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In-vivo investigation of dimethoate toxicity on serum enzymes, target organs and intestinal tissues of albino rats

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Abstract

Dimethoate is a broad spectrum organophosphate insecticide used to control agricultural, industrial and domestic pests. Despite its benefits, toxicities in non-target organisms have been widely reported. This study investigated acute and sub-chronic toxicities of dimethoate in rats. The arithmetic method of Karbar and a modification of repeated 8-week oral toxicity were adopted for the determination of acute and sub-chronic toxicities of dimethoate, respectively. Symptoms of acute toxicity evaluated include muscular weakness, respiratory distress, convulsion and death. Animals that were used for the sub-chronic toxicity studies were divided into 4 groups of 5. Groups 2, 3 and 4 were fed with feeds containing 200, 500 and 800 mg/kg dimethoate respectively, while group 1 served as the control. The weight of the animals were determined weekly for 8 weeks, and thereafter sacrificed. Blood samples were collected for biochemical tests, and the liver, kidney, lung, intestine and heart were excised and processed for histopathological analysis. The calculated median lethal dose (LD_{50}) was 176 mg/kg. Animals in all groups gained weight progressively. However, a dose-dependent significant rise in alanine transaminase and alkaline phosphatise, but not aspartate transaminase accompanied with significant focal necrosis in liver, eosinphilic casts in kidney and intestinal ulceration in group 3 and 4 in animals. In addition, dimethoate- induced inflammation was observed in the liver, kidney, lung and intestine tissues. The derangement of biochemical parameters and relevant histopathological alterations observed in rats exposed to dimethoate are suggestive of toxicity. Therefore necessary precautionary measures should be taking during handling and use.

Keywords: Albino rats; Dimethoate; Serum enzymes; Toxicity

1. Introduction

Dimethoate, IUPAC name, O, O-dimethyl-S(N-methylcarbomethyl) phosphorodithioate is a broad spectrum organophosphate insecticide and acaricide, with contact and systemic actions; available in emulsifiable concentrate, wettable powders, granules and ultra low-volume concentrate formulations [1]. It is rapidly absorbed from the gut and skin and rapidly excreted with no accumulation in fat tissue. Its dissipation and total residue in greenhouse celery conformed to first order kinetic equation with half-life of 2.42 days [2]. Dimethoate is very stable in acidic solution, but rapidly breakdown in alkaline pH [3]. Dimethoate is bio-transformed into five products, namely: Methyl diethanol amine, aspartylglycine ethyl ester, phosphonothioic acid propyl-O, S-dimethyl esther, O,O,O-Trimethyl thiophosphate and omethoate [4].

It is used to control wide range of agricultural, industrial and domestic pests including aphids, beetles, thrips, weevils, flies, scale insects, moths, spider mites and leaf hoppers. In agriculture particularly, it is used to control pests on cereals, fruits, pastures, vegetables and field crops such as corn, tomatoes and watermelons across the world [5, 6]. Despite the enormous benefits, dimethoate is potentially hazardous to other organisms such as birds, bees, fishes, mammals and

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even human beings that inhabit terrestrial or aquatic ecosystems [7]. Exposure of Juvenile *Clarias gariepinus* to sub-lethal levels of dimethoate in static bioassay system induced various behavioural changes in the catfishes which include skin discolouration (unusual lighter colour), sudden jerky movements, restlessness and erratic swimming, excessive secretions of mucous, bleeding from eyes and gills, rising and remaining unusually long at the surface [8]. Ozge Temiz *et al.,* [9] showed that sodium, potassium and calcium ions which play important roles in osmoregultory system in fish decreased while the serum alaninine aminotransferase, (ALT) and aspartate aminotransferase (AST) levels significantly elevated when the fishes, *Oreochromis niloticus* were exposed to dimethoate.

