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Ovarian Oxidative Damage and Histological Changes Induced by Dimethoate and the Protective Role of Vitamin C in Female Albino Rat (Rattus norvegicus)

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Abstract

The ovarian oxidative damage and histological changes induced by dimethoate and the protective role of vitamin C were investigated using the female albino rat (Rattus norvegicus) as test animals. During the studies, 64 female rats were 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 20.68 mg/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 low dose (LD) + 200mg/kg of vitamin C, group six was given the medium dose (MD) + 200mg/kg of vitamin C, group seven was given the high dose (HD) + 200mg/kg of vitamin C, group eight was given only 200mg/kg of vitamin C, while group one was given 1ml of distilled water per day, which served as the control (CT). All dimethoate and vitamin C doses were dissolved in distilled water and given to the rats via oral gavage for fourty two (six weeks) consecutive days. After treatment, some animals were sacrificed, dissected, and the ovary and uterus tissue samples were taken for oxidative stress and histological studies. Results of the ovarian oxidative stress molecules in all the dimethoate treated groups were significantly ($P<0.05$) different from the control while those in the dimethoate + vitamin C treated groups were insignificantly different from their respective dimethoate treated groups. The histological studies showed that ovarian follicles were severely (+++) damage while the endometrial glands and blood capillaries were extremely (++++) damaged in the uterine architecture, with no remarkable protective effect from the vitamin C treatment. It is hereby recommended, following the above results, that maternal exposure to insecticides should be avoided.

Keywords: Ovary, uterus, oxidative damage, histological changes, rat, dimethoate, vitamin c

INTRODUCTION

Oxidative stress is a state of an imbalance between reactive oxygen species (ROS) or free radicals and antioxidants in the body, which can lead to cell and tissue damage. Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen that play several beneficial roles for the organism [1]. At low/moderate concentrations, they are needed for physiological activities such as intracellular cell signaling and homeostasis, cell death, immune defense against pathogens and induction of mutagenic response [2, 3]. These free radicals are produced endogenously as natural by-products of the normal aerobic cellular metabolism. Additionally, they can be induced by exogenous sources such as ionizing radiation, lifestyle, diet, stress, pesticides and smoking.

Maintaining equilibrium between the reducing and oxidizing status is crucial for proper physiological functions; therefore, living organisms are equipped with antioxidant defense systems, consisting of both enzymatic and nonezymatic antioxidants to regulate the levels of these free radicals [4, 5]. An imbalance between the production of ROS and the ability of the antioxidant systems to readily detoxify these reactive intermediates results in oxidative stress. Free radicals generated in excessive and uncontrollable amounts under oxidative stress conditions cause damage to DNA, proteins and lipids, which can severely compromise cell health and contribute to disease development [6, 7]. Indeed in the

past years, considerable research has demonstrated that oxidative stress is involved in the natural process of aging as well as a wide variety of human diseases, including neurodegenerative disorders; multiple sclerosis, cardiovascular disease, rheumatoid arthritis and cancer [8, 9].

Reactive oxygen species (also known as free radicals) are highly reactive chemical molecules formed due to the electron acceptability of O2. The spectrum of reactive oxygen species that are considered responsible for biological oxygen toxicity include the intermediates of the partial reduction of oxygen such as superoxide (O2.), hydrogen peroxide (H2O2), and other reactive species like hydroxyl radical (HO.), peroxyl radical (ROO.), lipid peroxide (ROOH), alkoxy radical (RO.), hypochlorous acid (HOCl), nitric oxide (NO), peroxinitrite (ONOO.), nitrogen dioxide (NO2) and singlet oxygen (102). The reduction of molecular oxygen (O2) which produces superoxide (O2.), serves as the precursor of most other reactive oxygen species. Dismutation of superoxide in turn may be partially reduced, thus forming hydroxide ion and hydroxyl radical (HO.), or fully reduced to water [10, 11]. Free radicals also include the end products of lipid peroxidation, which are reactive aldehydes. Compared with other free radicals, aldehydes are highly stable and diffuse out from the cell and attack targets far from the site of their production. About 32 aldehydes were identified as products/metabolites of lipid peroxidation, which include: (a) saturated aldehydes e.g. propanal, butanal, hexanal, octanal, and decanal- the most important; (b) 2, 3-Trans- unsaturated aldehydes e.g. hexenal, octenal, nonenal and decenal; (c) a series of 4-hydroxylated, 2, 3- trans-unsaturated aldehydes e.g. 4-hydroxylalkenal (HNE) and malondialdehyde (MDA).

