رقم البحث (46)

MATERNAL AND FETAL TOXIC EFFECTS OF ABAMECTIN IN RABBITS

BY

Seleem F.R.; Abou Elmagd M.M. and Abdallah M.A.

Department of Forensic Medicine and Toxicology

Faculty of Veterinary Medicine, Mansoura University

ABSTRACT

To evaluate maternal and fetal toxicity induced by abamectin pesticide (Vertimec) in female rabbits, thirty pregnant New Zealand white rabbits were divided into five groups (6 for each). The first group used as control and received orally distilled water. The second group were orally administered 1mg/kg B.wt from 7th to 20th day of pregnancy. The third group orally administered 1mg/kg B.wt from 21st to 29th day of pregnancy. The fourth group orally administered 2mg/kg B.wt from 7th to 20th day of pregnancy. The fifth group orally administered 2mg/kg B.wt from 21st to 29th day of pregnancy. The five groups were sacrificed at 30th day of pregnancy. The uterine horns were examined for resorption, live and dead fetuses. Fetuses were examined for skeletal and visceral examination. Liver, kidney, spleen, brain, sciatic nerve and placenta were preserved in 10% formalin buffer for histopathological preparation. Blood samples were collected from all groups and serum was separated for biochemical analysis. The results revealed that a significant decreases in the fetal body weight and crown-rump in fetuses from treated dams compared to control. Abamectin induced dose-dependent skeletal malformations. Histopathological examination revealed pathological changes in the liver, kidney, spleen, brain in both dams and fetuses. Also, pathological changes were showed in placenta and sciatic nerve of dams. Biochemical analysis revealed that total count of white blood corpuscles (WBCs) was significantly decreased, while hemoglobin concentrations and the values of packed cell volume were not altered. Plasma levels of AST and ALP were significantly increased, while the activity of ALT not altered. Also, the levels of GST and SOD were significantly decreased, while the level of No was significantly increased. This study indicated that abamectin has deleterious effects on both mother and fetuses during pregnancy.

INTRODUCTION

Exposure during pregnancy to pharmaceutical and environmental chemicals remain a worldwide problem, assessing risk for human developmental toxicity is a major obstacle in drug development, as it relies on data from animal experiments with associated concordance problems. **(Kim et al., 2004)**

Abamectin (ABA) belongs to the family avermectins, which are the macrocyclic lactones produced by a soil actinomycete *Streptomyces avermitilis (Fisher and Mrozik, 1989).* Abamectin (avermectin B1) is a mixture of two components, with the major component avermectin B_{1a} 80% of the mixture, and the minor component avermectin B_{1b} 20% of the mixture, differing by a single methylene group. Abamectin is currently used in several countries as a pest control agent in livestock and as an active substance of nematicides and insecticides for agricultural use **(Kolar et al., 2008)**. This product is a potent insecticide and may be highly toxic to mammals (**Lankas and Gordon, 1989)**. The avermectins block electrical activity in nerve and muscle preparations by increasing the membrane conductance to ions of chloride **(Clark et al., 1995)**. The target for abamectin involves the gamma-amino butyric acid (GABA) receptor in the peripheral nervous system.

ABA poisoning can impair the function of hepatocytes. Research conducted by (**Hsu et al., 2001)** showed elevated levels of the enzyme aspartate aminotransferase (AST) in the blood serum of rats after exposure to ABA by gavage at doses between 1 and 20 mg/kg body weight. The maximum activity was obtained with a dose of 20 mg/kg of body weight 1h after ingestion. **Eissa and Zidan (2010),** using a commercial product, also observed signs of abamectin liver toxicity, with increased activity of the enzyme AST in rats treated with doses equivalent to 1/10 or 1/100 of the LD50 (18 mg/kg) in the diet of animals over 30 consecutive days. In addition, **El-Shenawy (2010)** undertook a comparative study of the in vitro toxic action of some insecticides, including ABA at concentrations of 10 and 100 μM, on isolated rat hepatocytes. There was a significant increase in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity when hepatocytes were incubated for 30 min with either concentration of ABA. This activity persisted after 120 min. Rats given 0.4 mg/kg/day of abamectin had increased still births, decreased pup viability, decreased lactation and decreased pup weight **(Lankas and Gordon, 1989)**, suggesting that abamectin may have the potential to cause reproductive effects. Abamectin produced cleft palate in the offspring of treated mice and rabbits, but only at doses that were also toxic to the mothers **(Lankas and Gordon, 1989**). The aim of this study was to evaluate the maternal and fetal toxic effects of abamectin when administered at different periods of pregnancy.

MATERIAL AND METHODS

Insecticide used:

The tested insecticide was abamectin, trade name (Vertimec) 1.8%EC, Syngenta Company; used as an acaricide. A mixture containing a minimum of 80% avermectin B_{1a} (5-O- demethylavermectin A_{1a}) and a maximum of 20% avermectin B_{1b} (5-O-demethyl-25-de-(1-methylpropyl)-25-(1-methylethyl) avermectin A_{1a}.

Experimental animals:

Thirty mature female New Zealand white rabbits, weighing 3 to 3.5 kilogram and aged about 4.5 to 5 months old were obtained from Animal Experimental Unit, Faculty of Agriculture, Mansoura University. The animals were apparently clinically healthy. Animals were housed in separate batteries and kept under controlled condition (23±1^{o}C), 12h light and 12h dark cycle. Rabbits were fed on standard laboratory pelleted diet and water ad. Libitum , they were accommodated for our laboratory condition for 2 weeks before starting the experiment.

