

PATHOLOGICAL, MALE REPRODUCTIVITY AND RESIDUES OF DIMETHOATE TOXICITY IN ALBINO RATS

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ABSTRACT □ The current study explore the effect of the organophosphorus compound (dimethoate) on male reproductive efficiency , tissue residues and it's pathological picture. Dimethoate was given either directly to albino rats at dose level 1/10 , 1/20, 1/40 of LD50 and also given green sprayed forage along 65 consecutive days. The results indicated that dimethoate altered the biochemical parameters of serum indicating liver and kidney dysfunction. Also semen picture and the weight of male sexual organs (testes , prostate and seminal vesicle) together with lower testosterone level gave indication for lower reproductive efficiency . Tissue dimethoate residues were detected in liver , testes and skeletal muscles. Concerning to pathological findings dimethoate induced degenerative changes in liver, kidneys and heart

in the form of cloudy swelling , vacuolar and hydropic degeneration. Focal areas of hemorrhages and necrosis were also seen in liver and kidneys. Vascular lesions in the form of congestion , thrombosis and necrosis of blood vessels as well as perivascular mononuclear infiltration were also pronounced. The mostly affected organs were brain and testes especially in rats given dimethoate contaminated feed. These changes included vesiculation in the brain tissue, encephalomalacia as well as satellitosis and neurophagia. The tests showed atrophy of seminiferous tubules together with fibrosis, intertubular edema and failure of spermatogenesis. We advise that great attention should be taken when dealing with such insecticides in order to avoid its various adverse action on different body tissue

of farm animals or human in contact.

Introduction

The wide application of organophosphorus insecticide in agriculture represent a great hazard to livestock. As the prolonged exposure to these contaminants even at low concentrations may lead to toxicity, immuno-suppression or reproductive failure (Nafstad, et al., 1983). In Egypt , Dogheim et al., (1996) recorded organochlorine and organo-phosphorus pesticides, including those have been prohibited from use , in human milk and environmental samples collected from Kafr El-Zayat governorate. Dimethoate is an organophosphorus insecticide used extensively in agriculture as a systemic insecticide and acaricide for gardens , vineyards and field crops (Humphreys , 1988). The toxic effects of dimethoate were studied by many authors. Metelov et al., (1977) stated that dimethoate have a neurotoxic effect in sheep , calves and fish. In human being , Krieger and Thongsinthusak , (1993) found that dimethoate is readily absorbed and its urinary metabolites are readily eliminated following to low doses. Afifi et al., (1991) recorded a dose -related decrease in the weight of the most genital organs in male albino rats. Also reduced sperm motility associated with increase in the percentage of dead and abnormal spermatozoa of treated rats. Level of plasma testosterone was lowered in treated groups and histological examination revealed that dimethoate caused moderate to severe de-

generative changes of spermatogonial cells. The highest tissue residues of dimethoate were recorded in liver and testes and the lowest was recorded in skeletal muscles. Institoris et al., (1995) studied the immunotoxicity of repeated doses of dimethoate and methyl parathion given to rat over three generations and found that dimethoate had a detectable effect on body weight , birth weight and number , organ weight , hematological parameters and immune function. Sirvastava and Raizada (1996) recorded that dimethoate produced enzymatic changes in liver of rat associated with pathomorphological changes in liver and brain. They also noticed reduced acetyl choline esterase activity in fetal brain and placenta when given to dams indicating possible transmigration of dimethoate from dams to fetuses.

Concerning to persistency of dimethoate , Pa-reek and Kavadia ,(1988) indicated that the waiting period for dimethoate was (5 to 6) days to avoid consumer risk after its application at 0.03 % on musk melon, long melon , and ridge gourd On the same aspect , Cabras et al., (1995) studied the persistency of some organophosphorus compounds on orange fruits and found that the residues of dimethoate was only found in the fruit peel and a very low concentrations were detected in the fruit pulp.

The current study was undertaken to study the toxopathological effect of dimethoate at different concentrations and to evaluate its effect on fertility of male albino rats. Furthermore to detect the tissue-residues of dimethoate after direct admin-

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istration to rats or when sprayed on edible plants and given to rats.

MATERIAL AND METHODS

* Materials:

Chemicals: Dimethoate * El-Naser. 2 * an organophosphorus insecticide was obtained as emulsifiable liquid concentration containing 40 % (W/V) active ingredient (product of El-Nasr Co. Egypt). Its chemical name is O,O dimethyl S-(N- methyl) carbamoyl methyl phosphorodithioate. LD₅₀ of dimethoate in rats is 250 mg / kg B. wt. (Ware , 1978).

Animals , dosing and grouping: One hundred and twenty mature albino rats of both sexes weighing 150-160 gm B. wt. were divided into two main groups

Group A:

One hundred rats were divided into four sub-groups a, b, c, d, each of 25 rats of both sexes. The animals were given daily doses of dimethoate diluted in distilled water at concentrations of 1/40 1/20 and 1/10 of LD₅₀ by stomach tube to the 1st three treated groups respectively. The fourth group was given distilled water and kept as control. Five rats from each subgroup were sacrificed after one, two , three and four weeks for histopathology and serum was collected at the end of the experiment " 4 weeks " for some biochemical analysis and blood was collected on anticoagulant for determination of choline esterase activity.

Group B:

Twenty male rats were classified into 4 sub-groups each of five rats, fed on a green forage sprayed with dimethoate at the concentration indicated in the pamphlet and offered to animals after 2, 4, 6 days of application in the 1st three treated subgroups respectively. The other control subgroup offered dimethoate free green forage.

* Methods:

For animals in group A:- serum samples were separated for determination of some biochemical parameters as follows:

* Serum total protein , albumin and globulin after Weichselbaum (1946), Daumas et al., (1971) and Coles , (1986) respectively.

* Alkaline phosphatase was estimated using method of Kind and King (1954).

* Serum transaminases (GPT and GOT) were estimated by the method of Reitman and Frankel (1957) and serum urea and creatinine were determined according to Hundan and Rapoport , (1968) and Chaney and Marbach , (1963) respectively.

* Cholinesterase was determined in whole blood using reagent kit of BioMerieux , according to Whittaker , (1984) .