Malondialdehyde (MDA) was considered for a long time as the most important lipid peroxidation metabolite [12]. However, recent studies have demonstrated that 4-hydroxyalkenal can also do the same. They cause cellular damage and produce different effects by acting as intracellular signals, able to modulate gene expression, cell proliferation, differentiation and apoptosis. The hydroxyl-group close to a carbonyl-group present in there chemical structure is related to this high reactivity with different targets.They are easily diffusible species, but the biological effect depends on the molecule target and behaviour as a signal to produce the damage [13].

The biological effects of excess levels of the spectrum of these species are quite similar, and that is the reason they are collectively known as reactive oxygen species (ROS) [14]. However, in specific terms, the denominations “reactive oxygen species” (ROS) is referring to the three chemical species (O2., H2O2 and HO.), the products of the partial reduction of oxygen. Similarly, the denominations “reactive nitrogen species” (RNS) is loosely referring to the three chemical species (NO, ONOO. and NO2) [15]. The reference as a whole to either group, ROS and RNS, is usually made to explain or to refer to their biological activity, what reflects the fact that each group is auto-propagated in biological systems from their promoters, O2 and NO [16]. However, the sources of ROS production in animals may be divided into endogenous and exogenous sources.

Endogenous sources of ROS (free radicals) in animal cells may be through respiration, immune cell signaling via NADPH-oxidase (Nox) pathway, lipid peroxidation and lipid nitration. Exogenous Sources include several lifestyle and environmental factors that promote excessive free radical formation and oxidative stress, which may include pollutants, ozone, cigarette smoke, alcohol intake, certain pesticides and cleaners, stress, radiation, heavy metals [17], diet high in sugar and fats, excessive sunbathing, bacteria, fungal and viral infections, intense and prolonged exercise, drugs and excessive intake of antioxidants [11]. Ionizing radiation, for instance, can generate damaging intermediates through the interaction with water, a process known as “radiolysis”. Since water comprises 55-60% of human body, the probability of radiolysis is quite high under the presence of ionizing radiation. In the process, water loses an electron and becomes highly reactive. Then through a three-step chain reaction, water is sequentially converted to hydroxyl radical ($^{\cdot}OH$), hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\cdot-}$), and ultimately oxygen (O_2). The hydroxyl radical is extremely reactive and immediately removes electrons from any molecule in its path, turning that molecule into a free radical and this propagating a chain reaction. However, hydrogen peroxide is actually more damaging to DNA than the hydroxyl radical, since the lower reactivity of the hydrogen peroxide provides enough time for the molecule to travel into the nucleus of the cell, subsequently reacting with macromolecules such as DNA [11].

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cell of organisms. Antioxidant terminates these chain reactions. Antioxidants are substances that neutralize or remove free radicals by donating an electron to a free radical without making themselves unstable. This causes the free radical to stabilize and become less reactive. The neutralizing effect of antioxidants helps protect the body from oxidative stress [18]. Antioxidants may be classified into two broad groups; depending on weather they are soluble in water (hydrophilic) or lipids (lipophilic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation [18].

These compounds may be synthesized in the body or obtained from the diet [19]. The different antioxidants are present in a wide range of concentrations in body fluids and tissues, with some such as glutathione mostly present within cells, while others such as uric acid are more evenly distributed [20]. The relative importance and interactions between these different antioxidants is a very complex question, with the various antioxidant compounds and antioxidant enzyme systems having synergistic and interdependent effects on one another [18]. The action of one antioxidant may therefore depend on the proper function of other members of the antioxidant system. The amount of protection provided by any one antioxidant will also depend on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts [19]. Some compounds contribute to antioxidant defense by chelating transition metals and preventing them from catalyzing the production of free radicals in the cell. Selenium and zinc are commonly referred to as antioxidant nutrients, but these chemical elements have no antioxidant action themselves, but are instead required for the activity of some antioxidant enzymes [21]. However, some examples of antioxidants include the antioxidant enzymes, uric acid, vitamin C and vitamin A.

