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IMMUNOTOXICITY OF MONOCHROTOPHOS AND PHOXIM ON ALBINO RATS

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ABSTRACT :

Two organophosphorus pesticides monochrotophos and phoxim have been tested for their immunotoxicity in rats using organ weight, hematological and biochemical tests on blood as well as histopathological assessment. One hundred albino rats, three months age, obtained from Experimental Animal House of Assiut University were used in the experiment. The animals were divided into five groups. Each group consisted of twenty animals and subjected to the following treatment: first group was given daily 2 mg monochrotophos/kg b. w. (1/10 LD_{50}), 2nd group was given daily 1 mg monochrotophos/kg b. w. (1/20 LD_{50}), 3rd group was given daily 200 mg phoxim/kg b. w. (1/10 LD_{50}), 4th group was given daily 100 mg phoxim/kg b. w. (1/20 LD_{50}) orally by stomach tube, 5th group was kept as control.

Exposure for one-month resulted in a significant decrease in total protein, albumin and globulin values of treated animals in comparison with controls. A significant decrease was also observed in IgG and IgM in animals treated with 1/10 LD_{50} of both pesticides. A significant increase was recorded in GST level of treated animals in comparison with control ones after exposure for one month except those received the low dose of monochrotophos.

A highly significant decrease in total protein as well as globulin values was observed in both concentrations of both pesticides after exposure for two months. IgM levels showed a highly significant decrease in animals treated with 1/10 LD_{50} of both pesticides but it was significantly decreased in animals exposed to the low dose. IgG levels was significantly decreased in animals exposed to the high dose only. GST level of all treated animals showed a highly significant increase in comparison with control group after exposure for two months.

The histopathological results revealed necrosis of the lymphocytic elements in both lymph node and spleen seen after one month of exposure and exaggerated at the end of the experiment. This was associated with decrease and even absence of B cell in transformation. The liver showed hydropic degeneration of the hepatocytes reached to focal area of necrosis at the end of the experiment.

In conclusions: this study indicated that humoral immune responses were decreased in a dose-time dependent pattern as the immunosupresive effects of monochrotophos and phoxim were increased in animals exposed to the higher doses of these pesticides for the longer duration.

INTRODUCTION :

Pesticides are the only toxic chemicals deliberately released into the environment in large amounts. Their potential to cause adverse effects to human and wildlife populations has been the subject of intense study and has led to the development of increasingly stringent and encompassing regulations for the risk assessment of novel formulations and to control the use of existing compounds. The organophosphorus pesticides (OPs) were introduced as a replacement for the persistent organochlorine pesticides after the tendency of DDT and its metabolites to bioaccumulate in ecosystems and to cause adverse health effects, particularly in top predators (Woodwell *et al*., 1967; Peakall *et al*., 1975; Murphy, 1986) led to the legal ban or restriction of their use in the 1970s. The increased use of OPs, originally seen as less of a threat to the environment due to their low persistence, has led to a different range of ecotoxicological problems associated with their high acute toxicity.

Over the last 20 years experimental evidence has accumulated that OPs can interfere with the immune system and exert immunotoxic effects in laboratory animals through both anticholinergic and non-cholinergic pathways (Wong *et al*., 1992; Barnett and Rodgers, 1994; Vial *et al*., 1996). These studies have included documentation of histopathological changes to immune tissues and organs, cellular pathology, altered maturation and changes in lymphocyte sub-populations and functional alterations to immunocompetent cells (Voccia *et al*., 1999). In some cases, these effects on immune components and functions have been linked to alterations in disease resistance in exposed organisms. However, the environmental relevance of these findings remains debatable as in spite of their widespread use, there is a lack of clearly documented examples of altered immune function in OP-exposed human and wildlife populations.

Monochrotophos (Nuvacron®) is a fast-acting organophosphorus insecticide with systemic and contact action. It is used for control of broad spectrum pests, including (sucking, chewing and boring insects and spider mites on cotton, citrus, olives, rice, maize, sorghum, sugar cane, sugar beet, peanuts, potatoes, soybeans, vegetables, etc. It is classified by WHO as I b (highly hazardous). Phoxim (Sebacil®) is a non-systemic insecticide effective against a broad range of insects, used particularly to control hemipteran or lepidopteran pests of man or stored products (Worthing and Hance, 1991). It is an ectoparasiticide of the organophosphate group used for the control of Psoroptes-, Sarcoptes- and Chorioptes mites, biting and suckling lice, sheep keds, flies, ticks and fly maggots in wounds. Species for which registrations have been granted include cattle, pigs, sheep, goats and horses, but some countries have excluded the use of phoxim in lactating animals (WHO/FAO, 2000). The commercial preparation, which used is marketed only for animal treatment. It is specific for the control of ectoparasites in livestock, sheep, goats and domestic animals except cats.

In the present study monochrotophos and phoxim pesticides have been tested for their immunotoxicity in rats using organ weight, hematological and biochemical tests on blood as well as histopathological assessment.

MATERIALS AND METHODS :

Materials:

Experimental animals:

One hundred albino rats, three months age, were obtained from Experimental Animal House of Assiut University. The animals were kept under hygienic measures provided with commercial ration and fresh water.

Insecticides:

1-Monochrotophos (dimethy (E)-1-methyl -2-(methyl carbamoyl) vinyl phosphate), commercially named Nuvacron®, 40. It is a member of a new group of organophosphorus produced by Ciba Geigy Limited, Basal, Switzerland. It is emulsifiable concentrate containing 400 mg monochrotophos /L. Acute oral LD50 for rats was 20 mg/kg B.W. (Worthing and Hance, 1991).

2-Phoxim (diethoxyphosphinothioyloxyimino (phenyl) acetonitrile), commercially named Sebacil® E.C.50% It is produced by Bayer AG, Leverkusen, Germany. It is emulsifiable concentrate containing 500 mg phoxim /ml. Acute oral LD50 for rats was 2000 mg/kg B.W. (Worthing and Hance, 1991).

Methods:

Experimental design:

The animals were divided into five groups. Each group consisted of twenty animals and subjected to the following treatment.

1-First group was given daily 1 mg monochrotophos/kg b.w. (1/20 LD_{50}) by stomach tube.