Method adopted in group B:

Semen evaluation:

This group offered green forage sprayed with dimethoate and offered to animals after 2, 4 and 6 days of application. Feeding continued for 65 days to cover the period of spermatogenic cycle which ranges from 56 - 60 days in rats (Hershberger et al., 1969) then animals were scarified and the testes , seminal vesicles and prostate

glands were desiccated and weighed. The cauda epididymides were minced in normal saline and a drop of this epididymal suspension was picked up for semen evaluation according to Zamjanis , (1970).

Testosterone estimations:

At the end of the experiment, serum was collected for estimation of testosterone by enzyme immunoassay and reagent kits supplied from Orion Diagnostica , Finland. Samples were measured for testosterone using ELISA reader.

Tissue residues:

After slaughtering , samples from testes , liver , and skeletal muscles were collected from each animal. One gram of each tissue was thoroughly homogenized in absolute alcohol at a rate of 1 gm tissue to 10 ml alcohol, then centrifuged at 3,000 r.p.m. for 15 minutes. The supernatant were filtered through 0.45 μ millipore filter paper and transferred to clean tubes and used for estimation of dimethoate concentration using high performance liquid chromatography with programmable u.v detector .

It was chromatographed on a reverse-phase C18 column. The appropriate volumes of samples were injected into column and retention time were measured at 254 nm with the mobile phase of 80:20 acetonitrile and water. (Zehra et al., 1995).

Histopathology:

Specimens from liver , brain , heart , kidney , lung and testes were taken and fixed in 10 % buffered neutral formalin solution . After proper fixation , these specimens were processed and then sections 5 microns thickness and stained

using hematoxylin and eosin after (Drury and Wallington , 1980) .

Statistical analysis was carried out according to Sandecore, (1971).

RESULTS

Table (1) revealed that choline esterase activity decreased due to dimethoate at a dose related pattern. a similar pattern have been recorded for the percentages of total protein, albumin , globulin while GPT, GOT , alkaline phosphatase , urea and creatinine showed a dose dependent increase. Concerning to the effect of dimethoate given with green forage to rats after 2 , 4, 6 days of spraying and continued for 65 consecutive days table (2) revealed reduction in the weight and testicle , seminal vesicle and prostate gland and this decrease was correlated with the time elapsed from spraying.

Table (3) indicates a decreases in sperm concentration , percentages of live sperm and motility due to green forage contaminated with dimethoate. Also the level of testosterone n mol/L decreased while the percentages of total sperm abnormalities increased compared with the control group.

Table (4) indicate tissue residues of dimethoate in liver, testes and skeletal muscles and higher in liver followed by testes and the lowest residues recorded in skeletal muscles. Also the table indicates a correlation between tissue residues and time elapsed after spraying.

Concerning to clinical signs , group A, showed

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Table (1) Effect of dimethoate on some biochemical parameters at different dose levels (1/40 LD₅₀, 1/20 LD₅₀, 1/10 LD₅₀) in serum of albino rat after administration for 1 month.

Biochemical parameters	Control	1/40 LD ₅₀	1/20 LD ₅₀	1/10 LD ₅₀
Choline esterase activity (U/L)	3262.25±22.91	2153.5±24.96 ^{**}	1925.62±22.81 ^{**}	1668.12±21.63 ^{**}
Total protein (gm %)	8.1±0.22	7.20±0.20 ^{**}	6.50±0.21 ^{**}	5.60±0.20 ^{**}
Albumin (gm %)	4.20±0.25	4.00±0.26 ^{**}	3.90±0.23 ^{**}	3.80±0.25 ^{**}
Globulin (gm %)	3.90±0.23	3.20±0.21 ^{**}	2.60±0.22 ^{**}	1.80±0.20 ^{**}
GPT (U/ml)	72.41±1.53	85.22±1.75 ^{**}	95.20±2.35 ^{**}	102.40±2.55 ^{**}
GOT (U/ml)	84.44±2.20	92.65±2.10 ^{**}	102.22±1.80 ^{**}	112.31±1.85 ^{**}
Alk. phosph. (U/100 ml)	24.20±0.60	32.22±0.66 ^{**}	33.32±0.80 ^{**}	35.21±1.20 ^{**}
Urea (gm %)	20.23±2.51	33.21±2.66 ^{**}	55.72±2.71 ^{**}	120.14±2.62 ^{**}
Creatinine (gm %)	2.12±0.62	2.40±0.91 ^{**}	4.12±0.92 ^{**}	5.20±0.90 ^{**}

Table (2) Effect of dimethoate on relative body weight (gm / 100 gm B. wt) of male reproductive organs after administration with green forage for 65 days after 2, 4 and 6 days of spraying (mean ±S.E).

organ	control	6 days after spraying	4 days after spraying	2 days after spraying
Testicle	1.5 ± 0.012	1.33 ± 0.029 ^{**}	1.07 ± 0.018 ^{**}	0.97 ± 0.016 ^{**}
Seminal vesicle	0.22 ± 0.008	1.33 ± 0.029 ^{**}	0.12 ± 0.006 ^{**}	0.10 ± 0.007 ^{**}
Prostate gland	0.18 ± 0.007	0.14 ± 0.006 ^{**}	0.08 ± 0.006 ^{**}	0.07 ± 0.006 ^{**}

Table (3) Effect of dimethoate on semen picture and serum testosterone of male albino rat after administration with green forage at different period of application for 65 consecutive days.

Semen picture	control	6 days after spraying	4 days after spraying	2 days after spraying
Progressive motility %	82± 1.31	66 ±2.1 ^{***}	51 ± 2.3 ^{***}	46 ± 2.6 ^{***}
Live sperm%	85± 0.95	65 ±3.2 ^{***}	52 ± 2.2 ^{***}	49 ± 2.1 ^{***}
Sperm Con. (X 106 /ml)	316.61± 10.97	296.14 ±10.12 ^{***}	275.42 ± 8.75 ^{***}	260.14 ± 7.61 ^{***}
Total sperm abnormalities (%)	1.50± 0.30	8.2 ±0.62 ^{***}	16 ± 0.78 ^{***}	20 ± 0.78 ^{***}
Serum testosterone nmol/L	3.80±0.32	3.25 ± 0.41 ^{***}	2.52 ± 0.35 ^{**}	2.31 ± 0.55 ^{**}

Table (4) Dimethoate residue (PPM) in tissue of albino rat after its administration with green forage and given to animal after (2 , 4 , 6) days of application for 65 consecutive days. (mean + S.E).

