Haematological and Hepatopathological Changes Induced by Dimethoate in *Rattus rattus*

Mohammad Yaqoob Lone*1, Bashir Ahmad Baba2, Prathvi Raj2, Vinoy K Shrivastava1, Mangla Bhide3

1Laboratory of Endocrinology, Bioscience department, Barkatullah University, Bhopal, (M. P.) India.
2Department of Botany Govt. Motilal Vigyan Mahavidyalaya, Bhopal (M. P.) - 462001 INDIA
31-3 Department of Zoology, Dr. H.S. Gour Central University, Sagar, (M. P.) India

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**ABSTRACT**
The present investigation was carried out on the toxic effects of the pesticide dimethoate (30 EC, 98.4% pure) on some blood parameters i.e., (red blood cells, white blood cells count and hemoglobin contents) and changes in the histoarchitecture of liver in rat *Rattus rattus*. Thirty adult *Wistar rats* were divided into three groups, each of ten. Group first was served as control and fed with pellets diet and 0.05 ml olive oil (vehicle). Rats of group II and III were fed with pellets diet and received dimethoate (1mg/kg b.w. alternate days) mixed with 0.05 ml olive oil orally for 30 days. In our result we find that exposed rats showed significant (p<0.001) decline in the haemoglobin content initially for 15 days and for later part of experiment more severe decline noticed as compared to control. The RBCs count decreases significantly (p<0.001) in 15 days and 30 days treated group as compared to control. W.B.Cs count was increased significantly (p<0.001) in 15 days experiment as compared to control, while it decreased after 30 days experiment as compared to 15 days treated rats. The histopathological changes in the liver of rat treated with dimethoate for 15 and 30 days revealed vacuolization, hypertrophy of hepatocyte and eccentric nuclei. The results showed that the degree of distortion of the liver and alteration in hematology were proportional to the exposure periods and concentration of the chemical was found during the experiment.

**Corresponding author:**
Mohammad Yaqoob Lone
C/O Dept. of Zoology,
Dr Harisingh Gour Central University Sagar,
M.P-India, 470003.
Contact no.09752788513,
Email id: yaqoob.azu@gmail.com

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INTRODUCTION

The common use of insecticides in public health and agricultural schedules has caused severe acute and chronic cases of human and animal poisoning [1]. The acute toxicity of organophosphorus compounds (ops) are believed to be due to primarily to the inhibition of acetylcholinesterase (AChE) resulting in an accumulation of acetylcholine(Ach) with a sustained overstimulation of Ach receptors in the clefts of central and peripheral neuronal synapses.[2].

Dimethoate is an insecticide referred to as organophosphates, which is cholinesterase inhibitor. Cholinesterase is an enzyme that is essential for the proper working of the nervous systems of both humans and insects. Administration of dimethoate to pregnant rats produced enzymatic changes associated with mild pathomorphological changes in liver and brain, [3]. Previous studies indicate that dimethoate intoxication causes cellular injury and oxidation stress which leads to lipid peroxidation and free radical production [4,5,6] Recent studies have shown that acute and sub chronic exposure to dimethoate alters the antioxidant status and histology of liver, brain and testes of rats [7,8,9] and human erythrocytes [10]. Also the effect of two organophosphorous pesticide were studied on the hepatic and intestinal metabolism in the rat and found that the metabolizing enzymes responsible for both the bioactivation and detoxification are present in the small intestine at lower level than the liver but still significant [11]. The pyrethroid insecticide, cypermethrin, significantly ($P < 0.05$) induced free radical production in plasma, liver, brain and testes [12].

The purpose of this study is to establish an account of whether low dose of dimethoate as pesticide has any non-target effect on humans or other animals. The rat was selected as the test species of mammals. So we focused to investigate the alteration in hepatopathology and hematological parameters which might occur as a result of dimethoate intoxication in Rattus rattus.

MATERIALS AND METHODS

Chemical: Dimethoate (30 EC, 98.4% pure S= CH$_3$NHCOCH$_2$SP(OCH$_3$)$_2$ under the trade name Rogar was brought from Hindustan chemical and pesticide faujdar compound ,ibs mark Kurla west Mumbai.