2-Second group was given daily 2 mg monochrotophos/kg b.w. (1/10 LD_{50}) by stomach tube.

3-Third group was given daily 100 mg phoxim/kg b. w. (1/20 LD_{50}) by stomach tube.

4-Fourth group was given daily 200 mg phoxim/kg b. w. (1/10 LD_{50}) by stomach tube.

5-Fifth group was kept as control.

All animals were weighted before the first dose and before sacrifaction. Ten animals from each group were sacrificed after one month of the first dose. The other ten animals were sacrificed after two months from the beginning of the experiment. Blood samples were collected from each rat in a clean sterile centrifuge tube with anticoagulant (EDTA) for cell count and plasma collection. Plasma samples were separated and stored at –20 °C for biochemical tests. The spleen from each animal was weighted.

Immunopathological study:

After postmortem examination of sacrificed rats, tissue specimens were taken from spleen, subscapular and axillary lymph nodes as well as the liver. Specimens were fixed in 10% neutral formalin. The fixed samples were dehydrated in alcohol, processed and embedded in paraffin blocks. Sections of 5-7µ were prepared and stained with Heamatoxelin and Eosin stain (Banchroft and Stevens, 1982).

Other specimens from spleen and lymph nodes were fixed in cold acetone, processed and embedded in paraffin blocks. Sections were taken and used for enzyme histochemical (alkaline phosphatase) study. Alkaline phosphatase used for detection of B cell in transformation and macrophagal activity (Gomeri, 1952, El-Sherry *et al*., 1994 and Abd Elghaffar, 1995).

Biochemical assay:

1-Total protein and albumin were measured photometrically using total protein and albumin kits, bioMérieux, France.

2-Glutathione-s-transferase (GST) activity were measured according to Habig *et al.*, (1974) with glutathione and substrate 1-chloro 2,4-dinitrobenzene (CNDB).

3-For determination of rat immunoglobulins, rat immunoglobulin kits, by radial immuno-diffusion (RID) methodology using Binding Site RID Products, England. This analysis was done in the Diagnostic Department, the Federal Institute for Veterinary Medicine Examinations, Mödling, Austria.

Statistical analysis:

Data were expressed as means ± SEM. Data were analyzed by analysis of variance (ANOVA) with Bonferroni's Post-test for multiple comparisons with confidence intervals at 90% as appropriate using Prism-3 computer program.

RESULTS:

The biochemical results are recorded in Tables (1&2). Table (1) shows that after exposure for one month there were significant decrease in total protein, albumin, and globulin values of treated animals in comparison with control ones. A significant decrease was also observed in IgG and IgM in animals treated with 1/10 LD_{50} of both pesticides. A significant increase was recorded in GST level of treated animals in comparison with control ones after exposure for one month except those received the low dose of monochrotophos.

Table (2) shows a highly significant decrease in total protein and globulin values of exposed animals in both concentrations of both pesticides after exposure for two months. IgM levels showed a highly significant decrease in animals treated with 1/10 LD_{50} of both pesticides but it was significantly decreased in animals exposed to the low dose. IgG levels was significantly decreased in animals exposed to the high dose only. GST level of all treated animals showed a highly significant increase in comparison with control group after exposure for two months.

Table (3) shows a losses of body weight gain in animals received a dose of 1/10 LD_{50} for one or two months of both pesticides. The animals received the low dose showed a decrease in body weight gain only in comparison with control. A decrease in splenic weight was noticed in treated animals. Total WBCs count showed a significant decrease after two months of exposure for both pesticides.

Immunopathological results were showed in Figures (1-19). The Immunopathological difference between the two compounds was minimal

Table (1): Effects of monochrotophos (Nuvacron®) and phoxim (Sebacil®) on some biochemical parameters of albino rats after daily exposure for one month.

Biochemical Parameters	Control Mean ± SE	Monochrotophos		Phoxim	
		1/20 LD_{50}	1/10 LD_{50}	1/20 LD_{50}	1/10 LD_{50}
Total protein (g/L)	71.5±1.07	66.2±0.84 ^{c}	63.9±0.58 ^{d,1}	60.4 ± 1.5 ^{d}	60.1±0.76 ^{d,I}
Albumin (g/L)	37.1±0.78	34.2±1.58 ^{a}	32.6±0.45 ^{b,1}	32.3 ± 1.2 ^{b}	31.1±0.31 ^{c,I}
Globulin (g/L)	34.4±0.29	32.0±0.96 ^{b}	31.1±0.13 ^{c,1}	29.1 ± 0.3 ^{d}	29.1±0.45 ^{d,I}
IgG (µg/L)	2645±131.24	2464.9±110.12^{a}	2165.5±137.06 ^{b,2}	2598.6±78.55 ^{a}	2106.3±70.75^{b,II}
IgM (µg/L	1225.9±26.28	1216.5±14.11^{a}	1092.8±33.45 ^{b,1}	1188.3 ± 45.2 ^{a}	1089.5±19.1 ^{b,I}
GST (µmol/min)	41.98±1.07	44.38 ± 0.93 ^{a}	48.11±1.03 ^{c,1}	46.5 ± 0.5 ^{b}	51.25±1.32 ^{d,II}

a–d: Versus control 1–4: versus Monochrotophos (1/20 LD_{50}) I–IV: versus Phoxim (1/20 LD_{50})
a, 1, I : non significant b, 2, II : P < 0.05 c, 3, III : P < 0.01 d, 4, IV : P < 0.001

Table (2): Effects of monochrotophos (Nuvacron®) and phoxim (Sebacil®) on some biochemical parameters of albino rats after daily exposure for two months.