N.D = not detected

Sample	control	6 days after spraying	4 days after spraying	2 days after spraying
Liver	N.D	1.52±0.07	2.44 ± 0.07	2.91± 0.08
Testes	N.D	0.87±0.04	1.25 ± 0.06	1.62± 0.06
Muscle	N.D	0.32±0.02	0.61 ± 0.03	0.92± 0.03

N.D = not detected

livation, lacrimation, tremors, watery diarrhea and partial loss of appetite after one and two weeks from administration in all sub-groups. Meanwhile, after three and four weeks, severe emaciation, roughened hair, excitation and loss of appetite were prevalent. The severity of signs tolerated by increasing the dose and time of exposure.

In group B, gradual emaciation, loss of hair and incoordination were the most clinical signs recorded.

Pathological findings: Post-mortem examination of rats received 1/10 LD₅₀ revealed severe congestion of liver, kidneys, heart and brain with the presence of petechiae on their surfaces after one and two weeks from administration. meanwhile, after three and four weeks, diffuse areas of hemorrhages with the presence of grayish white foci on the surface of liver, heart and kidneys were seen. The testes were congested and small in size (Fig.1).

Microscopically, after one and two weeks from administration, the liver showed severe congestion of blood vessels and sinusoids. Focal areas of hemorrhages with perivascular mononuclear cellular infiltration. The hepatocytes were suffered from mild degenerative changes in the form of vacuolar and hydropic degeneration (Fig.2). The kidneys showed congestion of the renal blood vessels and intertubular blood capillaries. The glomeruli showing congestion of the glomerular tuft. Periglomerular hemorrhages were also seen (Fig.3). The heart and brain showed congested blood vessels and focal areas

of hemorrhages. Meanwhile, after three and four weeks, focal areas of necrosis in the form of structureless eosinophilic substances infiltrated with mononuclear cells were detected in the liver and kidneys. The heart showed focal mononuclear cellular aggregations. The testes showed degenerative changes in the primary and secondary spermatocytes with the presence of sperm giant cells in the tubular lumen.(Fig. 4). The rats received a dose of 1/20 of dimethoate LD₅₀ showed congestion of parenchymatous organs with the presence of petechiae on their surfaces after one and two weeks from administration. But after three and four weeks the brain also showed severe congestion of blood vessels and the testes were congested and somewhat decreased in size. Microscopically, after one and two weeks severe congestion, hemorrhages and vascular thrombosis were seen in the liver, kidneys and heart. (Fig.5). Moreover, the kidneys showed intertubular mononuclear cellular infiltration (Fig.6). The renal tubules showed degenerative changes in the form of cloudy swelling and vacuolar degeneration. The heart showed focal areas of myelomalacia infiltrated with mononuclear cells (Fig.7). The testes showed intertubular edema with congestion of intertubular blood vessels.(Fig.8). The brain showed vesiculation in the brain substances with focal gliosis after three and four weeks.(Fig. 9 & 10). The rats received 1/40 LD₅₀ showed congestion of the liver, kidneys and heart with the presence of petechial hemorrhages on their surfaces. The brain showed congestion of its blood vessels. These gross findings

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Group B: The post mortem examination showed a marked decrease in the parenchymatous organs. Grayish white foci were detected in the liver , kidneys and heart. Some examined cases showed paleness or yellowish coloration of the liver and kidneys. The brain showed petechial hemorrhages. The testes were markedly decreased in size and become firm with thickened and wrinkled capsule. Microscopically, the liver showed congestion of blood vessels and sinusoids with focal mononuclear infiltration.(Fig.,12). The portal areas showed fibrous tissue proliferation together with hyperplasia of the epithelial cell lining the bile duct with newly formed bile duct. Portal mononuclear infiltration were also detected. The kidneys showed presence of hyaline casts in the renal tubules with focal areas of necrosis infiltrated with mononuclear cells .Focal replacement of the renal tissue by fibrous connective tissue (Fig,12). The heart showed focal areas of myelomalacia and hemorrhages . The brain showed multiple areas of encephalomalacia with satellitosis and neurophagia of the neuron.(Fig., 13).Diffuse hemorrhage in brain was

also detected (Fig,14). The testes showed atrophy of the seminiferous tubules with complete absence of spermatogonia (Fig., 15). Intertubular edema and fibrous tissue proliferation were also detected (Fig., 16).

DISCUSSION

Pesticides are double edged weapon , they are widely used to control pests which led to 30 - 40 % losses in crops allover the world. However, they leave some residues in crops and are incriminated in environmental pollution. Many investigations have been dealt with the harmful influence of insecticides used for controlling of animal parasites , but few studies were done on plant insecticides to find out their hazards in animals which may consume forage recently treated by such pesticides. The toxic effect of these pesticides may be greatly reflected on productivity and reproductivity of farm animals beside its immune potents against prevalent diseases.The current study deals with an organophosphorus compound (dimethoate) with the aim of exploring its effect on reproduction , pathological effects and its residues.

Table (1) shows the effect of dimethoate on some biochemical parameters of serum in intoxicated animals. like other organophosphorus compound it was expected that cholinesterase activity will reduced due to dimethoate. Similar results were recorded by Metelov et al., (1977) due to dimethoate in fish ; Hamza et al., (1991) due to carbofuran , carbamate insecticide ; and by El-

