Vet. Med. J., Giza. Vol. 58, No.3 (2010): 259 - 279

The Role of Enchinacea Purpurea against Immuno - Supressive Effect of Dimethoate on Male Albino Rat

Manal, SH. Hussein*·[a], Azza, H. Abd- El-Rahman·[a] and Walaa M.S. Ahmed****

***Forensic* Medicine & *Toxicology* Dep. Beni seuf university, faculty of vet.med. [a]Associate Professor of *forensic* medicine & *toxicology* , Taif university, medicine and medical science college (Saudi Arabia)**
**** Clinical Pathology Dept. Fact of Vet. Med. Beni-Suef University.**

Received: 06/02/2011
Accepted: 15/02/2011

SUMMARY

Dimethoate, an organophosphate pesticide, is used in controlling the pests of a variety of crops. Herbal medicine is the most widely used form of medicine in the world today where the medicinal plants contain many curative bioactive ingredients. The present work was planned to evaluate the potential protective effect of Enchinacea purpurea (EP) against the immunotoxic effect induced by dimethoate in adult male albino rats. Rats were classified into four groups (10 in each). Rats in the 1st group received no treatment and served as control. Rats in the 2nd group were orally administered dimethoate 40%EC in a dose level of 3mg / kg bw. Equivalent to 1/10 LD50. Rats in the 3rd group were orally administered EP (Immulant) in a dose level of 2.5 ml/kg bw. The fourth group was treated with dimethoate 40%EC as in group II in addition to EP in a dose level as in the third group. Administration of tested substances was carried out daily for successive 7 days. Rats from treated as well as from control group were injected IP with ($1-2\times10^8$) sheep RBCs as non specific antigen. After 7 and 14 days from injection of sheep RBCs (SRBCs) five rats from each group were taken, blood and tissue samples were collected. The present data revealed a significant decrease in WBCs count (leucopenia), neutrophillia and lymphocytpenia with lower haemagglutination inhibition antibody titre (HI) and significant decrease in IgM in serum samples from dimethoate treated rats. Also in the same group there was a significant decrease in serum

thymus were recorded in dimethoate treated group. EP supplementation induced appreciable improvement in all previous abnormal alterations observed in dimethoate treated rats. Therefore, this study revealed that EP exhibit marked protective role against the toxic effect of dimethoate on immune system of male albino rats.

Key words: *dimethoate, immunotoxicity, enchinacea purpurea, rat*

INTRODUCTION

Organophosphorous compounds are widely used in industry, agricultural and for public health purpose, they are among the toxic compounds employed for insect control. Exposure of organophosphorous compounds in agriculture is one of the occupational hazardous (Tsatsakis, et al., 1998). The main effect of organophosphorous pesticides (OPs) is neurotoxicity, which is caused by the inhibition of acetylcholinesterase. OPs also affect immune responses including effects on antibody production, Interleukin IL-2 production cell proliferation, decrement of CD5 cells, and increment of CD26 cells and auto antibodies, (Qing and Tomoyuki 2006 and Johnson et al., 2002).

Dimethoate (O, O-dimethyl S-N-methyl carbamoyl methyl phosphodithioate) is an insecticide refer to organophosphates and it is frequently used in agriculture. Dimethoate poisoning is usually associated with neuromuscular transmission block in both animals and humans because it act by interfering with the activities of cholinesterase an enzyme that is essential for the proper working in the nervous system (Dongren et al., 1999). Immuno- toxicological effects due to dimethoate have also been reported (Institoris et al., 1999).

The use of the immune system as a sub-lethal biomarker for xenobiotics has been of increasing interest in recent years (Fitzpatrick et al. 1992). Any impairment of the immune system can lead to increased susceptibility to infection from numerous sources, with potentially lethal consequences.

Echinacea purpurea L. (EP) is a plant originally used by native Americans to treat respiratory infections and have long been used to aid in wound healing and to enhance the immune system (Lee et al., 2010).

The EP have been proven to show good immunoregulation, antiinflammation and antioxidant capacity and with no hypersensitivity or other side effects during clinical trial stages (Lee et al 2009 and Zahi et al 2007)

Therefore this study was aimed to demonstrate the protective activity of

enchinacea purpurea against the immunotoxic effect of dimethoate on male treated rats.

MATERIALS AND METHODS

Materials

I- Tested substances:-

1- Dimethoate 40% EC is an organophosphorous insecticide with a chemical formulas || CH3NHCOCH2SP (OCH3)

Its chemical name is O, O-dimethyl S-N-methyl carbamoyl methyl phosphodithioate. It is available as an emulsifiable concentrate obtained from Sydon Cheminova Company or crop-protection Bazal _Swizerland.

2- Enchinacea purpurea, trade name (Immulant), where each 120 ml contain 2 gm Enchinacea purpurea dry extract obtained from Arab company pharmaceuticals and medicinal plants, Egypt.

3- Biochemical kits:- determination of some serum biochemical constituents were performed by using readymade kits from biodiagnosticc and cromatest linear chemicals company.

II –Experimental animals:-

Forty apparently healthy male albino rats with initial weight 90-100 gm were supplied from breeding unit of Egyptian organization for the biological vaccine production. Animals were left for one week before start of experiment in order to acclimatized the conditions. They were fed on balanced commercial rat diet with free access to food and water.

Experimental rats were classified into four equal groups (10 in each). The first group, rats received no treatment and served as control. The second group, rats in this group were orally administered dimethoate 40%EC in a dose level of 3mg / kg bw. equivalent to 1/10 LD50 of dimethoate (Hays and Laws 1990)., In the third group, rats in this group were orally administered Enchinacea purpurea as Immulant in a dose level of 2.5 ml/kg bw (paget and Barnes ,1964). In the fourth group rats was treated with dimethoate 40%EC as in group II in addition to Enchinacea purpurea (Immulant) as in the third group. Administration of tested substances was carried out daily for successive 7 days. Rats from treated as well as control groups were injected IP with ($1\text{-}2\times10^8$) sheep RBCs as non specific antigen according to Giang et al (2002).

After 7 and 14 days from injection of sheep RBCs (SRBC) five rats from each group were taken, blood sample were collected from control as well as treated groups from orbital venous plexus. Each sample was divided into two portion first one was collected into clean dry Epindworf tube containing disodium salt of ethylenediamine tetra acetic acid (EDTA) (1-

2mg/ml blood) as anticoagulant, this sample used for total and differential leucocytic count estimation. The second part was collected into plain centrifuge tubes and used for serum separation. Collected sera were used for biochemical and immunological studies. Tissue samples from liver, spleen and thymus gland were taken from all rats in all treated and control groups at 7 and 14 days after sheep RBCs inoculation and fixed in 10% neutral formalin and used for histopathological studies.

Methods

1- **Total and differential leucocytic count estimation:-** They were performed according to (Jain, 1986).

2- **Biochemical measurements:** - Determination of total protein and albumin concentration were performed according to (Gornal *et al.*, 1949 and Doumas, 1971).

3- **Immunological studies:-**

a- **Haemagglutination inhibition test (HI):-** This test was carried out according to standered procedure described by Majujab and Hitchner (1977).

b- **Determination of serum IgG and IgM:-** IgG and IgM were determines by enzyme linked immunosorbent assay (ELISA) according to Temple et al (1995).

c- **Electrophoretic pattern of serum protein:** - Serum protein electrophoresis was performed according to Ritzmann and Daniels (1979).

4- **Histopathological examination:** - Histopathological examination of liver, spleen and thymus gland specimens from contro nd treated group were performed according to the method of Banchroft *et al.*, 1996.