Biochemical Parameters	Control Mean±SE	Monochrotophos		Phoxim	
		1/20 LD_{50}	1/10 LD_{50}	1/20 LD_{50}	1/10 LD_{50}
Total protein (g/L)	70.86±1.08	63.04±0.4 [d]	60.5±0.51 [d,1]	61.35±1.5 [d]	59.66±0.65 [d,I]
Albumin (g/L)	37.56±0.67	34.04±0.58 [a]	31.5±1.11 [c,1]	31.7±1.46 [c]	30.5±0.85 [d,I]
Globulin (g/L)	33.3±0.41	29 ± 0.54 [d]	29.0±0.66 [d,1]	30.65±0.7 [c]	29.16±0.75 [d,I]
IgG (µg/L)	2688.9±43.37	2440.75±105.52 [a]	2360.5±75.79 [b,2]	2398.5±47.06 [a]	2226.25±74.09 [d,I]
IgM (µg/L)	1250±21.92	1062.4 ± 45.42 [b]	942.0 ± 48.02 [d,1]	1004.75±54.62 [c]	868.75±27.58 [d,I]
GST (µmol/min)	40.5±0.78	47.75 ± 0.75 [d]	48.63 ± 1.07 [d,1]	45.88 ± 0.64 [d]	49.75±0.8 [d,II]

Table (3): Effect of daily monochrotophos (Nuvacron®) and phoxim (Sebacil®) exposure on albino rat body weight gain or loss, splenic weight and total WBCs count.

Biochemical Parameters	Control Mean±SE	Monochrotophos		Phoxim	
		1/20 LD_{50}	1/10 LD_{50}	1/20 LD_{50}	1/10 LD_{50}
One month treatment:					
-% of body gain or loss	9.50	3.39	- 6.49	1.73	-8.00
-Splenic weight (gm)	0.48±0.35	0.46±0.065	0.41±0.09	0.45 ± 0.086	0.35 ± 0.024
-Total WBCs count	7.5±0.8 [a]	7.3±0.18 [a]	6.9±0.75 [a,1]	7.05 ± 0.17 [a]	7.1 ± 0.23 [a,I]
Two months treatment:					
-% of body gain or loss	7.40	2.33	-11.36	2.7	-5.70
-Splenic weight (gm)	0.49±0.075	0.42±0.089	0.32±0.096	0.41 ± 0.079	0.35 ± 0.12
-Total WBCs count	7.2±0.13	5.5±0.23 [d]	3.5±0.31 [d,4]	5.9 ± 0.26 [d]	3.7±0.19 [d,IV]

a–d: Versus control 1–4: versus Monochrotophos (1/20 LD_{50}) I–IV: versus Phoxim (1/20 LD_{50})
a, 1, I : non significant b, 2, II :P < 0.05 c, 3, III : P < 0.01 d, 4, IV : P < 0.001

Lymph nodes:

The lymph nodes of control animals showed normal histological articture (Fig. 1). The nodal cortex formed of lymphoid follicles contains germinal center. After one month the lymphoid follicle showed central necrosis (Fig. 2), in which the lymphoid cell nuclei undergo pyknosis and rhyxis with appearance of central empty spaces instead of the germinal center (Fig. 3). After two months, there was a dramatic fibrosis of the nodal cortex. The fibrocytes proliferate towards the cortex from the nodal capsules (Fig. 4). In some cases the nodal cortex was completely replaced by fibrocytic cell proliferation (Fig. 5).

Alkaline phosphatase reaction was very strong in the lymph nodes of the control animals. The B cells in transformation took the cytoplasmic black staining while the activated macrophage took both nuclear and cytoplasmic black staining (Fig. 6). After one month only few macrophagal cells could be seen in the nodal cortex (Fig. 7). The alkaline phosphatase reaction was completely negative after two months that indicating absence of both B cells and macrophage (Fig. 8).

