that the proportion of time spent indoors is greater for small children and the elderly than that spent outside (Whitmore et al., 1994, Lewis et al., 1994).

Some insecticides can persist in the indoor environment for months or years after application or track-in (Osibanjo and Adeyeye, 1997). This is particularly true for insecticides whose efficacy depends on persistence, e.g DDT, chlordane and dieldrin. Dews of insecticides found in the air inside the home and on floors and other interior surfaces contribute to the overall exposure to household occupants (Nebeker et al., 1994). Results from the Environmental Protection Agency's Non-occupational Pesticide Exposure Study (NOPES, 1990) showed that children spend much of their time on the floor, and thereby come into intimate contact with yard dirt and lawns. Analysis of the carpet dust collected from several homes in the study showed pesticides in concentrations from 1 to 100 µg/g carpet dust compared to mean air levels of 0.1 to 0.5 µg/m^3 (USEPA, 1990). Dislodgeable insecticides residues in carpets and/or on uncovered floors, therefore, may present a relatively important exposure route for infants and toddlers through oral ingestion.

Limited toxicity data in Nigeria for insecticide use in homes and the lack of suitable testing standards and protocol have not provided statistically defensible

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estimates of exposure levels in the population (Udoh, 1998). Both wildlife and humans are at risk from pollution in the environment because insecticides and other chemicals have been known to cause impaired reproduction (Dietz et al., 2008; Younglai et al., 2007), deformities and diseases (Ignarro et al., 2007), deficit in brain function (Brucker-Davis et al., 2008; Guzelian, 1982), or other adverse health effects (Swan, 2008; Wu et al., 2006; Bolaji and Sasikala, 1993). A study of this nature therefore requires the determination of the effect of such insecticides on basal biochemical functions as well as other toxicity parameters. Raid contains pynaminforte, a synthetic pyrethroid known to be toxic to fish, bees and causes tremor in mammals (Ansari et al., 2009). Other components include deltamethrin, alpha-cyano class of pyrethroid and neopynamin, a pyrethroid compound (Ansari et al., 2009).

Raid was introduced as ant and roach killer including flying insects. This led to its use as a mosquito repellant in homes. It comes with a synergizer and the exposure pathway is usually through inhalation, which most times cause paraesthesia (Guzelian, 1982). The relevance of this study was predicated by accidental and/or suicidal poisoning mostly by the oral route at doses estimated at 2 - 250 mg/kg. It is presumed that Raid inhibits enzymes, but nothing is really known of a biochemical lesion. Oral ingestion caused epigastric pain, nausea and vomiting, with higher doses causing coma within 15 - 20 min. (He et al., 1989). The present study, therefore, was designed to investigate the effects of a commonly used insecticide, (Raid®), on the survival, growth and activity of alkaline phosphatase (ALP), glutathione reductase (GR), catalase (CAT), superoxide dismutase (SOD) activities, as well as the effects on glutathione content GSH), lipid peroxidation (LPO) and utilization of basal energy in Wistar rats.

MATERIALS AND METHODS

Test facility

Adult male and female Wistar rats obtained from animal holding College of Health Sciences Obafemi Awolowo University, Ile-Ife, Nigeria were housed in ventilated aluminum boxes (41 x 28 x 15 cm). A total of 65 animals were used. Five animals were placed in each of the six boxes which contained a 3 cm layer mixture of sawdust shaves. The animals were kept in a room set at 23°C and a light: dark cycle of approximately 12 h. Control animals were fed Purina 5001 rodent laboratory chow in corn oil. Fresh tap water (temperature, 27 ± 1°C) was provided *ad lib.* Protocols describing the use of rats were approved by the Animal Care Committee of Obafemi Awolowo University, Ile-Ife, Nigeria, and in accordance with the American Physiological Society's "Guiding Principles for Research Involving Animals and Human Beings" (Anonymous, 1998).

Test procedure

The active constituent of the pesticide, trade name Raid (manufactured by Johnson Wax Nigeria) was mixed with dry food. Technical grade pyniminforte 20% (Tagros Chemical Ltd), cypermethrin 20% (Meghmani Organics Ltd) and deltamethrin (20% [AI] (Excel crop Care Ltd) were dissolved in acetone and thoroughly mixed with rat chow making 1% (AI) (wt:wt). The acetone was evaporated for 24 h in a fume hood before use. Animals in the different cages were fed active ingredient pyrethroid-spiked commercial feed at measured concentrations of 25.0 ± 2.4, 54.0 ± 4.2,108.0 ± 12.5, 216.0 ± 14.6, 430.0 ± 20.2 and 961.0 ± 80.6 µg/g (Nebeker et al., 1994) feed for 15 days. These six cages were each in duplicate (5 × 6 × 2 = 60 animals) while another cage served as the unexposed control group of animals. At test termination, blood was withdrawn by cardiac puncture into a tube containing EDTA or heparin, centrifuged at 4000 rpm for 15 min to obtain the plasma. Livers were dissected out for biochemical analysis of alkaline phosphatase activity, lipid peroxidation, glutathione reductase, catalase, superoxide dismutase glutathione and glucose contents.
Two rats from different boxes receiving the same concentrations of pyrethroid in the food were selected, weighed and were not fed within 24 h to allow clearing of the gastrointestinal tract. Tissue samples were taken at the end of feeding periods. Mean values were calculated from the two animal samples from each concentration on a given day. Differences in growth [total weight (g)], between exposed and control animals were used as an index of toxicity.

