Teratological and histopathological effects of Dimethoate 40 EC pesticides in albino rats

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ABSTRACT

In the present study we investigated the teratogenic and histopathological effects of Dimethoate 40 EC pesticide, in female rats given by two doses 16mg/kg (1/10 LD50) and 4mg/kg (1/40 LD50) over a period of 6-15 days of pregnancy during organogenesis of feti .The dams were sacrificed at day –twenty of gestation and their feti were subjected to morphological, visceral and skeletal examinations. The organ phosphorous was significantly decreased the number of viable feti and significantly increased the number of resorbed feti. No dead feti and induced retardation in growth of viable feti, some visceral and skeletal defects in these feti were seen.

Histopatholgical changes in organs are dose related and include ballooning degeneration of liver, diffuse degenerative changes of the cortex of the kidney ,haemosiderosis of spleen and the lung showed hyperplasia and desquamation of lining epithelium with sever congestion of the placenta.

Conclusively, Dimthoate caused some fetal defects and abnormalities particularly with increase the dose as well as some histopathological. Accordingly, it is advisable to avoid exposure of humane and animals to dimethoate especially during pregnancy.

It concluded that dimethoate have teratogenic and histopathological effect in fetus of pregnant rats which is dose dependant so extreme caution should be considered by pregnant women and animals to avoid its hazardous effects on their fetus.

Keywords: Dimethoate, Histopathological, teratological. organophosphorous

INTRODUCTION

Pesticides have contributed to dramatic increases in crop yields , and in the quantity and variety of diet they have helped to limit the spread of certain diseases, but can cause an jury to human health as well as, to the environment (Mansour, 2004).

Organophosphorus insecticides are generally short- lived and tend to accumulate in plant or animals tissue to many great extents. They are considered as anti cholinesterase insecticides in the target

tissues (Jayaratnam and Moroni., 1994). Recent studies have shown that acute and subchronic exposure to dimothoate alters the antioxidant status and the histology of the liver , brain and testes of rats (Saafi et al ., 2011) (Astiz et al ., 2009) and human erythrocytes (Garouri et al .,2011) . The liver is the primary organs involved in xenobiotic metabolism and is a target organs for chemicals and drugs. Hepatotoxicity is therefore an important endpoint in the evaluation of the effect of particular xenobiotics.

Also , pesticides have been implicated in various disorders and diseases including cancer, adverse reproductive outcomes, peripheral neuropathies, neuro behavior disorders, impaired immune function ,allergic sensitization actions, particularly of the skin , cumulative inhibition of cholinesterase activity as a result of long/ term low doses of exposure to organophosphorus compounds (WHO / UNEP. Public Health Impact of Pesticides used in agriculture. Geneva 1990).

Dimethoate has a stomach action and a cholinesterase inhibitor. It is of low persistence in the soil , water and environment (half –lives of 4 to 16 days) .Disappearance from open water is possibly due to microbial action or chemicals degradation as proteolysis and evaporation (Haward 1991).Dimethoate was dissolved and diluted to the required doses using sunflower oil.

The objective of this study was to examine the teratological and histopathological effect of dimethoate in dose of 16mg/kg (1/10 LD50) and 4mg/kg (1/40 LD50) on female albino rats during pregnancy.

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MATERIALS AND METHODS:

2.1 chemicals:

Dimethoate 40 EC is an organophosphorus pesticide with a chemical
formulaCH3NHCOCH2SP(OCH3)2
2.Agent type: 98% Tech,40%EC
3.CAS NO:60-51-5
4.Watre:0.5%
5.Characteristics:
Dimethoate is Cholinesterase inhibitor with contact and stomach action. Widely used to protect citrus, cotton, grape, olive potato, soybean, tobacco, vegetable to kill mites, aphids, etc. Flies in livestock shed can be
Controlled by the application of Dimethoate.
7. Delivery Time: In 20 days after confirmed L/C
Company:Shenzhen King Quenson Industry Co., Ltd.

Experiments were designed to examine the teratological and histopathological effect on the liver, intestine, stomach and kidney and changes in some parameters of albino rats following administration of dimethoate.

A total of 30 adult female and 5 male albino rats were used in the experiments. Animals were divided into three groups of 10 albino rats each. 8-10 months, 210-250 gm body weight, obtained from animal house colony of Faculty of Veterinary Medicine, Benha University. Rats were kept under hygienic and good condition of ventilation, and at room temperature 25 to 30C fed on standard balanced diet and water. Female rats were examined periodically using vaginal smear technique to ensure that they were in regular estrous cycle . Each female in estrous phase was paired with a male of proven fertility in a separate cage. In the morning, vaginal smear was taken to verify day of pregnancy. The female exhibiting a vaginal plug of coagulated ejaculate were considered pregnant and designed as zero day of pregnancy. Presence of spermatozoa in the obtained vaginal smear suspected pregnancy (Barcellona et al., 1977).

Pregnancy was confirmed by persistence of diestrous state for 5 days. After mating, physiological bleeding at 14th day of gestation and palpable fetal masses in the abdomen at 15th day after mating. They were maintained in the animal house on daily observation.

Weight of rat was registered before the beginning of treatment .LD50 value for dimethoate has been reported to be 160mg /Kg for rat (Howard., 1991) and was used in the present study.

2.2–Experimental design

Thirty pregnant dams were divided into three groups each of 10 rats. Rats within the 1st group were kept as a control, the dose of dimethoate were used from the result obtained from fish tissue which collected, the rats within groups 2 were given orally once daily with dimethoate at rate of 16mg/ Kg (1/10 LD50) and group 3 were given orally 4 mg/ Kg (1/40LD50) . The drug was given from 6th to 15th days of gestation during period of fetal organogenesis (Cook and Fairweather, 1968). All females were killed on the 20th day of pregnancy and their uteri were dissected in order to record the position and number of viable, resorbed or dead feti. The surviving feti were weighed and the length from crown to ramp was measured and examined for any external gross malformations, while others were stained by alizarin red for skeletal examination (Hays et al., 1988).