5- **Statistical analysis:** Data were compared across group using one way analysis of variance ANOVA followed by least significant difference (LSD) test at 5% and 1% within groups according to (Sendecor and Cochran, 1982).

RESULTS

Effect on WBCs and differential cell count

The data present in table 1 revealed significant decrease in WBCs count (leucopenia) , neutrophillia and lymphocytpenia in blood samples from dimethoate treated rats, while in rats treated with dimethoate and immulant the result showed significant increase in WBCs count when it compared with dimethoate treated group, but it is significantly decrease than that in control group, while neutrophile and lymphocyte percentage in this group nearly

similar to those in control one. Data represented in table 1 showed that WBCs and differential count in rats received immulant only, is closely related to those in control group.

Immunological studies

A-Haemagglutination inhibition (HI)

From table (2) it was clear that rat treated with dimethoate showed lower haemagglutination inhibition antibody titre (HI) against SRBC compared to control group after 14 days. EP as immulant caused significant increase in the anti-SRBC antibody titer. When EP was administered along with dimethoate, it significantly reversed the dimethoate-induced decrease in anti-SRBC antibody titer.

B- IgM and IgG determination

Results presented in Table 3 indicated that in dimethoate treated rats the mean values of serum immunoglobulin M (IgM) was significantly decreased at fourteen days post SRBC inoculation with no significant changes in immunoglobulin G (IgG), co administration of Immulant with dimethoate resulted in significant increase in IgM when it compare with dimethoate treated group . In the 3rd group that received immulant only, the mean values of IgG and IgM showed no alteration than that of control group.

C- Electrophoretic pattern of serum protein:-

From table (3) the obtained data revealed that there was a significant decrease in serum protein, albumin and gamma globulin concentration of rats that received 1/10 LD50 of dimethoate, while in the group that received Immulant in association with dimethoate the result showed significant increase in serum protein, albumin and gamma globulin concentration in comparison with the dimethoate treated group. Regarding to rats that treated with immulant alone, it was found no effect in total protein, albumin and globulin concentration when it compared with control group.

Histopathological finding:-

Microscopical examination of liver in dimethoate treated rats after 7 days from sheep inoculation showing inflammatory cells infiltration in the portal area with diffuse kupffer cells proliferation in between the degenerated hepatocytes (Fig. 1). In group of rats administrated dimethoate after 14 days from sheep RBCs inoculation, the histopathological examination of the liver showed sever dilatation and congestion in the portal vein with inflammatory cells infiltration in the portal area and degeneration in the hepatocytes (Fig. 4). The spleen of rats in this group showed depletion in the lymphoid cells

in the white pulps after7 and 14 days from sheep RBCs inoculation (Fig. 2, 5). The thymus of the rats in this group showed lymphoid depletion in the medullary portion at 7 and 14 days from sheep RBCs inoculation (Fig.3, 6).

Histopathological examination of the 4th group that received Immulant in association with dimethoate after 7 days post inoculation showed only congestion in the portal vein in liver samples and only congestion in the blood vessels of spleen (Fig.7, 8) while histopathological examination of liver, spleen and thymus gland specimens from rats in this group but after 14 days from sheep RBCs inoculation showing intact histological structure (Fig. 9, 10, 11)

Table (1): Mean values ± SE of total ($\times 10^3$ μ) and deferential leukocytic count (%) in control and treated rat groups.

Time	Group / parameter	Control	Dimethoate	immulant	Dimethoate +immulant
After 7 days from sheep RBCs inoculation	WBCs	10.2 ± 0.15	6.3 ± 0.42	8.9 ±0.61	7.9±0.46
	Neutrophil%	20.6±1.2	29.3±1.3	24.6±2.02	22.6±1.45
	Lymphocyte%	65±2.6	57.8±1.28	63±2.09	69±1.15
	Monocytes %	13±1.34	12.4±0.6	12±0.92	9±0.71
	Esinophil %	1.3±0.5	0.3±0.25	0.3±0.25	0.00±0.00
After 14 days from sheep RBCs inoculation	WBCs	10.41±0.31	7.41±0.24	9.1±0.33	8.4± 0.19
	Neutrophil%	22±2.00	29±1.5	28.5±1.3	23.5±1.5
	Lymphocyte%	69±3.21	61±2.08	66.6±2.3	71±1.5
	Monocytes %	9±0.37	10±0.60	4.9± 0.32	5.5±0.20
	Esinophil %	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

For WBCs the LSD at 5% is1.22 and at 1 % is 2.03
Neutrophils at 5% is 4.17 and at 1 % is 6.91
Lymphocyte at 5% is 6.45and at 1 % is10.69
Monocytes prob > f is 0.5928 (not significant)

Table (2): Mean values ± SE Haemagglutination antibody titer response to SRBC, IgM and IgG in control and treated rat groups.

Time	Groups / Parameter	Control	Dimethoate	immulant	Dimethoate +immulant
After 7 days from sheep RBCs inoculation	Total protein	7.55±0.017	6.4±0.01	8.43±0.00	7.3±0.1
	albumin	4.61±0.04	4.13±0.06	5.35±0.035	4.49±0.017
	Alpha	0.62±0.03	0.53±0.03	0.59±0.02	0.56±0.00
	Beta	0.30±0.03	0.31±0.02	0.28±0.01	0.28±0.00
	Gamma	2.04±0.09	1.64±0.00	2.3±0.11	1.96±0.03
After 14 days from sheep RBCs inoculation	Total protein	8.01±0.01	6.95±0.04	8.69±0.2	7.76±0.20
	albumin	5.02±0.02	4.41±0.01	5.37±0.01	4.84±0.01
	Alpha	0.59±0.01	0.56±0.02	0.75±0.02	0.60±0.02
	Beta	0.32±0.01	0.28±0.01	0.37±0.02	0.31±0.02
	Gamma	2.08±0.03	1.64±0.00	2.2±0.01	1.97±0.01

For IgM LSD at 5% is 4.741 and at 1 % is 7.183 IgG prob > F is 0.631 and F value is 634 with ¾ degree of freedom (insignificant)

Table (3): Mean values ± SE of total protein, albumin level and protein fractions g/ dl in control and treated rat groups.

Time	Groups / parameter	Control	dimethoate	immulant	Dimethoate +immulant
After 7 days from sheep RBCs inoculation	**HI antibody titre**	2.4± 0.24	2.00±0.31	2.6±0.24	2.2± 0.37
	IgM	18.48 ± 0.05	17.10±0.08	18.10±0.13	19.60±0.14
	IgG	13.50±0.11	10.94±0.23	12.58±0.17	11.95±0.21
After 14 days from sheep RBCs inoculation	**HI antibody titre**	4.6±0.4	2.4±0.15	5.4±0.34	4.00±0.23
	IgM	32.1±0.33	28.9±0.37	34.4±0.23	30.1±0.28
	IgG	14.48 ±0.24	13.32±0.18	15.43±0.12	14.25±0.09

For total protein the LSD at 5% is 0.870 and at 1 % is 1.44
albumin at 5% is 6.09 and at 1 % is 1.011
gamma globulin at 5% is 0.112 and at 1 % is 0.187
alpha globulins prob > f is 0.327 and F value is 1.574 with ¾ degree of freedom (insignificant)
Beta globulin prob > f is 0.352 and F value is 0.790 with ¾ degree of freedom (insignificant)