Animals and dosing

For present investigation 30 adult Wistar rats weighing 125 ± 5 gm were obtained from animal house of bioscience department, Barkatullah University, Bhopal, (M.P.) and they were acclimatized at 23±2°C with a 12 hours light-dark cycle for 15 days in the laboratory of endocrinology bioscience department, Barkatullah university to start the experiment. The animals were divided into three groups. Group I served as control consists of10 animals were fed with normal pellets diet and 0.05 ml olive oil. Group II and Group III consist of ten animals each fed normal diet with water ad libitum throughout experiment and received dimethoate (1mg/kg b.w on alternate days) mixed with 0.05 ml olive oil orally with the help of feeding canulla for 15 and 30 days . The animals were provided with rat feed and Weight of rats was recorded at the initiation and the termination of experiment period.

At the end of the treatment period, on 16$^{th}$ and 31$^{st}$ days the rats were sacrificed by cervical dislocation and dissected, the livers were removed and fixed for histopathological investigations using Carnoy’s fluid (6:3:1). Tissues were processed by routine histological techniques, sectioned at 5µm by a microtome (model: yorco spencer type), stained with Hematoxylin and Eosin (H&E) [13]. Finally, stained sections were examined under the light microscope and subsequently micrographs were taken. Blood samples were collected from the heart of rats for haematological investigations and were stored in EDTA vial. Blood samples with anticoagulant
EDTA were analyzed for blood parameters namely red blood cell counts, white blood cell counts and haemoglobin percentage [14].

**Statistical analysis:** All data obtained from the present investigation was analyzed by using statistical tools and the significance of findings were analyzed by using students ‘t’-test. [15]

**RESULTS**

**Haematological studies**

Haemoglobin and erythrocyte count was decreased in compared to control group significantly in 15 days treated rats and in later part of the experiment, Leucocytes count was increased significantly (**= p<0.001**) in 15 days treated rats as compared to control while showed significantly decrease in 30 days treatment as compared to 15 days treated rats.

**Table 1: Vaules of R.B.C.,W.B.C. and Haemoglobin content in blood of control and dimethoate exposed rat Rattus rattus after 15 and 30 days exposure**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Groups</th>
<th>R.B.C$^a$(10$^6$/mm$^3$)</th>
<th>W.B.C$^a$(10$^3$/mm$^3$)</th>
<th>Hb (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Control</td>
<td>6.76 ± 0.15</td>
<td>6.2 ± 0.25</td>
<td>9.41 ± 0.19</td>
</tr>
<tr>
<td>2.</td>
<td>Treated (15 days)</td>
<td>5.53 ± 0.13 ***</td>
<td>11.31 ± 0.31 ***</td>
<td>6.31 ± 0.12 ***</td>
</tr>
<tr>
<td>3.</td>
<td>Treated (30 days)</td>
<td>4.69 ± 0.19 ***</td>
<td>8.62 ± 0.22 ***</td>
<td>5.31 ± 0.18 ***</td>
</tr>
</tbody>
</table>

± = SEM value of ten animals, **= p<0.01, ***= p<0.001

**Hepatopathological observations:**

Liver sections stained with H & E were prepared from both control and treated rats and were examined under light microscope. The microscopic observations were noticed and the intensity of degenerative changes was presented by the Figs. 2 and 3. Sections from the control group showed radially arranged hepatic cords around the central vein. (Fig.1). the liver sections from treated rats showed moderate changes when compared with those from the control rats. Section of liver from 15 days dimethoate treated Rattus rattus showed dilated portal space, hypertrophy, mononuclear cell infiltration, degeneration of cells. (Fig.2). Section of liver from 30 days dimethoate treated Rattus rattus showed large area of necrosis, highly dilated sinusoidal spaces, vacuolated cells, eccentric nuclei with excessive degeneration and loss of radially arrangement of hepatocytes. Central vein and sinusoids between hepatocytes were dilated. (Fig.3).
Figure 1: T.S of Control *Rattus rattus* showing normal histoarchitectural features arranged around tributaries of the hepatic vein, cells were large in size contained homogenous cytoplasmic material with prominent nuclei.