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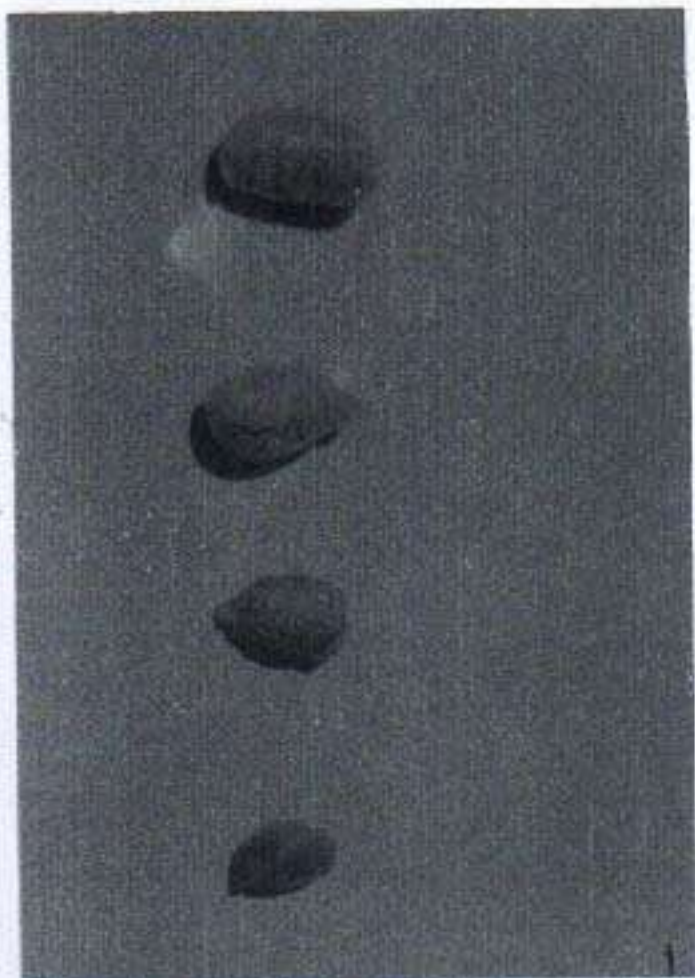
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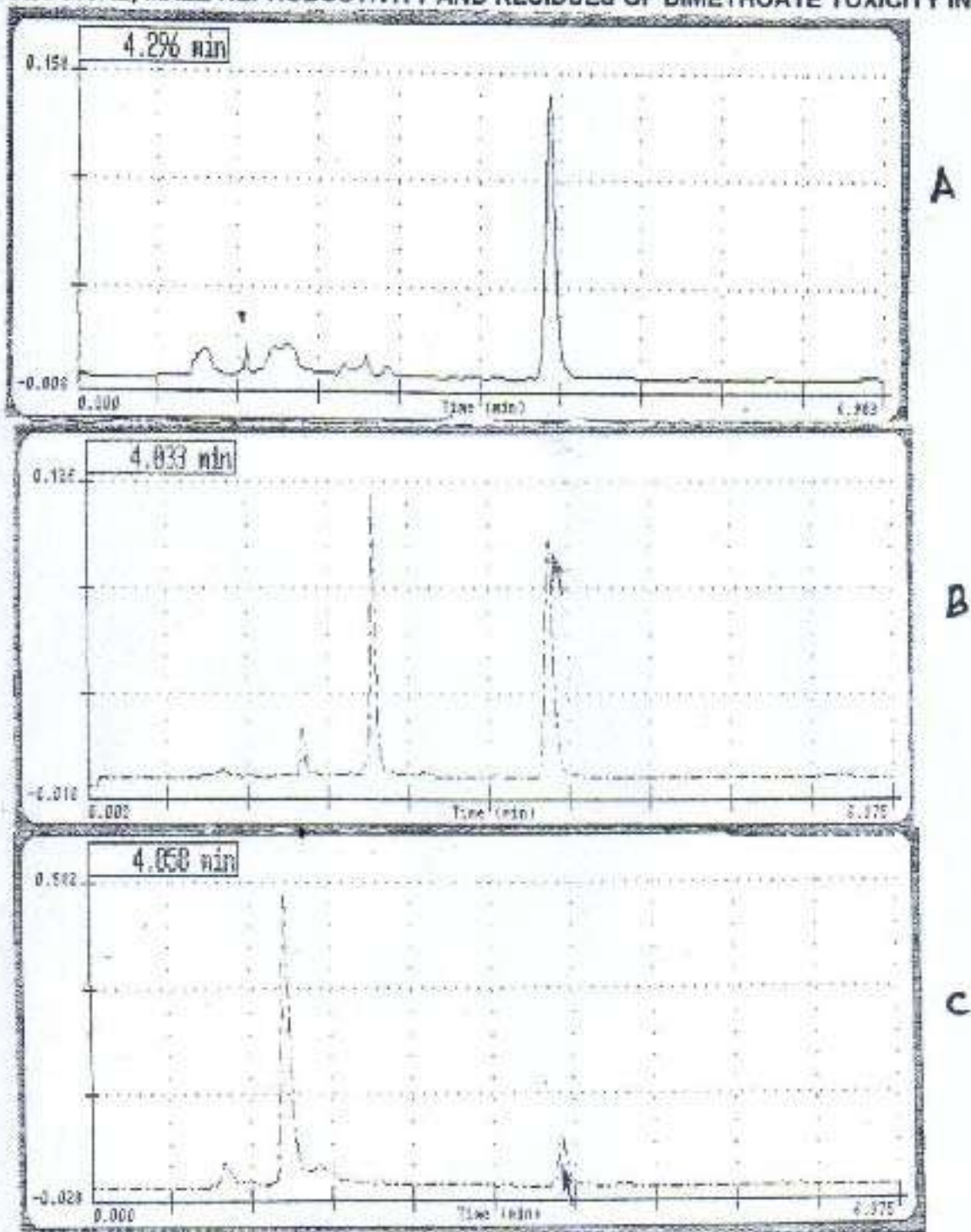


