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Full Research Paper

Use of biochemical biomarkers in the ecological risk assessment of permetrin pesticide exposure

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Biochemical biomarkers were used in ecological risk assessment of pesticide exposure. The experiments were carried out on six weeks old poultry birds (*Gallus domesticus*) weighing between 400 and 600 g (12 birds) and were divided into four groups of three birds each. The poultry birds were exposed for four weeks to different concentrations (1, 5 and 10%) of a locally manufactured insect powder called 'Rambo' which contains 0.6% permethrin. The control group had no pesticide added to their feed. Results of the experiment showed a significant increase ($p < 0.05$) in the activity of Alanine transaminase (ALT) at 5% (42.00 ± 1.00 IU/L) and 10% (42.67 ± 1.00 IU/L) pesticide contamination, when compared with the control (37.00 ± 1.00 IU/L). Aspartate transminase (AST) activity was significantly higher ($p < 0.05$) at 10% pesticide contamination (12.33 ± 0.66 IU/L) as against the control, whereas alkaline phosphatase (ALP) activity was significantly ($p < 0.05$) at all levels of pesticide contamination when compared with the control. There were no significant differences ($p> 0.05$) in the levels of total and conjugate bilirubin and creatinine between the treatments and the control, while urea showed significant difference at 5 (22.38 ± 0.42 mg/dl) and 10% (25.39 ± 0.50 mg/dl) pesticide contamination, respectively. Lipid peroxidation and lactate dehydrogenase showed significant differences at all levels of pesticide contamination, indicating possible oxidative stress. As such, results of this experiment support the use of biochemical biomarkers in the ecological risk assessment of pesticide contaminated environment.

Key words: Biochemical biomarkers, ecological risk assessment, pesticide contamination, non-target animals, poultry birds.

INTRODUCTION

The use of pesticides in agriculture has become important considering the huge loses farmers experience due to the ravaging effect of agricultural pests. The large scale use of pesticides in agriculture has proven effective in controlling and minimizing losses due to pests. Continuous and indiscriminate use of pesticides has been reported to cause severe environmental problems and is a health hazard for both humans and animals (Perring and Mellanby, 1975; Partanen et al., 1999). Bioaccumulation of pesticides in the food chain can lead to potentially adverse effect in humans and useful animals due to their putative toxic effect (Palmeira, 1999).

There is a need to devise an accurate and effective way of monitoring the effects of pesticide exposure to humans and the environment. Until recently, the most common end point measured when evaluating toxicity of chemicals were mortality values (Otitoju and Onwurah, 2007). Mortality values 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 (Neuhauser et al., 1984).

The use of biomarkers in accessing the risk of environmental exposure has been on the increase (Albertini, 1998). Biomarkers are an integral part of biomonitoring and they have characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathogenic processes or pharmacological responses to therapeutic intervention. This study was undertaken to investigate the effects of a pyrethroid insecticide marketed in Nigeria as 'Rambo' on some biochemical parameters of poultry birds

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Table 1. Effect of pesticide exposure on activities of liver enzymes.

Percentage of pesticide contamination	ALT (IU/L) (n = 3)	AST (IU/L) (n = 3)	ALP (IU/L) (n = 3)
0 (Control)	37.00 ± 1.00	9.67 ± 0.66	263.91 ± 1.01
1%	34.33 ± 6.70	10.33 ± 0.66	209.62 ± 5.57*
5%	42.00 ± 1.00*	11.67 ± 0.66	320.22 ± 5.65*
10%	42.67 ± 0.66*	12.33 ± 0.66*	356.93 ± 7.77

Values are expressed as mean ± standard error of means (SEM) (n = 3). * Significant at $p < 0.05$ when compared to the control value evaluated by student's t-distribution.

(*Gallus domestica*). As a non-target animal, poultry birds (domestic fowls) stand the risk of pesticide poisoning because of their tendency to pick up pesticide contaminated food and other items in the environment. This study will also examine the use of different biochemical parameters as biomarkers for the ecological risk assessment of pesticide contaminated environment.