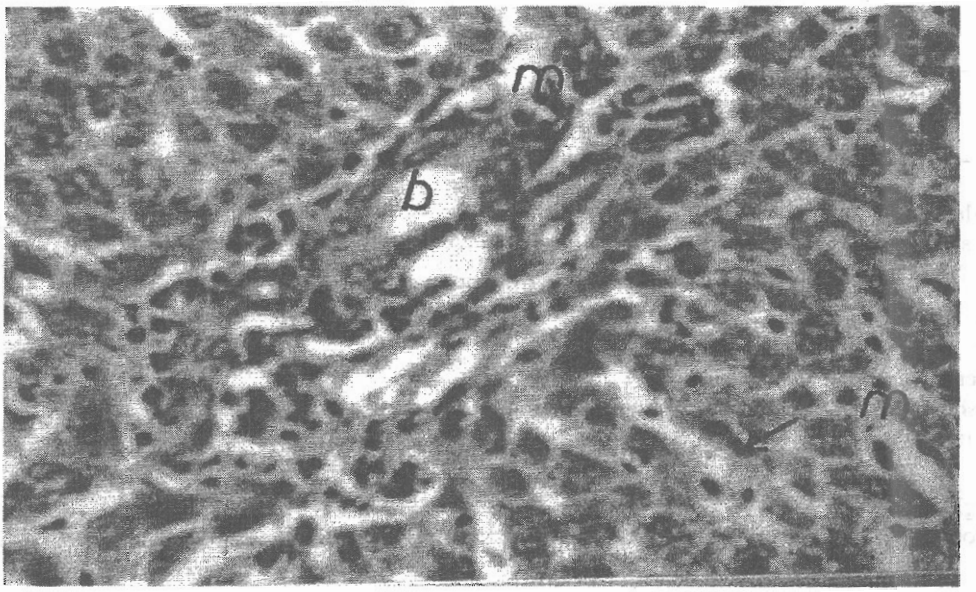

Fig (1):- Liver of rat treated with dimethioate after 7 days from sheep RBCs inoculation showing inflammatory cells infiltration (m) in the portal area between the newly formed bile ducts (b) with diffuse kupffer cells proliferation (arrow) in between the degenerated hepatocytes. H & E x 80.

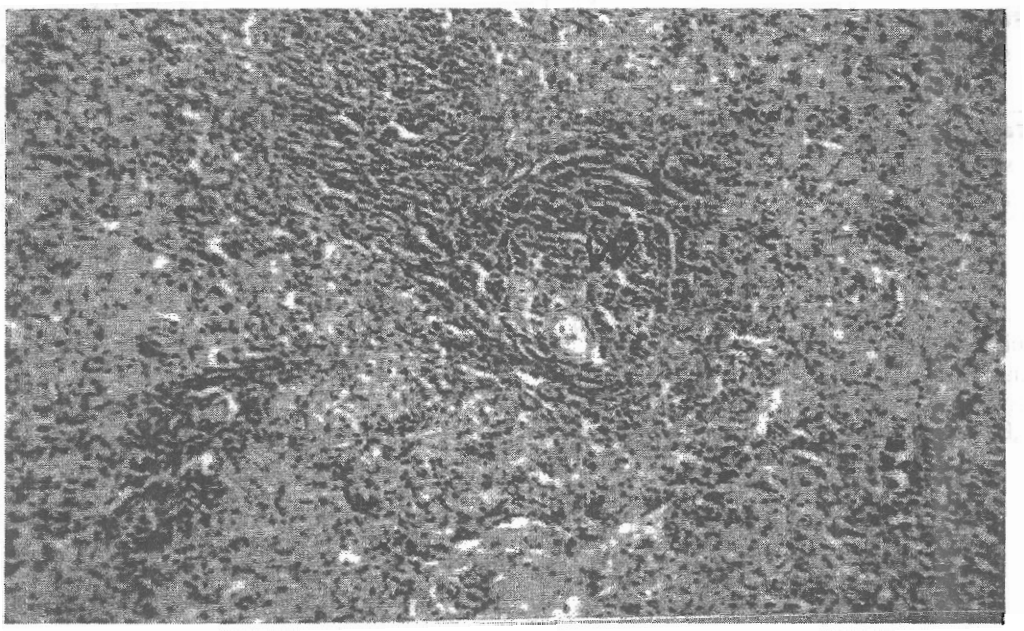

Fig (2):- spleen of rat treated with dimethioate after 7 days from sheep RBCs inoculation showing depletion in the lymphoid cells in the white pulps (w). H & E x 40.

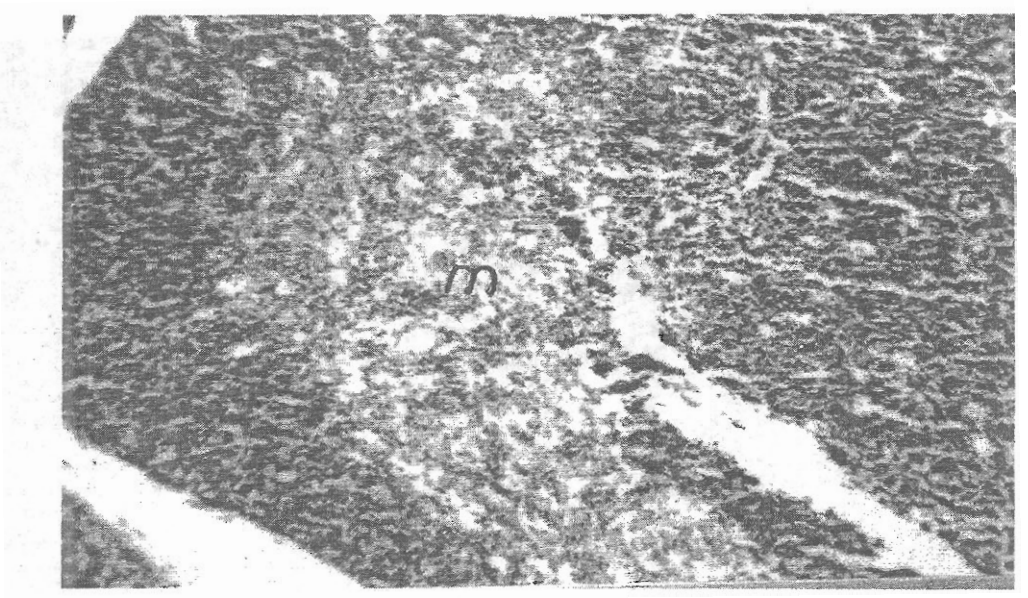

Fig (3):- Thymus of rat treated with dimethioate after 7 days from sheep RBCs inoculation showing lymphoid depletion in the medullary portion (m). H&E x 40.

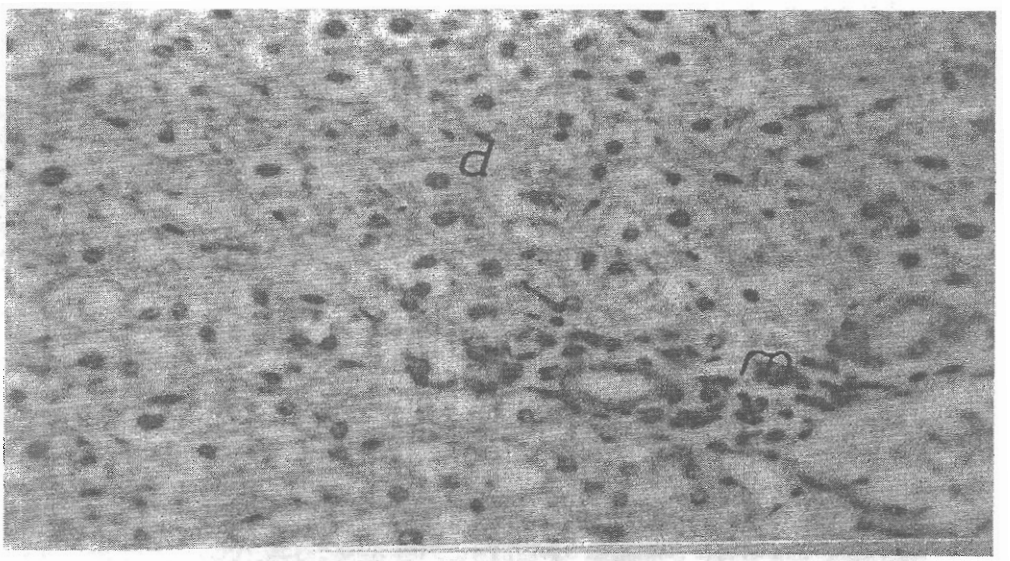

Fig (4):- liver of rat treated with dimethoate after 14 days from sheep RBCs inoculation showing sever dilatation and congestion in the portal vein (pv) with inflammatory cells infiltration (m) in the portal area and degeneration in the hepatocytes (d) H&E . x 80.

