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Oxidative Insults of 2, 2-dichlorovinyl-dimethyl phosphate (DDVP), an Organophosphate Insecticide in Rats Brain

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Abstract

The oxidative insult of 2, 2-dichlorovinyl-dimethyl phosphate (DDVP) in male rats brain was investigated. Rats were grouped into four: A, B, C and D where A, (the control) received orally 1 mL of distilled water; B, C and D (test groups) received orally 2.5, 5 and 10 mg/kg body weight of DDVP respectively for 28 days. DDVP administration caused significant decrease ($P<0.05$) in the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in the brain. Brain levels of glutathione (GSH), total thiol, vitamins C and E were also significantly reduced ($P<0.05$), while peroxide glutathione (GSSG) level increased significantly ($P<0.05$). Brain malonidialdehyde and lipid hydroperoxide also increased significantly ($P<0.05$) in all DDVP treated groups. The available data from this study revealed that DDVP brings about its toxicity through depletion of the antioxidant systems and thus exposing the cells and cellular macromolecules to oxidative assault by reactive oxygen species generated either from its metabolites or other *in vivo* means.

Keywords: 2, 2-dichlorovinyl dimethyl phosphate; redox homeostasis; enzymic antioxidant system; non-enzymic antioxidant system; lipid peroxidation.

Introduction

Redox status has proven to be an important tool in toxicological evaluation, mostly providing the cellular and biochemical mechanism of toxicity of chemicals and drugs (Ajiboye *et al.*, 2010a). These chemical compounds interact at both cellular and molecular level, bringing significant changes in the structure, function and metabolic transformation of all classes of biomolecules, enzymes and metabolic pathways (Ajiboye, 2010). These alterations which may be rapid or slow, may lead to different biochemical mechanisms providing similar pathological, clinical and laboratory findings (Ajiboye, 2010).

Organophosphate (OP) insecticides exhibit a high level of pest control ability combined with a relatively low degree of environmental toxicity. Hence, they are used widely around the world in agriculture and in households, which has led to a variety of negative effects in non-target species including human. However, the contribution of malaria to the high incidence of mortality and morbidity still encourages the use of pesticides and insecticides in the control of malaria vector in various homes. Among the pesticides, which is

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commonly used in Nigeria is DDVP under the brand name SNIPPERs.

DDVP is an organophosphate chemical used in domestic, agricultural and veterinary situations (Do, 2004). Due to its volatility, it is suitable for use as a fog, aerosol or mist for fumigation and disinfestation of machinery and confined areas, such as storage areas, warehouses, flour mills, silos, greenhouses and animal housing (Do, 2004). Other applications of DDVP include crop protection and pest control in domestic, commercial and industrial areas. The persistent of DDVP contributes to its efficacy in malaria control. The persistence could last for months and even year, causing a chronic exposure to children, mostly infants and toddlers through oral ingestion (Do, 2004).

The limited suitable standard and protocol for testing toxicity in Nigeria provide no statically defensible estimate of the level of exposure in human population. This study thus investigates the oxidative insults of DDVP in rats brain (as the brain is one of the primary target of DDVP), towards providing an insight to the mechanism of toxicity on human.

Materials and Methods

Materials

Chemicals

DDVP was procured from Saro Agrosciences Limited, Lagos, Nigeria. Glutathione peroxidase (GPx) assay kit was procured from Randox Laboratories Co-Atrim, United Kingdom. All other reagents used were supplied by Sigma-Aldrich Inc., St Louis, USA.

Animals

Apparently healthy, 3 months old, male albino rats (*Rattus norvegicus*) of Wistar strain, weighing 145 ± 2.52 g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were kept in clean metabolic cages of dimensions 33.0 x 20.5 x 19.0 contained in well-ventilated house conditions (temperature: 28- and 31°C; photoperiod: 12 h natural light and 12h dark; humidity: 50-55%) with free access to rat pellets (Bendel Feeds and Flour Mills Ltd., Ewu, Nigeria) and tap water.