Antioxidant enzymes include superoxide dismutases, catalases, glutathione and peroxidases [18]. Superoxide dismutases (SOD) are a class of closely related enzymes that catalyze the breakdown (dismutation) of superoxide into oxygen and hydrogen peroxide [22]. SOD enzymes are an important antioxidant defense in nearly all cells exposed to oxygen (aerobic cells) and in intracellular fluids [23]. Catalases (CAT) are enzymes that catalyze the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor [24]. Alutathione is an antioxidant in plants, animals, fungi and some bacteria. Glutathione is a cysteine-containing peptide found in most forms of aerobic life. It is not required in the diet and is instead synthesized in cells from its constituent amino acids [25]. Glutathione is capable of preventing damage to important cellular compounds caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides and heavy metals [26]. In cells, glutathione exists in reduced (GSH) and oxidized (GSSG) states. The ratio of reduced glutathione to oxidized glutathione within cells is a measure of cellular oxidative stress [27] where increased GSSG-to-GSH ratio is indicative of greater oxidative stress. In healthy cells and tissues, more than 90% of the total glutathione pool is in the reduced form (GSH), with the remainder in the disulfide form [28]. The oxidized state is converted to the reduced state by NADPH, which is catalyzed by glutathione reducetase [29]. GSH protects cells by neutralizing (i.e. reducing) reactive oxygen species [30]. This conversion is illustrated by the reduction of peroxides and free radicals. Glutathione is also employed for the detoxification of methylglyoxal and formaldehyde, toxic metabolites produced under oxidative stress. Glutathione 5-transferase enzyme catalyzes its conjugation to lipophilic xenobiotic, facilitating their excretion or further metabolism [31].

Ascorbic acid or vitamin C is a monosaccharide oxidation-reduction (redox) catalyst found in both animals and plants. Most animals including humans have lost by mutation during evolution, the ability to produce ascorbic acid in their bodies, so they must obtain it from their diet; it is therefore a dietary vitamin [32]. Ascorbic acid is a redox catalyst which can reduce, and thereby neutralize reactive oxygen species such as hydrogen peroxide [33]. In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the redox acerbates peroxidase, a function that is used in stress resistance in plants [34]. It is impossible to completely avoid free radical exposure and oxidative stress. However, the effects of oxidative stress can be minimized in the body by increasing the levels of antioxidants and decrease the formation of free radicals. This can be achieved by obtaining enough antioxidants through diets such as fruits and vegetables, and through health lifestyle choices. Examples of dietary antioxidant sources include berries, cherries, citrus fruits, carrots, tomatoes, turmeric, green tea, onion, garlic, cinnamon, fish, nuts, vitamin C, vitamin E and healthy lifestyles such as regular exercise [35].

Large number of xenobiotic has been identified to have the potential to generate free radicals in biological systems [36], as their means of toxicity. Some of these free radicals interact with various tissue compounds, resulting in dysfunction. The liver plays a central role in the detoxification process and faces the threat of maximum exposure to xenobiotic and their metabolic by-products. The susceptibility of liver on other body issues to this stress due to exposure to pesticides is a function of the overall balance between the degree of oxidative stress and the antioxidants capability [37]. Reactive oxygen species (ROS) have been implicated in liver and nervous system toxicity by several OPls [38] and is associated with lipid peroxidation and phospholipids degradation [39]. Previous studies indicate that OPls exert their biological effects through electrophilic attack on the cellular constituents of hepatic and brain tissues with simultaneous generation of reactive oxygen species [40]. The oral administration of dimethoate (DM) to quinoa pigs caused liver and other cells damage, leading to the secretion of hepatocytic and serum enzymes, which may serve as biomarkers of liver/cellular damage [41]. This study is aimed at evaluating the ovarian oxidative damage and histological changes induced by dimethoate and the protective role of vitamin c in female albino rats.

MATERIALS AND METHODS

Animals

Sixty four (64) adult virgin female Albino Wister rats (R. norvegicus) weighing 163.35±19.99g were purchased for the studies. Animals were purchased from the Animal House, Faculty of Pharmaceutical Sciences and housed in the Animal

and Environmental Biology Department Animal House, University of Port Harcourt. Animals were housed in plastic cages under controlled hygienic and environment conditions. Animals were fed with a standard laboratory rodent diet and water ad libitum, throughout the period of experimentation.

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 local chemist store at Choba, Port Harcourt, while all other chemicals were obtained from the local distributors of scientific materials in Rivers State, Nigeria.

Experimental Procedure

The 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 six weeks (42 days). At the end of the treatment (43^{rd} day), animals were sacrificed, dissected and ovary tissue samples were taken for the ovarian oxidative stress analysis and histological studies.

Preparation of Ovarian Homogenates

Ovarian homogenates were prepared from excised ovarian tissues. After weighing, the excised ovarian tissues were carefully washed with distilled water for the removal of blood and fatty parts. Tissues were then homogenized in 50mM of ice-cold sodium phosphate buffer (pH 7.4) containing 0.1mM of ice of ethylene-diamine-tetraacetic acid (EDTA), using the Potter-Elvehejem homogenizer. Thereafter, the homogenates were centrifuged at 3000 rpm at 4^0C for 15 minutes to remove debris, and the supernatant stored in the form of aliquots at -20^0c for the analysis of oxidative stress molecules including malondialdehyde (MDA), catalase (CAT), glutathione (GSH) and superoxide dismutase (SOD).