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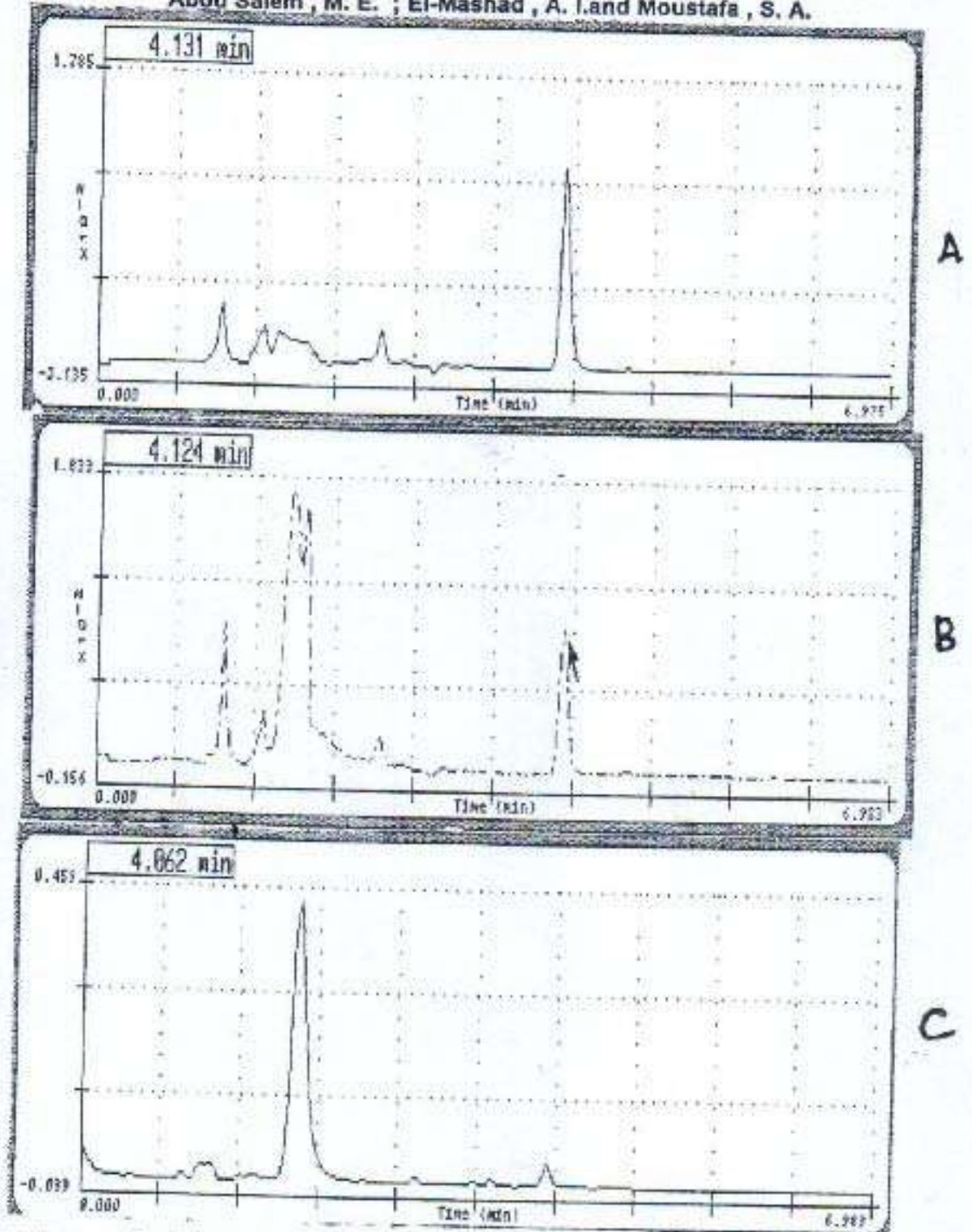


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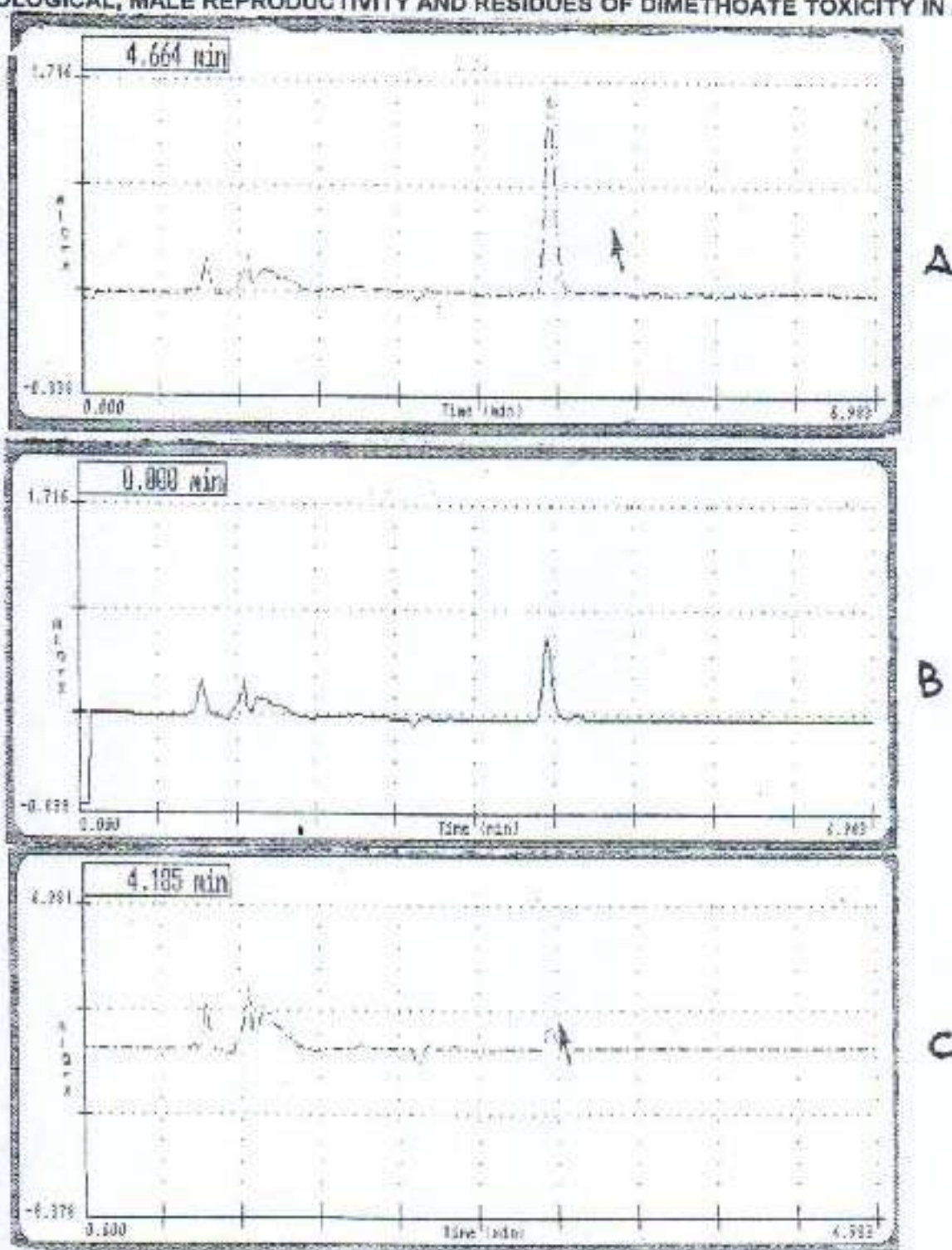


Chromatogram (1): Showing residues of dimethoat in liver (A), testes (B), and muscle (C) after 65 days of feeding cotaminated forage offered to animals after two days of application.



Chromatogram (2): Showing residues of dimethoat in liver (A), testes (B), and muscle (C) after 65 days of feeding contaminated forage offered to animals after four days of application.