Methods

Animal treatment

Male rats were completely randomized into four groups (A, B, C and D) of 15 animals each. Rats in groups B, C and D were administered once daily at 24 h interval for 28 days with 2.5, 5 and 10 mg/kg body weight respectively using stomach tube. Group A, which served as the control was treated like the test groups except that the animals received distilled water.

The animals were allowed free access to rat pellets and tap water. Five animals each were sacrificed from all the groups, 24 h after 1, 14 and 28 days of their daily doses. This study was carried out following approval from the Ethical Committee on the use and care of animals of the Department of Biochemistry, University of Ilorin, Nigeria. The animals were used according to the NIH Guide for the Care and Use of Laboratory Animals (NIH, 1985) in accordance with the principles of Good Laboratory Procedure (GLP) (WHO, 1998).

Tissue and Serum Preparation

The rats were sacrificed by placing them in jar containing wool soaked in ether. Their jugular vein was sharply cut with clean sterile scalpel blade after they were unconscious to collect blood. The blood samples collected were allowed to clot for 15 min and centrifuged at 300 g for 5 min for serum preparation using centrifuge. The clear supernatant was used for the estimation of serum enzymes. Brain excised from the rats was immersed in ice-cold 0.25 M sucrose solution to maintain the integrity of the organ. It was blotted with tissue paper, cut thinly with sterile scalpel blade and then homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v) (Akanji and Ngaha, 1989). The homogenates were centrifuged at 800 g for 10 min at 4°C; the resulting supernatant was frozen at -20°C to ensure the maximum release of the enzymes located in the cell before being used for the enzyme assay.

Enzyme Assay

Superoxide dismutase (SOD) and catalase (CAT) were assayed as reported by Misra and Fridovich (1972) and Beers and Sizer (1952) respectively. GSH-Px and glutathione reductase (GSH-Red) assays were also done according to Rotruck *et al.* (1973) and Mavis and Stellwagen (1968) respectively. Glutathione reduced (GSH) concentration was determined according to the method of Ellman (1959). GSSG level in the brain tissue was measured according to the method of Hissin and Hilf (1976). The total thiol (total sulfhydryl groups) content in the brain tissue was measured according to the method of Sedlak and Lindsay (1958) with some modifications. About 50 μL of the sample was mixed with 0.6 mL of Tris–EDTA buffer, 40 μL of 10 mM DTNB in methanol. The final volume was made up to 1 mL by adding MeOH. The reaction mixture was incubated at room temperature for 20 min, and the absorbance was measured at 412 nm. The total thiol content was calculated using molar extinction coefficient of 13,600 M^{-1} cm^{-1}. Serum Vitamins C and E were assayed according to the methods described by Omaye *et al.* (1979) and Desai (1984) respectively. Biuret method was used in the determination of protein concentration in the tissues (Gornall *et al.,* 1949). The concentration of lipid hydroperoxides and malonidialdehyde were assayed according to Reilly and Aust (1999).

Statistical analysis

Analysis of variance (ANOVA) followed by Duncan's Multiple Range Test was used to detect any significant differences among different means as well as the interactions between the variables used in this study using SPSS 15.0 Version. Differences were considered statistically significant at $P < 0.05$.

Results

Antioxidant enzymes

Antioxidant enzymes were significantly ($P< 0.05$) reduced following administration of DDVP in a dose dependent manner (Tables 1, 2, 3 & 4). The highest dose used produced 72.69, 83.27, 79.91 and 56.81 % decrease in the antioxidant enzymes of the brain tissue; SOD, CAT, GSH-Px and GSH-Red respectively at the end of the experimental period

Non-enzymic antioxidant

The level of non-enzymic antioxidant system, GSH, GSSG, total thiols, vitamins C and E were also significantly reduced (Tables 5, 6, 7 & 8). The highest dose of DDVP produced 81.74, 74.76, 72.58 and 74.38 % decrease in the brain GSH, total thiol, Vitamins C and E levels respectively, while DDVP produced a 86.23 % increase in GSSG in rat brain as compared to the control.