Ovarian Oxidative Stress Analysis

Ovarian level of malondialdehyde (MDA) was determined by the thiobarbituric acid reaction method, based on the principle that MDA reacts with thiobarbituric acid to produce a pink coloured product (chromatogen) which can be measured spectrophotometrically. Catalase activity was also determined by the spectrophotometric method. The activity of reduced glutathione (GSH) was determined according to (42) method, based on the principle that GSH react with Ellman's reagent to produce a yellow product. While the activity of superoxide dismutase was determined by the spectrophotometric method, based on the principle that SOD reacts with superoxide ($O_2^{\cdot}$) to inhibit the oxidation of epinephrine to form adrenochrome (a pink coloured compound that polymerize into brown or black melanin, due to further oxidation).

Histological Examination of the Ovary and Uterus

Ovaries and uteri of the control, dimethoate and vitamin C treated rats were carefully removed and fixed in 10% buffered formalin for 48 hours in properly labeled test tubes. The tissues were grossed and the best portions were put into labeled tissue cassettes and transferred to 95% alcohol for 2 hours for dehydration. Tissues were passed through xylene II for 2 hours to remove the alcohol and later embedded with paraffin wax and cut into sections of 2 to 3 µm thickness, using the microtome. Then sections were picked unto slides, allowed to drain, arranged on a slide rack and gently warmed for 10 minutes, using the hot air oven.

Thereafter, the slides were stained with haematoxylin for 10 minutes, rinsed in running tap water for 5 minutes and counter stained with eosin for 3 minutes. Slides were again dehydrated in absolute alcohol and then cleared in xylene and the ovary and uterus slides were examined for histological changes, if any, using the light microscope, while the microphotographs of the slides were taken, using the Moticam1000 camera at 100x magnification.

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 concentration levels of all the analyzed oxidative sress parameters in the vitamin C treated groups (groups five, six, seven & eight) with those of the dimethoate treated groups (groups two, three and four) and the control (group one). The histopathological changes of the ovary and uterus across the eight groups were also examined to find out whether the vitamin C administration was able to restore the dimethoate induced levels of the analyzed parameters and the histopathological changes of the ovary and uterus to normal levels.

Statistical Analysis

The data were expressed as mean±SEM. Statistical analysis were carried out using the SPSS (Satistical Package for Social Science) version 22 and Microsoft Excel version 10. Data from experiments with more than two independent variables were analysed using the One Way Ansylsis of Variance (ANOVA) followed by the Least Signaificant Difference (LSD) test. The significant diference was considered between the groups at a significant level of P<0.05.

RESULTS

Malondialdehyde (MDA) mean values in the ovarian homogenates showed a dose related increase (P< 0.05) in the dimethoate treated groups compared to the control whereas an insignificant decrease was observed in the dimethoate + vitamin C treated groups when compared to the groups that received only the dimethoate. There was no significant difference between the control (CT) and group eight (VC) with reported values of 2.10 nmol/ml and 2.24 nmol/ml respectively. The highest mean value of 3.15 nmol/ml malondialdehyde was recorded in group four (HD) whereas there was no significant difference between groups three (MD), five (LD+VC) and seven (HD+VC) (Table 1; Fig. 1). Catalase (CAT) activity was significant decreased (P< 0.05) in a dose dependent fashion in the dimethoate treated groups as compared to control, but significant increase in the groups that received the dimethoate + vitamin C as compared with those that received the dimethoate only. No significant difference was observed between groups two (LD) and six (MD+VC), and between the control (CT) and group eight (VC). The highest values of 181.40 nmol/mg and 189.83 nmol/mg were recorded in the control and group eight respectively, while the lowest value of 60.32 nmol/mg was reported in group four (HD). Activity of reduced glutathione (GSH) was significantly inhibited (P< 0.05) in the dimethoate treated groups when compared to the control group (CT), but insignificantly increased in the groups that received the dimethoate + vitamin C as compared with those that received dimethoate only. The highest mean value of 611.33 nmol/mg that was reported in the control group represents the normal activity level of glutathione in the control group, which was not significantly different from the glutathione activity level in group eight (VC) which had a mean value of 608.67 nmol/mg. There was significant decrease (P< 0.05) in the activity of superoxide dismutase (SOD) in the groups that received dimethoate as compared with the control whereas an insignificant increase in the activity level of superoxide was reported in the groups that received the dimethoate + vitamin C when compared with those that received only the dimethoate. A dose related decrease in the activity level superoxide dismutase was recorded between groups two (LD), three (MD) and four (HD) with the lowest activity level of 156.00 nmol/mg reported in group four (HD). There was no significant difference between the control CT) and group eight (VC) and between groups two (LD) and five (LD+VC). The highest activity levels were reported in the control and group eight with reported mean values of 244.00 nmol/mg and 253.00 nmol/mg respectively (Table 1; Fig. 2).

