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Plasma antioxidant enzymes, lipid peroxidation and hydrogen peroxide in wistar rats exposed to Dichlorvos insecticide

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ABSTRACT

Dichlorvos is an organophosphate insecticide used indiscriminately in Nigeria in various insecticide formulations due to its cheap production, accessibility, efficacy and affordability. Reactive oxygen species formation has been implicated in the mechanism of dichlorvos toxicity. Reports on antioxidant-prooxidant status in human users of dichlorvos are scarce and the few available reports are conflicting. 35 wistar rats were divided into 7 groups of 5 rats per group as follows; unexposed group, six groups exposed to dichlorvos for 1week, 2weeks, 3weeks, 4weeks, 5weeks and 6weeks in a poorly ventilated compartment. Dichlorvos was prepared in a dilution of 1:1 as recommended by the manufacturer for domestic use. Exposure to dichlorvos was done for 4hours daily. At completion of exposure, animals were anaesthetized and blood was drawn from the heart, plasma was separated and used for measurement of hydrogen peroxide (H_2O_2), malondialdehyde (MDA), superoxide dismutase (SOD) activity and catalase (CAT) activity. Results showed a significant decrease in plasma MDA concentration in rats exposed to dichlorvos for 4weeks, 5weeks and 6weeks when compared with unexposed control group. There was a significant increase of plasma CAT activity in rats exposed for 3weeks when compared to unexposed control. It could be concluded from this study that long term dichlorvos inhalation may alter plasma prooxidant-antioxidant balance, hence the need for cautious use.

Keywords: Enzymes, Organophosphates, Oxidative stress, Pesticides.

INTRODUCTION

Dichlorvos (O-O-dimethyl-O-2, 2-dichloro-vinyl phosphate; DDVP) is an organophosphate insecticide used widely in many Nigerian households [1]. DDVP (traded as Ota-piapia) is indiscriminately used because of its cheap production, efficacy, accessibility and affordability [2]. It has caused death in many Nigerian families [3] and worldwide [4], specifically through food contamination [5, 6]. Like other pesticides, dichlorvos is absorbed through the skin, ingested or inhaled. The general population is exposed to dichlorvos primarily through inhalation of contaminated indoor air, either during and/or immediately after application or through the use of polyvinyl chloride resin strips [7].

The mechanism for the toxicity of organophosphates is mainly by blocking of acetylcholinesterase – an enzyme which decomposes acetylcholine [8]. Immobilization of this enzyme results in an accumulation of excessive

amounts of acetylcholine in the nervous tissue and muscular motor plates, as well as in symptoms of endogenic poisoning by this neurohormone. Dichlorvos also causes disturbances in the flow of ions through these membranes by inhibition of enzymes which regulate this flow [9]. Other study supports a role of reactive oxygen species (ROS) in the mechanism of dichlorvos toxicity [10]. Excessive generation of ROS causes irreversible impairment of DNA and damage to membrane lipids leading to the production of Malondialdehyde (MDA) [11].

The biological mechanism of defense against oxidative stress includes antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) [12]. Superoxide dismutase (SOD) catalyzes the conversion of superoxide anion (O_2^-) a highly potent ROS into a less reactive species hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). Hydrogen peroxide formed by SOD activity is decomposed to water (H_2O) and molecular oxygen (O_2), a reaction catalyzed by the enzyme catalase (CAT) [13].

It was suggested by Sharma and Singh [10] that ROS are involved in the mechanism of DDVP toxicity. The reports on antioxidant-prooxidant status in human users of DDVP are scarce. Also the few available reports are conflicting. The study was therefore designed to investigate the effect of dichlorvos inhalation on biomarker of lipid peroxidation (MDA), reactive oxygen species (H_2O_2), and antioxidant enzymes (SOD and CAT) in wistar rats exposed for 1-6 weeks. This study aims to provide reasons for careful use of dichlorvos.

MATERIALS AND METHODS

Study design

This study was carried out in the Department of Chemical Pathology, University of Ibadan, Nigeria. Dichlorvos purchased from agrochemical shops was used for these studies. Dichlorvos was prepared at a concentration recommended by the manufacturer (50 ml of dichlorvos was mixed with 50 ml of clean water) for domestic use. This was placed in a poorly ventilated compartment in which animal cages were kept for 4 hours daily. Freshly prepared solution was used daily.

Male Wistar rats aged 2months were purchased from the animal house of Physiology Department, University of Ibadan, Nigeria and were quarantined for two weeks before the commencement of experiment. The animals were fed with standard fodder and watered *ad libitum.* The rats were divided into 7 groups with 3 rats in each group consisting of 6 experimental groups which were exposed to dichlorvos. Exposure was for 1week, 2 weeks, 3weeks, 4 weeks, 5 weeks, and 6weeks. The 7^{th} group comprised of unexposed rats.

Blood Sample Collection

Rats were anaesthetized with Ketamine Chloride administered intraperitoneally. Approximately 4 ml of blood was collected by cardiac puncture into lithium heparin bottles. Blood samples were centrifuged at 4000 x g for 10 minutes. Plasma was collected into Eppendorf bottles using Pasteur pipette and stored at -20^{o}C until analyzed.

Protein Determination

The protein concentrations of the various samples were determined using the Biuret method as described by Gornal et al [14]. The principle of the test is based on the formation of a coloured complex between proteins and cupric ions in an alkaline solution. The results were expressed in mg/ml.