Lipid peroxidation products

The level of lipid peroxidised product i.e. malonidialdehyde and lipid hydroperoxides (LPO) in the brain (Table 9) were significantly ($P < 0.05$) elevated following DDVP administration. The highest dose of DDVP (day 28) produced a 106.62 and 89.15 % in the brain malonidialdehyde and LPO levels respectively.

Table 1: Superoxide dismutase activity (SOD) in the brain of DDVP-treated rats

Doses/Days	1	14	28
Control	51.9 ± 1.8 [a]	50.3 ± 1.1 [a]	52.0 ± 2.3 [a]
2.5 mg/kg body weight	39.3 ± 2.4 [b]	32.1 ± 3.7 [c]	22.6 ± 1.4 [d]
5 mg/kg body weight	32.7 ± 1.3 [b]	26.3 ± 2.1 [c]	19.8 ± 2.6 [d]
10 mg/kg body weight	25.4 ± 1.2 [c]	20.1 ± 1.2 [d]	14.3 ± 0.3 [e]

The results are mean ± SD for 5 rats. Enzyme activities are expressed as nmol $min^{-1}mg^{-1}$protein. Values carrying superscripts different for each parameter are significantly different ($P<0.05$).

Table 2: Catalase activity (CAT) in the brain of DDVP-treated rats

Doses/Days	1	14	28
Control	25.1 ± 1.5 [a]	26.3 ± 3.2 [a]	25.7 ± 1.2 [a]
2.5 mg/kg body weight	20.8 ± 2.3 [b]	15.3 ± 1.3 [c]	8.8 ± 0.1 [e]
5 mg/kg body weight	16.3 ± 0.2 [b]	11.8 ± 0.1 [d]	6.3 ± 0.1 [e]
10 mg/kg body weight	12.1 ± 0.1 [c]	7.6 ± 0.2 [e]	4.3 ± 0.1 [e]

The results are mean ± SD for 5 rats. Enzyme activities are expressed as nmol $min^{-1}mg^{-1}$protein. Values carrying superscripts different for each parameter are significantly different ($P<0.05$).

Table 3: Glutathione peroxidase activity (GSH-Px) in the brain of DDVP-treated rats

Doses/Days	1	14	28
Control	47.1 ± 0.3 [a]	46.9 ± 0.7 [a]	46.3 ± 1.5 [a]
2.5 mg/kg body weight	35.3 ± 1.1 [b]	29.0 ± 2.3 [c]	18.9 ± 1.2 [d]
5 mg/kg body weight	30.1 ± 0.2 [b]	22.5 ± 0.5 [d]	15.3 ± 0.4 [e]
10 mg/kg body weight	27.9 ± 1.4 [c]	18.9 ± 0.3 [d]	9.3 ± 0.2 [e]

The results are mean ± SD for 5 rats. Enzyme activities are expressed as nmol $min^{-1}mg^{-1}$protein. Values carrying superscripts different for each parameter are significantly different ($P<0.05$).

Table 4: Glutathione reductase activity (GSH-Red) in the brain of DDVP-treated rats

Doses/Days	1	14	28
Control	2.01 ± 0.02 [a]	2.10 ± 0.01 [a]	2.13 ± 0.04 [a]
2.5 mg/kg body weight	1.72 ± 0.03 [b]	1.41 ± 0.01 [c]	1.01 ± 0.04 [d]
5 mg/kg body weight	1.57 ± 0.02 [b]	1.32 ± 0.03 [c]	1.08 ± 0.01 [d]
10 mg/kg body weight	1.34 ± 0.02 [c]	1.19 ± 0.01 [d]	0.92 ± 0.02 [e]

The results are mean ± SD for 5 rats. Enzyme activities are expressed as nmol $min^{-1}mg^{-1}$protein. Values carrying superscripts different for each parameter are significantly different ($P<0.05$).