Experimental Design:

Thirty pregnant New Zealand white rabbits were randomly distributed into equal five groups (6 for each). The first group used as control and received orally distilled water. The second group orally administered 1mg/kg B.wt from 7th to 20th day of pregnancy. The third group orally administered 1mg/kg B.wt from 21st to 29th day of pregnancy. The fourth group orally administered 2mg/kg B.wt from 7th to 20th day of pregnancy. The fifth group orally administered 2mg/kg B.wt from 21st to 29th day of pregnancy. All animals were observed twice daily for signs of treatment-related effects. At 30th day of pregnancy, all animals were sacrificed, the uterus were exposed and the uterine horns were examined.

Fetal observations

The total number of fetuses were counted for each animal, resorbed, live and dead fetuses were recorded. Fetuses were examined for weight and crown-rump length. Approximately one half of the fetuses were placed in Bouin□s fixative and subsequently sectioned for visceral examination **(Hayes, 1994).** Whereas, the other fetuses were eviscerated and stained with alizarin red S for skeletal examination.

Blood samples

Blood samples were collected immediately after slaughtering in dry clean centrifuge tubes. One portion was taken on EDTA as anticoagulant for haematological examination. The other portion collected without anticoagulant and left to clot at room temperature for about 20 min. and then centrifuged at 3000 r.p.m for 15 minutes; the serum was drown in dry clean-capped tubes and kept in deep freezer at-20ºC until conducting the biochmical analysis.

Haematological Examination

Total leukocytic count (TLC): Leukocytes were counted by hemocytometer according to **Feldman et al., (2000)**. Hemoglobin concentration (Hb): hemoglobin concentration (g\dl) was estimated spectrophoto-metrically using Cyanmethemoglobin method according to **Drabkin, (1949).** Packed cell volume (PCV): was estimated by using microhematocrite capillary tubes, and centrifuged according **to Coles, (1986).**

Biochemical Analysis

Aspartate and Alanine aminotransferase (AST, ALT) activities were determined according to the method of **Reitman and Frankel (1957).** Alkaline phosphatase (ALP) activity was determined as described by the method of **Belfield and Goldberg (1971)**. Glutathione-S-transferase (GST) was determined according to **Habig and Pabst (1974)**. Superoxide dismutase (SOD) was determined by colorimetric method according to **Nishikimi (1972)**. Nitric oxide (NO) was determined by colorimetric method according to **Montgomery and Dymock (1961).**

Histopathological study

Specimens from liver , kidney , spleen , placenta , brain and sciatic nerve were preserved in 10% neutral buffered formalin. Sections of 5 micron thickness were prepared from all specimens , stained by hematoxyline and eosin (H&E) and examined microscopically according to **Bancroff et al., (1990).**

Statistical Analysis

Data were subjected to statistical analysis using statistical software program (SPSS for Windows, version 15, USA). Means and standard error for each variable were estimated. Differences between means of different groups were carried out using one way analysis of variance (ANOVA). Data were expressed as the mean±SE. $P<0.05$ was considered as the level of significance.

RESULTS

I . Effects on developing fetuses:

Uterine examination of rabbit at 30th day of gestation revealed that abamectin resulted in 2 dead fetuses from dams orally administered abamectin at a dose of 2mg/kg B.wt from 21th to 29th day of gestation, one fetus died from dam orally administered abamectin at a dose of 1mg/kg B.wt from 21th to 29th day of gestation. Also, abamectin caused fetal resorption, two fetuses resorbed obtained from dam orally administered abamectin at a dose of 1mg/kg B.wt from 7th to 20th day of gestation. The obtained data clearly demonstrate that abamectin induced a significant decrease in the mean values of the fetal body weight and fetal crown-rump length of the obtained fetuses compared with the control group as shown in Table(1) and fig.(1).

Table (1): Fetal body weight and fetal crown rump of fetuses from dam rabbits orally administered different doses of abamectin at different periods of pregnancy (Mean ± SE) :

Group	Fetal B.wt (g)	Fetal crown-rump (cm)
Control	36.02 ^{a} ± 0.87	12.74 ^{a} ± 0.18
1 mg/kg (7th : 20th G.D)	35.01 ^{a} ± 1.06	12.84 ^{a} ± 0.17
1 mg/kg (21st : 29th G.D)	30.39^{bc} ± 0.75	12.24 ^{b} ± 0.15
2 mg/kg (7th : 20th G.D)	28.69 ^{c} ± 0.82	11.80 ^{c} ± 0.1
2 mg/kg (21st : 29th G.D)	31.86 ^{b} ± 0.86	11.98 ^{bc} ± 0.13

The means in the same column having the same superscript not significantly different ($p<0.05$).

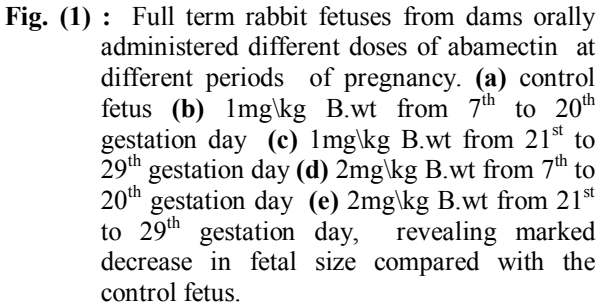

Fig. (1) : Full term rabbit fetuses from dams orally administered different doses of abamectin at different periods of pregnancy. **(a)** control fetus **(b)** 1mg\kg B.wt from 7^{th} to 20^{th} gestation day **(c)** 1mg\kg B.wt from 21^{st} to 29^{th} gestation day **(d)** 2mg\kg B.wt from 7^{th} to 20^{th} gestation day **(e)** 2mg\kg B.wt from 21^{st} to 29^{th} gestation day, revealing marked decrease in fetal size compared with the control fetus.

