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Effects of Dimethoate on Reproductive Organs, Reproductive Hormones and the Protective Role of Vitamin C in Female Albino Rat (Rattus norvegicus)

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INTRODUCTION

Background: Dimethoate (DM) (O, O-dimethyl-5-methyl carbomyol phosphorodithioate) is an organophosphate insecticide with a contact and systemic action. It is widely used against a broad range of insect pest and disease vectors such as mosquito and houseflies. The extensive use of dimethoate may pose a serious health hazards to animals and humans due to its persistence in soil and crops. In humans, the main risk groups to higher-dose dimethoate exposure include dimethoate producers, pesticide workers, farm owners and those that use it at home against mosquito and houseflies. Organophosphate pesticides may cause reproductive toxicity through several mechanisms, which may include direct damage to the structure of the cells, interference with biochemical processes necessary for normal cell function and biotransformation resulting in toxic metabolites (1). The complexities and chains of physiological conditions that surround the toxicity of organophosphates can elicit and maintain or exacerbate several pathological conditions (2). Hence the need arises for researchers to initiate and sustain researches of their effects on several organs and systems of the body.

Objective: The aim of this study was to evaluate the effects of dimethoate on reproductive organs, reproductive hormones and the protective role of vitamin c in female rats.

Experimental Chemicals

Dimethoate (40% EC) that was used in this study was manufactured by Jiangsu Tenglong Biological and Medical Co. Ltd., Jiangsu province, China and was procured from the Solutor Agro& Allied Chemicals Company, Shop A2, Mile 1 market, Port Harcourt. The vitamin C was purchased from a pharmaceutical store at Choba, Port Harcourt, while all other chemicals were obtained from the local distributors of scientific materials in Rivers State,s Nigeria.

Experimental Procedure

Sixty four (64) female rats were acclimatized for two weeks, weighed and later divided into eight groups of eight rats each and treated as follows: Groups two, three and four were treated with 8.86, 12.4 and 20.68mg/kg/day of dimethoate, and were later designated as low dose (LD), medium dose (MD) and high dose (HD) respectively. Group five was given the lose dose (LD) of dimethoate + 200mg/kg of vitamin C, group six was given the medium dose (MD) of dimethoate + 200mg/kg of vitamin C, group seven was given the high dose (HD) of dimethoate + 200mg/kg of vitamin C, group eight was given only the 200mg/kg of vitamin C, while group one was given 1ml of distilled water per rat (to serve as the control). All dimethoate (DM) and vitamin C (VC) doses were dissolved in distilled water and given to the rats via oral gavage for fourty two (42) consecutive days. After treatment, some animals were sacrificed, dissected, and the blood samples were taken for hormonal analysis, while vital reproductive/body organs were carefully collected, weighed and recorded.

Serum Collection

At the end of the exposure period (43rd day), the animals were anaesthesized by exposing them to excess dose of chloroform for about 2-3 minutes. Then with the aid of a syringe with needle, blood samples were collected by cardiac puncture into properly labeled lithium heparin bottles and taken to the laboratory. Within 20-30 minutes of blood

collection, the sera samples were drawn from the blood after centrifugation at 350 rpm for 10 minutes (using the Hereaeus Labofuge 400R, Kendro Laboratory products GmbH, Germany). The sera were then kept in a deep freezer at -20^{0}C for hormonal analysis.

Hormonal Analysis: The reproductive hormones that were assayed include estrogen (estradiol), progesterone, follicular stimulating hormone (FSH) and luteinizing hormone (LH). Serum hormones were determined by the solid phase Enzyme -Linked Immunosorbent Assay (ELISA), using specifically produced Enzyme Immunoassay Test Kit, based on the principle of competitive binding between the hormone in the test specimen and its conjugate for a fixed number of binding sites in rabbit & mouse antibody-coated microwells.

Organs Collection and Weighing

After the blood collection, the killed animals were dissected and vital reproductive and body organs were carefully removed, stripped from fatty tissues and blood vessels with the aid of a dissecting blade, forceps and scissor. Organs were then immediately weighed and recorded, using a digital weighing balance (Golden-Mettler USA: Model-MEAS: 217X115X317).

Protective Role of Vitamin C on Dimethoate Toxicity

The ameliorative properties of vitamin C (VC) on dimethoate toxicity of the female rats was determined by comparing the weight of vital reproductive/body organs and the concentration levels of all the analyzed hormones in the vitamin C treated groups (groups five, six, seven and eight) with those of the dimethoate treated groups (groups two, three and four) and the control (group one). They were examined to find out whether the vitamin C administration was able to restore the dimethoate induced levels of the analyzed hormones and the weight of reproductive/body organs to normal levels.