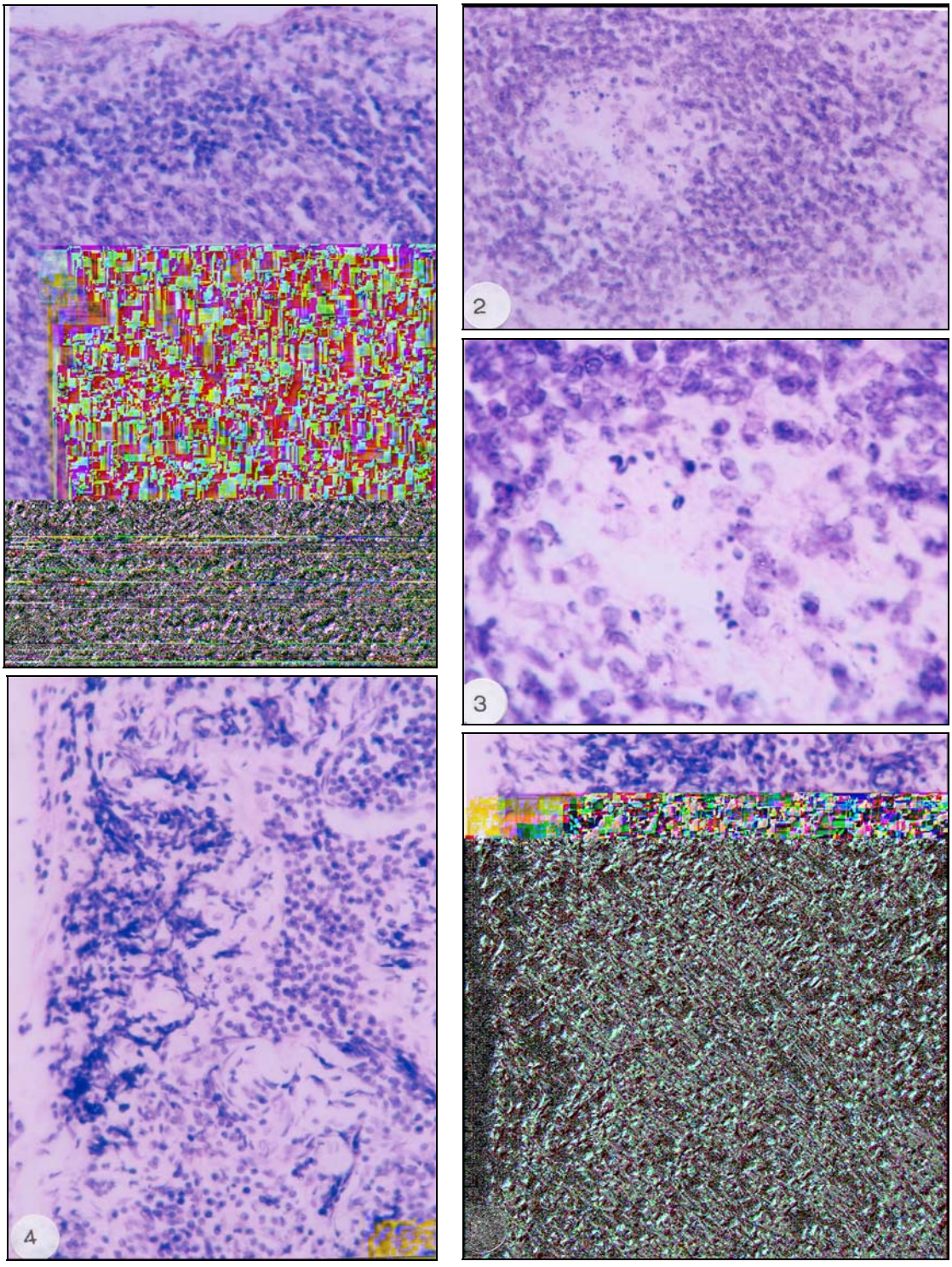

Plate (I): Lymph node showing:

Fig. (1): Normal lymph node from control rats. H&E. 10 X 10.
Fig. (2): Central necrosis of the lymphoid follicles in the nodal cortex. H&E. 10 X 25.
Fig. (3): High power of Fig.2 showing pyknosis of the lymphocytic nuclei. H&E. 10 X 40.
Fig. (4): Evidence of fibrosis, replacement of some cortical cells with fibrocytes. H&E. 10 X 10.
Fig. (5): Sever fibrosis of the nodal cortex. H&E. 10 X 10.

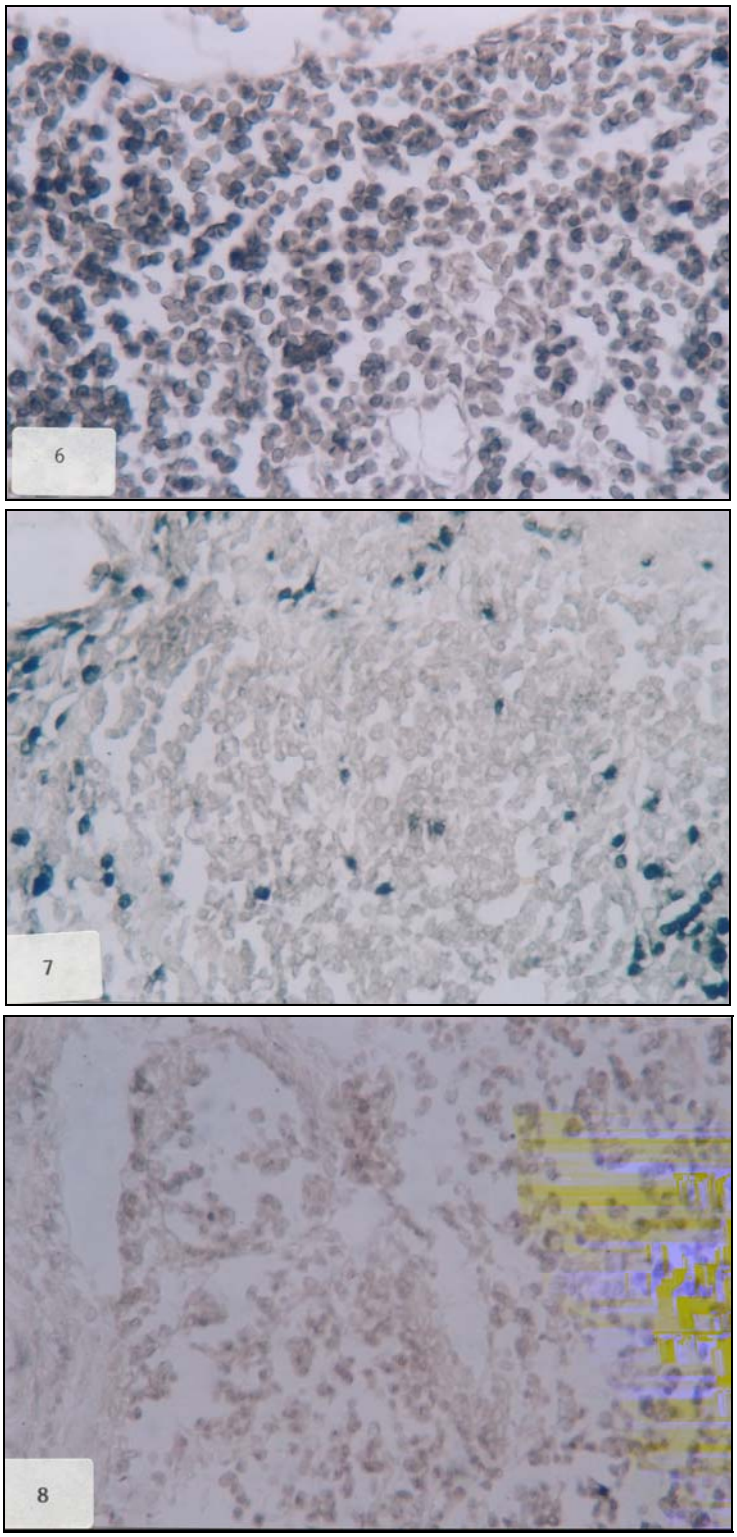

Plate (II): Lymph node stained with alkaline phosphatase:

Fig. (6): Presence of abundant amount of alkaline phosphatase positive cells which took the blackish stain. 10 X 25.
Fig. (7): Presence of few cells took the alkaline phosphatase reaction. 10 X 25.
Fig. (8): Absence of cells took the alkaline phosphatase reaction. 10 X 25.

Spleen:

Figure (9) shows the normal histological appearance of spleen from the control animals. After one month of the experiment, there was exhaustion of the lymphoid elements in both white and red pulp of the spleen with congestion of the vessels in the red pulp (Fig. 10). After two months the splenic follicle showed extensive coagulative necrosis of the lymphocytes (Fig. 11) and heamosidrosis in the red pulp (Fig. 12).

Alkaline phosphatase reaction showed B cells in transformation and activated macrophage in the splenic tissues of the control animals (Fig. 13). The reaction was moderate with appearance of few B cells after one-month exposure (Fig. 14). There was abundance of macrophage cells that might be represent the sidrophage after two months (Fig. 15).