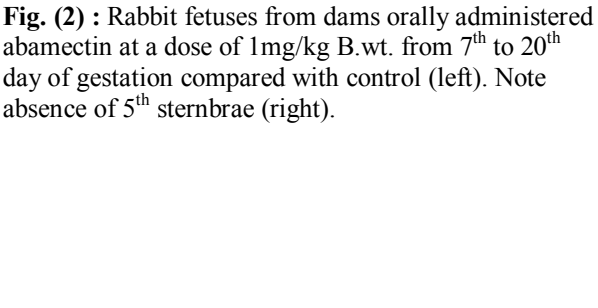

Fig. (2) : Rabbit fetuses from dams orally administered abamectin at a dose of 1mg/kg B.wt. from 7^{th} to 20^{th} day of gestation compared with control (left). Note absence of 5^{th} sternbrae (right).

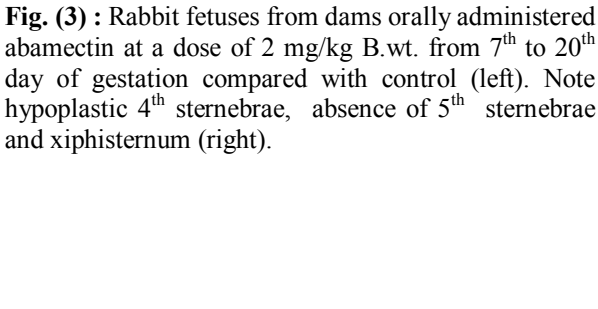

Fig. (3) : Rabbit fetuses from dams orally administered abamectin at a dose of 2 mg/kg B.wt. from 7^{th} to 20^{th} day of gestation compared with control (left). Note hypoplastic 4^{th} sternebrae, absence of 5^{th} sternebrae and xiphisternum (right).

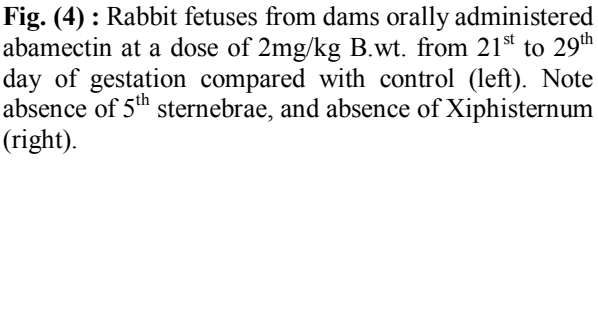

Fig. (4) : Rabbit fetuses from dams orally administered abamectin at a dose of 2mg/kg B.wt. from 21^{st} to 29^{th} day of gestation compared with control (left). Note absence of 5^{th} sternebrae, and absence of Xiphisternum (right).

Visceral examination of fetuses obtained from treated and control groups and later kept in Bouin's solution were macroscopically examined by the aid of a magnifying hand lens. No visceral abnormalities were detected among their fetuses.

Skeletal examination of fetuses stained with alizarin red S and obtained from both treated and control dams were examined and all treated groups elicited skeletal malformations evidenced by absence of ossifications of xiphisternebrae, incomplete ossification of sternebrae except group orally administered 1mg/kg B.wt from 21st to 29th day of pregnancy as shown in table (2) and figs (2,3,4).

Table(2): The skeletal malformations in fetuses from dam rabbits orally administered different doses of abamectin at different periods of pregnancy.

Group	Treatment	Dose mg / kg B.wt	Period of administration During G.P	Number of examined fetuses	Sternebrae					
					Xepho- str.			Str.		
						No.	%		No.	%
I	D.W.	0.5ml	7th : 29th	15	N	15	100	N	15	100
					I	0	0	I	0	0
					A	0	0	A	0	0
II	Abamectin	2mg/kg	7th : 20th	16	N	0	0	N	6	37.5
					I	0	0	I	10	62.5
					A	16	100	A	0	0
III	Abamectin	2mg/kg	21st : 29th	11	N	0	0	N	5	45.5
					I	0	0	I	6	54.5
					A	11	100	A	0	0
IV	Abamectin	1mg/kg	7th : 20th	14	N	0	0	N	7	50
					I	0	0	I	7	50
					A	14	100	A	0	0
V	Abamectin	1mg/kg	21st : 29th	12	N	12	100	N	12	100
					I	0	0	I	0	0
					A	0	0	A	0	0

N= Normal **I= Incomplete** **A= Absent**

II. Maternal toxicity :

1. Effect on Haematological Parameters:

Data recorded in table (3) revealed that PCV values not altered in all treated groups except group orally administered 1mg/kg B.wt from 21st to 29th day of pregnancy have significant increase. Also, Hb concentration not altered. While, abamectin induced a significant decrease of total leukocytic count of all treated groups compared with control group.

Table (3): Hematological parameters of rabbit dams orally administered different doses of abamectin at different periods of pregnancy (Mean±SE).