Srivastava and co-researchers [5] demonstrated that high concentration of dimethoate in water resulted in aquatic toxicity. They observed that fishes showed uncoordinated behaviour such as coming to the surface more frequently to gulp air, occasional attempt to jump out of water, increased opercula movement, sluggish and lethargic swimming, loss of buoyancy and muscular tetany, skin discoloration and mucous secretion along their opercula region prior to death. The histopathological alteration observed in the liver of freshwater fish, *Arius dussumieri* when exposed to sub-lethal concentration of dimethoate include hypertrophy of hepatic cells, disorganized cells forming homogenous mass, loss of polygonal shape of hepatocytes and loss of liver cord orientation [7]. Similarly, when adult male rats were exposed to dimethoate, the relative weights of the testes were decreased, and epididymal sperm concentration and motility decreased with increased doses [10]. Dimethoate has the potential to induce cytotoxicity, structural chromosome aberrations, bone marrow suppression as well as DNA damage in rats [11].

The wide spread poisoning, increased ecological imbalance, and by extension to non-target organism has been attributed to inappropriate method of handling during production and transportation, extensive and random application [12]. Most often than not, users do not make reference to the application guidelines in agriculture and public health operation, non use of personal protective clothing and indiscriminate disposal of pesticides containers, inadequate knowledge and awareness of the inherent dangers of pesticides, and absence of monitoring for pesticides residues on locally consumed food have being identified as additional reasons for the reported toxicity of the compound [13, 14]. Interestingly, it has been reported that melatonin protects against meiotic defects induced by dimethoate during porcine oocyte maturation, probably by preventing oxidative stress [15].

Like other organophosphates, the major mechanism of dimethoate toxicity in animals and human is the inhibition of acetyicholinestrase (ChE), an enzyme which hydrolysis the neurotransmitter, acetylcholine [16]. The inhibition of the ChE leads to accumulation of acetylcholine and over activation of its receptors at neuromuscular junction, autonomic and central nervous system. Hypotension is a common complication of acute organophosphorus poisoning which may progresses to shock and death within 12 - 48 h, post ingestion [17].

This study was therefore designed to investigate the enzymes, organ and tissue toxicities in rats following sub-chronic exposure to moderate and relatively high doses of dimethoate, using serum biochemical findings and histopathology of GIT, heart, liver, kidney and lungs parameters as indices. The finding may be extrapolated to assess the potential hazards in the human populations.

2. Materials and Method

2.1. Chemical

Dimethoate 40 EC applied as commercial emulsifiable concentrate formulation containing 40 % active ingredient.

2.2. Animals, treatment and ethical consideration

Male Albino rats weighing between 150 and 200 g were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University (ABU) Zaria. They were housed in clean cages in a well-ventilated room, under standard condition of temperature, relative humidity and natural light and allowed to acclimatize. The animals were fed with Vital® feeds and given water *ad-libitum* throughout the period of the study. The study performed in accordance with the principles for care and use of laboratory animals [18].

2.3. Preparation of Animal feeds

A 1000 g weight of feeds was mixed with graded doses of dimethoate (200, 400, and 800 mg/kg) dissolved in 20 ml of distilled water and compounded as pellets and served to the animals. Each group of the animals were given unrestricted access to the feeds.

2.4. Acute toxicity study

Acute toxicity study in rats was carried out using a modified arithmetic method of Karbar as described by Saganuwan [19]. The appropriate dosage range used in this study was pre-determined in two independent pilot studies. Five dosage levels (100, 140, 180, 220, and 260 mg/kg) were administered using intraperitoneal route and thereafter observed for symptoms of acute toxicity and death.

2.5. Sub-Chronic Toxicity Study

Twenty (20) male rats were divided into four groups, n=5 and tested according to the method of OECD-407 [20] described by Oloche *et al*. [21]. The rats in groups 2, 3 and 4 were fed with feeds containing graded doses, 200, 600 and 800 mg/kg of dimethoate, respectively, while group/ 1 served as control and were fed with feeds containing 0 mg/kg. To assess change in body weight, the animals were weighed weekly for 8 weeks. The rats were observed for signs of toxicity daily throughout the entire study period and thereafter sacrificed under anaesthesia.

Blood samples were collected into lithium heparinised sample bottles, left to stand on the bench for 2 hours, centrifuged at 4500 rpm for 20 minutes and the plasma used for biochemical analysis. Liver enzymes; alaninine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were determined using the method described by Reitman and Frankle, Habig *et al*. [22, 23].