The histopathological appearance of the liver after one month showed vacuolar degeneration of the hepatocytes (Fig. 16) as well as eosinophilic and mononuclear cell segregation on the sinusoids (Fig. 17&18). After two months there were focal areas of necrosis infiltrated with mononuclear cells (Fig. 19).

DISCUSSION:

Immunotoxic xenobiotics are not restricted to a particular chemical class. Compounds that adversely affect the immune system are found among drugs, pesticides, solvents, haloginated and aromatic hydrocarbons and metals. There is increasing evidence that certain pesticides can produce alterations in immune function in animal models (Penninks *et al.*, 1990, Barnett and Rodggers, 1994 and Voccia *et al.*, 1999, Roberta *et al.*, 2000).

This study indicated that there are no significant differences in most of the studied parameters of animals exposed either to monochrotophos or phoxim pesticides. The histopathological findings documented this result. The obtained results revealed also that animals exposed to these pesticides for two months showed exaggerations of biochemical, pathological, hematological changes and body weight loss. Galloway and Handy, (2003) reported that chronic toxicity with OPs will enhance immunosuppression through altered protein metabolism and malnutrition.

Our data revealed that total protein, albumin and globulin values of treated animals showed a significant or highly significant decrease in comparison with control group depending on the dose of pesticide and duration of exposure. Such findings were reported by Hazarika and Sarkar (2001) and Hazarika *et al.*, (2003).

Organophosphorus compounds are reactive and labile and can directly damage cell membranes, proteins and DNA (Mennear, 1998; Videira *et al.*, 2001) and post exposure oxidative damage has been reported in vertebrate blood cells and other tissues (Shishido *et al.*, 1972; Handy *et al.*, 2002). Direct damage to protein may be augmented by the increase in protein metabolism (Ceron *et al.*, 1996) and decrease in protein synthesis (Marinovich *et al.*, 1994), which can occur in response to OP exposure in vivo.

The obtained data indicated that the high dose of both pesticides had more suppressive effect on IgG and IgM than the lower one. A significant decrease was observed in IgG and IgM in animals treated with 1/10 LD_{50} of both pesticides for one month. Moreover after two months exposure, IgM levels showed a highly significant decrease in animals treated with 1/10 LD_{50} of both pesticides but it was significantly decreased in animals exposed to the low dose.

IgG levels was significantly decreased in animals exposed to the high dose only.

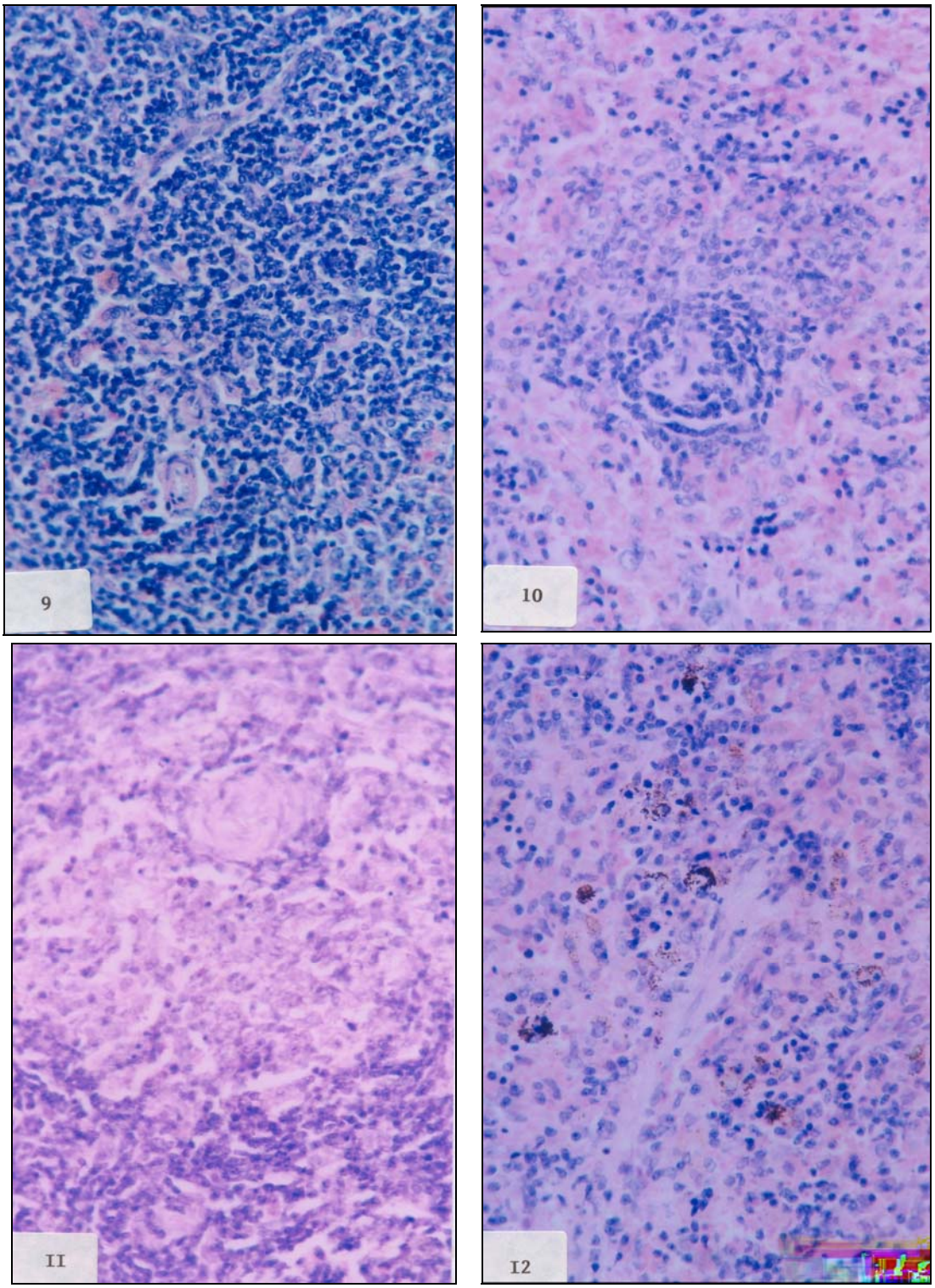

Plate (III): Spleen showing:

Fig. (9): Normal histology from the spleen of the control rats. H&E. 10 X 25.
Fig. (10): Exhaustion of the lymphocytes in both white and red pulp. H&E. 10 X 25.
Fig. (11): Sever exhaustion of the lymphocytes with evidences of necrosis. H&E. 10 X 25.
Fig. (12): Hemosidrosis with presence of sidrophage cells. H&E. 10 X 25.