Group	PCV %	Hb g /dl	TLC 10^3/µl
Control	$34.60^{bc} \pm 1.43$	$10.42^{a} \pm 0.04$	$12980.00^{a} \pm 226.71$
1 mg/kg ($7^{th} – 20^{th}$)	$39.40^{ab} \pm 2.04$	$10.56^{a} \pm 0.58$	$6710.00^{b} \pm 407.86$
1 mg/kg ($21^{st} – 29^{th}$)	$44.20^{a} \pm 1.68$	$11.83^{a} \pm 0.73$	$4800.00^{c} \pm 146.63$
2 mg/kg ($7^{th} – 20^{th}$)	$37.00^{bc} \pm 0.71$	$9.49^{a} \pm 0.39$	$7000.00^{b} \pm 460.98$
2 mg/kg ($21^{th} – 29^{th}$)	$34.00^{c} \pm 2.61$	$11.47^{a} \pm 0.78$	$5240.00^{c} \pm 438.58$

The means in the same column having the same superscript not significantly different ($p<0.05$).

2. Effect on biochemical parameters:

Data in table (4) illustrate that abamectin caused a significant increase in the activity of AST and ALP in serum of treated rabbits, whereas ALT activity remained unaltered.

Table (4): Serum transaminases (ALT&AST) and alkaline phosphatase (ALP) of rabbit dams orally administered different doses of abamectin at different periods of pregnancy(Mean±SE).

Group	ALT U/ml	AST U/ml	ALP IU/ml
Control	$13.15^{c} \pm 0.32$	$15.67^{b} \pm 0.22$	$32.33^{c} \pm 1.40$
1 mg/kg ($7^{th} – 20^{th}$)	$13.30^{c} \pm 0.35$	$30.07^{a} \pm 0.72$	$36.50^{b} \pm 0.96$
1 mg/kg ($21^{st} – 29^{th}$)	$13.35^{c} \pm 0.37$	$29.52^{a} \pm 0.08$	$34.67^{bc} \pm 0.66$
2 mg/kg ($7^{th} – 20^{th}$)	$13.08^{c} \pm 0.25$	$29.43^{a} \pm 0.38$	$44.67^{a} \pm 1.84$
2 mg/kg ($21^{th} – 29^{th}$)	$13.67^{c} \pm 0.09$	$30.20^{a} \pm 0.52$	$36.17^{b} \pm 0.79$

The means in the same column having the same superscript not significantly different ($p<0.05$).

Data in table (5) showed that abamectin induced a significant inhibition of GST activity in all treated groups compared with the control one, also a significant decrease in the activity of SOD of all treated groups compared with the control group. However, abamectin caused a significant increase in the levels of nitric oxide (NO) of all treated groups compared to control, except group orally administered 2mg/kg B.wt from 7^{th} to 20^{th} gestation day.

Table (5): Oxidative enzymes from rabbit dams orally administered different doses of abamectin at different periods of pregnancy(Mean±SE).

Group	GST U/L	SOD U/ml	NO mmol /L
Control	3108.2 ^{a}±110.14	344.83 ^{a}± 2.12	8.18 ^{c}±0.29
1 mg/kg (7^{th} – 20^{th})	1587.2 ^{bc}±36.58	264.17 ^{d}± 2.91	12.70 ^{b} ±0.25
1 mg/kg (21^{st} – 29^{th})	1710.8 ^{b}±4.45	289.33 ^{c}± 9.08	16.02 ^{a}±0.63
2 mg/kg (7^{th} – 20^{th})	1469.7 ^{c}±58.71	319.00 ^{b}± 3.61	9.43 ^{c}±0.65
2 mg/kg (21^{st} – 29^{th})	1686.7 ^{b}±43.76	276.00 ^{cd}± 6.91	16.82 ^{a}±0.71

The means in the same column having the same superscript not significantly different ($p<0.05$).

Histopathological changes

Histopathological examination of mother tissues indicated that liver showed congestion in the central vein and hepatic sinusoids, oedema and hemorrhage of portal area with vacuolation and necrosis of hepatocytes (fig.5). Kidney showed swelling and necrosis of renal tubular epithelium beside proliferative glomerulonehpritis (fig.6). Spleen showed hyperplasia in the wall of splenic arterioles with mild necrosis of lymphocytes and atrophy of lymphoid follicles (fig.7). Placental tissues showed hyperplasia of chorionic villi, haemorrhage and vacuolar and hydropic degeneration of trophoblost (fig.8). Brain showed necrotic neuron, neuronophagia with astrocytosis and satelletosis of glial cells (fig.9). Sciatic nerve showed slight vacuolation of neurolemmal sheath (fig.10).

Histopathological examination of fetal tissues indicated that liver showed haemorrhage, vacuolatoin in hepatocytes and haemopoietic stem cell were seen (fig.11). Kidney showed extramedullary hematopoiesis with embryonic cell differentiated into renal tubules and renal glomenuli (fig.12).

Fig. (5): Section from liver of dam received 2mg\kg B.wt from 21st to 29th gestation day, showing oedema and hemorrhage of portal area (arrow).

Fig. (6): Section from kidney of dam received 2mg\kg B.wt of abamectin from 7th to 2oth gestation day, showing proliferative glomerulonephritis (arrow) in renal glomeruli and necrosis of renal tubular epithelium.

Fig.(7): Section from spleen of dam received 1mg\kg B.wt of abamectin from 7th to 2oth gestation day, showing hyperplasia in the wall of splenic arterioles.

Fig.(8): Section from placenta of dam received 2mg\kg B.wt of abamectin from 7th to 20th gestation day, showing massive hemorrhage (arrow head) and hyperplasia of chorionic villi of trophoblast (arrow).