MATERIALS AND METHODS

Test sample

The pesticide used for this study was a locally made insect powder called 'Rambo' which is indicated by its manufacturer to contain 0.60% permethrin as its active ingredient. Rambo insect powder is a product of Gongoni Company Limited, 89A Sharade Industrial Estate, Phase III, Kano, Nigeria.

Formulation of contaminated poultry feeds

Commercially available poultry feed was contaminated by weighing out a definite amount of the feed and mixed with a graded percentage of the insecticide to give 1, 5 and 10% (w/w) contamination, respectively. The doses were carefully chosen to ensure that the birds were not exposed to lethal concentrations of the pesticide. As such, feed for the control contained no 'Rambo' insect powder.

Experimental animals

Twelve, four weeks old, poultry birds (*Gallus domesticus*) weighing 400 to 600 g were bought from a poultry farm market in Owerri, Nigeria. The birds were maintained on commercial poultry feed (starter feed) for about two weeks before the commencement of the experiment. They were then randomly divided into four groups containing three birds per group in a battery cage where they were fed *ad libitum* with 0, 1, 5 and 10% (w/w) contaminated feeds, respectively. The group fed with uncontaminated feed served as the control. As such, the birds were provided with adequate water supply.

Biochemical assays

Total bilirubin and conjugate bilirubin were determined by the use of diazolized sulphanilic acid as described by Pearlman and Lee (1974) and Zoppi et al. (1976).

Aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were assayed based on the method of Reitman and Frankel (1957). Lactate dehydrogenase (LDH) activity was determined using a modification of the method of Wacker et al. (1956). However, the levels of creatinine and urea were spectrophotometrically analyzed using biochemical kits.

Lipid peroxidation

Lipid peroxidation was carried out by assessing the concentration of thiobabituric acid reacting substances (TBARS) determined by measuring the absorbance at 532 nm using a spectrophotometer (Tuner Model 390 Digital spectrophotometer) as described by Wallin et al. (1993).

Statistical analysis

Results were statistically analyzed using student's t-test and were considered to be significant at $p < 0.05$.

RESULTS

Table 1 shows the effects of pesticide exposure on the activities of liver function enzymes, namely: aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). As such, there was significant increase ($p < 0.05$) in ALT activity at 5% (42.00 ± 1.00 IU/L) and 10% (42.67 ± 1.00 IU/L) pesticide contamination when compared to the control (37.00 ± 1.00 IU/L). AST activity was also found to be significantly higher ($p < 0.05$) at 10% pesticide contamination (12.33 ± 0.66 IU/L) as against the control. However, there were significant increases in ALP activity at 1, 5 and 10% pesticide contamination when compared with the control.

Results of the biochemical assay indicate that there was no significant difference ($p > 0.05$) in the level of both total and conjugate bilirubin between the treatments and the control (Table 2). The level of urea was significantly higher only at 5% (22.38 ± 0.42 mg/dl) and 10% (25.39± 0.50 mg/dl) pesticide concentration than the control. There were no significant differences ($p > 0.05$) in the level of creatinine between the treatments and the control.

Lipid peroxidation increased significantly at all levels of pesticide contamination (5.67 ± 0.33, 7.67 ± 0.33 and 9.67 ± 0.33 Nmol/ml for 1, 5 and 10% pesticide

Table 2. Effect of pesticide exposure on biochemical parameters.

Percentage of pesticide contamination	Total bilirubin (mg/dl) (n = 3)	Conjugated bilirubin (mg/dl) (n = 3)	Urea (mg/dl) (n = 3)	Creatinine (mg/dl) (n = 3)
0 (Control)	0.61 ± 0.36	0.21 ± 0.00	14.55 ± 0.41	0.21 ± 0.01
1	0.13 ± 0.01	0.03 ± 0.00	14.78 ± 0.73	0.24 ± 0.01
5	0.23 ± 0.00	0.04 ± 0.01	22.38 ± 0.42*	0.32 ± 0.43
10	0.36 ± 0.01	0.05 ± 0.00	25.39 ± 0.50*	0.63 ± 0.03

Values are expressed as mean ± standard error of means (SEM) (n=3). * Significant at p<0.05 when compared to the control value evaluated by student's t-distribution.