Table 1: Effects of dimethoate and vitamin C on oxidative stress molecules in the ovary

Treatment groups	Oxidative stress molecules			
	MDA (nmol/ml)	CAT (nmol/mg)	GSH (nmol/mg)	SOD (nmol/mg)
GRP 1 (CT)	2.10± 1.18^{a}	181.40± 2.59^{cd}	611.33± 64.84^{a}	244.00± 59.43^{a}
GRP 2 (LD)	2.01± 0.20^{a}	148.33± 15.70^{b}	492.33± 24.37^{ab}	207.67± 49.19^{bc}
GRP 3 (MD)	2.38±1.13^{c}	70.30± 10.32^{ab}	454.00± 22.65^{b}	194.67± 41.70^{ab}
GRP 4 (HD)	3.15± 1.91^{ab}	60.32± 5.96^{a}	454.00± 12.08^{b}	156.00±23.44^{b}
GRP 5 (LD+VC)	2.41± 1.42^{c}	159.23± 10.83^{bc}	509.67± 14.38^{ab}	215.00±49.49^{bc}
GRP 6 (MD+VC)	2.26±0.32^{a}	150.97± 8.10^{b}	498.00± 8.50^{ab}	197.00± 46.29^{ab}
GRP 7 (HD+VC)	2.39±0.55^{c}	170.80±8.26^{bcd}	440.33± 70.05$^{b`}$	175.33± 28.75^{c}
GRP 8 (VC)	2.24±0.49^{a}	189.83± 3.90^{cd}	608.67± 22.24^{a}	253.00± 67.36^{a}

Each value represents the mean±SEM, values with same superscript letters are not significantly different at P<0.05 compared with the control group. MDA = Malondialdehyde, CAT=Catalase, GSH = Glutathione, SOD = Superoxide dismutase.

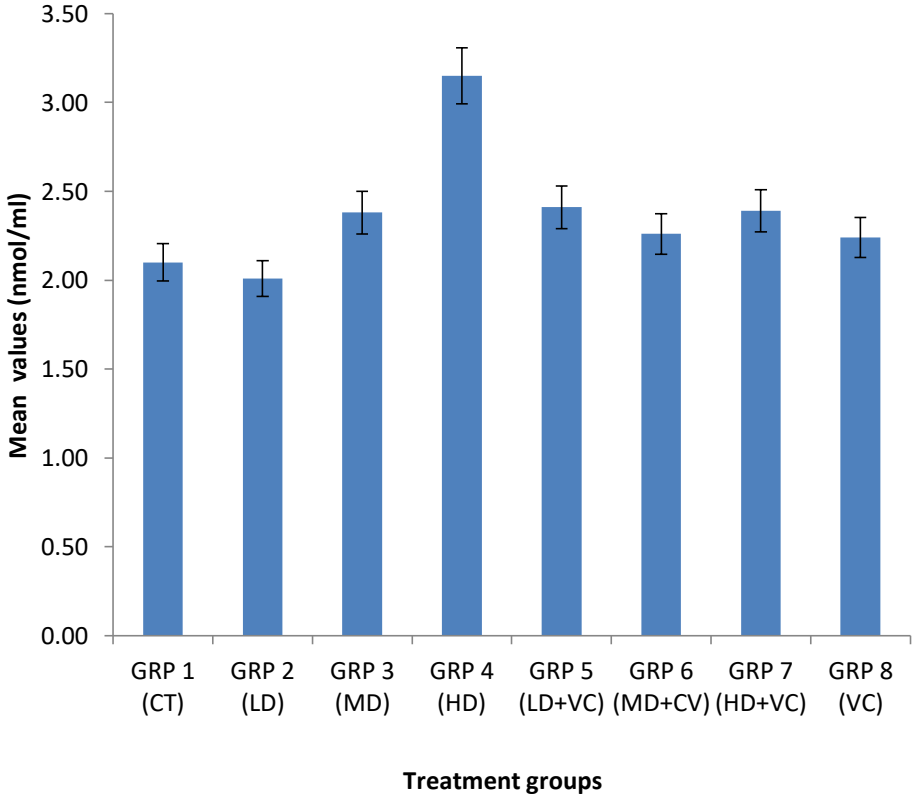

Fig. 1: Effects of dimethoate and vitamin C on the mean values of malondialdehyde (MDA) in the ovary.