RESULTS:

2-Teratological studied

Oral administration of dimethoate by dose of 4 and 16 mg/Kg.B.WT to pregnant female rats from 6th to 15th days of pregnancy induced changes in number of viable, dead, resorbed feti and fetal body

weight & crown – rump length (Table 1 and Fig 1-3). Visceral- abnormalities of feti were recorded as diverticulm dilatation of the brain, hypoplasia of the lung, hyperplasia of the heart, enlargement of the suprarenal gland and hypoplasia of the kidney (Table 2 and Figs. 4-11). While skeletal examination of alizarin red stained feti obtained from dams given oral administration of dimethoate in doses of 4 and 16 mg /Kg.b.wt. from 6th to15th days of gestation showed different abnormalities defect in ossification of the skull, absence of some bone of sternebre absence of digit bones(Table 3 and Figs 12 - 18).

The fetus showed :

1-decrease in body gain

2- Decrease food consumption

3-emaciation

The liver and kidney of fetus showed :

1-Enlarged liver

2-with double therapeutic dose presence area of necrosis

3- Dilatation of bile duct

4- enlarged and congested kidney

5- Presence area of hemorrhage

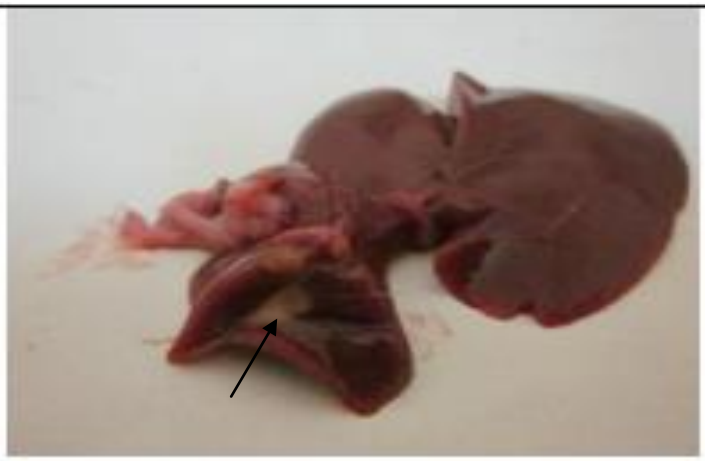


Fig (27) :The liver of the rat administered dimethoate by dose of 16mg /Kg.b.wt from 6th to 15th days of pregnancy showed sever congestion and enlargement and some area of necrosis

Table (1): Effect of dimthoate on feti obtained from pregnant female rats after oral administrations of dimethoate in maximum dose16mg (1/10 LD50) and minimum dose 4 mg (1/40 LD50 Kg.b.wt from 6th to 15th days of pregnancy once daily (n=5).

Parameters	Control group		Dimethoate group			
			4 mg/Kg. b.wt (mimimum dose)		16mg/Kg.b.wt (maximum dose)	
Number of female rats	10		10		10	
Number of viable feti	No	%	No	%	No	%
	97	100%	72	80.89%	52	67.53%
Number of dead feti	-	-	-	-	-	-
Number of resorbed feti	-	-	17	19.10%	25	32.46%
Fetal body weight (gm)	3.97±0.03988	-	3.51±0.0597	-	2.25±0.1941**	-
Fetal crown- rump lengthcm (cm)	4.16±0.04566	-	3.73±0.04409	-	3.43±0.07848*	-

Table (2): Visceral abnormalities in feti obtained from pregnant female rats after oral administrations of dimethoate in maximum dose16mg (1/10 LD50) and minimum dose 4 mg (1/40 LD50)/Kg.b.wt from 6th to 15th days of pregnancy once daily (n=10).

