Effects of Dichlorvos on Reduced Glutathione, Glutathione S-Transferase and Lipid Peroxidation of Egg Laying Hens

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ABSTRACT: Reduced glutathione (GSH) and Glutathione S-transferase (GST) play important roles in the detoxification of several xenobiotic compounds, including pesticides. This study was carried out to access the effect of the organophosphate pesticide, dichlorvos on the activities of GSH, GST as well as lipid peroxidation of egg laying hens. Seven weeks old pullets weighing between 400-600 g divided into four groups of 10 birds each were fed on commercial poultry feeds contaminated with 0.01, 0.02 and 0.04% of dichlorvos. The control group had no pesticide added to the diet. The birds were maintained on the above diet for 20 weeks after which their blood samples were collected for analysis. There was a significant reduction ($p<0.05$) in serum GSH at 0.04% pesticide contamination (10.64 ± 1.50 µg/l) compared to the control (17.87 ± 0.37 µg/l). Similarly, there was also a significant reduction in serum GST activity at 0.04% pesticide contamination as against the control. Lipid peroxidation increased significantly from 1.17 ± 0.52 MDA/mg protein in the control to 4.34 ± 1.22 MDA/mg protein in the birds fed on 0.04% contaminated diet. There were significant increases ($p<0.05$) in serum protein at all the levels of pesticide contamination when compared with the control. The result of this study suggests the use of GST, GSH and lipid peroxidation as biomarkers of dichlorvos exposure.

KEY WORDS: Reduced glutathione (GSH), Glutathione S- transferase (GST), Organophosphate pesticide, Dichlovos.

1. INTRODUCTION

Continuous and indiscriminate use of pesticides for agriculture, industrial and domestic purposes has the potential of causing adverse effects on both humans and other non-target organisms. Organophosphates are the most widely used pesticides and the cause of more incidences of pesticide poisoning than any other class of pesticides [1]. The mode of action of organophosphorus pesticides (OPs) is mainly through the inhibition of the enzyme acetylcholinesterase resulting in the accumulation of acetylcholine at the neuromuscular junctions [2, 3]. Dichlorvos (DDVP) is a highly volatile organophosphate, widely used as an insecticide to control household pests, in public health, and protecting stored product from insects. It is also used to treat a variety of parasitic worm infections in dogs, livestock and humans [4]. Glutathione is a cysteine-containing peptide found in most forms of aerobic life [5]. Due to their high sensitivity to environmental pollutants, glutathione (GSH) and glutathione S-transferase (GST) have been used as markers of toxic effects of exposure to various xenobiotics [6, 7]. Lipid peroxidation has also been used as a bioindicator of oxidative damage in organisms exposed to environmental pollutants. This study examines the effect of dichlorvos on GSH, GST and lipid peroxidation of domestic fowl (layers).

MATERIAL AND METHODS

Test Sample: Dichlorvos (2, 2 – dichlorovinyldimethyl phosphate) –DDVP, was purchased from an agrochemical shop in Owerri.

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 pesticide to give 0.01, 0.02 and 0.04 % (w/v) contamination respectively. Feed for the control contained no pesticide

Experimental Animals

Day old black pullets were obtained from Zartec Farms, Ibadan, Nigeria. The birds were brooded under appropriate conditions until they were seven weeks old. The seven weeks old pullets with an average weight of 557.5 ± 9.5 g were divided into four groups containing 10 birds each and housed in poultry pens at the livestock unit of the Department of Animal Science and Technology, Federal University of Technology, Owerri, Nigeria. Three groups received a diet containing 0.01, 0.02 and 0.04 % dichlorvos respectively, while the control was fed on pesticide-free diet. The experiment lasted for a period of ten weeks.

The birds were maintained on the above diet for 20 weeks after which their blood samples were collected for analysis.

Analytical Procedures

Reduced glutathione was measured according to the method of Ellman [8] as described by Bulaj et al. [9]. Glutathione S-transferase activity was determined according to the procedure described by Habig et al. [10]. The specific activity of GST was expressed as µmol GSH-CDNB (1-chloro-2, 4-dinitrobenzene) conjugate formed/min/mg/protein using an extinction coefficient of 9.6 mM-1 cm-1. Lipid peroxidation was determined by assessing the concentration of Thiobarbituric acid reactive substances (TBARS) as described by Liu et al. [11]. Serum protein was assayed by the Biuret method as described by Gornall et al., [12]. Bovine serum albumen (B.S.A) was used a standard protein.