Statistical Analysis

The data were expressed as mean±SEM. Statistical analysis were carried out using the SPSS (Statistical Package for Social Science) version 22 and the One-Way Analysis of Variance (ANOVA). The significant differences were considered between the groups at a significant level of $P<0.05$.

RESULTS AND DISCUSSION

Weight of body organs

The present study revealed that the treatment of rats with dimethoate insecticide for 42 days at doses of 8.86, 12.4 and 20.68mg/kg/day caused significant ($P<0.05$) reduction in the mean weight of body organs including the kidney, heart, liver, lungs and spleen as compared with the control. The study also showed that the reduction in mean weight of body organs was more drastic with increase in dose level of the dimethoate. These results are in accordance with those of (3) and (4) who posited that the reductions in the weight of body organs and body weight were sensitive indices of toxicity after exposure to toxic substances. However, there was significant increase ($P<0.05$) in the mean weight of body organs in the groups treated with dimethoate + vitamin C whereas there was no significant difference between the control (CT) and group eight (VC) (Table 1).

The increase in the mean weight of body organs in the dimethoate + vitamin C treated groups may be due to the ameliorative effect of the vitamin C on dimethoate toxicity. High mean weights of body organs were recorded in the control (CT) and group eight (VC) whereas the least mean weight was reported in group four (HD). The high weights reported in the control indicates the normal weight of the body organs while the high weight recorded in group eight (VC) may be due to the non-toxic/ ameliorative effect of the vitamin C (5). Also, the drastic reduction in the mean weight of body organs in group four (HD) indicates a dose dependent toxic effect of dimethoate on the body organs (6).

Table-1: Effects of dimethoate and vitamin C on the weight of body organs

Treatment groups	Weight of body organs (g)				
	Kidney	Heart	Liver	Lungs	Spleen
GRP 1 (CT)	0.68± 0.08^{c}	0.78± 0.03^{d}	7.78± 0.51^{c}	0.73± 0.05^{d}	0.60±0.00^{c}
GRP 2 (LD)	0.58± 0.03^{bc}	0.60± 0.04^{b}	6.65± 0.13^{cd}	0.58± 0.05^{bc}	0.53± 0.03^{bc}
GRP 3 (MD)	0.53± 0.03^{ab}	0.60± 0.00^{b}	5.53± 0.28^{ab}	0.53± 0.03^{ab}	0.48± 0.03^{b}
GRP 4 (HD)	0.43± 0.030^{a}	0.45± 0.03^{a}	4.60± 0.17^{a}	0.40±0.04^{a}	0.38± 0.03^{a}
GRP 5 (LD+VC)	0.65± 0.05^{bc}	0.70± 0.04^{bc}	6.98± 0.52^{cb}	0.65± 0.03^{cd}	0.53± 0.03^{bc}
GRP 6 (MD+VC)	0.60± 0.00^{bc}	0.68± 0.03^{bc}	6.48± 0.21^{bc}	0.55± 0.03^{bc}	0.48± 0.03^{b}
GRP 7 (HD+VC)	0.58± 0.03^{bc}	0.65± 0.03^{bc}	6.43± 0.15^{bc}	0.50± 0.00^{ab}	0.45± 0.03^{b}
GRP 8 (VC)	0.70± 0.06^{c}	0.73± 0.03^{d}	7.65± 0.39^{de}	0.73± 0.05^{d}	0.58± 0.03^{c}

Each value represents the mean±SEM, values with same superscript letters are not significantly different at $P< 0.05$ compared with the control group. GRP = Group, CT = Control, LD = Low dose, MD = Medium dose, HD = High dose, VC = Vitamin

Weight of reproductive organs

Significant decrease (P<0.05) in mean weights of the ovary, uterus and fallopian tube were reported in the dimethoate treated groups compared to the control whereas a significant increase was recorded for the groups that received the dimethoate + vitamin C as compared with the groups that received only the dimethoate. The decrease in the weight of reproductive organs suggests the toxic effect of dimethoate on the reproductive organs. These results are in agreement with the result of (7) who observed that the weight of female reproductive organs of mice were significantly decreased with thiocarb treatment, and that of (8) who recorded a reduction in the weight of pituitary gland and ovary in parental and FI females treated with methoxychlor.

