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HISTOPATHOLOGICAL AND HEMATOLOGICAL EFFECT OF DIMETHOATE ON THE LIVER AND KIDNEY OF ALBINO MICE

C.B.Jamdade[1], R.D.Bodare[2]
[1]*Sadguru Gadage Maharaj College, Karad, Maharashtra, India.*
[2]*Prof Dr ND Patil Mahavidyalaya, Malkapur Maharashtra, India.*

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ABSTRACT

Dimethoate is the widely used organophosphorous pesticide in agriculture land for controlling pests of variety of crops like citrus, cotton, tea, tobacco, fruits, olives, potatoes and vegetables. The aim of present work was to study toxic effect of pesticide dimethoate on some blood constituents and some organs of Swiss albino mice. In present investigation animals were treated with 1/10th of LD_{50} of dimethoate (DM) via oral gavage. The DM was administered to the animals at a dose 15mg/kg b.w. for 20 consecutive days. After completion of the dose, following hematological parameters were studied ; Hemoglobin (Hb),Red Blood Cells(RBCs), White Blood Cells (WBCs), Packed Cell Volume(PCV) and after that animals were scarified by cervical dislocation; liver and kidney dissected out and fixed for histopathological investigations. The sections were examined under light microscope. Histopathological changes observed in the liver were vacuolation, hepatic pycnosis, blood congestion, hemorrhage, nuclear death, enlargement of portal vein and high lymphatic infiltration throughout the central vein .The kidney showed some changes in cellular lining of the Bowman capsule, glomerular degereration, hemorrhage, compress blood vesslses, tubuar degeration, glomerular shrinkage ,cell rupture and hydropic changes.

KEYWORDS- ***Dimethoate, Histopathological, Hematological, pycnosis, lymphatic infiltration.***

1. INTRODUCTION

The pesticides are one of the most potentially harmful chemicals liberated in the environment in an unplanned manner. There are so many types of pollutants that affect our life both directly and indirectly. Pesticides are biologically active chemical and it may spread from food chain to lead widespread contamination in the environment ((El-Sebae, 1993; Zaahkouk et *al*., 2000).The misused of pesticide may be harmful to humans, animals and environment. Dimethoate is a insecticide widely used in agricultural land(Sharma et al. 2005). Dimethoate (IUPAC name O,O-ndiethyl S-methyl carbamoyl methyl phosphorodithioate) is a broad use systemic organophosphate insecticide in agriculture against a wide range of insects like mites and fungal diseases of fruits, vegetables, and field crops as both systemic and contact pesticide; as well as indoor to control houseflies [Rome, 1999]. It is applied as spray in agriculture, horticulture and viticulture for control of insects on a variety of field, fruit and vegetable crops.

The use of dimethoate is very important in affecting many diseases in plants and animals as well as human [M. Al-Haj et al.,2005]. Toxicity of systemic pesticides dimethoate results in deleterious effects on many organs and systems in human and other mammals particularly the reproductive system , immune system, nervous system, and sexual hormones, liver [J. Gomes et al.1999], [F.Sayim 2007], Kidney [F.A.Khogali et al.2005], pancreas , brain. The primary mechanism of action of Organophophosphate pesticides (OP) is based on inhibition of both mammalian brain and plasma acetylcholinesterase (Ache) activity (De-bleecker *et al.*, 1993).

Many studies have been carried out on the effect of dimethoate on reproductive and endocrine functions, it suggested that it could affect serum concentrations of reproductive and metabolic hormones.(Aprea, C et al.,1998). Some studies have shown that biochemical changes that might occur in the kidney of albino rat as a result of dimethoate intoxication. (R. Penchalamma*et.al. 2014)* and the effect of dimethoate on reproductive system and fertility of adult male rat which disrupted spermatogenesis and reduced the fertility (Ferdinand Ngoula *et al.*, 2014)

The liver and Kidney are vital organ of vertebrates & same other animals. The liver is the primary organ involved in xenobiotic metabolism and is a major target organ for chemicals

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and drugs. Hepatotoxicity is therefore an important endpoint in the evaluation of the effect of a particular xenobiotic. The kidney is one of the most target organ of experimental animals attacked by pesticides Sivapiriya *et al*., 2006; Mansour and Mossa, 2010).Few studies have been made on the histopathological effects of dimethoate on some organs (Thangavel, 1994 and Persis, 2001). The present study aimed to investigate histopathological and hematological effects of the organophosphorous pesticide dimethoate that is extensively used in some agricultural areas in India on some organs of albino mice.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals used for this experiment were obtained from sigma chemicals CO. USA, including Dimethoate.