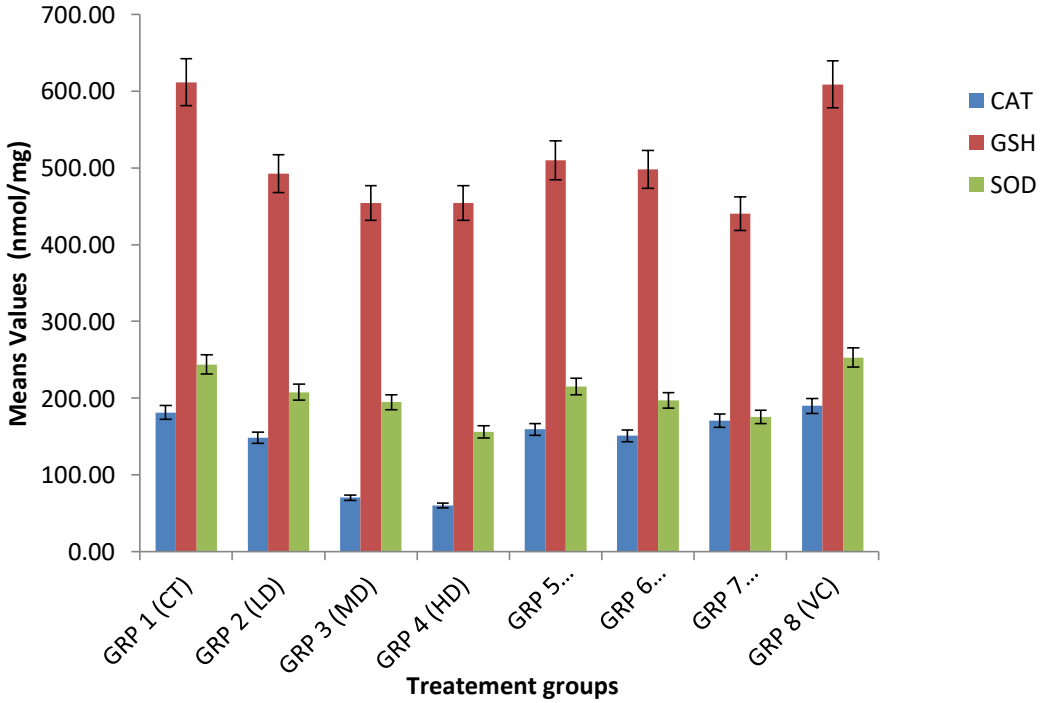

Fig. 2: Effects of dimethoate and vitamin C on the mean values of antioxidant enzymes in the ovary

There was no reported effect (-) on the examined ovarian parameters in the control (CT) and group eight (VC), except the mild effects (+) on graafian and secondary follicles that were reported in group eight (VC). There was no effect on the ovarian cortex, medulla and blood vessels for all the groups. However, the corpus lutea were severely (+++) affected in groups three (MD) and five (LD+VC) while atretic follicles were severely (+++) affected in groups six (MD+VC) and seven (HD +VC). Graafian follicles were severely (+++) affected in groups four (HD) and six (MD+VC). The effect on primary follicles was extremely severe (++++) in group four (HD) and mildly severe (++) in groups two (LD), five (LD+VC) and seven (HD+VC) (Table 2). There was no reported effect (-) for the examined parameters in the uterine architecture in groups one (CT), two (LD), six (MD+VC) and eight (VC). There was also no reported effect on the uterine endometrium and myometrium across the eight groups. However, an extremely severe (++++) effect for endometrial glands was reported in group four (HD) followed by group five (LD+VC) which was severely (+++) effected. Blood capillaries were extremely affected (++++) in group seven (HD+VC) only, but without any effect for all the other groups for the blood capillaries (Table 3; Fig. 3).

Table 2: Severity of histological effect of dimethoate and vitamin C on the ovarian parameters

Ovarian parameters	**Treatment groups**							
	GRP 1 (CT)	**GRP 2 (LD)**	**GRP 3 (MD)**	**GRP 4 (HD)**	**GRP 5 (LD+VC)**	**GRP 6 (MD+VC)**	**GRP 7 (HD+VC)**	**GRP 8 (VC)**
Corpus lutea	–	+	+++	+	+++	+	+	–
Atretic follicles	–	–	+	++	+	+++	+++	–
Graafian follicles	–	+	++	+++	++	++	++	+
Primary follicles	–	+	+++	++++	+	++	+	–
Secondary follicles	–	+	+	+++	+	+++	+	+
Ovarian cortex	–	–	–	–	–	–	–	–
Medulla	–	–	–	–	–	–	–	–
Blood vessels	–	–	–	–	–	–	–	–

GRP = Group, CT = control, LD = Low dose, MD = Medium dose, HD High dose, VC = Vitamin C. –= Normal, + = Mild, ++ = Moderate, +++ = Severe, ++++ = Extremely severe.