Table 5: Glutathione reduced (GSH) levels in the brain of DDVP-treated rats

Doses/Days	1	14	28
Control	23.6 ± 1.2 [a]	23.1 ± 0.3 [a]	23.0 ± 0.2 [a]
2.5 mg/kg body weight	18.4 ± 0.3 [b]	16.3 ± 0.2 [b]	9.2 ± 0.5 [d]
5 mg/kg body weight	16.3 ± 0.7 [b]	13.1 ± 0.5 [c]	7.9 ± 0.2 [d]
10 mg/kg body weight	13.5 ± 0.4 [c]	10.0 ± 0.3 [d]	4.2 ± 0.1 [d]

The results are mean ± SD for 5 rats. GSH concentrations are expressed as nmol mg^{-1}protein. Values carrying superscripts different for each parameter are significantly different ($P<0.05$).

Table 6: Peroxidised glutathione (GSSG) in the brain of DDVP-treated rats

Doses/Days	1	14	28
Control	2.3 ± 0.01 [a]	2.4 ± 0.03 [a]	2.3 ± 0.09 [a]
2.5 mg/kg body weight	4.7 ± 0.06 [b]	5.9 ± 0.08 [c]	8.0 ± 0.1 [d]
5 mg/kg body weight	6.1 ± 0.03 [c]	8.4 ± 0.1 [d]	11.6 ± 0.7 [e]
10 mg/kg body weight	8.7 ± 0.1 [c]	12.1 ± 0.3 [d]	16.7 ± 0.4 [e]

The results are mean ± SD for 5 rats. GSSG concentrations are expressed as nmol mg^{-1}protein. Values carrying superscripts different for each parameter are significantly different ($P<0.05$).

Table 7: Total thiol levels in the brain of DDVP-treated rats

Doses/Days	1	14	28
Control	63.5 ± 0.2 [a]	63.8 ± 0.7 [a]	63.8 ± 0.2 [a]
2.5 mg/kg body weight	52.4 ± 0.1 [b]	45.9 ± 0.3 [c]	31.3 ± 0.1 [d]
5 mg/kg body weight	48.1 ± 1.0 [b]	32.2 ± 0.1 [d]	28.9 ± 0.2 [d]
10 mg/kg body weight	35.8 ± 0.1 [c]	27.2 ± 1.2 [d]	16.1 ± 1.1 [e]

The results are mean ± SD for 5 rats. GSSG concentrations are expressed as nmol mg^{-1}protein. Values carrying superscripts different for each parameter are significantly different ($P<0.05$).

Table 8: Vitamin C and Vitamin E levels on the brain of DDVP-treated rats

	Vitamin C ($mgdL^{-1}$)			Vitamin E ($mgdL^{-1}$)		
Doses/Days	1	14	28	1	14	28
Control	35.2 ± 1.2 [a]	35.2 ± 0.2 [a]	36.1 ± 0.5 [a]	20.6 ± 0.1 [a]	20.0 ± 0.2 [a]	20.3 ± 0.1 [a]
2.5 mg/kg body weight	30.1 ± 0.1 [b]	23.6 ± 0.4 [c]	16.0 ± 0.3 [d]	15.3 ± 0.2 [b]	12.2 ± 0.1 [c]	9.6 ± 0.1 [d]
5 mg/kg body weight	28.3 ± 0.2 [c]	20.5 ± 0.3 [d]	13.2 ± 0.1 [e]	12.7 ± 0.1 [b]	10.3 ± 0.5 [d]	8.9 ± 0.2 [d]
10 mg/kg body weight	25.7 ± 0.4 [c]	19.0 ± 0.1 [d]	9.9± 0.3 [e]	10.9 ± 0.1 [d]	8.7 ± 0.4 [d]	5.2 ± 0.1 [e]

The results are mean ± SD for 5 rats. Values carrying superscripts different for each parameter are significantly different ($P<0.05$).