Figure 2: Rats treated with dimethoate for 15 days showing hepatic cells with eccentric nuclei hypertrophy & cytoplasmic degeneration.

Figure 3: Rats treated with dimethoate for 30 days showing cytoplasmic vacuolization of hepatocytes & eccentric nuclei with devoid of fat within the cell.

DISCUSSION

Present experiment was designed to evaluate the acute toxicity of dimethoate because of the importance of the compound e.g. its large scale use as an insecticide in agriculture. Many studies have shown that acute and subchronic exposure to dimethoate generates lipid peroxidation and alters the antioxidant status of several tissues in rats. [7].

Haematological and histopathological effects of dimethoate on rats have been studied in present study. Haematological parameters are sensitive index of the physiological changes of an animal to any environment pollutants and toxic stress of any nature shows significant changes in the blood. The decrease in Hb value is due to an increase in the rate at which Hb is destroyed. Iron is obtained from stored ferritin and a dietary source which is essential for synthesis of Hb. Intoxicated rats reduce the food intake capacity and there is no other source of iron intake, might be the reason for iron deficiency. The poisoning by pesticide residues leads to the development of anemia due to interference of Hb biosynthesis and shortening of the life span of circulating erythrocytes [16,17]. Due to toxicity there is destruction of erythrocytes or inhibition of erythrocytes production, which decrease the R.B.Cs count in Wistar rats. Deficiency of vitamin B$_{12}$ and folic acid leads to impaired synthesis of nucleic acid resulting in defective maturation of erythrocytes and their nuclei [18]. A similar decrease of R.B.Cs and the haemoglobin has been reported in rats given sub-acute dose of organophosphate. [19].

Our findings were supported with the data provided by [17], showed that pesticides decrease R.B.C. count and Hb% levels, while [20], noticed the decrease of R.B.C. count, Hb %, and increase in erythrocytes sedimentation rate in rabbits exposed to orally dose of 10mg/kg b.wt. of the organophosphorus pesticide Methidathion. The differential count of white blood cells (WBC) was also investigated. The present enhancement of WBC count following dimethoate intoxication might be due to leucocytosis as in leucocytosis hematopoietic cells proliferate leading to progressive infiltration in peripheral blood. The haematological changes were mainly due to mal absorption of nutrients or the hyperactivity of the animal. Similarly, [21] reported similar results when exposing the rat to dimethoate. As a result, it was apparent that dimethoate caused the negative alteration on some hematological parameters.

The histopathological changes investigated in the selected organ reflected some significant effects with variable intensities. The liver seemed to be mostly affected by all doses of the pesticides. Our results are also due to similar as earlier results of [22] who had revealed that the treatment of rats with endosulfan, causes liver damage which included dilation of sinusoidal spaces with irregular nuclear shape, degenerative changes included binucleated cells, hypertrophy of hepatocytes and lymphocytic infiltration in the central vein. In support of our findings, there were changes in large-sized parenchymatous cells, cytoplasmic vacuolization and...
condensed nuclei, disruption of hepatic architecture, dilated congested blood vessels with proliferative lining epithelia, an increase in the number of Kupffer cells and lymphocytes infiltration [23]. In rats, treated with permethrin and DDT separately causes liver damage whereas DDT causes cytoplasmic vacuolization and hepatocyte necrosis [24].

This change in the morphology of liver cells might be due to chemical induced that can express itself through different ways. The acute effect consists of lipid peroxidation in the cell membrane and accumulation of lipids (fatty liver) leading to the death of the cell. The necrotic process can affect small groups of isolated parenchymal cells, groups of cells located in zones or virtually all the cells within a hepatic lobule.

In conclusion, the results of the present study suggested that the dimethoate has adverse effects on some blood parameters and liver dysfunctions leading to histological impairment, these changes may be induced by dimethoate directly or indirectly to tissues depending on dose and duration. More over extensive studies are needed to evaluate the toxicity of dimethoate at molecular level because genotoxic chemicals such as insecticides have common chemicals and physical properties that enable them to interact with genetic materials.

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