Analytical procedures

The hepatic homogenate fraction was prepared by the method described by Siegler and Kazarinoff (1983), and re-suspended in 0.15 M (KCL) to an approximate final concentration of 20 mg of protein per millimeter (approximately 1 g of liver per millimeter). Fractions of the plasma and liver homogenate were used for the determination of alkaline phosphatase (ALP) activity using a cellular enzyme assay, which was based on the conversion of para-nitrophenylphosphate (p-NPP) to para-nitrophenol and the colorimetric determination of the resulting colored product. The test system was optimized with respect to substrate concentration, reaction time and the number of cells used as a source of enzyme. The obtained values were converted to quantitative results through a standard curve created using commercial ALP.

The level of lipid peroxidation in homogenate was determined as malondialdehyde (MDA) content by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). Liver (0.2 g) were homogenized in 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 5 min. To 1 ml supernatant, 4 ml of 20% TCA containing 0.5% TBA was added. The mixture was added and heated at 90°C for 30 min and then quickly cooled on ice. After centrifugation, the absorbance of the non specific absorption at 600 nm was subtracted. The concentration of MDA was calculated using an extinction coefficient (ε) of 155 mM^{-1} cm^{-1}.

Glutathione (GSH) level was determined by using aliquots of the plasma and liver homogenate separately (20%) diluted with 5% (w/v) Trichloroacetic acid (TCA) containing 3 mM EDTA. After centrifugation, 1 ml of 0.5% DTNB (5, 5-dithiobis-2-nitrobenzoic acid) was added to the supernatant fractions and absorbance was measured at 412 nm as described by Reed (1980).

Glutathione reductase (GR) activity was determined by the methods of Shi et al. (2009) by monitoring the glutathione-dependant oxidation of NADP at 340 nm wavelengths. 1 ml reaction mixture containing NADP, GSSG and enzyme extract was allowed to run for 3 min at 25°C. Corrections were made for any GSSG oxidation in the absence of NADP. Activity was calculated by using extinction coefficient (ε) 6.2 $mM^{-1}cm^{-1}$ and expressed in enzyme units (mg protein)$^{-1}$. One unit of enzyme is the amount necessary to decompose 1 µmol of NADP per min at 25°C. Catalase activity

in the tissue was estimated by the method given by Aebi (1984) with slight modification, 0.2 g of tissue was homogenized in 2 ml of extraction buffer under cold conditions. The homogenate was centrifuged at 10,000 rpm for 20 min at 40°C. The supernatant was used for quick assay.

Catalase activity was determined by observing the disappearance of H_2O_2 by spectrophotometer. Reaction, carried out in total volume of H_2O_2, was allowed to run for 3 min. Activity was calculated by using extinction (ε) 0.036 $mM^{-1}cm$.

Superoxide dismutase (SOD), activity was measured by the method described by Makay et al. (2009). The liver (0.2 g) was homogenized in 2.0 ml of extraction buffer, centrifuged at 15,000 rpm at 4°C and the supernatant was assayed by its ability to inhibit photochemical reduction nitroblue tetrazolium. The test tubes containing assay mixture were incubated in light under 15 W inflorescent lamps for 15 min, illuminated and non-illuminated reactions without supernatant served as calibration standard. Absorbance was read at 560 nm wavelength. One unit of enzyme activity was defined as the quantity of enzyme that reduced the absorbance reading of samples to 50% in comparison with blank. The colorimetric method of Nelson (1994) was used for the determination of residual glucose. Protein was determined by the Lowry et al. (1951) method.

Statistical analysis

In all the experiments, three replicates were performed for each concentration. Mean and standard deviations were calculated from triplicate measurement of three preparations. The two-way analysis of variance in a completely randomized block design involving six treatments (one control and five treatments) and five durations was performed to confirm the validity of the data. Critical difference was calculated to compare between various treatments. Significant differences of the means were accepted for $p < 0.05$.