Table 9: Levels of lipid peroxidised products in the brain of DDVP-treated rats

	Malondialdehyde ($nmol^{-1}$mgprotein)			Lipid hydroperoxide ($nmol^{-1}$mgprotein)		
Doses/Days	1	14	28	1	14	28
Control	35.3 ± 0.2[a]	35.2 ± 0.7[a]	36.1 ± 0.9[a]	41.6 ± 1.7[a]	41.9 ± 1.0[a]	41.9 ± 1.9[a]
2.5 mg/kg bodyweight	39.2 ± 0.8[a]	47.8 ± 2.3[b]	56.0 ± 1.9[c]	48.6 ± 1.5[b]	55.1 ± 1.8[c]	72.1 ± 1.3[e]
5 mg/kg bodyweight	48.7 ± 0.3[b]	51.1 ± 0.2[c]	70.3 ± 1.8[d]	50.9 ± 1.2[b]	61.1 ± 3.3[d]	77.5 ± 5.3[e]
10 mg/kg bodyweight	52.1 ± 2.3[c]	67.5 ± 1.2[d]	74.6 ± 2.1[e]	55.3 ± 1.8[c]	62.7 ± 1.3[d]	79.3 ± 5.2[e]

The results are mean ± SD for 5 rats. MDA and lipid hydroperoxide concentrations are expressed as nmol mg^{-1}protein. Values carrying superscripts different for each parameter are significantly different

Discussion

Antioxidant enzymes

Maintenance of normal cellular functions in the presence of oxygen largely depends on the efficiency of the defence mechanisms against free-radical-mediated oxidative stress (Sinha *et al.,* 2008). The enzymic antioxidant systems, which includes SOD, CAT, GSH-Px, GSH-Red, as well as glucose 6-phosphate dehydrogenase (Glc-6-PD), plays a coordinated role in the prevention of oxidative damage by ROS (O_2^- and H_2O_2) (Ajiboye *et al.,* 2010b). However, natural defences of the organism (enzymatic, non-enzymatic or dietary origin) are overwhelmed by an excessive generation of ROS.

Recent findings indicated that toxic

manifestations induced by pesticides may be associated with the enhanced production of reactive oxygen species (ROS), which give an explanation for the multiple types of toxic responses (EL-Gendy *et al.,* 2010), among which is oxidative damage of tissues and cellular macromolecules. Also, insecticides have been reported to induce production of reactive oxygen species and oxidative tissue damage **(**Bagchi *et al.,* 1995). Others have provided additional evidence for the occurrence of OP-induced oxidative tissue damage evidenced by DNA-strand breaks (Bagchi *et al.,* 1995), increased activities of antioxidant enzymes, and down-regulation of glutathione peroxidase activity and glutathione (Hai *et al.,* 1995).

The administration of DDVP at all doses produced a significant ($P < 0.05$) decrease in the activity of SOD (Table 1) in the brain all through the experimental period. This could result in gross accumulation of O_2^-, which can either initiate lipid peroxidation directly (Yen and Duh, 1994) or indirectly, through the product of its metabolism [singlet oxygen ($O^{\cdot}$) and hydroxy radical ($^{\cdot}OH$]. O_2^- accumulation could also inhibit catalase (Kono and Fridovich, 1982), exposing the cells and the cellular macromolecules to H_2O_2 mediated oxidative attacks. It could cause functional decline of oxidative phosphorylation, an increase in oxidative stress, and an increase in the rate of apoptosis (Lukaszewicz-Hussain *et al.,* 2004).