Fig.(9): Section from brain of dam received 1mg\kg B.wt of abamectin from 21st to 29th gestation day, showing neuronal necrosis(arrow), satellitosis and neuronophagia (arrow head).

Fig.(10): Section from sciatic nerve of dam received 1mg\kg B.wt of abamectin from 21st to 29th gestation day, showing slight vacuolation in neurolemmal sheath (arrow).

Fig.(11): Section from liver of fetus from dam received 2mg\kg B.wt of abamectin from 21st to 29th gestation day, showing hemorrhage (arrow), vacuolation in hepatocytes (arrow head) and haemopoietic stem cell.

Fig.(12): Section from kidney of fetus from dam received 1mg\kg B.wt of abamectin from 21st to 29th gestation day, showing embryonic cell differentiated into renal glomeruli (arrow) and renal tubules.

DISCUSSION

Exposure of the embryos to environmental chemicals can result in congenital malformation or abortion. Although experimental teratology data are considered sufficient for risk assessment, only knowledge of mechanisms of action permits justifiable. **(Giavini and Menegola, 2004).** In the present study the results showed that abamectin caused fetal resorption. This results were in agreement with **(Tuchman-Duplesis, 1975; and Ali et al., 1988)** reported that rats given ivermectin during the early stage of pregnancy revealed death in embryos and teratogenic changes in the fetuses of the treated rats during the late stage of pregnancy. **Persaud and Henderson (1969)** provided attractive clues for fetal resorptions. The authors reported that during the first twelve days of embryonic development, certain teratogenes may kill the embryo by damaging all or most of its cells by preventing implantation of the blastocyst or by producing several chromosomal changes and consequently its resorption. **Haschek and Rousseaux, (1993)** provided attractive suggestion for fetal resorption, where they recorded that the critical point of intrauterine development, the first interferes with the implantation of the embryo or destroy their chromosomes. Furthermore, **Collins and Collins (1979)** reported that the mechanisms of action of most teratogens occurred through interference with nucleic acid replication\transcription or RNA translation, deficiency of energy supply for metabolism of the organism by restricting the availability of substrates either directly or through the presence of analogs or antagonist of vitamins, essential aminoacids and others.

The obtained data clearly demonstrate that oral administration of different doses of abamectin at different periods of pregnancy induced a significant decrease of the body weight and the crown-rump length of the obtained fetuses. It is well known that, the development of mammalian embryo is controlled by complex factors, maternal, placental and autogenous; these factors include hormones, protective mechanisms (immune system) and nutritional factors. Changes in these factors might be expected to lead to developmental abnormalities **(Saxen, 1976)**. Accordingly, increased accumulation of abamectin may alter any of the previously mentioned factors causing fetal abnormalities. Furthermore, many authors explain causes of the decrease in fetal weights and their viability. **Tuchman-Duplesis(1975)** attributed the decrease in the fetal body weight to the accumulation of the drugs in the fetal body than the maternal body. Such accumulation could be enhanced by the very simplified

structure of rat's placenta which allowed the passage of drugs from their circulation and concentrated in fetal tissues or act as inhibitors of membrane enzymes involved in embryonic nutrition. It is a fact that the fetal body weights reflect the fetal development and neonatal mortality coupled with the concept that many chemicals may destroy cellular active DNA and so reduced biosynthesis of essential components, like protein and energy source (ATP and NAD\NADP) and consequently the fetal growth **(Haschek and Rousseaux, 1993).** It is a fact that the fetal body weight and the crown-rump length faithfully echo the fetal development and neonatal mortality coupled with the concept that chromosomal abnormalities induced by many chemicals affect cellular DNA and consequently the fetal growth **(Lubchenco, 1970).**

The present study indicated that abamectin induces skeletal abnormalities in all group exposed to abamectin at two doses used (1 and 2 mg\kg) at different periods of pregnancy, but the pronounced effects were observed in a dose 2mg\kg at a period of 7^{th} to $2o^{th}$ day of pregnancy, these effects may be due to during this period the placenta not full term and its role as a barrier for detoxification not completed and the toxin have direct effect on embryo cells, also the embryo cells at high degree of differentiation and more sensitive to the toxic effects of chemicals. The effects were observed in other groups were less dangerous may be due to the placenta become full term and the possibility for detoxification may be considered.

The present study demonstrated that abamectin caused a significant decrease in leukocyte counts (WBCs). A significantly reduced total count of white blood cells could be indicative of immune-suppressionm **(Schroder et al., 2007)**.The obtained results are in agreement with those found by **(Ali, 1990; Anubama et al., 2001)** who stated that avermectins reduced leukocyte counts in rabbits and rats.

The results of the present study showed that oral administration of abamectin significantly increased the levels of plasma AST and ALP in treated rabbits, compared to the control group. While, the levels of ALT not altered. These findings were in agreement with the results obtained by **Hsu et al., (2001).** They indicated that the activity of AST level was elevated in abamectin-dosed rats in a dose-dependent manner at 1, 3, and 12 h, respectively. Activity of serum enzymes like AST and ALT, represent the functional status of the liver **(Cremer and Seville, 1982).** As certain hepatic damage is considered pathologically irreversible (**Helling et al., 1995**), the elevation of AST may render the liver to be more susceptible to other toxicants **(Chamulitrat and Spitzer, 1996; Nayak et al., 1996).** Aspartate aminotransferase is an important indicator of liver damage in clinical studies.