The increase in mean weight of reproductive organs that was observed in the dimethoate + vitamin C treated groups as compared to the dimethoate treated rats can be attributed to the protective effect of vitamin C, which has also been reported by other investigators (5, 9). No significant difference (P<0.05) was reported between the control (CT) and group eight (VC) (Table 2 and Figure 2) and this could be due to the non-toxic effect of the vitamin C to the reproduction organs.

Table-2: Effects of dimethoate and vitamin C on the weight of reproductive organs

Treatment groups	Weight of reproductive organs (g)		
	Ovary	Uterus	Fallopian tube
GRP 1 (CT)	0.38± 0.03^{d}	1.70± 11^{d}	0.90± 0.04^{c}
GRP 2 (LD)	0.23± 0.03^{b}	1.43± 0.14^{cd}	0.83± 0.05^{ab}
GRP 3 (MD)	0.18± 0.01^{b}	1.13± 0.09^{b}	0.58± 0.11^{b}
GRP 4 (HD)	0.13± 0.01^{a}	0.83± 0.05^{a}	0.16± 0.02^{a}
GRP 5 (LD+VC)	0.33± 0.03^{cd}	1.60± 0.09^{d}	0.60± 0.17^{b}
GRP 6 (MD+VC)	0.28± 0.03^{bc}	1.50± 0.04^{cd}	0.65± 0.10^{bc}
GRP 7 (HD+VC)	0.28± 0.03^{bc}	1.25± 0.10^{bc}	0.63± 0.09^{bc}
GRP 8 (VC)	0.38± 0.03^{d}	1.73± 0.09^{d}	0.90± 0.04^{c}

Each value represents the mean±SEM, values with same superscript letters are not significantly different at P< 0.05 compared with the control group. GRP = Group, CT = Control, LD = Low dose, MD = Medium dose, HD = High dose, VC = vitamin C.

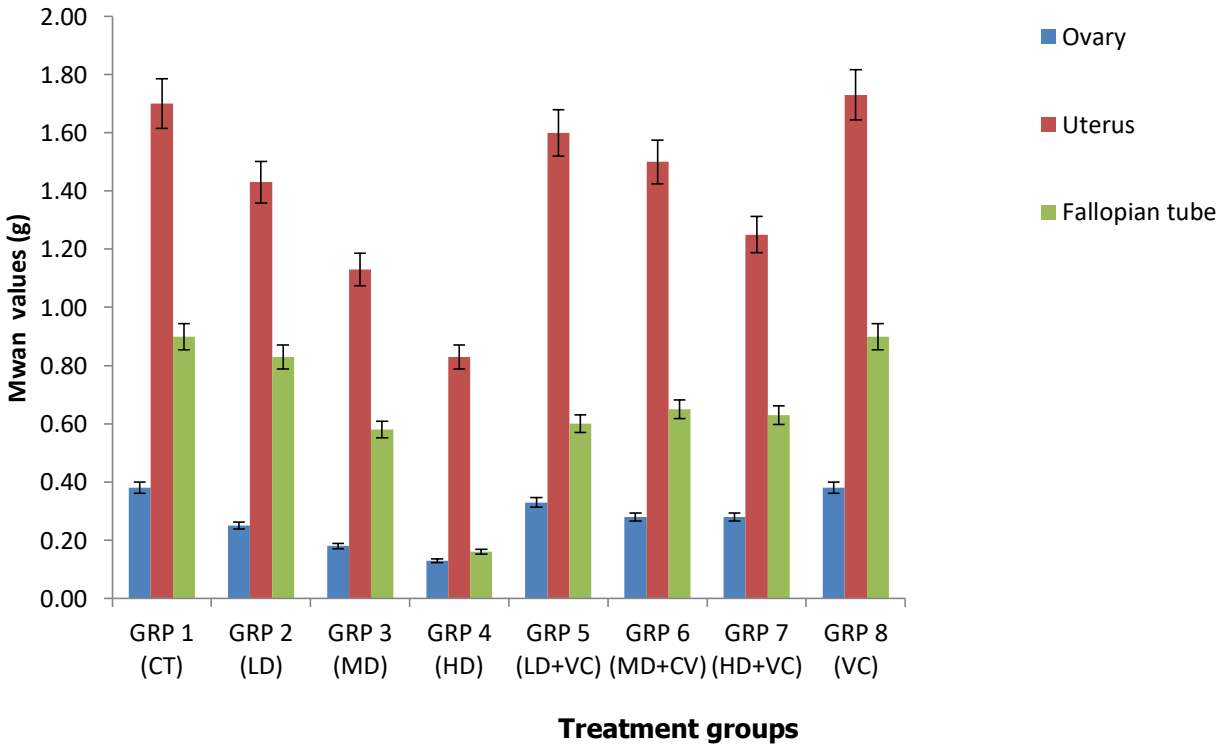

Figure-1: Effects of dimethoate and vitamin C on weight of reproductive organs.