2.2 Animals and experimental design

Healthy swiss albino mice *Mus musculus* Linn. were used for present investigation. The mice were obtained from Rajarambapu College of Pharmacy, Kasegaon, Tal -Walwa; Dist -Sangli 415 404 (1290/PO/Re/S/09/CPCSEA,16th Mar.2019) Adult mice 30 to 35 ± 2 gm/BW were used for present investigation throughout received Amrut mice feed (Pranav Agro Industries, Pvt. Ltd, Sangli) and water *ad libitum*. All animals were housed in plastic cages with daily observations. Animals were maintained under controllrd laboratory conditions consisting of a 12h dark/light cycle,24-25°C temperature and 35-60 % relative humidity.

Animals were divided into two groups (n = 6)

1. Control group

The adult mice were given 0.5 ml of distilled water/ day/ animal for 20 days

2. DM Toxicated group

The adult mice were given 15mg/kg bw/ day/ animal for 20 days by orallywith oral gavage.

After completion of the treatment mice were taken in the batches dissected and liver and kidney were removed and fixed for histopathological studies using 10 % neutral formalaline for 24 hrs. After routine processing, paraffin –embedded (58-60°C) tissue samples were sectioned at 4-5µm thickness and stained with harries haematoxylin and eosin. Finally stained sections were observed under light microscope and photographs were taken.

Blood samples were collected by retro-orbital eye method of mice for hematological investigations. The parameters studies were the hemoglobin, the hematocrit, RBCs, WBCs.

2.3 Statistical Analysis

The statistical analysis was performed using student s t -test

3. RESULTS

3.1 Histopathological study of Liver

Histopathological examination of the liver sections in the control mice showed a normal histological structure .The central vein located in the center of the lobule and it surrounded by hepatic cells. The distinct nuclei and hepatic sinusoids are also observed.

The sections from treated mice showed changes in structure when compared with control mice.(Fig.1-A&B).These changes include the liver congestion and Vasodilation, Lymphatic infiltration and cell pycnosis(Fig.1-D), dead nuclei (Fig.1-C), congestion, necrosis and hemorrhage(Fig.1-E), Parenchyamal cells shows degerneation in nuclei and vacuolization and enlargement of hepatic sinusoids(Fig.1-F). Moreover, an increase in number of Kuffer cells was observed. These histopathological changes due to dimethoate exposure were observed in the Liver.

3.2 Histopathological study of Kidney

Histopathological examination of the Kidney sections in the control mice showed a normal histological structure, normal renal tubules, renal corpuscles, proximal convoluted tubules and distal convoluted tubules. Further, the Glomerulus, Bowman's capsule, urinary space, podocytes, medulalry rays were noticed as shown in (Fig.2-A &B).

The treated Kidney section with dimethoate showed Glomerular, Bowman's capsule with swollen cells and hypoplasia, glomerular shrinkage, Vacuolization (Fig.2-E),Compressed blood vessels and hemorrhage (Fig.2-D),tubular degeneration and Tubular Widened Lumen(Fig.2-F) Cell rupture and swollen proximal convoluted tubule. (Fig.2-C). These histopathological changes due to dimethoate exposure were observed in the Kidney.