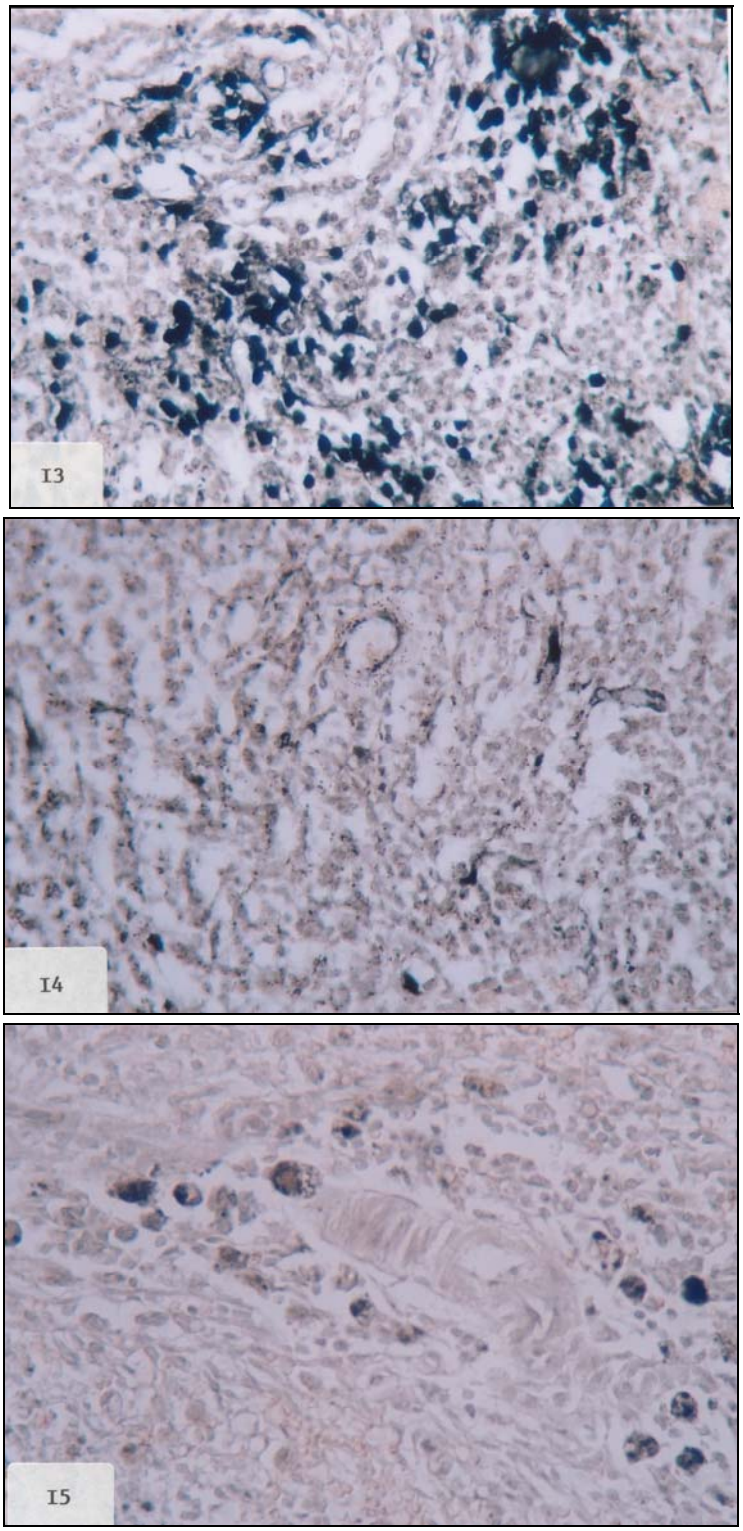

Plate (IV): Spleen stained with alkaline phosphatase showing:

Fig. (13): Prominent alkaline phosphatase positive B lymphocyte and macrophage cells. 10 X 25.

Fig. (14): Fewer number of alkaline phosphatase positive B cells. 10 X 25.

Fig. (15): Fewer number of alkaline phosphatase positive macrophagal cells represented the sidrophages. 10 X 25.