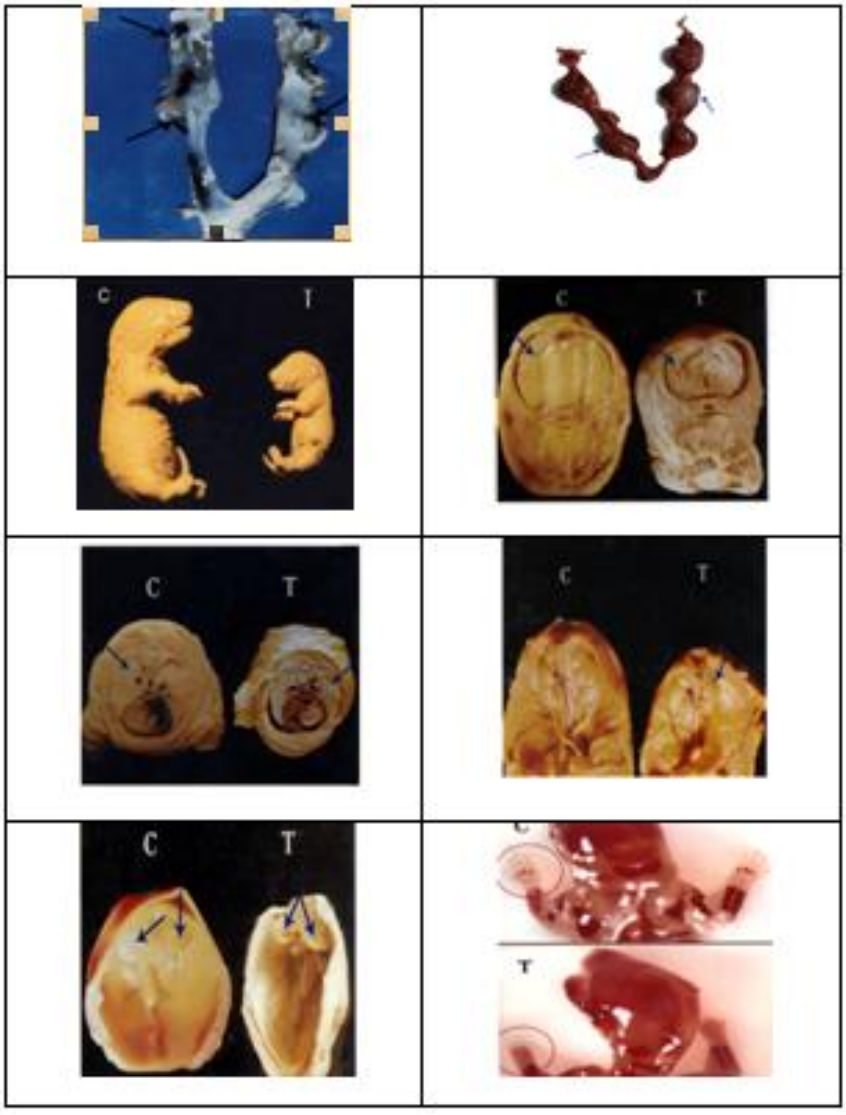
Groups	Dose	Number of examined mother	Abnormalities													
			Brain diverticulum		Thymus hypoplasia		Lung hhypoplasia		Heart enlargment		Liver enlargment		Kidney hypoplasia		Supra renal gland enlargmentrnal	
			No	%	No	%	No	%	No	%	No	%	No	%	No	%
Group 1	Control	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Group 2	4 mg/Kg. b.wt (mimimum dose)	10	2	10%	3	15%	4	20%	3	15%	4	20%	3	15%	3	15%
Group 3	16mg/Kg.b.wt maximum dose)	10	4	20%	5	25%	7	35%	5	25%	8	40%	6	30%	5	5%

% Percent of total abnormalities in relation to the number of examined feti.

Table (3): Skeletal abnormalities in feti obtained from pregnant female rats after oral administrations of dimethoate in maximum dose16mg (1/10 LD50) and minimum dose 4 mg (1/40 LD50)/Kg.b.wt from 6th to 15th days of pregnancy once daily (n=10) .

Groups	Dose	Number of examined mother	Abnormalities													
			Skull		Sternebra		Ribs		Digit's bone		Metatarsal bone		Metacarpal bone		Caudal vertbrae	
			No	%	No	%	No	%	No	%	No	%	No	%	No	%
Group 1	Control	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Group 2	4 mg/Kg. b.wt (mimimum dose)	10	3	20%	4	15.78%	2	13.3%	4	26.7%	3	20%	3	20%	4	31.57
Group 3	16mg/Kg.b.wt (maximum dose)	10	6	40%	7	46.7%	4	26.7%	8	53.3%	6	40%	6	40%	7	46.15