Target organs such as liver, kidney, lung, heart and intestine were excised, weighed and examined for any histopathological alteration. About 4 to 5 μm section of each organ was made, fixed in 10 % neutral buffered formalin for 24 h, washed under running tap and dehydrated in ascending grades of alcohol. The prepared tissues were then cleared in xylene and embedded in paraffin wax at 58 ^{o}C. The paraffin embedded sectioned tissues were stained with haematoxylin and eosin and subsequently examined under light microscope (Axiophot, Germany) fitted with an automatic photo micrographic system. The Banff criteria and the Knodel semi-quantitative scoring systems were used to evaluate histopathological alterations [24].

2.6. Statistical analysis

The data obtained were expressed as mean $\pm$ SEM. Statistical analysis were performed by one way analysis of variance (ANOVA) and student's test. Confidence interval of 95% ($p<0.05$) was considered significant.

3. Results

3.1. Median lethal dose (LD_{50})

The experimental animals exhibited acute dose-related symptoms such as hypersalivation, muscular weakness, swaying gaits, constricted pupils, respiratory distress, with convulsions and deaths, especially at 600 and 800 mg/kg. The LD_{50} after peritoneal administration of dimethoate to Albino rats was determined to be 176 mg/kg.

3.2. Weight gain by experimental animals

A steady weekly increase in weight in all groups of rats fed with meal containing varying doses of dimethoate and in the control group that received feeds that did not contain dimethoate was observed. However, the percentage weight gain over weeks 2, 3, 4, 5, 6 and 8 in groups 3 and 4 animals, respectively was significantly ($p<0.05$) lower relative to the control. In addition, animals in these groups exhibited low appetite observed as decreased feed intake (Table 1).

Table 1 Average weight gain of experimental animals over eight (8) weeks

	% Weight gain per week			
Week	**Group 1**	**Group 2**	**Group 3**	**Group 4**
0	0 (104)	0 (102)	0 (108)	0 (110)
1	6.7 (111)	4.9 (107)	5.6 (114)	4.5 (115)
2	14.4 (119)	11.7 (114)	4.6 (113)	6.4 (117)
3	25.6 (130)	20.6 (123)	8.3 (117)	5.5 (116)
4	31.7 (137)	22.5 (125)	17.6 (127)	9.1 (120)

5	36.5 (142)	25.5 (128)	19.4 (129)	10.0 (121)
6	41.3 (147)	32.4 (135)	18.5 (128)	12.7 (124)
7	43.3 (149)	33.3 (136)	20.4 (130)	18.2 (130)
8	47.1 (153)	34.3 (137)	21.3 (131)	16.4 (128)

Key: number of animals/group (n) = 5, data in parenthesis represent average weight of animals (g)

3.3. Effects of dimethoate on liver enzymes

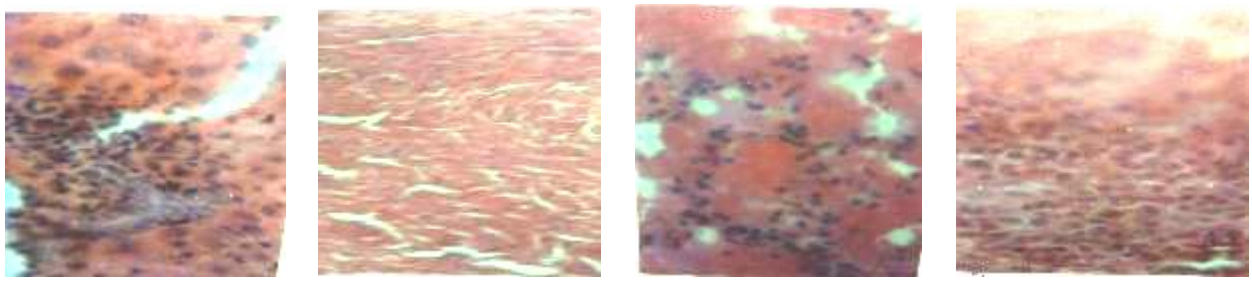
Table 2 shows a dose-dependent significant ($p<0.05$) increase in the levels of alanine transaminase and alkaline phosphatase in the group of animals that were fed meal containing graded doses of dimethoate relative to the control. However, the serum levels of aspartate transaminase were not significantly different ($p>0.05$).