Reproductive hormones in the serum

There was significant decrease (P< 0.05) in serum levels of follicular stimulating hormone, luteinizing hormone, estrogen and progesterone in the dimethoate treated groups as compared to the control. The observable decrease may be due to the direct influence of dimethoate on the hypothalamic and pituitary function, which affects the secretion of these hormones (10). Similarly, hormonal balance, which is the proper level of hormones, is important to preserve female reproduction and maintain fertility. This balance can be disturbed by the changing levels of estrogen or progesterone. The results of this study are consistent with the results of other previous studies on pesticides (11, 12).

Table-3: Effects of dimethoate and vitamin C on reproductive hormones in the serum

Treatment groups	Reproductive hormones			
	FSH (mlu/ml)	LH (mu/ml)	Estrogen (pg/ml)	Progesterone (ng/ml)
GRP 1 (CT)	11.13± 2.04^{c}	30.37± 3.06^{c}	62.33± 6.12^{c}	17.40± 2.20^{c}
GRP 2 (LD)	9.17± 0.49^{bc}	22.97± 2.58^{b}	50.33± 2.03^{b}	15.03± 0.97^{cd}
GRP 3 (MD)	6.03± 0.67^{b}	17.03± 0.84^{a}	43.67± 3.18^{ab}	10.57± 0.76^{ab}
GRP 4 (HD)	2.10± 0.06^{a}	13.27± 0.69^{ab}	39.67± 1.20^{a}	7.30± 0.52^{a}
GRP 5 (LD+VC)	9.87± 0.81^{bc}	24.8± 2.34^{bc}	51.33± 2.40^{b}	15.63± 0.97^{cd}
GRP 6 (MD+VC)	5.73± 0.37^{b}	17.23± 0.41^{a}	47.00± 2.52^{ab}	11.60± 0.86^{ab}
GRP 7 (HD+VC)	4.77± 0.15^{ab}	15.27± 1.16^{a}	45.33± 0.88^{ab}	11.07± 0.74^{b}
GRP 8 (VC)	10.8± 1.70^{C}	28.27± 1.95^{bc}	62.33± 4.26^{c}	16.77± 1.10^{c}

Each value represents the mean±SEM, values with same superscript letters are not significantly different at P<0.05 compared with control group. FSH = Follicular stimulating hormone, LH = luteinizing hormone.

The present study also showed a slight increase in serum levels of these hormones in the dimethoate + vitamin C treated groups when compared with their respective dimethoate treated groups, and the slight increase may be due to effort of the vitamin C to normalize the serum level of these hormones with that of the control (13). Also, the lowest values of the reproductive hormones were reported in the high dose (HD) group of the dimethoate treated groups (Table 3), which showed a dose dependent toxicity of the dimethoate. This is in agreement with the results obtained by (4).

Methoxychlor insecticide has also been shown to reduce luteinizing hormone secretion and reforms serotonin and norepinephrine at the hypothalamic level, which indicates that methoxychlor possess the potential to alter the hypothalamic-pituitary-gonadal axis which inhibits progesterone synthesis in granulosa cells. This inhibits estradiol-17$_{\beta}$ stimulated progesterone synthesis (6).

CONLCUSION

This study revealed that the sub chronic exposure to dimethoate insecticide at dose levels of 8.86, 12.4 and 20.68mg/kg/day induces reproductive toxicity in female rats manifested by decreases in weight of vital body organs, reproductive organs and serum level of reproductive hormones of female rats. However, the ultimate effects were observed in the high dose (HD) group. In contrast, co-administration of vitamin C with the insecticide antagonizes the reproductive toxicity. Based on the study observations, it is therefore proposed that vitamin C may provide a cushion for prolonged therapeutic options against insecticide-induced reproductive toxicity without harmful side effects.

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COMPETING INTERESTS

The authors have decleared that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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