% Percent of total abnormalities in relation to the number of examined feti.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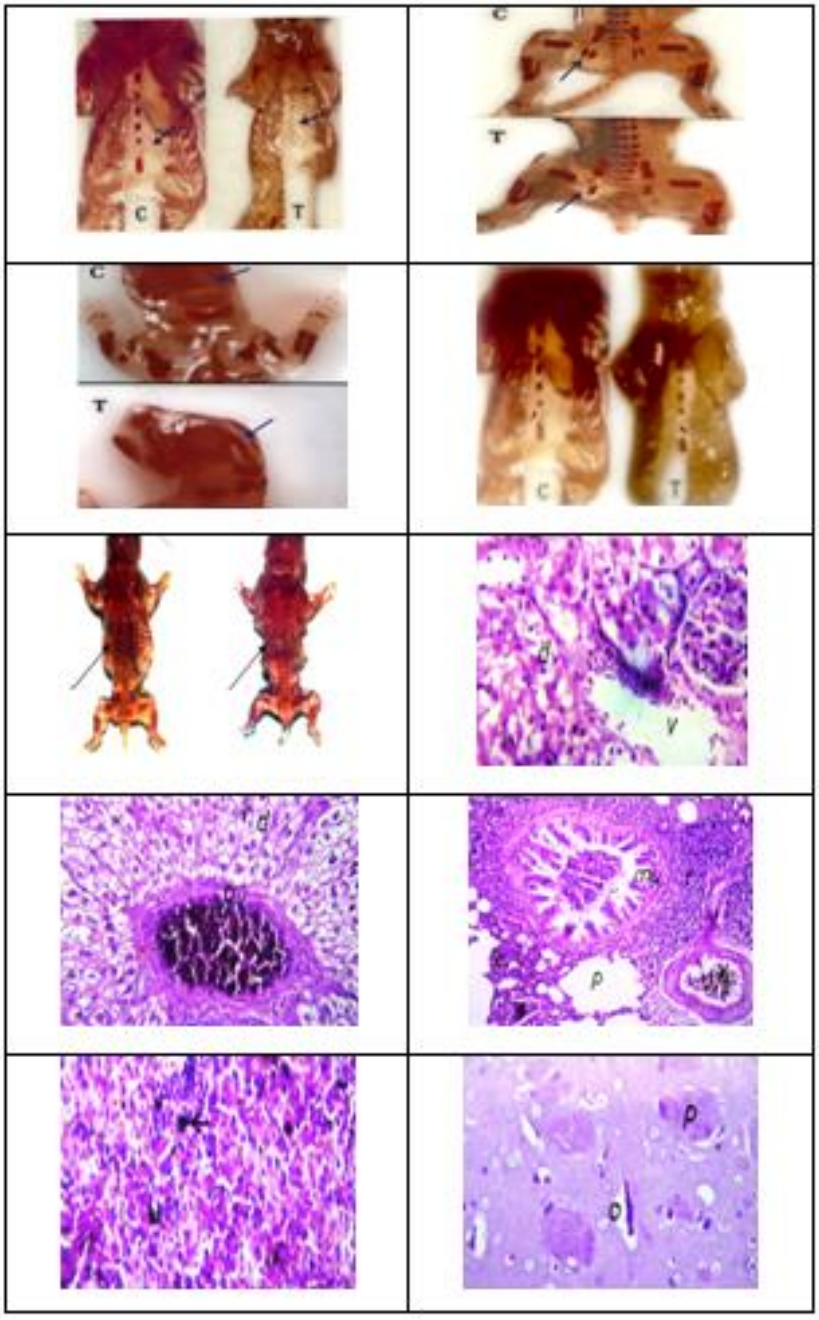
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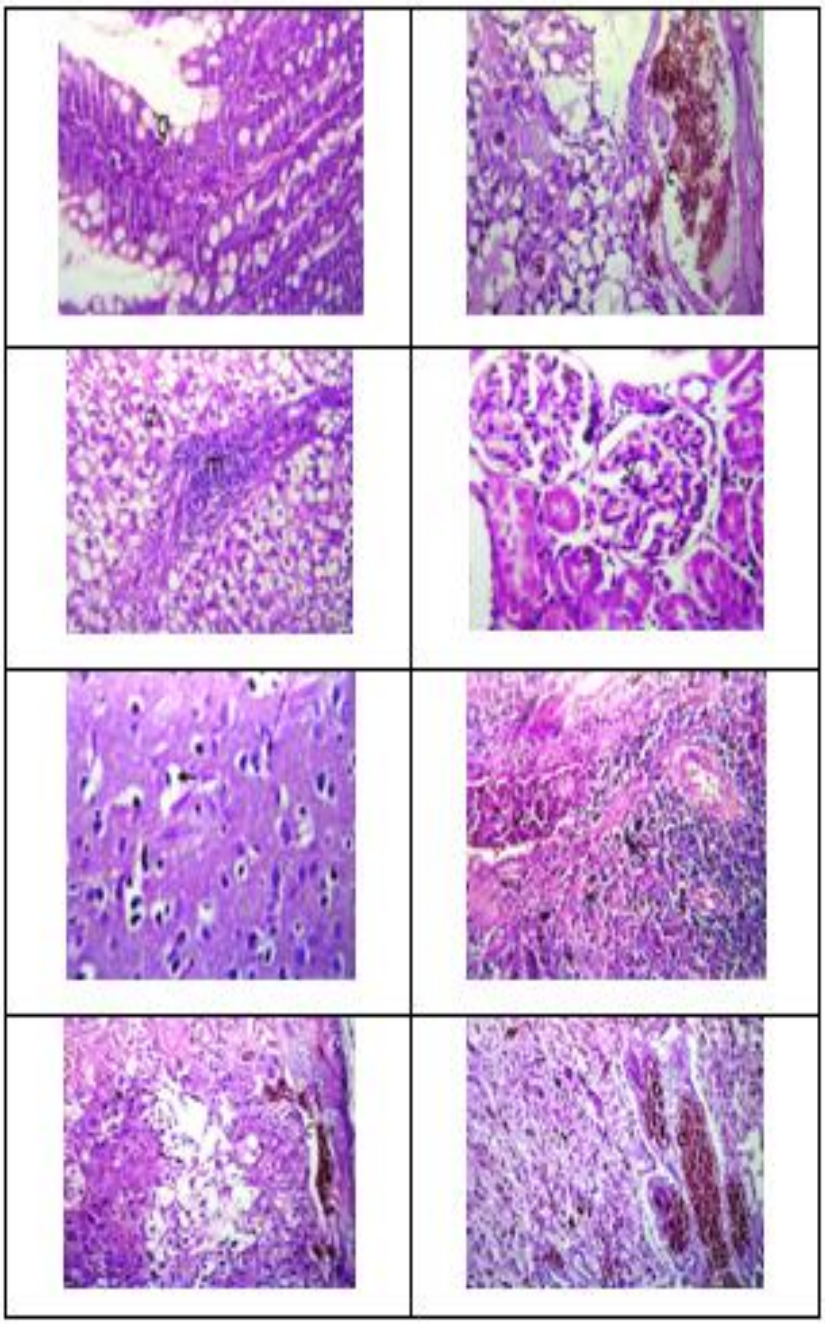