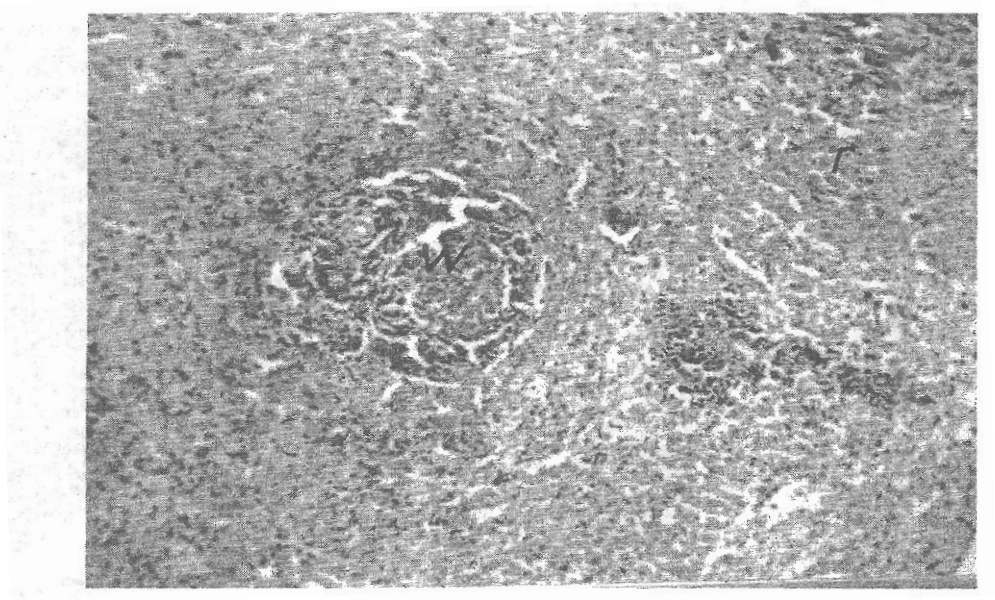

Fig (5):- spleen of rat treated with dimethioate after 14 days from sheep RBCs inoculation showing sever depletion in the white pulps (w). H& E. x 40.

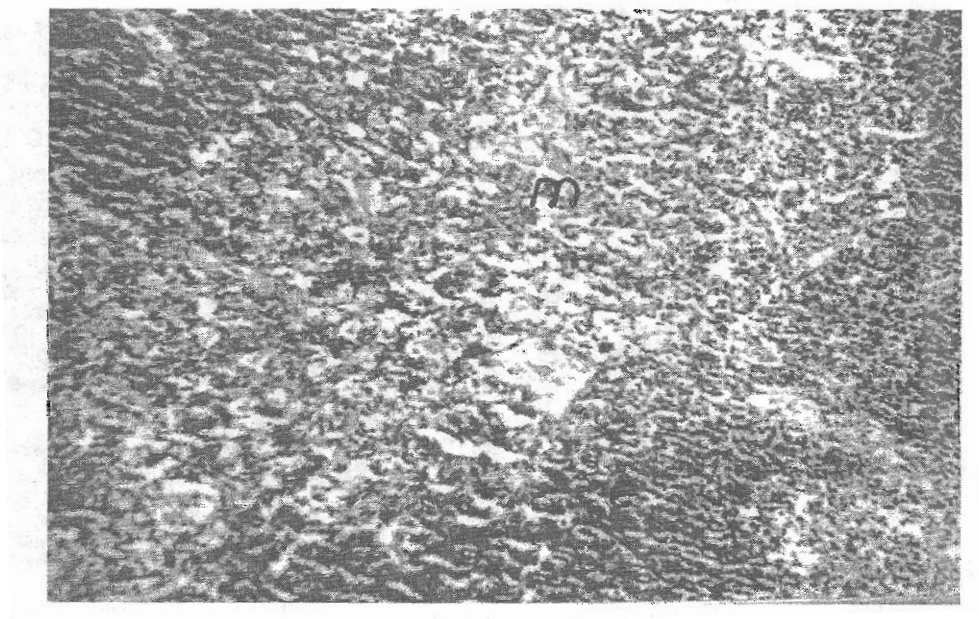

Fig (6):- Thymus of rat treated with dimethioate after 14 days from sheep RBCs inoculation showing mild lymphoid depletion in the medullary portion (m) H&E. x 40

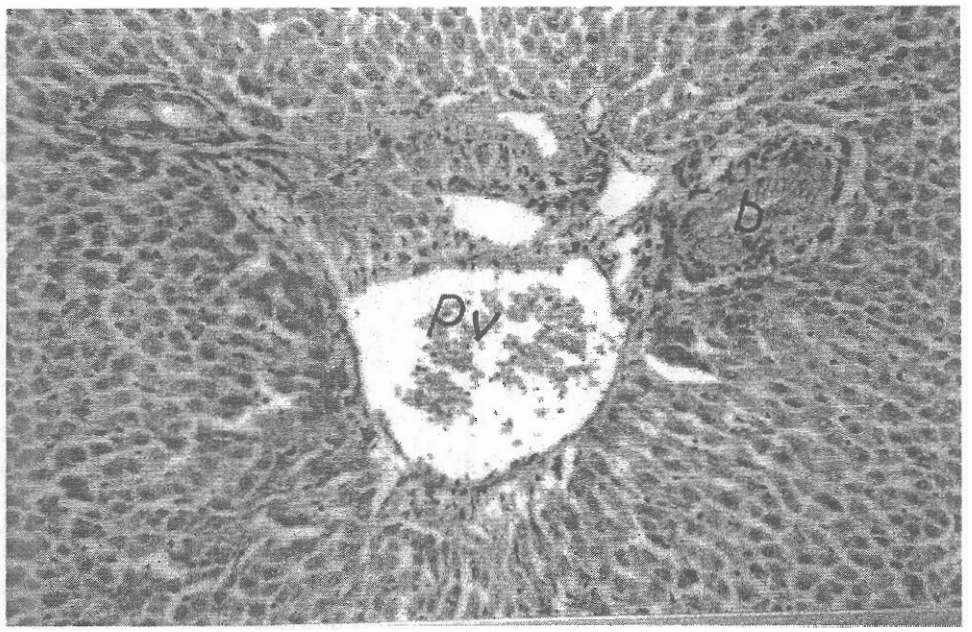

Fig (7):- Liver of rat treated with dimethioate & immulant after 7days from sheep RBCs inoculation showing showing congestion in the portal vein (pV) with newly formed ductules (b) in portal area. H&E x 40.