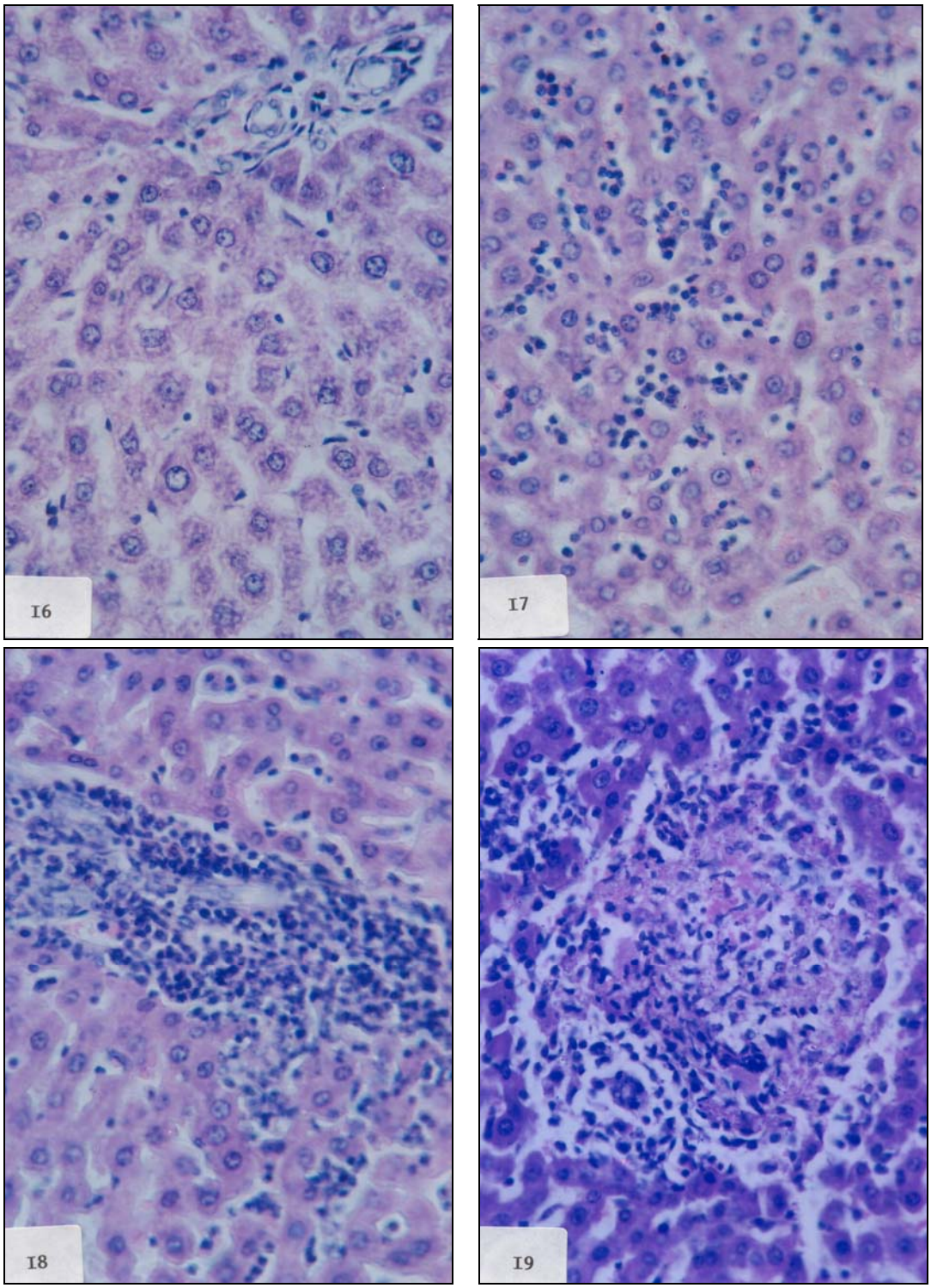

Plate (V): Liver showing:

Fig. (16): Vacuolar degeneration of the hepatocytes. H&E. 10 X 25.
Fig. (17): Infiltration of eosinophiles and mononuclear cells in the hepatic sinusoids. H&E. 10 X 25.
Fig. (18): Infiltration of eosinophiles and mononuclear cells in the portal area. H&E. 10 X 25.
Fig. (19): Focal area of necrosis associated with hemorrhage and cellular infiltration. H&E. 10 X 25.

A decrease of total IgG or IgM was also reported in mouse and humans exposed to organophosphorus (Barnett *et al*., 1980; Stiller-Winkler *et al*., 1999). Prolonged exposure of low doses of malathion (a cumulative high dose) results in decreased humoral immunity (Burns-Nass *et al*., 2001). In contrast, subchronic oral administration of leptophos and malathion did not suppress generation of antibody forming cells (Koller *et al*., 1976 and Rodgers *et al*., 1986). Both cellular and humoral immune responses were reported to be suppressed by sub acute administration of parathion (Wiltrout *et al*., 1978).

The effects of sub-chronic doses of malathion exposure on humoral and cell-mediated immune (CMI) responses were studied in male albino mice, rats, and rabbits (Banerjee *et al*., 1998). They found that sub-chronic malathion exposure induced differential degrees of humoral and CMI suppression in these experimental animals. However, both cellular and humoral immune responses were decreased in a dose-time dependent pattern and a consistent trend was observed. They concluded also that the threshold level of the malathion for inducing immune suppression depends on the animal species, type of antigen used and the method of immunological assay.

Continuously exposed individuals to a mixture of OPs, carbamates, phenoxy herbicides and pyrethroides and compared to non-exposed controls showed significantly decreased odd ratio for IgM indicating enhanced macrophage activation and impaired humoral defense (Stiller-Winkler *et al*. 1999). When the same immunological parameters were measured before and after one application period, significant decreases were noted in IgG and complement C3c, indicating that both specific and non-specific humoral defenses were impaired by recent exposure.

The glutathione S-transferases (GSTs) are a family of enzymes that catalyze the neucleophilic attack of the sulfur atom of glutathione on the electrophilic center of a variety of chemical compounds (Daniel, 1993). The GSTs were believed to play an important role in the protection of cellular macromolecules from attack by reactive electrophiles. El-Mouelhi and Kauffman (1986) found that GST activity in both periportal and pericentral zones was much greater than other liver detoxifying enzymes. Besides, GSTs via their GSH dependent peroxidase activity may participate in protecting tissues from organic hydroperoxides produced during oxidative stress (Coles and Ketterer, 1990).

In this study, a significant increase in GST levels was recorded of exposed animals in comparison with controls after exposure for one month except those received the low dose of monochrotophos. Moreover, its levels showed a highly significant increase in all doses after exposure for two months. Hendrich *et al*., (1987) reported that glutathione S-transferase (GST) plays an important role in the cellular detoxification of xenobiotics. Dramatic increase in this enzyme has been observed in preneoplastic and neoplastic lesions during multistage hepatocarcinogenesis. Such findings were reported (Banerjee *et al*., 1999; Ahmed *et al*., 2000).

The obtained results indicated that total WBCs count was significantly decreased in rats exposed to both pesticides for two months. In a recent study of 64 pest and termite control operators exposed to chlorpyrifos and other Ops, abnormalities were found in the white blood cell count for four workers coincident with a decrease in peripheral cholinestrase activities and in sensory nerve conductance velocity. Individuals with depressed enzyme activity had sprayed chlorpyrifos daily for 5

days before blood samples were taken (Gotoh *et al*., 2001).