Fig. (1): Gravid rat uterus obtained from mother after repeated orally administrations of 16 mg dimethoate/Kg.b.wt from 6th to 15th days of pregnancy showing early uterine resorption.
Fig. (2): Gravid rat uterus obtained from mother after repeated orally administrations of 16 mg dimethoate/Kg.b.wt from 6th to 15th days of pregnancy showing late uterine resorption
Fig. (3): Retardation of growth of a fetus obtained from mother after repeated orally administrations of 16 mg dimethoate/ Kg.b.wt from 6th to 15th days of pregnancy
Fig. (4): Diverticulum dilatation of a fetus obtained from mother after repeated orally administrations of 16 mg dimethoate / Kg.b.wt ncy
Fig. (5):Pulmonary hypoplasia with cardiac enlargement of a fetus obtained from mother after orally administrations of 16 mg dimethote / Kg.b.wt from6th to 15th days of pregnancy
Fig. (6): Hypoplesia of left kidney with enlargement of right supra renal gland of a fetus obtained from mother after orally administrations of 16 mg dimethote / Kg.b.wt from6th to 15th days of pregnancy
Fig. (7): Unilateral dilatation of renal pelvis of a fetus obtained from mother after orally administrations of 16 mg dimethote / Kg.b.wt from6th to 15th days of pregnancy
Fig. (8): Absence of digits of a fetus obtained from mother after orally administrations of 16 mg dimethote / Kg.b.wt from6th to 15th days of pregnancy
Fig. (9): Absence of 4th, 5th and 6th sternebra of a fetus obtained from mother after orally administrations of 16 mg dimethote / Kg.b.wt from6th to 15th days of pregnancy
Fig. (10): Absence of caudal vertebrae of a fetus obtained from mother after orally administrations of 16 mg dimethote / Kg.b.wt from6th to 15th days of pregnancy
Fig. (11): Impaired ossification of skull of a fetus obtained from mother after orally administrations of 16 mg dimethote / Kg.b.wt from6th to 15th days of pregnancy
Fig. (12): Absence of 5th sternebra of a fetus obtained from mother after orally administrations of 16 mg dimethote / Kg.b.wt from6th to 15th days of pregnancy
Fig. (13): Increase intercostal space of the ribs of a fetus obtained from mother after orally administrations of 16 mg dimethote / Kg.b.wt from6th to 15th days of pregnancy
Fig (14):The kideny of rat adminestered dimethoate orally by dose of 4mg /Kg.b.wt from 6th to 15th days of pregnancy showed degeneration of the linning epithelium cell of the tubules with dilatation of the blood vesseles H&E × 80.
Fig. (15): Liver of rat administered dimethoate orally by dose of 4mg /Kg.b.wt from 6th to 15th days of pregnancy showed balloning degeneration in hepatocytes (d) and sever congestion in the portal vein(pv) H&E ×64.
Fig (16) : the lung of the rat administered ntestine of rat administered dimethoate by dose of 4mg /Kg.b.wt from 6th to 15th days of pregnancy showed hyperplasia withcellular desqumation in the linning epithelial cells associated with peribronhciolar inflammatory cells aggregation, dilatation in the blood vesseles and collapse as well as emphysma in the alveoli H& Estain ×40.
Fig (17) :The spleen of rats administered dimethoate by dose of 4mg /Kg.b.wt from 6th to 15th days of pregnancy showed hemosiderosis in the red pulps H&E × 80.
Fig (18): the brain of rat administered dimethoate by dose of 4mg /Kg.b.wt from 6th to 15th days of pregnancy showed sever congestion of blodd cappilaries of odema and degernaration. H&E stainX64.
Fig (19) :The small intestine of the rat administered dimethoate by dose of 4mg /Kg.b.wt from 6th to 15th days of pregnancy showed diffuse goblet cell formation allover the lining mucosal epithelium cells (g) H&E stain × 46.
Fig (20) : The placenta of the rat administered dimethoate by dose of 4mg /Kg.b.wt from 6th to 15th days of pregnancy showed sever congestion (C) H&E stain × 40.

Fig (21): Liver of rat administered dimethoate orally by dose of 16mg /Kg.b.wt from 6th to 15th days of pregnancy showed focal inflammatory cells aggrgation in the portal area with diffuse ballog degeneration in hepatocytes H&E stain ×64.
Fig (22): The kideny of rat adminestered dimethoate orally by dose of 16 mg /Kg.b.wt from 6th to 15th days of pregnancy showed swelling and vacuolization in the lining endothelium of the glomerular tuft (g)with swelling in the linning epithelium of the tubules (S) H&E stain × 80.
Fig (23) : The brain of rat administered dimethoate by dose of 16mg /Kg.b.wt from 6th to 15th days of pregnancy showed cellular oedema(arrow) in the cerebrum H&E 80
Fig(24): The spleen of rat administered dimethoate by ose of 16 mg /Kg.b.wt from 6th to 15th days of pregnancy showed Congestion was noticed in the blood vessels and red pulps associated with focal hemosiderosis H&E stain ×40
Fig (25) :The placenta of the rat administered dimethoate by dose of 16mg /Kg.b.wt from 6th to 15th days of pregnancy showed congestion of blood vessels in chorioallantioic membrane (C) H&E 40
Fig (26): The placenta of the rat administered dimethoate by dose of 16mg /Kg.b.wt from 6th to 15th days of pregnancy showed sever congestion of blood vessels between the trophospongium and labyrinth H&E 40.
Fig (27) :The liver of the rat administered dimethoate by dose of 16mg /Kg.b.wt from 6th to 15th days of pregnancy showed sever congestion and enlargement and some area of necrosis

DISCUSSION

Teratological effect:

The exposure of organophosphorus to pregnancy is an important factor because it affects two organism, a mother and a fetus. Oral administration of dimethoate in minimum dose (4 mg / Kg. b. Wt) and maximum dose (16mg/Kg.b.wt) during the period of organogenesis induced significant decrease in the number of feti as well as viable feti per mother, decrease in body weight and increase in implantation loss . The result of the current study revealed significant decrease in body weight of rats treated with dimethoate especially with high dose treatment .These results are in good agreement with those found by many authors .In concern , Pant et al , (1995) observed a significant decrease in body weight of rats treated with 0.2-0.8 mg carbofuran /Kg.B.wt . Sharma et al . (2005) found that a significant decrease in body weight gain at high dose (90mg/Kg/day)of chloropyrifos. Abed twab and mostafa 2012 studied the adverse effects of exposure to formulated chlorpyrifos-ethyl (9.60 mg kg-1 b.wt.), chlorpyrifos-methyl (300 mg kg-1 b.wt.) and methomyl (1.70 mg kg-1 b.wt.). Changes in the body weight after insecticide dosing was used as a valuable index of insecticide-related organ damage (Lu, 1996; Mansour and Mossa, 2010a; Mossa et al., 2011). Other authors revealed that the tested insecticide caused significant decrease in body weights of treated rats and may be due to the overall increased degradation of lipids and proteins as a result of the direct effects of anti-cholinesterase compound (Goel et al., 2005; Mansour and Mossa, 2011; Mossa et al., 2011). Other investigations have reported the reduction in body weight and change in relative organs weights in rats (Woolliams et al., 1983; Mansour et al., 2001; Mossa et al., 2011) and mice (Ambali et al., 2007) after exposure to anti-cholinesterase insecticides..These results are in good agreement with those found by many authors