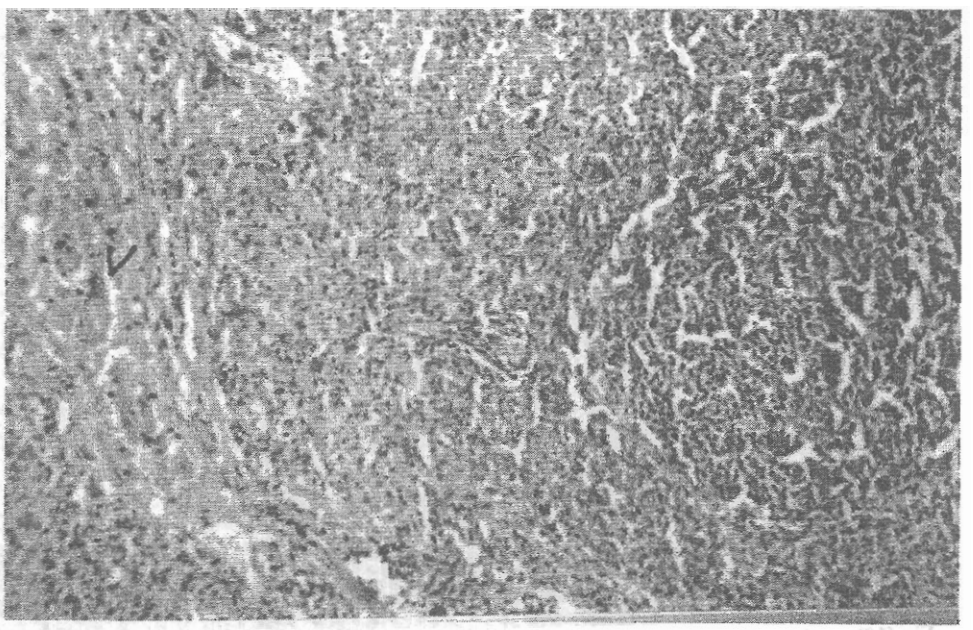

Fig (8):- spleen of rat treated with dimethioate after 7 days from sheep RBCs inoculation showing only congestion in the blood vessels (v) H&E x 40

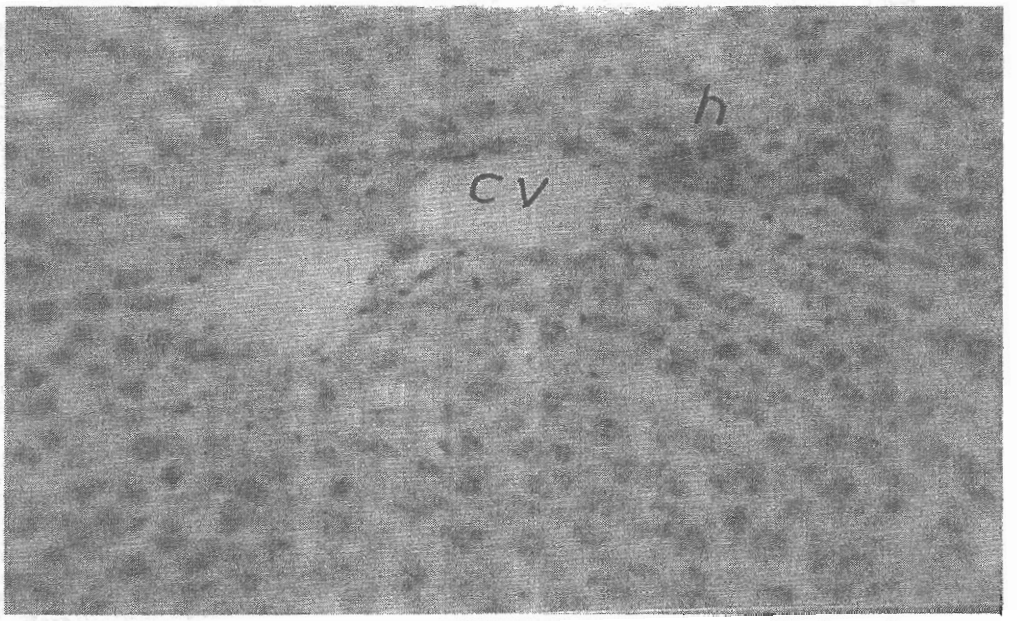

Fig (9):- liver of rat treated with dimethioate and immulant after 14 days from sheep RBCs inoculation showing intact histological structure of the central vein (cv) and surrounding hepatocytes (h). H&E x 64.

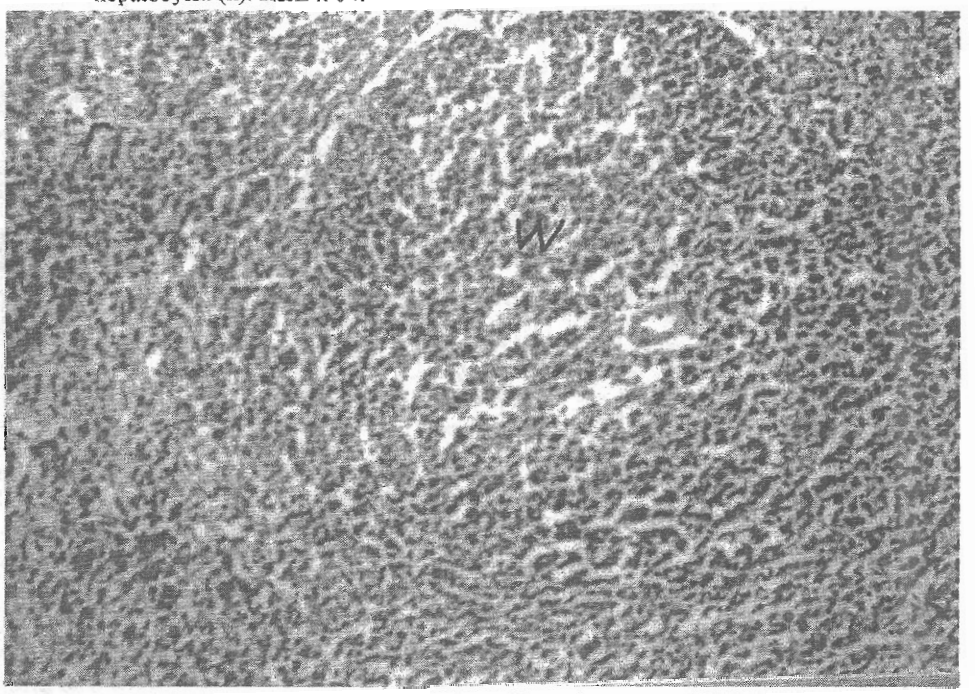

Fig (10):- spleen of rat treated with dimethoate and immulant after 14 days from sheep RBCs inoculation showing intact histological structure H&E x 40.