Statistical Analysis

The results were statistically analyzed using Student's t-test with the program statistical package for social sciences (SPSS) version 17. The data means were considered different at $p < 0.05$.

Table 1: Effect of Dichlorvos on Serum Glutathione and Glutathione S-transferase

Pesticide Concentration	GSH (µg/L)	GST (mg/ml x 10^{-4})
Control	17.87 ± 0.37	23.74 ± 0.55
0.01%	15.39 ± 0.44	9.76 ± 2.69*
0.02%	14.68 ± 1.84*	3.94 ± 1.57*
0.04%	10.64 ± 1.50*	3.87 ± 0.17*

Values are expressed as mean ± standard error of means (SEM) (n = 3). * Significant at $p < 0.05$ when compared to the control.

Table 2: Effect of Dichlorvos on Serum Protein and Lipid Peroxidation

Pesticide Concentration	Protein	Lipid peroxidation (MDA/mg protein x 10^{-9})
Control	28.73 ±0.00	1.17 ± 0.52
0.01%	33.63 ± 12.87*	2.47 ± 0.59*
0.02%	70.86 ± 34.64*	3.01 ± 0.71*
0.04%	64.65 ± 12.76*	4.34 ± 1.22*

Values are expressed as mean ± standard error of means (SEM) (n = 3). * Significant at $p < 0.05$ when compared to the control.

RESULTS AND DISCUSSION

Glutathione (GSH) and Glutathione S-transferase are among the major antioxidant defenses. Glutathione plays a very important role in the detoxification and elimination of xenobiotics like pesticides. There was a significant reduction ($p<0.05$) in serum GSH at 0.04% pesticide contamination (10.64 ± 1.50 µg/l) compared to the control (17.87 ± 0.37 µg/l) (Table 1). A similar result was observed when poultry birds were exposed to permethrin insecticide [13]. Kumar et al. [14] had earlier reported a decrease in glutathione levels in poultry birds exposed to xenobiotics. El-Demerdash [15] also reported a reduction in GSH content of rats exposed to a mixture of synthetic pyrethroids and organophosphate

insecticides. Reduction in the level of glutathione is a sign that the pesticide is actively being detoxified. The activity of GST was significantly lowered as result of pesticide exposure. There was a reduction in GST activity with increase in pesticide concentration: 9.76 ± 0.55, 3.94 ± 1.57 and 3.87 ± 0.17 mg/ml x 10-4 for 0.01, 0.02, and 0.04 % pesticide contamination respectively as against the control (23.74 ± 0.55 mg/ml x 10-4). Glutathione S-transferases (GSTs) are ubiquitous multifunctional enzymes which play a key role in cellular detoxification [16] and especially chemical xenobiotics like pesticides [17]. The major activity of GST is the conjugation of compounds with electrophilic centers to the tripeptide glutathione [18]. The glutathione conjugates are metabolized further to mecapturic acid and then excreted. Our study revealed a significant ($p < 0.05$) reduction in GST activity as pesticide concentration was increased. Otitoju and Onwurah [19] reported a reduction in plasma GST activity in rats exposed permethrin insecticide. Elevated levels of GST activity have been associated with resistance to all the major classes of insecticides [20]. Lipid peroxidation increased significantly from 1.17 ± 0.52 MDA/mg protein in the control to 4.34 ± 1.22 MDA/mg protein in the birds fed on 0.04% contaminated diet (Table 2). Several pesticides have been shown to stimulate peroxidation of cellular membranes [21]. Lipid peroxidation has been suggested as one of the molecular mechanisms involved in pesticide induced toxicity [22]. The increase in lipid peroxidation observed in this study is an indication that there is oxidative stress occasioned by exposure to pesticide. Others have also reported elevated lipid peroxidation in animals exposed to dichlorvos [23, 24, and 25]. Ezeji et al. [26] observed an increase in lipid peroxidation in poultry birds fed on permethrin contaminated diet.

CONCLUSION

Results of the above study show that glutathione and glutathione s-transferase activities in laying hens are both affected by dichlorvos exposure, further endorsing their use as biomarkers of pesticide exposure. Furthermore, increase in lipid peroxidation in the laying hens shows the presence of oxidative stress occasioned by exposure to dichlorvos.

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