as Farag et al ., (2007) who found that administration of dimethoate in dose of 15 and 28 mg/kg.b.wt associated with a decreased number of implantations and live fetuses, and an increased number of dead and early resorptions at 28 mg/kg/day treated group.40LD50 causes detritus effect of fetus in form of reduction in fetal body weight and preimplantation loss . Mahadevaswami and kaliwal (2004) reported that administration of dimethoate by dose of 24 and 28 mg/Kg .b.wt induced significant decrease in number of implantation ,and live fetus. Amina et al (2002) reported that dimethoate cause maternal toxicity that included reduction in body weight and feed consumption was observed only in the treated group of 28 mg/kg/day. Kimbrough & Gaines [1998] found the deaths and resorption was increased in pregnant rats when they were given a single high dose of parathion or diazinon on the 11th day of gestation. The decrease in number of feti per mother might be attributed to the lack of oval production or of the basic cell constituent as a result of drug administration (Tuchmann, 1975). Decrease in number of viable feti might be explained on the basis of histopathological changes of placenta as sever congestion was observed in between the giant trophoblasts in minimum dose Congestion was noticed in the blood vessels in the chorioallantoic membrane as well as in the deep layer between the trophospongium and labyrinth in maximuim dose which affect in transmission of nutrition to fetous.

Repeated oral administrations of 4 and 16 mg/Kg.b.wt of dimethoate to pregnant female rats during the period of organogenesis induced many fetal visceral abnormalities as diverticuluim dilatation which might be attributed to the lake of placental transfusion of amino acid arginine metabolism in fetus (Tuchmann 1975),lung hyperplasia , thymus hypoplasia or due to neurotoxic effect as dimethoate which cause inhibition of acetyl cholinesterase an enzyme which found in free state mainly in brain, nerve cells muscle ,lung and erythrocytes this enzyme strongly inhibited in case of poisoning with organophosphorus compound. Mohammed al (2004). Organophosphates have shown the ability to penetrate the placental barrier and thus could potentially affect the developing fetus. Pesticides like Organophosphates have been detected in amniotic fluid, umbilicord blood, (Umbilical cord blood is blood that remains in the placenta and in the attached umbilical cord after childbirth. It is a reservoir of stem cells which can be used to treat hematopoietic and genetic disorders), meconium and infant urine, indicating exposure of the human fetus to pesticides [Bradman et al 2003) and Abu-Qare et al (2000). Abou-Qare and Abou Donia [2001] reported that a single cutenous dose of methyl parathion significantly inhibited maternal and fetal brain acytelcholine and plasma butyrylcholinesterase in rats.

Dimethoate is an organophosphate insecticides known to produce oxidative stress in human and animal cells .As a lipophilic molecule, it can easily pass through the cell membrane into the cytoplasm. Once inside the cell, dimethoate can induce a high level of damage in several tissues and activate the formation of free radical Ahmed et al (2012), also this result was agree with that reported by Uzun et al (2010) and Cemek et al (2010), who found an induction of oxidative stress in lung tissue after oral ingestion of chlorpyrifos and fenthion. The generation of reactive oxygen species

may be result of organophosphates metabolism by cytochrome P450s ,monooxygenase which catalyze oxidation by addition of molecular oxygen atom into a substrate (organophosphate) through electron transport pathway (Jakoby and Zigler 1990), Chamber et al 2001.

Repeated oral administrations of 4 and 16 mg/Kg.b.wt of dimethoate to pregnant female rats during the period of organogenesis induced many fetal skeletal malformations as impaired ossification of skull, absence of sternabre, absence of caudal vertebrae, absence of digit′s bone of fore and hind limbs and absence of some metatarsal and metacarpal bones. These results agreed with those reported by Dimethoate is possibly a human teratogen Hallenbeck and Cunningham-Burns. (1985) It was teratogenic in cats and rats (Hayes and Wayland 1982.). A dosage of 12 mg/kg/day given to pregnant cats increased the incidence of extra toes on kittens. The same dosage given to pregnant rats produced birth defects related to bone formation, They are considered as anti cholinesterase insecticides in the target tissues (Jayaratnam and Moroni., 1994) leads to accumulate endogenous acytelcholine in nerve tissue and other effector organs (Mayers et al 1990) . Acytelcholine may also have a functional role in bone (Compaston 1999) , Genevar, 1999, Greisaru 1999) Genever et al, 1999) identified that acytelcholine expression in the bone by osteoblasts, this fact suggested that acytelcholine might have noncholinergic capacity of the bone through its ability to mediate cell function as demonstrate in various tissue (Downes and Granto ,2004) (Silman and sissman, 2005) .Organophosphorous is potent inhibitor of acetylcholine caused significant reduction in bone mass and density in individuals following low level exposure (Compston, 1999) ,also causes several skeletal deformity (Misawa, 1982). diazinon-induced inhibition in growth of some skeletal elements, such as femur, tibia, metatarsi and digits of the leg in chick embryos were demonstrated in the study by Misawa et al. (1982).