Table 3: Severity of histological changes in the uterine architecture of rats

Uterine architecture	**Treatment groups**							
	GRP 1 (CT)	**GRP 2 (LD)**	**GRP 3 (MD)**	**GRP 4 (HD)**	**GRP 5 (LD+VC)**	**GRP 6 (MD+VC)**	**GRP 7 (HD+VC)**	**GRP 8 (VC)**
Endometrium	–	–	–	–	–	–	–	–
Myometrium	–	–	–	–	–	–	–	–
Endometrial glands	–	–	++	++++	+++	–	–	–
Blood capillaries	–	–	–	–	–	++++	–	–

GRP = Group, CT = Control, LD = Low dose, MD = Medium dose, HD = High dose, VC = vitamin C. –=Normal, + = Mild, ++ = Moderate, +++ = Severe, ++++ = Extremely severe.

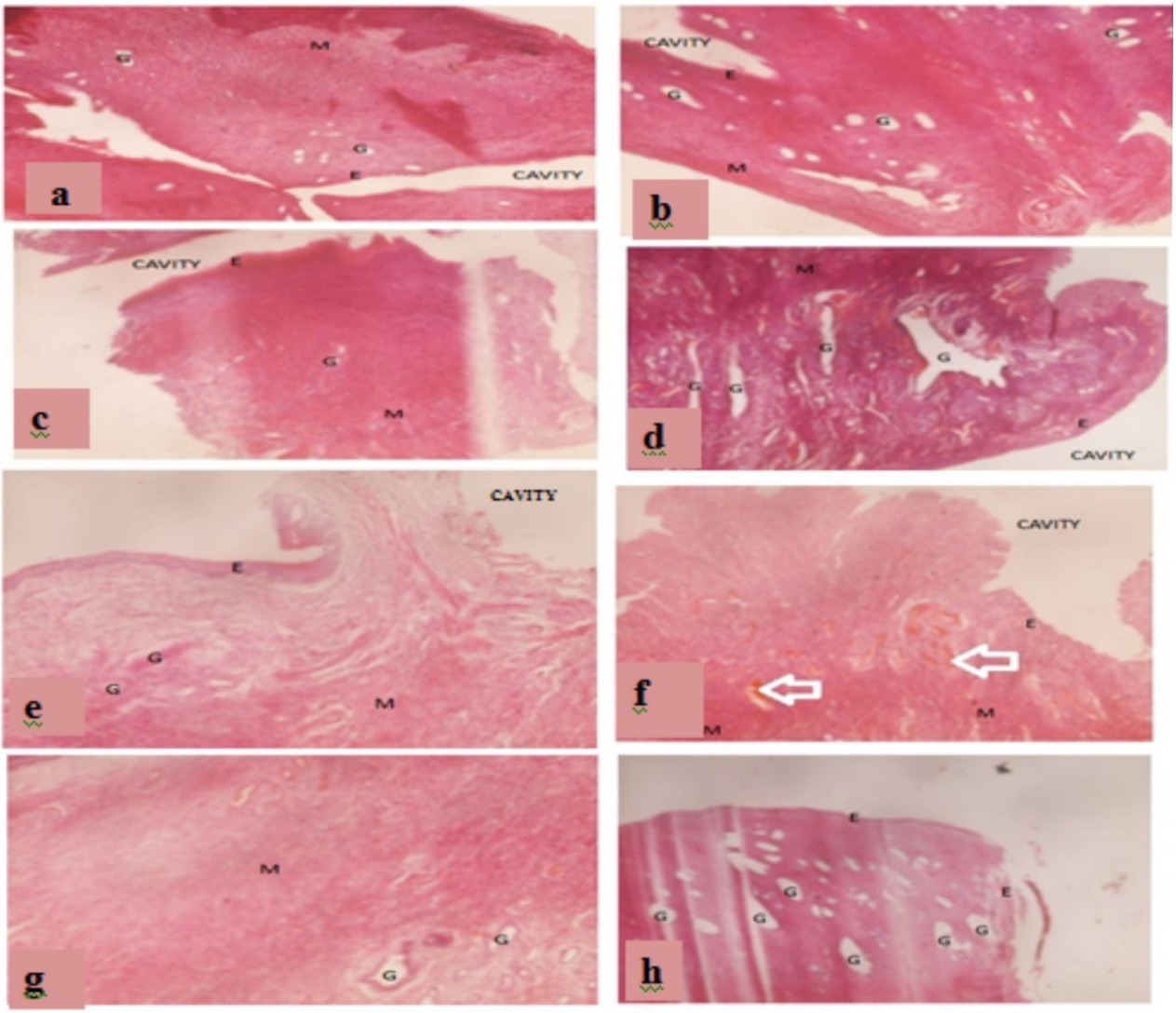

Figure 3a – h: photomicrographs of cross sections of the uterus control, dimenthoate and vitamin c treated groups for histological changes (H & E X100) (a) control (CT), (b) low dose (LD), (c) medium dose (MD), (d) high dose (HD), (e) low dose + vitamin C (LD+VC), (f) medium dose + vitamin C (MD+VC), (g) high dose + vitamin C (HD+VC), and (h) vitamin C (VC) treated groups. Note the normal appearance of uterus with intact endometrium (E), myometrium (G), endometrial glands (G) and endometrial cavity in control, vitamin C, some groups that received dimethoate only, and some groups that received dimethoate+ vitamin.

Discussion

The oxidative stress biomarkers examined in the present study include malondialdehyde (MDA), catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD). The results from the ovarian homogenates showed a dose related increase for MDA while a dose related decease was reported for CAT, GSH and SOD in the dimethoate treated groups as compared with the control. The increase in MDA with the concomitant decrease in the activities of CAT, GSH and SOD in the dimethoate treated groups indicates the present of a dimethoate-induced oxidative stress/damage in the ovary. This result is in agreement with that of [43] who reported an increase in MDA with a decrease in the levels of CAT, SOD and GSH in tissue homogenates of animals treated with cypermethrin. [44] Also showed that dimethoate intoxication produces radicals and the alternation of the antioxidant defense system.

The present study also reported a decrease in MDA with associated increase in the activities of CAT, GSH and SOD in the groups that received the dimethoate + vitamin C when compared with their respective dimethoate treated groups, whereas no significant difference was reported between the control (CT) and group eight (VC). The decrease in MDA with the associated increase in the activities of CAT, GSH and SOD in the dimethoate + vitamin C treated groups may be due to the effort of the vitamin C to restore the levels of these oxidative stress biomarkers to normal level. Both animal [45] and human [46] studies have shown vitamin C to be a potent antioxidant which mediates its antioxidant effects by

scavenging free Reactive Oxygen Species (ROS). Therefore, results of the present study suggests vitamin C ameliorating effects to be likely mediated by the inhibition of free radicals generation, free radicals scavenging and the enhancement of antioxidant enzymes activity [43].