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3) Showing residues of dimethoat in liver (A), testes (B), and muscle (C) after 65 days of feeding cotaminated forage offered to animals after six days of application.

t al., (1993) due to confider organophosphorus compound in albino rat and it was also recorded in fetal brain and placenta when dimethoate was given to dams in albino rat (Sirvastava and Ralzada , 1996).A pronounced reduction was recorded due to dimethoate on the levels of total protein and globulin with no remarked effect on the level of albumin. The decrease of total protein may be due to damage effect on protein biosynthesis machinery and or inhibition to m RNA transcription (El-Sheikh et al ., 1993).

The effect of dimethoate on the enzyme indices of liver function shows a remarkable dose related increase in the activity of GOT , GPT and alkaline phosphatase. It is known that transaminases are important enzymes in all biological processes as they found mainly in liver and their level in blood serum is a good indicator for diseased and damaged liver (Wilkinson , 1970 and Garb, 1971) .

Our results concerning the pathology confirmed this damage in liver (Fig. 2 , 5) which agree the results recorded by hamza et al., (1991)due to carbofuran , carbamate insecticide. However and in contrast to the present investigations , El-Sheikh et al ., (1993) recorded that a single dose of confider caused a gradual decrease in both GOT and GPT and also disagree with the result of Eisa and Bayomy (1992) using cyanophos pesticide in albino mice. This alteration may be due to difference in potency and structure of examined pesticide and may also be due to various experimental condition. As indices for kidney function , urea and creatinine levels

showed a persistence elevation with the dose given. This finding indicates damaging effect of dimethoate to the kidney a process that confirmed in our histopathological investigations (Figs. 3, 6 , 12).

The effect of dimethoat on the relative weight of male reproductive organs after administration with green forage for 65 consecutive days and at various period of application on plant is shown in table (4). Plant sprayed with dimethoate reduced the weight of testicie , seminal vesicle and prostate gland at various period of application (2, 4, 6 days) .The reduction was more pronounced at fewer period after application on plant. This picture indicated that the insecticide is metabolised by time, any how it was still effective during the examined period after application. The recorded decrease in the weight of sexual organs may be attributed to a direct destructive effect of dimethoate on male sexual organs especially tests as recorded in our study (Figs. 4, 8, 15, 16). In this respect our results agree that recorded previously by Afifi et al., (1991) and Institoris et al., (1995).

Table (3) shows another indication for the role of dimethoate on male fertility where the deleterious effect of dimethoate given with green forage was pronounced and manifested by reduction in the percentages of progressive motility , live sperm and sperm concentrations synchronized with detectable increase in the percentage of total sperm abnormalities. The alterations in sperm picture in treated animals were inversely correlated with the time elapsed of dimethoate spraying on the

green forage.

The reduction in sperm cell concentration could be attributed to reduction in meiotic index of testicular cells as being recorded for pyrethroid in albino rat (El-Ashmawy et al., 1993). The manifestation of testis in our histopathology may add another explanation or the present status of semen picture. Our results shows a complete agreement with pervious studies dealt with pesticide e.g. Afifi et al., (1991) ; Hamza et al., (1991) and El-Ashmawy et al., (1993) for dimethoate , carbofuran and Matox (pyrethroid), respectively. Table (5) shows also that serum testosterone reduced in the groups fed on green forage sprayed with dimethoata. The decrease in the level of testosterone might explain the significant depression in the testicular , epididemis , seminal vesicle and prostate gland relative weight and a significant reduction in sperm concentrations previously explained by El-Ashmawy et al., (1993) Our results coincided with that recorded by Afifi et al., (1991).

Table (4) and chromatograms (1&2&3) shows the residues of dimethoate in tissue of albino rats given in green forage at different period of spraying for 65 consecutive days. Liver showed the highest concentrations of dimethoate followed by testes and the lowest concentrations was recorded in skeletal muscles. Our data means that dimethoate is stable compounds and leave various concentrations in (liver , testes and skeletal muscles) even after 6 days of application on plants. In this respect our results agree with metlov et al., (1977) who found dimethoate as a

parent compound in fish tissues for 40 days and attributed the remained concentration in fish to the reduction in acetyl choline esterase activity in the brain. Also we refer that Afifi et al., (1991) recorded different residues due to dimethoate in albino rat. However, Krieger and Thorngsthusak, (1993) stated that dimethoate is readily absorbed and its urinary metabolites are readily eliminated following to low doses. We have to mention also that Pareek and Kavadia , (1988) indicated that the waiting period for dimethoate was 5-6 days to avoid consumer risk . This work seems to be in agreement with our findings.

Concerning to histopathological investigations our results revealed that the administration of different concentrations of dimethoate had adverse effect on various body tissues according the dose and time of administration. As we found that most of parenchymatous organs (liver, kidneys and heart) showed degenerative changes , hemorrhage and focal areas of necrosis. These findings were in complete agreement with Abol-Gharr , et. al., (1963) , Krasuse and Homola (1974) ; Clark and Clark , (1975) ; El-Mansoury , (1983); ElSwak , (1988); El-Sawak, (1990) ; Afifi et al., (1991) and El-Swak, et al., (1992) who found similar changes in some organophosphorus insecticides. Meanwhile , testicular changes in our results were considered the most important lesions especially in rats given the green forage sprayed with dimethoate at different period of application and given for 65 successive days. The testes were small in size , tense and showed wrinkled capsule. Microscopically, degenerative

changes in primary and secondary spermatocytes with complete absences of spermatogenesis were seen. Moreover, atrophy of seminiferous tubules with fibrous tissue proliferation were detected. These findings were in a partial agreement with that mentioned by Jackson and Jones (1968) ; Krause and Homola, (1977) and Afifi et al., (1991) who found degenerative changes and hemorrhage in the testes of rats received dimethoate. These degenerative changes especially in the reproductive organs returned to the infertility action of dimethyl groups as previously mentioned by Afifi et al., (1991). Our results revealed that the nervous manifestation in rats received dimethoate at different concentrations as confirmed microscopically by congestion of brain blood vessels , perivascular mononuclear infiltration together with multiple areas of encephalomalacia, satellitosis and neurophagia. These findings were in a partial agreement with that mentioned by Abbassy et al., (1989) who found fragmentation of axons, degeneration in neurons as well as demyelination of sciatic nerve in cases of sulphos treated hens.