Histopathological finding:

Histopathological examination of female organs (liver, kidney, brain, spleen, lung, heart, intestine, skeletal muscle, and placenta) showed changes in female organs after administration of dose 4mg/Kg.b.Wt. As show in fig (14-26) The liver showed congestion with ballooning degeneration in the hepatocytes in diffuse manner and focal inflammatory cells aggregation in the portal area, this result are agree with that reported by dermal exposure to dichlorvos resulted in the appearance of mononuclear cell infiltrates in the lungs, liver. After administration of a higher dichlorvos dose, In these animals the total amounts of leukocytes and lymphocytes were significantly higher when compared to the control group [Nurulain and Shafiullah 1990] . Liver is a target organ , primary site of detoxification and is generally the major site of intense metabolism and is therefore prone to various disorders as a consequence of exposure to the toxins of extrinsic as well as intrinsic forms and plays important role in metabolism to maintain energy level and structural stability of body (Guyton and Hall, 2002). It is also site of biotransformation by which a toxic compound has been transformed in less harmful form to reduce toxicity (Hodgson, 2004). However, this will damage the liver cells and produce hepatotoxicity .Dimethoate caused dose-

related histopathological changes .Thease results are in agreement with that reported by many authers ; Selmanoglu and Akay (2000) they reported similar histopathological changes including mononuclear cell infiltration, congestion, hydropic degeneration and hepatocellular damage in the liver of male rats treated with dimethoate, endosulfan and carbaryl. Also Sharma et al. (2005) who found that 30 day exposure of male rats to technical grade dimethoate at dose of 6and 30 mg/Kg caused portal inflammation.

The kidney showed diffuse degenerative change was detected in the epithelial cells lining the tubules at the cortex associated with dilatation in the intertubular blood vessels, while female administered dose of 16 mg/Kg.b.Wt., the kidney showed swelling and vacuolization in the lining endothelium of the glomerular tuft as well as swelling in the lining epithelium of the tubules at the cortex , this result agree with that reported by Ullman et al., (1977) who reported that the histopathological changes of the kidney causing renal degeneration which may be related to the role played by the kidney in excretion of insecticides, also Evans et al ., 1988 who reported that hydropic degeneration of the kidney with leucocytic infiltration indicator of nephrotoxic effect of organophosphorus pesticides of ievamisole and khattab 1994 recorded the same effect caused by dimethoate, also Khogali et al 2005 found histopathological changes of the kideny of mice treated by 60 mg /kg dimethoate pesticides showed blood congestion in between tubules.

The spleen showed hemosiderosis in the red pulps this result agree with that reported by (Hekmate et al 2005) she found hemosiderin granules found in the spleen under the effect of low concentration of fenthion after administration to Cyprinus carpio with proliferation of lymphocytes with high concentration this may be due to increase in the rate of destruction of erythrocytes after exposure to pesticides (Hibiya 1982) . It was thought that these changes were due to an increase rate of breakdown of red cells and/or the toxic effect of pesticides on bone marrow. (Mossa, 2004). Shakoori et al. (1990) .

The lung showed hyperplasia of bronchioles with cellular desquamation in the lining epithelial cells associated with peribronhciolar inflammatory cells aggregation, dilatation in the blood vessels and collapse as well as emphysema in the alveoli, the lung showed diffuse goblet cells formation all over the lining mucosal epithelium associated with administration of dimethoate by dose of 4 mg/Kg . b.wt, while administration of 16mg /Kg B.Wt the lung showed hyperplasia in the lining epithelium of the bronchioles associated with peribronciolar lymphoid cells aggregation, haemorrhage, and emphysema of the air alveoli histopathological changes in lung tissue were noted as emphysema, hemorrhages and hemosiderin deposits as result of dimethoate-induced lung oxidative damage (Ibtesam ben amare et al .,2011) dimethoate is an organophosphate produce oxidative stress in human and animal cells as a lipophilic molecules, it can easily pass through the cell membrane into the cytoplasm .Once inside the cell, dimethoate can induce a high level of damage in several tissues including lung as exposure to dimethoate resulted in significant increase in lipid peroxidation and protein oxidation indicated by increase level of malondialdehyde suggesting that dimethoate activated formation of free radicals as discussed by uzun et al 2010and semek et al 2010) this result are agree with that

reported by dermal exposure to dichlorvos resulted in the appearance of mononuclear cell infiltrates in the lungs, liver, kidneys and heart. After administration of a higher dichlorvos dose, changes in the lungs were manifested as widened interalveolar spaces infiltrated with macrophages and lymphatic cells, as well as by hyperemia. Similar histological changes in the lung of rats exposed to dermal absorption of a single dose of dichlorvos for 4 hours were observed earlier. In these animals the total amounts of leukocytes and lymphocytes were significantly higher when compared to the control group [Nurulain and Shafiullah 1990]

The brain of rat administered dimethoate by dose of 4mg /Kg.b.wt from 6th to 15th days of pregnancy showed Plague formation was noticed in the cerebral matrix of the brain, and sever congestion of blood capillaries, odema and degeneration. while the brain of rat administered dimethoate by dose of 16mg /Kg.b.wt from 6th to 15th days of pregnancy showed cellular oedema . Eskanse et al (1999) showed that exposure to chemical during different stages of development like pre and peri-conception, fetal and preinatal that organophosphorous pesticides affect nervous system. Nigar et al (2011) showed that the treatment with dizinon induced significant increase in malondildehyde in rat brain as result of oxidative stress which contribute to dizinon induced brain toxicity. Mohamed et al (2004) studied the teratogenic effect of dimethoate on chicken embryo leads to decrease body weight and embryo mortality and malformations of featus ,deformity mainly in the brain and this suggests the defective cell proliferation, central nervous system and heart development were target of dimethoate even at low concentration. Inhibition of acetycholinesterase suggests disruption of nerve function during the embryo development

Diffuse goblet cells formation all over the lining mucosal epithelium of the intestine after administration of 16mg/ Kg.B.Wt another author revealed that low doses of diazinon insecticide caused different histopathological changes in the small intestine of male guinea pig. These changes were manifested in infiltration and hypertrophy of the lymphocytes, Rady (2009) haemorrhage in the submucosa, erosion in lining epithelium, pyknotic nuclei and necrotic cells. These lesions were more evident with the high doses. Similarly, desquamation, haemorrhage and necrosis of the epithelial cells of the stomach and intestine were noticed post α-cypermethrin insecticide oral administration in rats (Manna et al., 2004 a). In vitro study showed that the cultured intestinal and colonic cell proliferation was decreased by diazinon insecticide (Greenman et al., 1997).