During hepatocellular injury, AST was found to be secreted into the blood **(Kalender et al., 2005).** In dying or damaged cells, these enzymes leak into the blood stream **(Mansour and Mossa, 2010).** However the results were disagreed with (**Ewies et al., 1995** and **Abd El-Wahab et al., 2002)** who found that abamectin caused a decrease in ALT and AST activities in rats. In addition, **(Gomes et al., 1999**) revealed marked decrease in ALT and AST activities as a result of treatment with a mixture of organophosphorous pesticides.

The results of the present study showed that oral administration of abamectin induced a significant decrease in the levels of GST and SOD enzymes. Our results are in agreement with **(El-Shafey et al., 2011)** due to that GST involved in detoxification of abamectin to non-toxic products or by rapidly binding and very slowly turning over the insecticide. Also, with **(El-Shenawy, 2010)** who reported decreased GST activities in rat liver following exposure to insecticides fenitrothion, endosulfan and abamectin and he added that organophosphorous insecticides consume GSH through a detoxification reaction and that GST catalyzes this reaction between GSH and xenobiotic regulating possible harm **(Mulder et al., 1990)**. The present results are coincident with **El-Demerdash (2011)** who reported that significant decrease in the antioxidant enzyme activity (GST) in liver proved the failure of antioxidant defense system to overcome the influx of reactive oxygen species generated. However, the inhibition of enzymes involved in free radical removal led to the accumulation of H_2O_2 which promoted lipid peroxidation of DNA, altered gene expression and cell death **(Halliwell and Gutteridge, 1999).** The decline in the enzyme levels may be due to an excessive formation of superoxide anions, thus resulting in an inactivation of H_2O_2 scavenging enzyme.

In the present study, abamectin caused a significant increase in the NO levels. This finding agree with (**Hsu et al., 2001**) where NO increased in rats orally given 1.5 to 20 mg\kg of ivermectin. On the other hand, our findings disagrees with (**Zhang et al., 2009)** where 2µg\ml and 4µg\ml of ivermectin resulted in decrease in NO by 10% and 30% at 24hr period in lipopolysaccharide treated RAW 264.7 cell culture model.

The present study revealed congestion in the central vein and hepatic sinusoids, oedema and hemorrhage of portal area with vacuolation and necrosis of hepatocytes. These results were in agreement with those of **(Waleed M.S., 2010)** who reported area of hepatic necrosis and hepatic cell degeneration with marked vacuolation of hepatocytes in pigeons injected s\c with ivermectin. While, these results were in disagreement with those **(Kim E.C., 1995)** who did not report any histological changes in chicken injected s\c with ivermectin. This may be

explained by the fact that pathway and primarily ivermectin is metabolized in the liver via oxidative, and excreted in feces while less than 5% of ivermectin is excreted in urine. A very large numbers of etiological agents are capable of causing necrosis such as medications**.(Hafidh A. and Omad A. 1999).** Concerning the kidney, abamectin induced swelling and necrosis of renal tubular epithelium beside proliferative glomerulonephritis. These results are agree with those **(Elissa and Zidan, 2010)** who reported interstitial nephritis in male rat's kidney after receiving 1\10 and 1\100 LD_{50} of abamectin. Histopathologic effects of abamectin revealed that lesions in the liver, sciatic nerve and brain, these results reported in pigeons **(Waleed M.S. 2010**). The lesions in the liver and placenta may be have a role in embryo and fetal toxicity. The main conclusion of the present study that abamectin has teratogenic effects at different periods of pregnancy and also has maternal effects which reflected on the fetal growth. The evaluation of teratogenic effects of abamectin will need further studies with different doses and different animal species, so the use of abamectin must be restricted in uses during pregnancy.

REFERENCES

Abd El-Wahab I.; Eissa A.I.; Khalil S.M. and Hoda E. (2002): Effect of the biopesticide, abamectin on the liver and kidney functions in albino rats. *Egypt. J. Biochem.,* **20:** 183-195.

Ali A.; Fahmy F.M. and Edress M. Nareman, (1988): Pathological and clinico-pathological studies on antiparasitic drug "ivermectin" toxicosis in albino rats. Zag. Vet. J. 16 (14): 19-31.

Ali B.H., (1990): The effect of ivermectin on some haematological indices in rabbits: influence of vitamin k treatment. Clin. Exp. Pharmacol. Physiol., 17: 735-738.

Anubama V.P., H. Honnegowda K. Jayakumar G. Krishnappa K. Narayana and Y.B. Rajeshwari, (2001): Effect of doramectin on immune response of rats to SRBC antigen. India Vet J., 78: 779-782.

Bancroff J.P.; Stevenes A. and Turner D.R. (1990) : Theory and practice on histological techniques, third edition, Clurechill Livingston, Edinburgh, London.

Belfield A. and Goldberg D.M.(1971) : A colorimetric method for determination of serum Alkaline Phosphatase enzyme 12, 561.

Chamulitrat W., Spitzer J.J. (1996): Nitric oxide and liver injury in alcohol-fed rats after lipopolysaccharide administration. Alcohol Clin. Exp. Res. 20 (6): 1065–1070.

Clark J.M., Scott J.G., Campos F., Bloomquist J.R. (1995): Resistance to avermectins: extent, mechanisms, and management implications. Annu. Rev. Entomol. 40: 1-30.

Coles E.H. (1986) : Veterinary clinical pathology. 4th Ed. W.B.Saunders Company, Philadelphia, 279-297.