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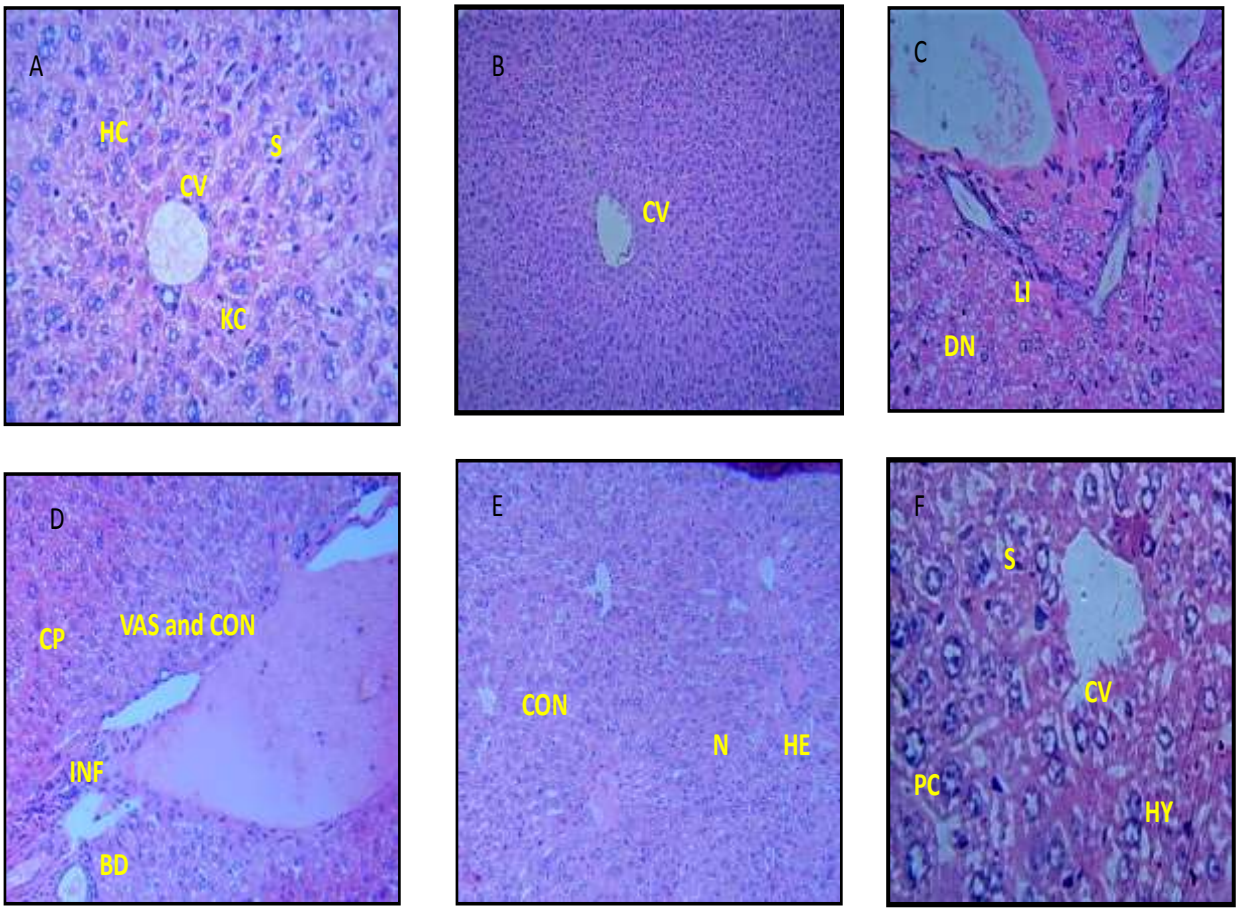

Figure 1: Photomicrograph Of The Liver Sections Of adult Mice Treated With 15 mg/Kg B.W Of Dimethoate (H&E Stain X100 and X400). A&B] Control group C,D,E,F] DM Toxicated group -central vein(CV), hepatic cells (HC),Sinusoids (S) , Kupffer Cells (KC), liver congestion (CON) ,Infiltration (INF), Vasodilatation and Congestion (VAS and CON,hypertrophy (HY). Bile duct(BD),Necrosis(N),Parenchymal cells (PC), Dead nuclei (DN),Cell pycnosis(CP),Liver hemorrhage(HE)