The placenta showed Sever congestion in between the giant trophoblasts after administration of dimethoate by dose of 4 mg/Kg.B.Wt while after administration of 16 mg/Kg.B.Wt placenta showed congestion was noticed in the blood vessels in the chorioallantoic membrane as well as in the deep layer between the trophospongium and labyrinth .Organophosphates have shown the ability to penetrate the placental barrier and thus could potentially affect the developing fetus (Abu-Qare et al (2000). Pesticides like Organophosphates have been detected in amniotic fluid, umbilical cord blood, (Umbilical cord blood is blood that remains in the placenta and in the attached umbilical cord after childbirth. It is a reservoir of stem cells which can be used to treat hematopoietic and genetic disorders, meconium and infant urine, indicating exposure of the human fetus to pesticides [Bradman et al 2003) .

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تأثير الديموسوات علي التطور الجنيني والأحشاء الداخلية في إناث الفئران

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تـم هـذا العمـل لمعرفـة تـأثير الـديموثوات وهـو أحـد المبيـدات الحديثـة مـن مجموعـة (الاورجانوفوسفور) علي التطور الجنيني والأعضاء الداخلية في اناث الفئران وذلك علي النحو التالى:

أولا- تأثير الديموثوات علي التطور الجنيني

تمت هذه الدراسة علي عدد ثلاثون من إناث الفئران الحوامل التى قسمت إلي ثلاث مجموعات متساوية (١٠ فئران لكل مجموعة): المجموعة الأولى تركت كمجموعة ضابطة أما المجموعة الثانية والثالثة فتم تجريعها عن طريق الفم – علي الترتيب بجرعة ٤ ملجم 1/10 (لجرعة السامة ، ١٦ ملجم ١٠/١ الجرعة المميتة) ديموثوات /كجم من وزن الجسم مرة يوميا ابتداءا من اليوم السادس وحتى اليوم الخامس عشر من الحمل (فترة تكوين الأعضاء في الأجنة).وقد لوحظ نقص فى وزن الفئران وفقد الشهية و أخذت الأجنة من أرحام أمهاتها في اليوم العشرون من الحمل وتم فحصها ظاهريا كما فحصت الأحشاء الداخلية بثلثى عدد الأجنة المأخوذة من كل أم بعد حفظها في سائل البوان وفحص الهيكل العظمي للثلث الأخر من الأجنة بعد صبغها بصبغة الألزرين الحمراء وقد دلت النتائج علي الأتي:

تجريغ الديموثوات المتكرر بجرعتي ٤ ، ١٦ ملجم/كجم من وزن الإناث الحوامل مرة يوميا ابتداءا من اليوم السادس حتى اليوم الخامس عشر من الحمل سبب نقص في عدد الأجنة الحية ولم يسبب وفاة في الأجنة مع زيادة في عدد الأجنة المجهضة وسبب اتساع تجاويف المخ وضمور الغدة التيموسية وصغر حجم الرئة مع كبر حجم القلب وزيادة في حجم الكبد مع ضمور في الكلى..

وبفحص الهيكل العظمي تبين تمعظم غير تام في عظام الجمجمة واختفاء عظام المشط والأصابع في الأطراف الأمامية والخلفية واختفاء عظام القص والفقرات العجزية والخلفية .

ثانيا- تأثير الديموثوات علي الأعضاء الداخلية للإناث الحوامل

استخدم لهذا الغرض عدد ثلاثون من إناث الفئران البالغة وقد قسمت إلي ثلاث مجموعات متساوية (١٠ لكل مجموعة)، استخدمت المجموعة الأولى كمجموعة ضابطة أما المجموعة الثانية والثالثة فقد تم حقنها على النرتيب في العضل بالجرعة العلاجية ٤ ملجم/كجم والجرعة المضاعفة ١٦ ملجم/كجم مرة يوميا لمدة ١٠ أيام متتالية – بعدها ذبحت الفئران وأخذت عينات من الكبد والكلى والمخ والرئة والطحال والامعاء وتم وضعها في الفورمالين ١٠% وتم فحصها هستوباثولوجيا ومن الفحص الهستوباثولوجي أوضحت الدراسة استسقاء مائى فى المخ وتكسر فى الخلايا المكونة للطحال. لوحظ تخثر فى خلايا الكبد ويشمل على انتفاخ غروى وتكون فجوات مائية فى الخلايا. تخثر وتجمع فى الخلايا البلعمية فى جدار الأمعاء . وقد لوحظ مع زيادة الجرعة زيادة بعض التغيرات الباثولوجية وتشمل على موت بعض البقع النسيجية فى الكلية والكبد وكذلك سقوط بعض الخلايا المبطنة للرحم والمشيمة.

ونستنتج من هذه الدراسة أن استخدام الديموسوات بجرعته العلاجية وضعف العلاجية كمبيد حشرى للأمهات الحوامل يسبب تشوهات جنينية مثل امتصاص مبكر وتشوهات واضحة في الأجنة

كما يؤدي إلي بعض التشوهات علي الأحشاء الداخلية والعظام للأمهات الحوامل لذا ينصح بعدم إستخدامة أثناء فترة الحمل خاصة بجرعات عالية.