Collins T.F., Collins E.V., (1979): Current methodology in teratology research in : new concepts in safety evaluation. Part 1, first ed. Bailliere Tindall, London.

Cremer J.E., Seville M.P. (1982): Comparative effects of two pyrethroids, deltamethrin and cismethrin on plasma catecholamines and on blood glucose and lactate. Toxicol. Appl. Pharmacol. 66 (1): 124–133.

Drabkin D.L. (1949) : Standarization of hemoglobin measurement in fish medicine Stockopf, K.M. (ed), 1st ed; W.B. Saunders Co. Philadeiphia. 114-115.

El-Demerdash F.M. (2011): Lipid peroxidation, oxidative stress and acetylcholinesterase in rat brain exposed to organophosphate and pyrethroid insecticides. Egypt Food and Chemical Toxicol., 49 (6): 1346-1352.

Elissa F.I. and Zidan N.A. (2010): Haematological, biochemical and histopathological alterations induced by abamectin and Bacillus Thuringiensis in male albino rats. J. of Basis & Applied Sci., 3 (3): 2497-2505.

El-Shafey A.A.M.; Selim M..E.; El-Mahrouky F.; Gabr W.M. and Kandil R.A.(2011): Some Physiological and Biochemical Effects of Oshar Extract and Abamectin Biocide on male albino rats. Journal of American Science,7(12) : 254-261.

El-Shenawy N.S. (2010): Effects of insecticides fenitrothion, endosulfan and abamectin on antioxidant parameters of isolated rat hepatocytes. J. Toxicol. In Vitro, 24 (4): 1148-1157.

Ewies E.; El-Hwashy N.; Said H. and Kandil M. (1995): Acute toxicity of ivermectin in laboratory animals. 1st Int. Conf. of Pest Control, Mansoura, pp. 89-95.

Feldman B.F.; Zinkl J.G. and Jain V.C. (2000) : Schalm's Veterinary hematology. Fifth Edition. Lippincott Williams and Wilkins. Canda; P.P. 1145-1146.

Fisher M.H. & Mrozik H. (1989): Chemistry, p.1-23. In: Campbell W.C. (ed.) Ivermectin and Abamectin. Springer Verlag, New York.

Ghoniem H.; Mervat A. and Mansour H. (1992): Some studies on antiparasitic drug ivermectin in rabbits. *Zag. Vet. J.,* **20:** 753-761.

Giavini E. and Menegola E. (2004): Gene-teratogen interaction in chemically induced congenital malformation. Biol. Neonate. 85 (2): 73:81.

Gomes J.; Dawodu A.; Lioyd O.; Revitt D. and Anilal S. (1999): Hepatic injury and disturbed amino acid metabolism in mice following prolonged exposure to organophosphorous pesticides. *Hum. Exp. Toxicol.*, **18(1):** 33-37.

Habig W. and Pabst M.; Jakoby W.J. (1974) : A UV method for determination of Glutathione-S-Transferase. Biol. Chem. (249), 7130-7139.

Hafidh A. and Omad A. (1999): Veterinary pathology handbook. Ministery of higher education and scientific research. University of mousl.

Halliwell B. and Gutteridge J.M.C. (1999): Glutathion in metabolism. In *Free Radical in Biology and Medicine*, edited by: Halliwell, B. and Gutteridge, J. M. C. 146-150. Oxford, UK: Claredon Press.

Haschek W.M., Rousseaux C.G. (1993): Fundamentals of toxicological pathology. Academic Press, London, pp. 515-541.

Hayes, A.W. (1994) : Principles and Methods of Toxicology, 3rd edition. Raven Press Let, New York.

Helling T.S., Wogahn B.M., Olson S.A., Evans L.S., Reddy B.R., VanWay C. (1995): The effect of prostaglandin E1 on liver adenine nucleotides and cytoplasmic enzymes in a porcine model of normothermic hepatic ischemia. Hepatology 22 (5): 1554–1559.

Hsu D.-Z., Hsub C.-H., Huangc B.-M., Liu M.-Y., (2001). Abamectin effects on aspartate aminotransferase and nitric oxide in rats. Toxicology 165 (2–3), 189–193.

Kalender S., Ogutcu A., Uzunhisarcikli M., Acikgoz F., Durak D., Ulusoy Y., Kalender Y.(2005): Diazinon-induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes. Toxicology 211 (3): 197–206.

Kim E.C., (1995): Clinical signs of ivermectin toxicity and the efficacy of antigabaergic convulsants as antidote for ivermectin poisoning in epileptic chickens. Vet. Hum. Toxicol.; N; 37(2) : 122-126.

Kim K.; **Anna-Maja N.; Birger S.; Anne-Lee G.; Lennart D. and Michael S. (2004):** Valproic acid teratogenicity : A toxicogenomics approach. Environmental health perspectives 112(12): 1225-1235.

Kolar L., Erzen N.K., Hogerwerf L., Van Gestel C.A.M. (2008). Toxicity of abamectin and doramectin to soil invertebrates. Environ. Pollut. 151 (1): 182-189.

Lankas R.G. and Gordon L.R. (1989) : Toxicology. In : "Ivermectin and Abamectin" (Campbell. W.C., ed.) Springer Verlag. New York. NY., 10-142.

Lubchenco L.O. (1970) : Assessment of gestational age and development at birth. Ped. Clin. N.A.17: 125-145.