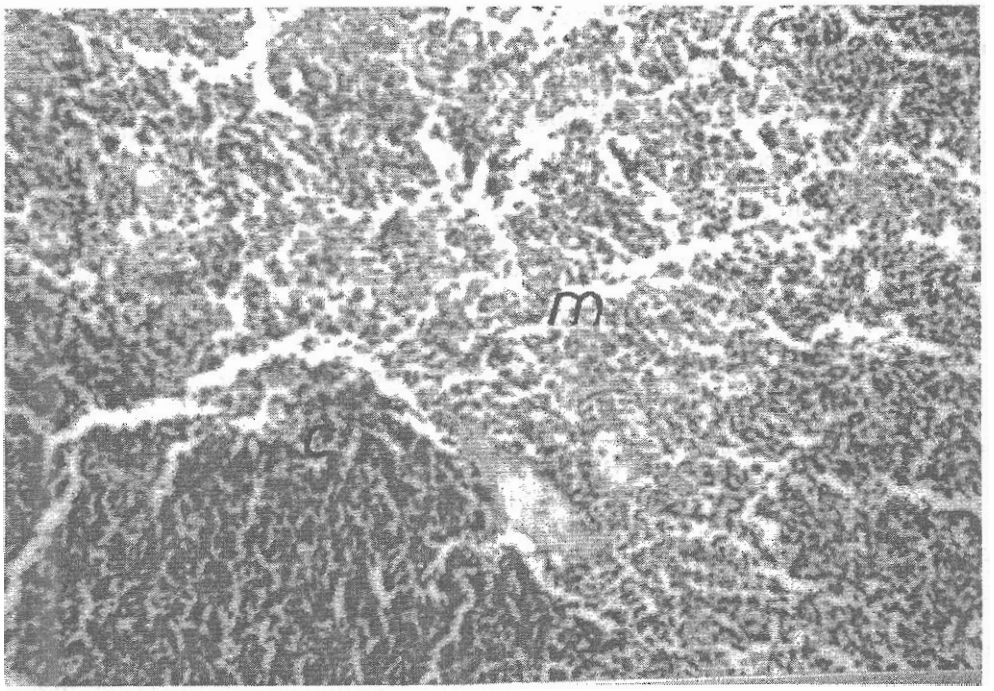

Fig (11):- Thymus of rat treated with dimethoate and immulant after 14 days from sheep RBCs inoculation showing intact histological structure H&E x 40.

DISCUSSION

During the last decades, the extensive use of different pesticides in agriculture and for public health purposes, has led to drastic effects especially in animals and human (Pesticides residues in foods, 1996). Most of these chemicals are not highly selective but generally they proved to be toxic to many non-target species including man and other desirable forms of life that co inhabit the environment, therefore, their improper application may result in serious illness and even death.

Dimethoate, the insecticide used in this study, is a widely used organophosphate compound which has a significant contact and systemic action against a wide variety of insects and pests of both plants and animals (Westcott et al., 1987).

In this study, the recorded results revealed significant decrease ($p<0.05$) in leukocytic count of dimethoate treated male rates. This result was parallel to those recorded by Institóris et al (1999). They observed significant decrease in WbCs count in rat treated with dimethoate. A low white blood cell count may be attributed to the effect of dimethoate in hematological tissues (spleen and kidney) this attribution is in agreement with Banaee et al 2008 and supported by the

histopathology in this study which confirmed the effect of dimethoate in the spleen.

Decrease percentage of lymphocytes (lymphopenia) was recorded in dimethate treated group and that can be an indicator of immune system deficiency Poisonous substances treatments can also deplete the body's supply of lymphocytes, as can exposure to dimethoate. Marked lymphopenia was observed by Ambwani et al 2006 in avian when treated with thousand times dilution of No Observable Effect Level (NOEL/103) dose of dimethoate and also by Nath and Banerjee (1996) in heteropneustes fossilis

The present data recorded significant increment ($p < 0.05$) of neutrophile percentage in dimethoate treated group after exposure to 3mg / kg bw. dimethoate 40%EC equivalent to 1/10 LD50 of dimethoate. This result is in accordance with Ghosh and Banerjee (1993) who reported lymphopenia and increased in both neutrophile and eosinophile in heteropneustes fossilis, after an effect of dimethoate in 96h LC50 concentration. The most common and important cause of neutrophilia is infection, but tissue damage from other causes as toxin raises the neutrophile for similar reasons. Poisonings, and severe disease, like kidney failure all cause neutrophilia (Holland, et al., 1997).

The effect of dimethoate on humoral immune response was evaluated via measuring antibody titre to SRBC by haemagglutination inhibition (HI) assay. In the present study, exposure of the animals to dimethoate produced a significant reduction in HI antibody response at 14 days post SRBC inoculation. This result is parallel to finding of Pramod et al. (2008). Also the present result correlated with findings of other workers who observed reduction in antibody titre on exposure to organophosphate pesticides (Banerjee et al, 1998). The reduction of HI antibody titre may be attributed to the effect of dimethoate on the immune system of rats this speculation is supported by the histopathological examination of spleen and thymus gland of dimethoate treated rats which revealed marked depletion and degeneration of lymphoid tissue in spleen and thumus.

The statistical analysis of the present results revealed significant decrease in immunoglobulin M (IgM),in this aspect our results were correlate with those mentioned by Aly and el-Gendy 2000 who found a significant decrease in serum total immunoglobulins (Ig) and IgM in female mice exposed to single oral dose of dimethoate (16 mg/kg). It was suggested that humoral immunosuppression of dimethoate may be due to direct action of acetylcholine on the immune system or secondary to toxic chemical stress

associated with cholinergic poisoning (Rishi and Garg, 1997). Pruett (1992) suggested that the immunotoxicity of organophosphorus pesticides observed in vertebrates may result from direct action on the cells or from excessive cholinergic stimulation, thus affecting lymphocyte or macrophage function.

The mechanism of chemically induced immunosuppression is not completely understood, but it appears that in some cases hemotoxicity, direct damage to the organs and progenitor immune cells, is partially responsible. Also pesticides can affect the process of hematopoeises, the production and maturation of blood cells, including immune cells (Luster, 1995). Also Institoris et al (2002) mentioned that pesticides may exert an indirect action on the immune system as well they may be metabolically activated to their metabolites may also have effects on other organ system (e.g liver damage) which then impacts the immune system, or may induce alterations in hormonal homeostasis.

Administration of dimethoate 40%EC in a dose level of 3mg / kg bw. equivalent to 1/10 LD50 of dimethoate induced significant decrease in total protein values with hypoalbuminemia and decreasing in γ globulin which may be due to liver damage .This result in agreement with that found by Uzunhisarcikli (2008), who reported decrease in total protein and albumin levels after 4 and 7 weeks following methyl parathion application(0.28 mg/kg day) .Also Mohamed et al (2010) they mentioned that repeated doses of profenofos (17.8 mg/Kg body weight/day) daily for 15 days produced marked decrease in albumin level α2, β1, γ1 content . Such changes in t. protein, albumin and protein fractions reflect hepatocellular injury and disturbed amino acid metabolism induced by dimethoate (Gomes et al., 1999). Exposure to organophosphorus insecticides has been shown to inhibit all the cytoplasmic proteases and some of the lysosomal proteases in the liver tissue, the major site for insecticide metabolism (Mantle, 1997).

Exposure to dimethoate causes marked histophathological alterations in liver, spleen and thymus gland of dimethoate treated rats either at 7 or 14 days after sheep RBCs inoculation. This alterations in the form of degeneration of hepatocytes with inflammatory cells infiltration in the portal area in liver with sever depletion of white pulp of spleen while sever lymphoid depletion in medullary portion were observed in thumus glands. These findings are in agreement with those record by Sharma et al., 2005 and Sayim , 2007 they reported that Dimethoate caused dose-related histopathological changes including mononuclear cell infiltration, congestion, an

enlargement of the veins and sinusoids, hepatocellular damage, necrotic changes, increase in the number of Kupffer cells, cytoplasmic vacuolization and degeneration in nuclei in the liver of exposed rat.

Histopathological examination of the spleen in dimethoate treated rats revealed sever depletion in the white pulps. Regarding to the effect of dimethoate on thymus gland the obtained data revealed a marked histopathological changes, these changes are in accordance with that reported by Tiefenbach and Lange 1980. Who reported that after dimethoate adminstration histological examinations indicate reduction in the cortex of the thymus and disruption of the thymocytes and the number of rosettes forming cells in rats was reduced.