In conclusion, the current study proved that dimethoate is an organo-phosphorus compound has toxopathological action especially on brain and testes leading to male reproductive failure and leave residues in animal tissue with predicted consumer risk. Therefore great attention should be taken during field application of dimethoate and similar insecticides to avoid the possible adverse action on various body tissue of farm animals and occupationally exposed hu-

man.

List of figures:

(Fig. 1) Showing a marked decrease in size of testes of rats received 1/10, 1/20 and 1/40 from LD₅₀ after four weeks from administration .A control, B, 1/10 C, 1/20 D 1/40 LD₅₀.

(Fig. 2) Liver of rat received 1/10 LD₅₀ after two weeks showing congestion of central vein and vacuolar degeneration of hepatocytes. (H & E stain X 600)

(Fig. 3) Kidney of rat received 1/10 LD₅₀ after two weeks showing intertubular hemorrhage. H & E stain X 250

(Fig. 4) Testes of rat received 1/10 LD₅₀ after four weeks from administration showing degenerative changes in spermatocytes with presence of sperm giant cells in their lumen. (H & E stain X 450)

(Fig. 5) ^{Heart} Liver of rat received 1/20 LD₅₀ after two weeks from administration showing intermuscular hemorrhage and hyalinization of the cardiac muscles. (H & E stain X 630)

(Fig. 6) kidney of rat received 1/20 LD₅₀ after two weeks showing degenerative changes in the renal tubules together with intertubular

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mononuclear infiltration. (H & E stain X 600)

(Fig. 7) showing focal area of myomalacia with mononuclear infiltration in the heart of rat received 1/20 LD₅₀ of dimethoate after four weeks. (H & E stain X 400)

(Fig. 8) Testes of rat received 1/20 LD₅₀ after four weeks showing intertubular edema and degeneration of seminiferous tubules.(H & E stain X 630)

(Fig. 9) showing vesiculation of the brain substance in rat received 1/20 LD₅₀ of dimethoate after four weeks from administration. (H & E stain X 450)

(Fig. 10) Brain of rat received 1/20 LD₅₀ after four weeks showing focal area of gliosis. (H & E stain X 450)

(Fig. 11) kidney of rat received 1/40 LD₅₀ of dimethoate after four weeks from administration showing degeneration and cast formation in the renal tubules.

(H & E stain X 300).

(Fig. 12) kidney of rat fed on dimethoate sprayed forage for 65 days by showing focal fibrosis of the renal tissues. (H & E stain X 450)

(Fig. 13) Brain showing encephalomalacia in rat received forage sprayed by dimethoate

and fed for 65 days. (H & E stain X 300)

(Fig.14) Brain showing focal area of hemorrhage in rat fed on dimethoate sprayed forage by for 65 days. (H & E stain X 350)

(Fig.15) Testes of rats showing atrophy of seminiferous tubules which fed on dimethoate sprayed orage for 65 days . (H & E stain X 300)

(Fig.16) showing degeneration in the epithelial lining the seminiferous tubules in rat fed on dimethoate sprayed forage for 65 days . (H & E stain X 450)

REFERENCES

Abbassy , A. M. , El-Swak, A.A. and Tag-Eldin, H. M. (1989): Side effects on environmental toxicants III neurotoxic effects of the organophosphorus insecticides sulporfos.Egypt. J. Comp. Path. Clin. Path. Vol. II , No. 1 , 126 - 143.

Abol-Gharr, M.; Shaaban, K. ; El-Sergary, M. and Doghaim, R.(1968): Histological lesions resulted from the toxicity of endrin and parathion in guinea pigs.Proceeding of the 3rd Arab animal Vet. Congress.

Affi, N. ; Ramadan, A.; AbdEl-Aziz , M.; and Saki, E. (1991): Influence of dimethoate on testicular and epididymal organs , testosterone plasma level and their residues in rats.DTW-DTsch-Tierarztl-Wochensche, Vol. 98(11) ; 419 -

423.

Cabras, P.; Garau, V. ; Melis, M.; Pirisi, F. ; Sapsedda, L.; Cabitza, F. and Cubeddu, M.(1995):Persistence of some organophosphorus insecticides in orange. Italian Journal of food Science. , Vol. 7, No. 3, 291 -298.

Chaney, A. and Morbach, A. (1963) : Modified reagents for determination of urea and ammonia. Clin. Chem. 8. 130.

Clarke, E. G. and Clarke, L.M.(1975):Veterinary toxicology. 1st ed , Bailliere, Tindall.

Coles, E. H. (1986): Veterinary clinical pathology. 4th ed. W.B. Saunders Comp. West Washington square Philadelphia and London.

Daumas, B. ;Watson, B. and Biggs, H. (1971): Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. 81 : 87.

Dogheim, S. M. ; Mohamed, El-Z.; Gad-Alla, S.; El-Aaled, S.; Emel, S.; Mohsen, A. and Fahmy, S. (1996): Monitoring of pesticide residue in human milk, soil, water, and food samples collected from Kafr El-Zayat Governorate. J.A.O.A.C. Int., 79 (1) : 111 - 116.

Drury, R. A. B. and Wallington, A. E. (1980) : Carleton's histological technique, 5th ed. , Oxford

Univ., Press, London.

Eisa, A. and Bayomy, M. (1992): Changes in blood chemistry of albino mice to induced intoxication with organophosphorus pesticides cyanophos. J. Pest. Control Environ. Sci. 4 : 135 - 158.

El-Ashmawy, I. ; Zakaria, A.; Hemed, S. ; El-Fikey, S. and Husselny (1993): Cytotoxic effects of the pyrethroid insecticide (Matox)^R with reference to its influence on the reproductive hormones. Vet. Med. J. Giza., Vol. 41, No. 3 (125 - 130).

El-Mansoury, A.A. (1983): Toxicological study on the hazards occurring as a result of application of herbicide " Gesaprim". M.V.Sc. Thesis, Fac. Vet. Med. , Cairo Univ.

El-Sheikh, A. ; Ammar, I. and Ragaa, A. Eissa. (1993): Genotoxicity of systemic pesticides confider in male albino mice. A Blood serum enzymes as an index. J. Pest. Control and Environ. Sci., Vol. 5, No. 2 P (75 - 89).

El-Swak, A.A (1989): Toxic and pathological effects in hens treated with single oral dose of organophosphorus insecticides Leptophos. Egypt. J. Comp. Path. Clin. Path. Vol. 2 , No. 2 , 183-201.