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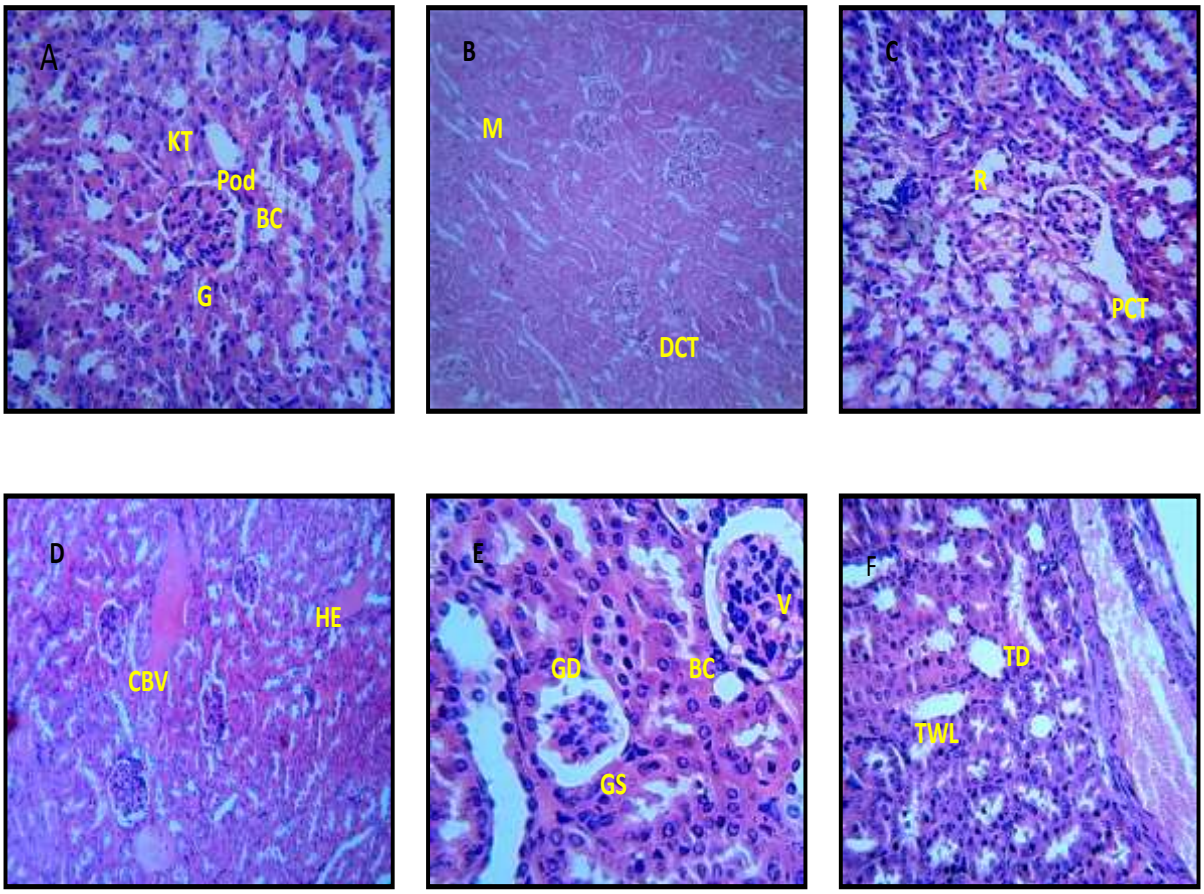

Figure 2: Photomicrograph Of The Kidney Sections Of adult Mice Treated With 15 mg/Kg B.W Of Dimethoate (H&E Stain X100 and X400). A&B] Control group C,D,E,F] DM Toxicated group - Glomerular Degeneration(GD), Tubular Degeneration (TD) Hemorrhage(H) ,Compressed Blood Vessel (CBV),Glomerular Shrinkage(GS), Vacuolation in the glomerulus. Hypoplsia in cells of Bowman's capsule, Rupture(R), Tubular widened lumen (TWL),Normal kidney tubules(KT),Podocytes(POD),Medullary rays(M),Distal convoluted tubule (DCT), Proximal convoluted tubule (PCT)

3.3 Hematological Studies

After completion of the the dimethoate dose,the blood parameters investigated were hemoglobin content, hematocrit (PCV) and blood cell count(RBcs & WBCs) .The obtained results were analyzed by using student t -test.

Parameters	Control (D/w)	Dimethoate (15mg/kg bw)
Hb (gm)	13.83 ±2.13	12.07 ±2.10*
PCV (%)	40.41 ± 3.11	37.82 ±4.71**
RBCs (Cumm)	8.41 ±0.30	8.81 ±1.18
WBCs(Cumm)	3.61 ± 0.31	4.54 ± 1.71

*= significant, **=highly significant

Table 1- Hematological values of control and dimethoate treated mice

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Table showed that some hematological values of mice treated with dimethoate ,When compared with control values, the hemoglobin content and packed cell volume values showed significant decrease in the treated group (P≤0.05)and were highly significant. The red blood cell count in dimethoate treated mice showed no significant difference as compared to the controls (P≥0.05). The white blood cell count showed varying changeable values but was not significant.