Table 2 Dimethoate induced alteration of liver enzymes in Albino rats fed graded doses of dimethoate

Dose (mg/kg)	AST (IU/L) (n =5)	ALT (IU/L) (n =5)	ALP (IU/L) (n =5)
0	82.4 ± 1.5	77.8 ± 1.5	20.2 ± 0.2
200	91.2 ± 1.6	134.2 ± 2.1	22.8 ± 0. 7
600	96.0 ± 1.8	148.8 ± 1.7	30.0 ± 0.8
800	105.8 ± 2.2	166.2 ± 2.0	35.0 ± 0.9

3.4. The histopathological changes of target organs of Albino rats fed with graded doses of dimethoate

Significantly higher ($p<0.05$) congestions and round cell infiltration were observed in the liver, kidney, lung and intestine of rats that received 600 and 800mg/kg relative to the control. The liver tissues showed focal necrosis, while increased ulceration and haemorrhage were observed in the intestine, kidney and lungs at 800 mg/kg. Granular and eosinophilic casts in kidney were observed at the highest test concentration of 800 mg/kg (Figure 1).



A **B** **C** **D**

Photomicrograph of liver of Albino rats fed with 800 mg dimethoate/kg feeds showing round cell infiltration and ulceration (H & E X 100); Photomicrograph of heart of Albino rats fed with 800 mg dimethoate/kg feeds showing normal heart muscles (H & E X 100); Photomicrograph of lung of Albino rats fed with 800 mg dimethoate/kg feeds showing round cell infiltration (H & E X 100) ; Photomicrograph of intestinal mucosa of Albino rats fed with 800 mg dimethoate/kg feeds showing extensive round cell infiltration and ulceration (H & E X 100).

Figure 1 Photomicrograph of excised tissues showing histopathological changes in Albino rats fed with graded doses of dimethoate

4. Discussion

Pesticides are biologically active chemicals which are generally used in agriculture or against harmful and insects and vectors in human health protection programs [25]. In recent times, the non-target toxicity of these chemicals has assumed unacceptable dimension that requires investigation. This negative development might be attributed to increased usage in agriculture for enhancement of improved crop yield in medical settings for improved quality healthcare delivery. It is therefore imperative to embark on conscientious continuous enlightenment campaign and to educate the populace on their proper and best usage procedure.

Lethal dose (LD_{50}) is a common measure of acute toxicity. The calculated LD_{50} of 176 mg/kg intraperitoneally indicated that dimethoate was moderately hazardous [26]. The observed dose related symptoms such as muscular weakness, swaying gaits, respiratory distress, convulsions which in severe cases resulted in death could be due to irreversible inhibition of acetylcholinesterase enzyme resulting in accumulation of acetylcholine and over activation of acetylcholine receptors at neuromuscular junction in the autonomic and central nervous system [16].

The weight loss, although not significantly different ($p<0.05$) observed at the highest tested dose might have been due to the loss of appetite evident as decreased feed intake resulting low nutrient available for normal growth [27]. Observations in this study slightly differ from that of Nqoula *et al* [9] on rats exposed to dimethoate. They reported significant weight loss, and suggested that the decrease in food consumption could be due to the decrease in metabolism or inhibition of hunger resulting in lack of appetite or anorexia. It was also suggested by other researchers that the decrease in body weight might also be as a result of the combined action of oxidative stress and/or increase degradation of lipids and proteins as a direct effect of exposure to organophosphorous compound [28]. However, animals in the group of rats fed with 200 mg/kg feeds and the control group which received feeds that did not contain dimethoate progressively gained weight, indicating that the animals had unsuppressed appetite resulting in their normal growths and physical developments.