El-Swak, A.A (1990): Histopathological changes due to the effect of organophosphorus

PATHOLOGICAL, MALE REPRODUCTIVITY AND RESIDUES OF DMETHOATE TOXICITY IN AL-

Insecticides profenfos in hens. Egypt. J. Comp. Path. Clin. Path. Vol. 3, No. 1, 129-158.

El-Swak, A.A; Hussein, A. Y. and El-Manakhly (1992): Histopathological changes in rats intoxicated with organophosphorus insecticides Lep-tophos.

Garb, S. (1971): Laboratory tests in common use. Springer Publ. Co. Inc. New York.

Hamza, S; Ali, F.; Hussein, Y; Gounim, M and Ashry, K. (1991): The toxic effect of repeated administration of carbamate insecticides carbfuran in rats. Benha. Vet. Med. J., Vol. 2, No. 2, (171-177).

Hershberger, L.; Hansen, D. and Hansen, L. (1969): Effect of antifertility agents on male mice and rats as determined by a serial mating technique. Proc. Soc. Exp. Biol., 131, 667 - 669.

Humphreys, D. J. (1988): Veterinary toxicology. 3rd ed. Bailliere, Tindall, London, Philadelphia, Toronto, Sydney, Tokyo, Japan. P. 136-184.

Hundan, H. and Ropoport, A. (1968): estimation of creatinine. Clin. Chem. 14 : 22.

Institoris, L.; Siroki, O. and Desi, I. (1995): Immunotoxicity study of small doses of dimethoate and methyl parathion administered to rats over three generations. Hum. Exp. Toxicol., 14 (11) : 879 - 883.

Jackson, H. and Jones, A. R. (1968): Antifertility action and metabolism of trimethyl phosphate in rodents. Nature, 220, 591 - 592.

Kind, P and King, E. (1954): Estimation of plasma phosphates by determination hydrolyzed phenol with amina-antipyrine. J. Clin. Path., 322-326.

Krause, W. and Homola, S. (1977): Alteration of the seminiferous epithelium and the Leydig cells of the rat testis after application of dichlorvos. Bull. Environ. Contam. Toxicol., 11, 429 - 433.

Krieger, Rand Tongsinthusak, T. (1993): metabolism and excretion of dimethoate following ingestion of over tolerance peas and a bolus dose. Food-Chem-Toxicol, 31 (3) : 177 - 182.

Metelov, V. V.; Brichko, V. F. and Korzhevenko, G. N. (1977): Residues of some organophosphorus compounds and their effect on fish. Veterinary, Moscow, USSR, 1-100-103.

Nafstad, I.; Berge, Sanmes, E. and Lyngest, A. (1983): Teratogenic effects of the organophosphorus compound fenchlorphos in rabbits. Acta Vet. Scand., 24, 295 - 304.

Pareel, B. and Kavadia, V. (1988): Residues of carteryl, dimethoate and phosalone on different Cucurbits. J. Entomol. Res., Vol. 12, No. 1 (16-20).

Reitman, S. and Frankel, S. (1957) : A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase, Am. J. Clin. Path. 23 : 56.

Sendecora, G. (1971): Statistical method. 14th ed. The Iowa state collage press, Amer., Iowa.

Sirvastava, M. and Raizada, R. (1996): Development effect of technical dimethoate in rats : Maternal and fetal toxicity evaluation. Indian J. Exp. Biol. 34 (4) : 324 - 333.

Ware, W. G. (1978) : The pesticide Boned[®] Copyright by Freeman , W. H. San Francisco, U.S.A.

Weichselbaum, T. (1946): A buret colorimetric

method for determination of total protein. Am. Clin. Path. 16 : 40.

Wilkinson, J. (1970) : Clinical significance of enzyme activity measurements.

Clin. Chem. 16, 882 - 891.

Wittaker, M. (1984): In methods of enzymatic analysis. Cited from bioMerieux reagent kit.

Zehra, R.; About, M. and Masood, A. (1996): Mutagenic activity of the ganges water with special reference to the pesticide pollution in the river between Kachla to Kannauj (U.P) India. Mutation Research 343 (137 - 144).

Zernjanis, R. (1970): In animal reproduction. 2nd ed. The Williams and Wilkins company, Baltimore.

التأثير الباثولوجي للتسمم بالديميثويل و تأثيره على الذكورة

و متبقيات في أنسجة الغنران البيضاء

دا محمد/صحي أبو صام دا عبد البسط ابراهيم محمد السيد دا شوقي احمد احمد مصطفى

استهدف هذه الدراسة تأثير المبيد الحشري الفسفوري (ديمثويل) على الكفاءة التناسلية للذكور و متبقيات في الأنسجة و كذلك تأثيره الباثولوجي حيث تم إعطاء المبيد بجرعات 100 و 200 و 400 مللي الجرعة نصف المميتة مباشرة عن طريق الفم كما تم رش المبيد على علائق خنزراء و أعطيت الفحص بعد 65 يوما متتالية.

وقد أظهرت النتائج أن الديمثويل أدى إلى تغير الصفات الكيميائية لتصل الغنران بما يدل على تأثير وظائف الكبد و الكلى. هذا بالإضافة إلى أن صورة السائل المنوي و وزن الأضغاء الذكورية (الخصية و البروستاتا و الحويصلة المنوية) مع انخفاض معدل التستوستيرون أعطى مؤشرا على انخفاض الكفاءة التناسلية. كما ظهرت متبقيات للمبيد في أنسجة الكبد و الخصية و العضلات.

كما أظهر الفحص الباثولوجي وجود تغيرات فسادية في الكبد و الكلى و القلب على هيئة تورم غيبي و استحداث مائية. كما ظهرت مناطق انزفة و تنكز في الكبد و الكلى. وتوجد احتقان و خلخلة و تنكز في الأوعية الدموية مع انتشار لبعض الخلايا الالتهابية. وقد أفادت النتائج إلى أن الملح و الحمضيات كانت أكثر الأعضاء تأثرا خاصة في الغنران التي أعطيت غذاء ملوثا بالمبيد حيث ظهرت مناطق تنكز بالمخ مع تغيرات فسادية في الخلايا العصبية. كما لوحظ ضمور في الأنبيبات المنوية و الخصية مع ارتشاحات و تلف و فشل في إنتاج الحيوانات المنوية.

مما سبق ينصح بالعناية التامة عند تداول هذا المبيد لتجنب آثاره المختلفة على الصحة.