Although, the least reactive of the ROS, H_2O_2, diffuses throughout mitochondria and crosses cell membranes enabling it to inflict many types of cellular injury (Ray and Husain, 2002). Upon entry into cytosol, it interacts with cytosolic metal ions, such as iron or copper, to form highly toxic $^{\cdot}OH$, which often causes DNA alteration (Banmeyer *et al.,* 2005). It directly causes damage to the cell membrane by releasing arachidonic acid, which may be responsible for the prolonged damage in H_2O_2-treated cells even after H_2O_2 has been scavenged (Banmeyer *et al.,* 2005). The significant ($P<0.05$) reduction in the activity of catalase (Table 2) could have resulted from either accumulation of O_2^- or exhaustion of the enzyme. The consequential effect of this reduction includes: lipid peroxidation of cellular macromolecules, DNA alteration and redox homeostasis perturbation.

H_2O_2 and hydroperoxides are reduced by GSH-Px at the expense of GSH. Oxidized glutathione (GSSG) is reduced by GSH-Red in a NADPH-dependent reaction. The normal functioning of this closed system (redox cycle) maintains a high intracellular GSH:GSSG ratio and protects cells against oxidative damage (Kozer *et al.,* 2003). Thus, a significant ($P < 0.05$) reduction in the activities of GSH-Px and GSH-Red (Tables 3 & 4) following the treatment with DDVP could alter the normal functioning of redox cycle and expose the cellular macromolecules to oxidative attack of the ROS. Łukaszewicz-Hussain (2004) reported a similar decrease in the antioxidant enzymes activities in organophosphate toxicity.

Non-enzymic antioxidant

Non-enzymic antioxidant system is available to complement the activity of the enzymic antioxidant system in excessive oxidative stress by acting as a free radical, $O^{\cdot}$, O_2^-, $OH^{\cdot}$, as well as H_2O_2 scavenger and modulating enzymes *in vivo* (Ajiboye *et al.,* 2010b). Vitamins C and E play a significant role in the prevention of oxidative stress of cells (Pesh-Imam and Recknagel, 1977), as well as cellular macromolecules by acting as free radical scavengers. GSH functions by scavenging $O^{\cdot}$, O_2^- and $OH^{\cdot}$ (Singh and Ahluwalia, 2003). It protects lipids, proteins, as well as nucleic acids; from the attack of electrophilic compounds by attacking the electrophilic centre through its thiol group (-SH) (Ahluwalia *et al.,* 1996). The levels of these molecules (GSH, GSSG, total thiols, Vitamins C & E) in brain decreased significantly ($P< 0.05$) following the treatment of rats with DDVP. This decrease is similar to what was reported obtainable with Profenofos (Mansour *et al.,* 2009). The reduction in GSH level could affect the GSH: GSSG ratio, giving room for oxidative attack of cellular macromolecules.

Lipid peroxidation product

The brain has relatively poor antioxidant defense (Mates, 2000). It contains large amount of polyunsaturated fatty acids and consumes 20% of the body's oxygen (Travacio *et al.,* 2000). LPO

is the process of oxidative degradation of polyunsaturated fatty acids (PUFA) and its occurrence in biological membranes causes impaired membrane function, structural integrity, decrease in membrane fluidity and inactivation of a several membrane bound enzymes. It is plausible to speculate that DDVP treatment may result in peroxidation of PUFA, leading to the degradation of phospholipids and ultimately result in cellular deterioration. The present result strengthens this hypothesis and suggests that induction of oxidative stress is perhaps the central mechanism by which such tested pesticide exerts their cytotoxic effects (Khan and Kour, 2007). The dose dependent increase in MDA and LPO concentration of the DDVP treated animal's brain (Tables 7) may be the consequence of enhanced lipid peroxidation possibly by DDVP due to the high content of polyunsaturated fatty acid in the brain. This lipid peroxidation could be the consequential effect of antioxidant systems (enzymatic and non-enzymatic) depletion by DDVP.

Conclusion

The available data from this study revealed that DDVP brings about its toxicity through depletion of the antioxidant systems (enzymatic and non-enzymatic) and thus exposing the cells and cellular macromolecules to oxidative attacks by reactive oxygen species generated either from its metabolite or other *in vivo* means. It is thus recommended that the use of DDVP should be discourage because of the underlining toxicological implications.

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