Biochemical and histopathological tests are evaluation tools to detect organ-specific effect related to exposure to toxic chemical. Serum enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) considered to be among the most sensitive biochemical markers employed in the diagnosis of hepatotoxicity [29] were measured and used as indices of liver toxicity. Liver is responsible for several functions including primary detoxification and excretion of various xenobiotics and metabolites [30]. This makes it one of the major target organs for the dimethoate damages. The result showed dose-dependent significant ($p<0.05$) increase in serum level of ALT and ALP in dimethoate treated rats compared to negative control indicated that the pesticide caused hepatocellular injury, resulting in an increased serum levels of these enzymes. This finding is in consonance with those of AL-Awthan *et al.* [31] who demonstrated significant increases in the levels of AST, ALT and ALP in response to oral administration of dimethoate to guinea pigs. Exposure to dimethoate causes oxidative stress in lung by producing reactive oxygen species and the decreasing biological activities of some liver antioxidant enzymes such as catalase activity and glutathione -5- transferase [31]. Observations from this research strongly suggests hepatotoxic effects of dimethoate to the experimental animals and possible human..

Liver histopathology is the gold standard for diagnosis, assessing liver damage and identification of cause of the disease [32]. Congestion and round cell infiltration observed in the liver of rats treated with 600 or 800 mg/kg dimethoate and the focal necrosis at 800 mg/kg which were significantly higher than those of the control were signs of toxic damage to hepatocytes. This correlates with the elevation in the liver enzymes. Phusate [33] in a 60 day sub-chronic toxicity testing had reported a similar disarray of hepatocytes, cellular infiltration, karyomegaly, granular degeneration and haemorrhages in the liver of Wistar rat exposed to Gramoxone, a Paraquat based herbicide.

Similarly, the congestion and round cell infiltration observed in the kidney of 600 and 800 mg/kg treated rats which were significantly higher than the control are suggestive of toxicity. Tissue changes in liver were closely linked with histological abnormalities of kidney. Once absorbed, the toxicant is transported by blood circulation to liver for biotransformation and/or storage, and if transformed in the liver it may be excreted through the bile or by the kidney. Kidney therefore plays critical role in the excretion of pesticide and was thus constantly exposed to the pesticide and its toxic derivatives. The granular and eosinophilic casts in kidney at concentration of 800 mg/kg could be due to direct action of the pesticide. The result in this study was in agreement with the finding of [34] Afshar *et al*. who also observed marked tubular dilation, congestion and hemorrhage in the cortical and medulla part of the kidney of Wistar albino rats exposed to fenitrothion, an organophosphate pesticide.

The intestinal ulceration observed at 800 mg/kg might have resulted from sustained direct exposure to the toxic effect of the pesticide. There was significant relevant hemorrhage in the intestine of rats that received dimethoate. Damages to the blood vessel could be responsible for the hemorrhage observed in these organs [35]. The result of this study also supports histopathological finding of Ateeq [36] who observed that there were congested blood vessels, hemorrhage and infiltration in the intestinal tissues and changes such as degeneration, tubular degeneration, hemorrhage, infiltration, tubular cast and compressed blood vessel in the lungs, kidney and liver of dimethoate treated mice. The result of this study was similar to the histopathological findings of Sanbel and Ameer [37] who observed congestion in the blood vessel, hemorrhage and necrosis in the bronchioles of pregnant rat's lung exposed to monosodium glutamate.

5. Conclusion

Dimethoate widely used as a pesticide exhibited toxicity typical of organophosphate insecticides and produced a dose-dependent significant elevation of ALT and ALP that correlates with histopathological alterations observed as focal necrosis in liver, eosinphilic casts in kidney and ulceration in intestine suggestive of moderate toxic effect. It is therefore recommended that precautionary measures be taken during handling and use. The users should also be educated on the correct handling methods, safe dosage and correct interval between application to food material and their consumption. It is also recommended that specialized poison treatment referral centers be provided in communities that use this pesticide to manage accidental and chronic poisoning.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they conflict of interest.

Statement of ethical approval

Ethical approval for the use of experimental animals was obtained before the commencement of the study.

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