Echinacea purpurea (EP) is one of the most important medical herbs and is a kind of Asteraceae natively perennial grown in North America. Varieties of EP all contain similar main ingredients including caffeic acid derivatives, alkamides and flavonoids, and medical activities of which are yet to be exactly identified (Thygesen et al, 2007). The present data demonstrated that the oral administration of EP with dimethoate alleviated its harmful effect and induced marked improvement in immune status of dimethoate treated group that manifested by significant increase in WBC count, lymphocytosis with significant increase in anti-SRBC antibody titer . On the other hand EP induced marked increase in IgG, serum protein, albumin and gamma globulin. Similar result were mentioned by Aly and Mohamed (2010) who stated that the lymphocytic counts were significantly elevated with a significant increase in the total leucocytic count in groups administered echinacea for 1 and 2 months when compared with the control group. Regarding to the effect of EP on anti-SRBC antibody titer Bodinet et al. (2002) reported that the oral administration of a herbal immunomodulator, consisting of an aqueous ethanolic extract of the mixed herbal drugs Thujae summitates, Baptisiae tinctoriae radix, Echinaceae purpureae radix and Echinaceae pallidae radix caused a significant enhancement of the antibody response against sheep red blood cells. Jaless et al (1999) stated that Echinacea produced a significant augmentation of primary and secondary IgG response to the antigen with increase in the primary IgM response during the first 2 weeks of treatment. Eteghada et al (2010) investigated that Ep alone or in combination with levamisole induce significant increase in total protein, albumin, gamma globulin level, WBC, neutrophil and monocyte counts and phagocyte activity.

The effect of EP on the dimethoate may be attributed to two effects , either the

antioxidant effects of EP or the increase of over all immunity in dimethoate treated rat .The antioxidant effect of EP is due to its active ingredients especially flavonoids, as indicated by Lee et al (2010) who demonstrated that total flavonoid contents of EP extracts contain hydroxyl functional groups, are responsible for antioxidant effect in the plants, has been recognized, and the mechanisms of action of flavonoids are through scavenging or chelating process of free radicals produced by toxic agents..

The immune enhancing effect of EP may be attributed to active the ingredients in EP as caffeic acid derivatives, alkamides and polysaccharides. Echinacoside and chlorogenic acid were the main caffeic acid derivatives. Also the *E. purpurea* extract contained high levels of amides and cichoric acid, which proven to have stronger immunostimulatory effects than echinacoside. Several bioactive compounds have been reported from Echinacea. Numerous reports documented the immunomodulatory effects of alkamides (Woelkart and Bauer ,2007) which have been shown to work by binding to CB2 receptors which are G-protein coupled receptors expressed primarily by leukocytes Immunomodulatory and antioxidant properties have also been attributed to the caffeic acid derivatives and polysaccharides(Matthias et al. 2008). Polysaccharides may work through both TLR4-dependent and –independent pathways, ultimately activating NK-κB in macrophages (Sullivan 2008). Also Echinacea extract stimulates the immune system through activation of macrophage, polymorphonuclear leukocytes and natural killer cells (Barrett, 2003).

From this present results, we can concluded that exposure to dimethoate markedly affect immune system. Also the use of immulant that contain EP will gain good results and reduced the immunoinhibitory effect of dimethoate.

REFERENCES

Aly, S. M. and Mohamed, M.F.(2010):Echinacea purpurea and Allium sativum as immunostimulants in fish culture using Nile tilapia (Oreochromis niloticus)*J Anim Physiol Anim Nutr (Berl). Apr 23*

Aly, N. M. and El-Gendy, K.S. (2000): Effect of dimethoate on the immune system of female mice.*J. Environ.Sci. health B; 35(1):77-86.*

Ambwani,S.; Singhal,l. and Chauhan,R.(2006) Immunomodulatory effects of Cow urine on dimethoate induced immunotoxicity in avian Lymphocytes. *International Journal of Cow Science: 2, 1*

Banaee, M., Mirvagefei, A. R. Rafei, G. R. and Majazi Amiri, B.(2008) Effect of sub-lethal Diazinon Concentrations on Blood Plasma Biochemistry. *Int. J. Environ. Res., 2(2): 189-198.*

Banchroft, J.D.; Stevens, A. and Turner, D.R.

(1996): Theory and Practice of Hisological Techniques. Fourth Ed. Churchil Livingstone, New York, London, San Francisco, Tokyo.

Banerjee, B. D.; Pash, S. T., Hussain, Q. Z.; Koner, B. C. and Ray, A. (1998): A comparative evaluation of immunotoxicity of malathion after subchronic exposure in experimental animals. *Indian J Exp Biol;* 36: 273–282.

Barrett, B. (2003). Medicinal properties of Echinacea. *Phytomedicine; 10: 66-86.*

Bodinet, C., Lindequist U, Teuscher E, Freudenstein J. (2002): Effect of an orally applied herbal immunomodulator on cytokine induction and antibody response in normal and immunosuppressed mice. *Phytomedicine. Oct; 9 (7):606-13.*

Dongren, Y., Taol, L., Fengsheng, H., (1999). Electrophysiological studies in rats of acute dimethoate poisoning. *Toxicol. Lett. 107 (1–3), 249–254.*

Doumas B.T (1971): Colorimetric methods for determination of albumin.*Clin.Chim.Acta.31-87.*

Eteghada,S.S.; Nurij,h. Abadic,A. Ghavamia,S. Golabia , M. and Shanebandi,D.(2010): Synergetic effects of oral administration of levamisole and Echinacea purpurea on immune response in Wistar rat. *Research in Veterinary Science Article in Press, Corrected Proof - Note to users*

Fitzpatrick, L.C; Sassani, R.; Venables, B.J. and Goven AJ (1992): Comparative toxicity of polychlorinated biphenyls to earthworms Eisenia foetida and Lumbricusterrestris. *Environ Pollut 77: 65-69.*

Giang, T. T.; Suzanne, J.; Hodgkinson, N. C.; Murray ,K. S. ;Timothy, s. and Bruce ,M. Hall. (2002).Attenuation of Experimental Allergic Encephalomyelitis in Complement Component 6-Deficient Rats Is Associated with Reduced Complement C9 Deposition, P-Selectin Expression and Cellular Infiltrate in Spinal Cords. *J. Immunol. ;168;4293-4300.*

Gornal A.C; Baradawill C.J. and David M.M. (1949): Colorimetric methods for determination of total protein. *J. Biol. Chem. 177-751.*

Ghosh, K. and · Banerjee, V., (1993). Alteration in blood parameters in the fish Heteropneustes fossilis exposed to dimethoate., *Environ, Ecol., 11, 979-981.*

Gomes, J.; Dawodu, AH.; Lloyd, O.; Revitt, DM. and Anilal, SV. (1999): Hepatic injury and disturbed amino acid metabolism in mice following prolonged exposure to organophosphorus insecticides. *Hum Exp Toxicol.. 18(1):33-7.*

Hays, W.J. and Laws, E.R. .(1990). Handbook o pesticide toxicology, vol.3,classes o pesticides. Academic Press,Inc.,NY.

Holland, M. M., Steven, M., and Gallin, J. I., (1997). Disorders of Granulocytes and Monocytés. In Harrison's Principles of Internal Medicine, edited by Anthony S. Fauci, et al. New York, McGraw-Hill.