RESULTS

The toxicity of the insecticide, Raid, consumed in feed diet was monitored using several indicators of cellular damage. There were no significant effects of Raid on growth of the rats at low concentrations. However, at higher concentrations, survival was threatened. The mean total body weight of animals fed diets with concentrations of 430 and 961 µg Raid/g feed were significantly lower ($P < 0.05$) than the control by the 10th day (Table 1). The animals tried to avoid the Raid contaminated feed, but somehow all the feed was usually eaten each day. Animals fed with higher concentrations became more subdued and huddled in the darker part of the boxes as the day passed and were not as active as the controls. Of all the animals (65) used, six died (representing ~9% of animals) four from test boxes (A) and two from duplicate samples (B). Statistically, the mortality is not significant. The results indicate that mortality was only observed at higher concentrations of the insecticide.

The effects of Raid on alkaline phosphatase activity, glutathione and glucose levels are shown in Table 2. Raid increased alkaline phosphatase activity in both plasma and liver homogenate compared to the control at concentrations of 430 µg/g, the lowest concentration producing adverse effects significantly different from controls (LOAEL) µg/g, and 961 µg/g the highest concentration producing adverse effects significantly different from control (NOAEL). Alkaline phosphatase (ALP) in serum and lipid peroxidation (LPO) in the liver tissue significantly increased (Table 2), compared to control, whereas glutathione (GSH), glutathione reductase (GR), catalase (CAT), and superoxide dismutase (SOD) (Table 3) were simultaneous decreased in the liver tissue, while there is substantial residual glucose level, in both plasma and liver.

DISCUSSION

The adverse effects of chronic exposures to certain insecticides have been documented, but there is no such information on the insecticide that is the subject of this study. For example, long-term exposure of insecticide has been reported to result in systemic toxicity such that may impair the function of the nervous system and increase the risk of acute leukemia in children (Menegaux et al., 2006). Also, pesticides including organo-phosphorus insecticides used against crawling and flying insects in homes have the potential of being carcinogens (Peter and Cherion, 2000).

Results of this study showed clearly that Raid in concentrations of 430 and 961 µg/g has significant effects on mortality and weight loss in Wistar rats (Table 1). This adverse effect was demonstrated in this study by increase in alkaline phosphatase activity in both plasma and liver which is a known measure of hepatic toxicity, and confirms Raid as a hepato- toxicant. The significant increase in alkaline phosphatase activity (Table 2) may be due to hepatocellular necrosis which causes increase in permeability of cell membrane resulting in the release of this enzyme into the blood stream. The insecticide Raid significantly decreased reduced glutathione levels especially in the liver and this has implications for the ability of the animal to withstand oxidative stress. Studies have shown that GSH deficiency in cells is associated with markedly decreased survival (Kohlmeier et al., 1997). Thus, chemically stable, lipid-soluble, organopho-sphorus insecticides are hazardous to health through mechanisms including depletion of GSH (Menegaux et. al., 2006). Glutathione deficiency is associated with impaired survival in HIV disease (Herzenberg et al., 1997). Glutathione may be consumed by conjugation reaction, which mainly involve metabolism of zenobiotic agent. However, the principle mechanisms of hepatocyte glutathione turnover are known to be by cellular efflux (Sies et al., 1978).

Glutathione reductase is a known defense against oxidative stress, which in turn needs glutathione as co-factors. Catalase is an antioxidant enzyme which destroys H_20_2 that can form a highly reactive radical in the presence of iron as catalyst (Gutteridge, 1995).

Achudume et al. (2008) showed that bioaccumulation

Table 1. Growth of Wistar rats exposed to different concentrations of Raid in feed for 10 days.

Raid concentrations in feed (µg/g)	Test box	Mortality	Body weight after 10 days (g)*
Control	A	0/5	135 ± 5.4
25.0 ± 2.4	A	0/5	134 ± 21.7
	B	0/5	135 ± 3.2
54.0 ± 9.2	A	0/5	137 ± 2.9
	B	0/5	134 ± 17.0
108.2 ± 12.5	A	0/5	128 ± 3.
	B	0/5	130 ± 23.2
216.2 ± 14.6	A	0/5	129 ± 19.8
	B	0/5	132 ± 33.4
430.0 ± 20.2	A	2/5	128 ± 20.5
	B	1/5	126 ± 20.4
961.2 ± 70.5	A	2/5	118 ± 20.7a
	B	1/5	120 ± 5.3a

* Mean size (± SD) of rats body weight (n =5); a, growth significantly less than control (p < 0.05).

Table 2. Effect of Raid concentrations in feed on hepatic enzyme ` activity, reduced glutathione and glucose levels.