4. DISCUSSION

From several years the excessive use of different pesticides on agriculture land and for household pest has led to drastic effect on many non-target species like man ((WHO/PCS, 1996; Chantelli-Forti et al.,1993; Chaudhuri et al., 1999). The present study was performed to investigate histopathological and hematological effect of commonly used organophosphorus pesticide, dimethoate on albino mice. The results showed mild to severe effects on the target organs and some notable results were found. The histology of liver sections showed more severely affected by pesticide dimethoate. The changes reported includes Congestion, lymphatic infiltration, nuclear death, cell rupture, hemorrhage, increase in Kuffer cell number, enlargement of hepatic sinusoids, degeneration of nuclei, parenchymatous cells showing vacuolation, nuclear death or pycnosis. The mammalian organ liver is most target organ of toxic impact regarding its function in biotransformation and in xenobiotic metabolism (Roganovic and Jordanova, 1998). Khogali *et* al. (2005), reported that Dimethoate-induced vacuolation, blood congestion, hepatic pycnosis, and high lymphatic infiltration around the central vein. These results are agreed with many authors; Sharma *et al.*(2005) reported that a exposure of technical grade dimethoate caused portal inflammation, focal hepatocyte necrosis and centrizonal congestion in the liver of rats . Sayim, 2007; Gokcimen *et al.*, 2007; Elhalwagy *et al*., 2008 and Muthuviveganandave *et al*., 2011 showed that it may occur hemorrhage, inflammatory cell infiltration. Thangevel *et al.*, 1994 investigated that toxic effect of an organophosphate pesticide dimecron and acarbamate fungicide cumin on the histopathology of liver showed that protrusion of nuclei and destruction of hepatocytes. Sivapiriya *et al*, 2006 reported that acute and sub-chronic exposure to Dimethoate alters the antioxidant status and the histology of the liver in the mice. Persis and Kalairasi, 2001 showed the evidences of liver damage like hypertrophy, disintegration of hepatocytes showing different sizes of nuclei, lymphocytic infiltration, hemorrhage and sinusoidal blood congestion.Cholinesterase is important enzyme in the body and it's activity has been traditionally monitored as a biomarker of organophosphate pesticide exposure.The rats when exposed to dimethoate, showed the significant decrease in liver cholinesterase activities and effect on esterases. Organophosphate pesticides are known as cholinesterase inhibitors (Ware, 1983; Ecobichon, 1991). Some Physicians noticed that cholinesterase readings helpful for detecting the early effects of occupational organophosphate poisoning in human beings.

Present study demonstrated that exposure to organophosphate pesticide, dimethoate lead to changes in histopathology of hepatic tissue, it could be possible that prolonged exposure may lead to such disease like chronic liver failure or acute liver damage.

The kidneys are most important organ in the body as they remove waste products from the body and maintain homeostasis in the body. Kidneys are most sensitive to external factors which might be able to produced histopathological change and functional deficit also. The histopathological examination of kidney tissues in mice treated with dimethoate showed Tubular degeneration, glomerular degeneration, infiltration, hemorrhage, Infiltration, Tubular widened lumen, glomerular shrinkage,Vacuolation, swollen proximal convoluted tubule, swollen lining of Bowman's capsule, compressed blood vessel. These results are agreed with Khogali *et al.* (2005). Kerem *et al.,* (2007) and Afshar *et al.,* (2008) they reported tubular dilation, hemorrhage, hydropic degeneration and moderate congestion in the cortical male rats exposed to some organophosphate pesticides.

Also Al-Sharqi *et al*., (2012) reported large haemorrhagic areas, congested blood vessels, lobulated glomeruli, , degenerative changes and infiltration in kidneys of mice treated with insecticide (actara). Present study demonstrated that exposure to organophosphate pesticide, dimethoate lead to changes in histopathology of Kidney, it could be lead to such disease like kidney failure or damage.

The blood is important body fluid in humans and it brings oxygen and nutrients to all part of the body via circulatory system. The results of this study showed that the hemoglobin and hematocrit values were decreased as compared to control. Bhatnagar (1980) and Ray(1992), reported that the effect of organophosphorus pesticides on the hemoglobin (Hb) of several workers.The pestisides leads to development of anemia due to interference of pesticide poisoning in hemoglobin synthesis(Betrosin1995).Elias (2010) showed that dimethoate affect on the blood parameters ,Hb and RBCs values significantly decresed and ESR value increased when compared with control Rabbits

CONCLUSION

The results of the current study clearly demonstrate that organophosphate dimethoate is capable of inducing dose dependent histopathological and hematological changes in liver and kidney of exposed mice. According to above these results, it is suggested that dimethoate exposure might cause hazardous effects to man and environment.

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