Mansour S.A., Mossa A.H. (2010): Oxidative damage, biochemical and histopathological alteration in rat exposed to chlorpyrifos and the role of zinc as antioxidant. Pest. Biochem. Physiol. 96 (1): 14–23.

Montgomery H.A.C and Dymock J.F. (1961) : A colorimetric method for determination of serum nitric oxide. Analyst, 86, 414.

Mulder G.J.; Adang A.E.P.; Brussee J.; Ketterer B.; Meyer D. and Van de Gen A. (1990): The Glutathione binding site of glutathione-s-tranferase isoenzymes from the rat: selectively towards tripeptide analogues of glutathione. Glutathione-S-transferase and drug resistance. Taylor and Francis, Great Britain. p. 75

Nayak N.C., Sathar S.A., Mughal S., Duttagupta S., Mathur M., Chopra P. (1996): The nature and significance of liver cell vacuolation following hepatocellular injury-an analysis based on observations on rats rendered tolerant to hepatotoxic damage. Virchows Archiv. 428 (6): 353–365.

Nishikimi M.; Roa N.A. and Yogik (1972) : A colorimetric method for determination of serum Superoxide dismutase. Biochem. Bioph. Res. Common., 46, 849-854.

Persaud T.V.N. and Henderson W.M. (1969): The teratogenicity of barbital sodium in mice. Burg Res. 19 : 1309-1310.

Reitman S. and Frankel S.(1957) : A colorimetric method for determination of serum ALT, AST. Am. J. Clin. Pathol., 28:56.

Saxen L., (1976): Mechanisms of teratogensis. J. Embryol. Exp. Morphol. 36, 1-12.

Waleed M.S. (2010): Clinical and Histopathological investigation of Ivermectin toxicity in pigeons. Bas.J. Vet. Res. 9 (1): 130-140.

Zhang X.; Song Y.; Xiong H.; Zhang L.; Deng X. (2009): Inhibitory effects of ivermectin on nitric oxide and prostaglandin E2 production in LPS- stimulated RAW 264.8 macrophages. Int. Immunopharmacol; 9: 354-359.

الملخص العربى

التاثير السمى للابامكتين علي امهات و اجنة الارانب

فتحي رضوان سليم، ممدوح محمد ابوالمجد، مروة عبدالسلام عبدالله

قسم الطب الشرعي والسموم-كلية الطب البيطرى-جامعة المنصورة

لتقييم التاثير السمى للمبيد الحشرى (الابامكتين) علي امهات و اجنة الارانب ، استخدم عدد ٣٠ من الارانب النيوزلندى البيضاء الحوامل قسمت الي ٥ مجموعات كل مجموعة مكونة من ٦ حيوانات. المجموعة الاولي مجموعة ضابطة اعطيت عن طريق الفم بواسطة ابوبة معدية ماء مقطر. المجموعة الثانية اعطيت عن طريق الفم ١ مجم\كجم من وزن الجسم مادة الابامكتين من اليوم السابع حتى اليوم العشرين من فترة الحمل. المجموعة الثالثة اعطيت ايضا عن طريق الفم ١ مجم\كجم من وزن الجسم مادةالابامكتين من اليوم الحادى والعشرون حتى اليوم التاسع والعشرون من فترة الحمل. المجموعة الرابعة اعطيت عن طريق الفم ٢ مجم\كجم من وزن الجسم مادة الابامكتين من اليوم السابع حتى اليوم العشرين من فترة الحمل. المجموعة الخامسة اعطيت ٢مجم\كجم من وزن الجسم مادة الابامكتين من اليوم الحادى والعشرون حتى اليوم التاسع والعشرون من فترة الحمل. فى اليوم الثلاثون من الحمل تم ذبح جميع الارانب في كل المجموعات و تم فحص الرحم لمعرفة حدوث امتصاص الاجنة، و لمعرفة الاجنة الحية والميتة. وكذلك تم فحص الهياكل العظمية وفحص احشاء الاجنة. وقد تم استخراج الكبد، الكلي، الطحال، المخ، العصب الوركى وكذلك المشيمة وتم حفظها فى ١٠٪ من الفورمالين لدراسة التغيرات الباثولوجية بها. تم تجميع عينات من الدم من كل المجموعات، وتم فصل مصل الدم للتحليل الكيميائى الحيوى. وقد كشفت النتائج عن وجود نقص في وزن وطول الاجنة بنسبة ذات دلالة احصائية عن المجموعة الضابطة. و بفحص الهياكل العظمية للاجنة وجد ان الابامكتين تسبب في تشوهات الهيكل العظمى. و قد كشفت الدراسة الباثولوجية عن وجود تغيرات باثولوجية في الكبد و الكلي و الطحال والمخ في كلا من الامهات و الاجنة وايضا تغيرات باثولوجية في المشية والعصب الوركى في الامهات. و قد دلت صورة فحص الدم علي انخفاض ملحوظ في العدد الكلي لخلايا كرات الدم البيضاء بينما تركيز الهيموجلوبين و قيم حجم الخلية لم يتغير. و بفحص مصل الدم وجدنا زيادة ملحوظة في كلا من نشاط الانزيمات الخاصة بوظائف الكبد بينما مستوى انزيم الالانين امينو ترانس امينا ز لم يتغير كما وجد نقص ملحوظ في نشاط الانزيمات المضادة للاكسدة كما وجدت زيادة معنوية في مستوى اكسيد النيتروجين. نستخلص من هذه الدراسة ان الابامكتين له اثار ضارة علي كل من الامهات والاجنة اثناء فترة الحمل.