Raid concentrations in feed (µg/g)	Alk pas tissue activity (µgml-min^{-1})	GSH level (mg/ml)	Glucose level (mg/g liver)
430.0 ± 20.2			
Control	0.08 ± 0.04	0.18 ± 0.02	0.96 ± -0.04
Plasma	0.16 ± 0.09*	0.15 ± 0.06	0.99 ± 0.04
Control	0.18 ± 0.04	0.19 ± 0.02	1.84 ± 0.01
Liver	0.25 ± 0.02*	0.15 ± 0.01	1.95 ± 0.12
961.2±70.5			
Control	0.09 ± 0.05	0.18 ± 0.05	0.96 ± 0.52
Plasma	0.16 ± 0.01*	0.11 ± 0.05	0.99 ± 0.52
Control	0.18 ± 0.08	0.19 ± 0.02	1.94 ± 0.06
Liver	0.26 ± 0.08 *	0.09 ± 0.03*	2.16 ± 0.04*

Data values are mean ± SD; *Statistically significant compared to control (p < 0.05).

factor of insecticide raid was observed in lipid, up to three times that of the feed at the first concentration and gradually decreases as the concentration increases. The ability of Raid to induce oxidative stress was indicated in the present study by increased amount of hepatic lipid peroxides (Table 3). Lipid peroxidation is a chemical mechanism capable of disrupting the structure and function of the biological membranes that occurs as a result of free radical attack on lipids. This study confirms that Raid increased lipid peroxidation, oxidative stress and hepatotoxicity due to reduced antioxidant system (Table 3). In addition, SOD is family of metallo enzyme, which is considered to be stress protein which decreases in response to oxidative stress (McCord, 1990). It is evident that decrease of SOD in the tissue is a confirmation of its protection from damage caused by Raid.

While the animals may have been adversely affected

Table 3. Effect of insecticide Raid on MDA, GR, CAT, SOD, on the liver of rat.

Control (µg/g)	MDA (nmoles/g wet tissue)	GR (µmoles/g tissue)	CAT (µmoles/g tissue)	SOD (unit/mg protein)
Control	0.40 ± 0.5	4.54 ± 0.4	7.60 ± 0.6	6.62 ± 0.7
430.0 ± 20.2	1.25 ± 0.3	0.33 ± 0.9*	5.91 ± 0.1	5.50 ± 0.1
961.2 ± 70.5	3.56 ± 0.6*	0.20 ± 0.8*	4.00 ± 0.4*	4.20 ± 0.5*

Data values are mean ± SD*; Statistical significant $p < 0.05$ from control; MDA = malondialdehyde, GR = glutathione reductase, CAT = catalase, SOD = superoxide dismutase.

mainly by ingestion of the active ingredients, the effect of propellant chemical cannot be ignored. Inflammatory activation might be an important mechanism underlying toxicity effects in the tissue (Mense et al., 2006). The role of propellant in the toxicity of Raid is not clear. A comprehensive assessment of the risk associated with environmental use of Raid was determined in various tissues as it affects the basal biochemical molecules of cells (Achudume et al., 2008). The study showed that insecticide products contain a variety of other ingredients that are known to either be toxic or have not been adequately tested for toxicity. Surgan et al. (1996) were of the same view that pesticides contain hazard ingredients.

Various studies indicated that unregulated chemicals released into the environment are causing male animals and humans to take on feminine characteristics (Chem, 2008; Dietz et al., 2008; Brucker-Davis et al., 2008). Other studies showed that pesticides like endosulfan induced biochemical changes (Kumar et al., 2008), paraquat and deltamethrin exposure affect tropical responses of microorganisms (Leboulanger et al., 2009). The insecticide Cyfluthrin and Chlorpyrifos alter the expression of a subset of genes with diverse functions in primary human astrocytes (Mense et al., 2006). Similarly, glufosinate has profound effects on antioxidant enzymes (Qian et al., 2008a) while atrazine has inhibitory effect on polymerase chain reaction (Qian et al., 2008b).

The safety margins that members of the public may be exposed to and the amount that may have the potential to inhibit glucose utilization is a major concern. The liver being an important site of detoxification of many endogenous compounds requires basal energy. The increased level of glucose in the presence of Raid indicates interference in the uptake of tissue glucose following ingestion of the contaminated food.

Conclusion

The safety margins between the amount of insecticide 'Raid®' that members of the public may be exposed to and the amount that may have the potential to cause health effect is uncertain. Though, the effect or the safety margins are not sufficiently large enough to satisfy modern regulatory standards. The major concerns in this case were of the toxicological effects reflected in alkaline phosphatase activity, the low level of antioxidants after insecticide-contaminated feed and the decrease in basal energy level utilization.

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