Lipid peroxidation (LPO) Assessment

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation. This was carried out by the method of Varshney and Kale [15]. The method was based on the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde (MDA). The MDA level was calculated according to the method of Adam-Vizi and Seregi (1982). Lipid peroxidation in units/mg protein was computed with molar extinction coefficient of 1.56 x 10^5 $M^{-1}cm^{-1}$

Hydrogen Peroxide (H_2O_2) generation

H_2O_2 was measured as described by Wolff [16].

Superoxide Dismutase (SOD) activity

SOD activity was determined using the method of Misra and Fridovich [17]. In this method, SOD present in the sample competes with the detection system for superoxide anion. A unit of SOD is defined as the amount of enzyme

that inhibits the rate of adrenaline oxidation by 50%. Adrenaline oxidation leads to the formation of the coloured product, adrenochrome, which is detected by spectrophotometer. SOD activity was determined by measuring the rate of adrenochrome formation, observed at 480 nm, in a reaction medium containing glycine-NaOH (50 mmol/L, pH 10) and adrenaline (1 mmol/L). The result was expressed in Unit/mg protein.

Catalase (CAT) activity

CAT activity was determined according to the method of Sinha [18]. The method is based on the fact that dichromate is reduced to chromic acetate when heated in the presence of H_2O_2. Chromic acetate produced was measured colorimetrically at 570 nm. Result was expressed in µmole/mg protein.

Statistical analysis

The data was analyzed using statistical package for social sciences (SPSS) version 17.0. The Student's *t*-test was used to compare plasma mean values of MDA, H_2O_2, SOD and CAT in control group and various groups of dichlorvos exposed wistar rats. P-value less than 0.05 was considered significant.

RESULTS

Table 1 shows that there were no significant changes in plasma H_2O_2 and SOD activity in dichlorvos exposed groups when compared with unexposed groups. Increase in CAT activity was observed in rats exposed to dichlorvos for 3 weeks when compared to unexposed rats. Significant decreases in MDA were observed in rats exposed to dichlorvos for 4 weeks, 5 weeks and 6 weeks compared with unexposed control.

Table 1: Mean plasma levels of MDA, H_2O_2, and plasma activities of SOD and CAT

Groups	MDA (x±SD) (U/mg/protein)	H_2O_2 (x±SD) (µmol/ml)	SOD (x±SD) (U/mg/protein)	CAT (x±SD) (µmol/mg protein)
Control (n=5)	0.17±0.06	9.40±2.59	14.24±3.16	145.89±8.99
1 wk (n=5)	0.11±0.01	6.50±2.66	16.00±2.42	146.12±19.35
2 wks (n=5)	0.15±0.03	7.50±2.05	16.58±2.32	153.26±17.92
3 wks (n=5)	0.11±0.07	10.55±2.01	12.07±4.55	180.21±26.81*
4 wks (n=5)	0.08±0.03*	11.20±3.42	13.07±5.26	146.75±14.54
5 wks (n=5)	0.06±0.02*	5.90±2.45	14.77±4.70	153.44±11.58
6 wks (n=5)	0.05±0.12*	7.50±2.65	13.82±5.31	149.60±14.05

** Significant from control p < 0.05*

DISCUSSION

Studies have shown that excessive free radical production resulting in oxidative stress could be an important mechanism of organophosphate toxicity [19,20]. Dichlorvos, a volatile organophosphate compound with strong pesticide activity has been reported to alter the biological pro-oxidant – antioxidant balance in various toxicity studies. Hai et al [21] reported an increase of MDA level, SOD and CAT activities in the liver of dichlorvos-exposed fish. A recent study also showed that dichlorvos induced oxidative stress in rats through abnormal production of ROS [10].

Significant gradual decreases in plasma MDA levels were observed in rats exposed to dichlorvos for 4weeks, 5weeks and 6weeks when compared to unexposed control rats. This observation might be a result of increase in activities of antioxidant enzymes which clears ROS, thus decreased lipid peroxidation and low MDA synthesis in exposed rats. This is supported by the finding of this study where increased catalase activity (an antioxidant enzyme) was found in exposed rats especially for 3 weeks compared with unexposed rats. It might therefore be conjectured that the mechanism of dichlorvos induced toxicity did not involve lipid peroxidation. However, the present result contradicts earlier reports [10, 22 23]. The disparities might be due to differences in dichlorvos exposure routes and concentrations.

Antioxidant protection against oxidative damage consists of many enzymatic (SOD, CAT) and non-enzymatic (vitamin C, A, and E) factors which maintain physiological balance of reactive forms of oxygen [24]. SOD plays a major role as first line of the antioxidant defense system by catalyzing the dismutation of superoxide radical to form hydrogen peroxide (an oxidant) and molecular oxygen [25]. CAT is an ubiquitous enzyme present in cells of aerobic organisms. CAT converts two molecules of the hydrogen peroxide to molecular oxygen and two molecules of water [26]. There was elevated CAT activities in dichlorvos exposed rats especially rats exposed for 3 weeks when compared with unexposed rats. This increased CAT activity might be due to increased H_2O_2 production in exposed rats. However, some other studies [10, 23] reported that dichlorvos decreases CAT activity. The disparities in exposure routes, doses or exposure duration might account for differences in the results.

In conclusion, long term dichlorvos inhalation may alter plasma prooxidant-antioxidant balance, hence the need for cautious long term use.

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