Table 3. Effect of pesticide exposure on lipid peroxidation and lactate dehydrogenase activity.

% of pesticide contamination	Lipid peroxidation (n = 3)	Lactate dehydrogenase (n = 3)
0% (Control)	2.33 ± 0.33	4495.74 ± 44.62
1%	5.67 ± 0.33*	3205.18 ± 573.03*
5%	7.67 ± 0.33*	4748.44 ± 26.85*
10%	9.67 ± 0.33*	4045.29 ± 198.94*

Values are expressed as mean ± standard error of means (SEM) (n = 3). * Significant at p < 0.05 when compared to the control value evaluated by student's t-distribution.

contamination, respectively) when compared with the control (2.33 ± 0.33 Nmol/ml). Likewise, LDH activity showed significant difference ($p < 0.05$) at all levels of pesticide contamination (3205.18 ± 73.03 IU/L for 1%; 4748 ± 26.85 IU/L for 5% and 4045.29 ± 98.94 IU/L for 10%) when compared with the control (Table 3).

DISCUSSION

In the last decades, biomarkers have been widely used to diagnose environmental contamination and have proven to be very efficient in detecting early effects of pollutants that may have reflexes latter in time at higher levels of biological organization (Cunha et al., 2007; Lehtonen et al., 2007; Moreira et al., 2006). Biomarkers in birds have been used in the past as a part of biomonitoring programme (Cordi et al., 1997). These have included several enzymes, DNA studies and biochemical aspects of the organisms. As such, biochemical biomarkers have, among its potentials, a rapid early warning signal against potentially damaging effects of stressors (Otitoju and Onwurah, 2007).

The highest activity of alanine transaminase (ALT) was recorded at 10% pesticide exposure which was significantly higher than the control. ALT is mostly used as a specific indicator of hepatic injury that represents a marker of hepatocellular necrosis (Deepak, et al., 2000). This high level of ALT activity shows that the cells of the liver have been inflamed or have undergone cell death. As the cells are damaged, the ALT leaks into the blood stream leading to a rise in serum levels. Also, the activity of aspartate transaminase (AST) increased with its highest contamination at 10% (12.33 ± 0.66 IU/L). This implies that there was a damage in the hepatic cell. Alkaline phosphatase activity showed great significance as compared to the control ($p < 0.05$) with the highest activity recorded at 10% (w/w) contamination (3.56 ± 7.77 IU/L) which suggests an effect in the biliary tract with less specificity. Total and conjugate bilirubin values in the pesticide exposed birds were higher than the control, but the differences were not significant ($p > 0.05$). An altered bilirubin level is indicative of liver damage when compared to plasma activities of serum enzymes in the liver (Deepak et al., 2000). The study also revealed significant increases in the level of serum urea. However, the increase in creatinine level was not significant. Lipid peroxidation is a well established mechanism of cellular injury in both plants and animals and is used as an indicator of oxidative stress and tissue damage (Esterbarner et al., 1992). Lipid peroxidation increased significantly at all levels of pesticide contamination, when compared with the control, indicating that cells are under stress. Dargel (1992) reported that ingestion of pesticide contaminated diet has been shown to elicit toxic cell damage mediated by xenobiotic metabolism, free radical formation and lipid peroxidation. Otitoju and Onwurah (2007) also reported an elevated lipid peroxidation in rats exposed to permethrin. The activity of lactate dehydrogenase (LDH) was significantly higher at all levels of pesticide contamination studied compared to the control. Other studies have demonstrated that dehydrogenase enzymes activity of microorganisms is among the most sensitive parameter for evaluation of toxicity (Nweke et al., 2006; Alisi et al., 2008). In conclusion, this study has shown that biochemical parameters can be used in the ecological evaluation of pesticide exposure.

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