There was a loss of body weight gain in animals received a dose of 1/10 LD_{50} for one or two months of both pesticides. The animals received the low dose showed a decrease in body weight gain only in comparison with control. A decrease in splenic weight was also noticed in treated animals. Casale *et al*., (1983) and Day *et al*., (1995) reported that there were significant decreases in the weight of lymphoid organs and histologic lesions were evident in both thymus and spleen of mouse and pheasant exposed to OPs (parathion and malathion).

The histopathological results revealed necrosis of the lymphocytic elements in both lymph node and spleen seen after one month of exposure and exaggerated at the end of of the experiment. This was associated with decrease and even absence of B cell in transformation.

Histopathological damage to the lymphoid organs associated with decrease in immunocytic count, inhibition of proginator cell development and altered lymphocyte subpopulation were also encountered in different species of animals due to exposure to OPs (Parentmassin and Thouvenot, 1993; Thrasher *et al*., 1993; Jeong *et al*., 1995; Fairbrother *et al*., 1998; Khalaf-Alah, 1999; Blakley *et al*., 1999; Gotoh *et al*., 2001 and Handy *et al*., 2002).

Malathion, 0,0 dimethyl-0-2,2-dichloro-vinyl phosphate (DDVP), sarin, tabun and soman were significantly inhibited the generation of antibody forming cells when administrated at neurotoxic dosages (Casale *et al*., 1983, 1984 and Clement, 1985).

Structural and functional changes in immunocytic population may occure in OPs exposure due to inhibition of esterases asssociated with the cell membrane of lymphocte and monocyte (Becker and Hansen, 1973; Stepanovic *et al*., 1998). However, histopathological damage to lymphoid tissues resulting from phosphorylatiuon, oxidative damage, or altered neural function could also hinder the development and viability of lymphocytes (Handy *et al*., 2002).

Oxidative stress associated with the organo-phosphorus diazinon metabolism (Shishido *et al*., 1972) may have caused some of the pathological changes noted. Quantitative image analysis identified organ specific changes in the proportions of fixed lymphoctes considered sufficient to severely compromise immune function.

The pathological damage to the liver cells as well as the direct damage and decrease in the biosynthesis of protein due to OPs compounds will share in the decreased level of globulin fraction that important for immunoglobulin synthesis.

In conclusions: this study indicated that humoral immune responses were decreased in a dose-time dependent pattern as the immuno-supression effects of monochrotophos and phoxim were increased in animals exposed to the higher doses of these pesticides for the longer duration.

Acknowledgment

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التسمم المناعي لمبيدي المونوكروتوفوس والفوكسيم في الفئران البيضاء

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تم استخدام مائة فأراً أبيضاً تبلغ من العمر ثلاثة أشهر، قسمت إلى خمس مجموعات متساوية . أعطيت المجموعة الأولى والثانية 1/20 و 1/10 من الجرعة نصف المميتة من مبيد المونوكروتوفوس والثالثة والرابعة 1/20 و 1/10 من الجرعة نصف المميتة من مبيد الفوكسيم على التوالي يومياً مذابة بماء الصنبور، بينما استخدمت المجموعة الخامسة كمجموعة ضابطة تعطى نفس الكمية من الماء بدون المبيد. وقد تم وزن الفئران قبل وفي نهاية التجربة، وبعد مضي شهراً من بداية التجربة تم ذبح عشر فئران من كل مجموعة وعند مضي شهرين تم ذبح باقي الفئران، وقد تم جمع عينات من الدم للفحص البيوكيميائي وكذلك الأعضاء مثل الطحال والغدد الليمفاوية والكبد للفحص الهستوباثولوجي .

أوضحت النتائج حدوث انخفاض معنوي في مستويات البروتين الكلي، الألبيومين والجلوبين، في الفئران المتعرضة للمبيدين بدون اختلاف معنوي بينهما سواء بعد التعرض لمدة شهر أو شهرين مقارنة مع المجموعة الضابطة. كما ثبت من التجربة حدوث انخفاض معنوي في مستوى الأجسام المناعية (IgG & IgM) بعد التعرض للجرعة الأعلى فقط من هذه المبيدات بعد شهر من بداية التجربة ، وكان الانخفاض أكثر معنوية بعد التعرض لمدة شهرين وبخاصة IgM في الجرعة الأعلى.

وتبين من النتائج حدوث زيادة معنوية متفاوتة في نشاط خميرة الجلوتاثايون إس ترانسفيريز بعد التعرض لمدة شهر، إلا أن هذه الزيادة كانت في أعلى درجاتها بعد التعرض للمبيدين لمدة شهرين سواء في الجرعة الأقل أو الأعلى . وكانت نسبة الفقد في وزن الجسم واضحة نتيجة للتعرض للجرعة الأعلى سواء بعد شهر أو شهرين من بداية التجربة مقارنة مع المجموعة الضابطة، وأحدثت الجرعة الأقل من هذه المبيدات انخفاضاً في معدل زيادة وزن الجسم عند التعرض لمدة شهر أو شهرين مقارنة مع المجموعة الضابطة، كما حدث انخفاض في وزن الطحال في جميع الحيوانات وإن بدا أشد في الجرعة الأعلى، وسلك العدد الكلي لكرات الدم البيضاء نفس الإتجاه في الانخفاض والشدة .

أظهر الفحص الهستوباثولوجي وجود تنكرز في الغدد الليمفاوية بعد شهر من بداية التجربة وبعد شهرين لوحظ وجود تليف في الغدد الليمفاوية، كما أظهر الفحص المناعي قلة في عدد الخلايا الليمفاوية واختفائها تماماً في نهاية التجربة، وكذا في الطحال لوحظ تنكرز في الخلايا الليمفاوية في الكريات البيضاء والحمراء، أما في الكبد فقد لوحظت استحالات في خلايا الكبد مع زيادة في الخلايا المحببة الحمراء ووحيدة النواة بعد شهر من بداية التجربة ووجدت مناطق تنكرزية مصحوبة بنزيف دموي ووجود خلايا التهابية في نهاية التجربة .

ودلت النتائج علي التأثير المثبط الواضح لهذه المبيدات على الجهاز المناعي وإن بدا أشد مع طول فترة التعرض ومع زيادة الجرعة المتعرض لها، الأمر الذي يزيد من خطورة هذه المبيدات في إحداث نقص المناعة وضعف المقاومة للأمراض المختلفة .