Ovarian histological examinations showed that ovarian parameters like corpus lutea, primary, secondary, graafian and atretic follicles were severely (+++) damaged in both the dimethoate and the dimethoate + vitamin C treated groups as compared to the normal histological structures of these parameters that were observed in the control. The severity of damage observed in the dimethoate treated groups, particularly in groups three (MD) and four (HD) could be due to the dimethoate toxicity through Reactive Oxygen Species (ROS) production within the ovary, and consequently, the execution of apoptosis. This result is consistent with other previous studies on pesticides [44, 47]. However, the observable severe damage of some of the ovarian parameters in the groups that received dimethoate + vitamin C could be concluded that the vitamin C was unable to restore/ameliorate the severity of histological damage caused by the dimethoate on the affected parameters in the ovary.

Histological examination of the uterine architecture for the severity of damage showed that the endometrial glands and blood capillaries were extremely (++++) damaged in groups four (HD) and six (MD+VC) as compared to the control. Results for the uterine histological changes (Fig.3a-h) showed that the subchronic exposure of dimethoate to female rats can lead to scanty and small sized endometrial glands (Fig.3c), enlarged endometrial glands (Fig.3d), dilated and congested blood capillaries (Fig.3f). These results are in agreement with other previous studies with various insecticides.[48]Reported a decrease in endometrial gland density and vacuolated myometrium in animals treated with cypermethrin, while[49] reported an enlarged endometrial gland and myometrium in rats treated with chlorpyrifos. However, the reported dilated and congested blood capillaries in group six (MD+VC) (Fig.3f) could be due to the inability of the vitamin C to normalize the level of damage and histological changes caused by the dimethoate in the uterine architecture.

CONCLUSION

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 uterine and ovarian damage manifested by induction of oxidative stress and depletion of antioxidant enzymes in the ovary of rats. However, the ultimate effects were observed in the high dose (HD) group. In contrast, the co-administration of vitamin C with the insecticide antagonizes the ovarian oxidative damage but could not effectively ameliorate the histological changes in the uterus and ovary. Based on the study observations, it is therefore proposed that vitamin C may provide a cushion for prolonged therapeutic options against insecticide-induced ovarian oxidative damage without harmful side effects.

COMPETING INTERESTS

Authors declared that no competing interests exist between the authors and the producers of the products used for this study because we do not intend to use these products as an avenue for any litigation but for knowledge advancement only. Also, the study was solely funded by the authors.

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