Institóris,L.: Olga Siroki, Ülkü Ündeger, Nursen Basaran and Illés Dési(2002) Immunotoxicological investigation in rats dosed repeatedly with combinations of cypermethrin, As(III), and Hg(II). *Toxicology,Volume 172, Issue 1(1), Pages 59-67*

Institoris, L., Siroki, O., Desi, I., Undeger, U., (1999). Immunotoxicological examination of repeated dose combined exposure by dimethoate and two heavy metals in rats.Hum. *Exp. Toxicol. 18 (2), 88–94.*

Jalees R. Jennifer M. D., Steve M. C., James C., Brian L. and Maise,A.(1999) Increased production of antigen-specific immunoglobulins G and M following in vivo treatment with the medicinal plants Echinacea angustifolia and Hydrastis Canadensis. *Immunology LettersVolume 68, Issues 2-3, 1 June, Pages 391-395.*

Jain, N.C. (1986): "Schalm's Veterinary Hematology". 4th Ed. Loe and Febiger, Philadelphia, USA.Johnson VJ, Rosenberg AM, Lee K, Blakley B.R. (2002) Increased T-lymphocyte dependent antibody production in female SJL/Jmice following exposure to commercial grade malathion. *Toxicology.;* 170:119-129.

Lee, T. L. Chen, Z. H. Shieh, J. C. Lin and B. Yu.(2009) Study on antioxidant activity of Echinacea purpurea L. extracts and its impact on cell viability. *Afr. J. Biotechnol.* 8: 5097-5105.

Lee,T., Chiang Huang,C. Shieh,X. Chen,C.; Chen,L.; and Yu,B.(2010) Flavonoid, Phenol and Polysaccharide Contents of Echinacea Purpurea L. and Its Immunostimulant Capacity In Vitro. *International Journal of Environmental Science and Development, Vol.* 1, No. 1, 5-9.

Luster, M. (1995) Enviromental assault on immunity. *Enviromental health perspectives, 103 (3).*

Majujab, K.A. and Hitchner, S.B. (1977): Antibody response to strain combination of Newcastle disease virus as measured by haemagglutination inhibition. *Avian Dis., 21: 576-584.*

Mantle, D. (1997): Effects of pirimiphos methyl on proteolytic enzyme activities in rat heart, kidney, brain and liver tissues in vivo. *Clin Chim Acta 262: 89 - 97.*

Matthias, L.; Banbury, K.; M. Bone, D.; Leach,N and Lehmann,R(2008) Echinacea alkylamides modulate induced immune responses in T-cells. *Fitoterapia., 79: 53-58.*

Mohamed A.M., Metwally N.S., Mohamed S. M. and Hassan E.M. (2010): Protective capacity of butanolic extract of myoporum laetum against oxidative stress and immune disorder induced tissue damage in profenofos intoxicated rats. *International Journal of Academic Research Vol. 2. No. 2.*

Nath, R. and Banerjee, V., (1996). Effect of pesticides methylparathion and cypermethrin on the air-breathing fish Heteropneustes fossilis, *Environ. Ecol. 14, 163-165.*

Paget, G.E. and Barnes, J.M. (1964): Evaluation of drug activitis and pharmacometric, p 135-165. Academic Press. London and New York." Edited Lowernce and Al- Bachrach Chapter 6, Toxicity test

Pesticides residues in foods. 1996 evaluations. Part II, Toxicological Geneva, Publication no., *WHO/PCS/97.1.1997*

Pramod K. Mediritta.A, Krishna T.;, Reeta, K.;RajimnaT., Basu, D. Beneerje, Surender, S. and Krishna,K.(2008). Attenuation of the effect of lindane on immune responses and oxidative stress by ocimum sanctum seed oil (OSSO) in rats.*Indian J Physiol Pharmacol* 52(2): 171–177.

Pruett SB (1992) Immunotoxicity of organophosphorous compounds. In Chambers JE, Levi PE (eds) Organophosphates: chemistry, fate and effects.*Academic Press, New York.*

Qing.L; and Tomoyuki,K. (2006):The mechanism of organophosphorus pesticide-induced inhibition of cytolytic activity of killer cells. *Cellular & molecularimmunology*, 3(3):171-8.

Rishi ,S.and Garg BD.(1997) Effect of malathion induced cholinergic stimulation on humoralimmune response in WLH chicken. *Indian J Toxicol;* 4:27-31.

Ritzmann, S.E. and Daniels, J.C. (1979): Diagnostic proteinlogy separation and characterization of proteins, "Qualitative Assays in Laboratory Medicine", Harper and Row Inc, Hagerstown, Maryland.

Sayim (2007): Dimethoate-induced biochemical and histopathological changes in the liver of rats. *Exp. & Toxicologic Pathol. (59) 3-4, 237-243.*

Sharma Y, Bashir,S.; Irshad, M. Gupta S. D. and Dogra T.D.(2005):Effects of acute dimethoate administration on antioxidant status of liver and brain of experimental rats. *Toxicology* (206) 1 :49-57.

Snedecor, G .W. and Cochran, W. C. (1982): Statistical methods. 7th ed., Ames.: Iowa State Univ. Press .

Sullivan AM, Laba JG, Moore JA, and Lee TD (2008). *Echinacea-induced macrophage activation. Immunopharmacol Immunotoxicol* 30: 553-57.

Temple, L., Butterworth, L., Kawabata, T.T., Munson, A.F.,White, K.L., Jr, 1995. ELISA to measure SRBC-specific IgM: method and data evaluation. In: Burleson, G.R.,Dean, J.R., Munson, A.E. (Eds.), *Methods in Immuntoxicology,vol. I. Wiley-Liss, New York*, pp. 137–157.

Tiefenbach, B., and Lange, P. (1980). Studies on the action of dimethoate on the immune system. *Arch. Toxicol.* 4, 167-170.

Thygesen, J.; Thulin, A.; Mortensen, L.; Skibsted,H and P. Molgaard(2007).Antioxidant activity of cichoric acid and alkamides from Echinacea purpurea, alone and in combination. *Food Chem. 101: 74-81.*

Tsatsakis, A.M., Manousakis, A., Anastasaki, M.,Tzatzarakis, M., Katsanoulas, K., Delaki, C., Agouridakis,P., 1998. Clinical and toxicological data in fenthion and dimethoate acute poisoning. *J. Environ. Sci. Health* 33 (6), 657–670.

Uzunhisarciki M (2008). Methyl parathion'un ratlarda hepatotoksik etkisi ve vitamin C ve vitamin E'nin koruyucu rolü. *Doktora Tezi, Gazi Üniversitesi*, 1-111, *Ankara.*

Westcott, B., Lee, N.D., McKinlay, Y.W., 1987. Persistent and toxicity of dimethoate on wheat herbage and sweet clover herbage. *J. Environ. Sci. Health B* 22 (4), 379–390.

Woelkart K, and Bauer R.(2007) The role of alkamides as an active principle of Echinacea. *Planta Med* 73: 615-623.

Zhai, Y. Liu, L. Wu, D. S. Senchina, E. S. Wurtele, P. A. Murphy, M. L. Kohut and J. E. Cunnick (2007). Enhancement of innate and adaptive immune .functions by multiple Echinacea species. *J. Med. Food,10 (3): 423-434.*

دور مستخلص نبان الانشيسا بروورا (الاميولانت) ضد الأثر المثبط للمناعة لمركب الدايمثويت فى ذكور الفئران البيضاء

منال شعراوى حسين*[a] عزة حسن عبد الرحمن**[a] ولاء محمد سيد**

*قسم الطب الشرعي والسموم كلية الطب البيطرى - جامعة بنى سويف [a] جامعة الطائف كلية الطب و العلوم الطبية

**قسم الباثولوجيا الاكلينيكية كلية الطب البيطرى – جامعة نى سويف

يعد مبيد الدايميثويت من المبيدات الحشرية الفسفورية التى تستخدم للتخلص من الحشرات والافات و خاصة افات المحاصيل كما انتشر أستخدام النبات الطبى حديثا لاحتوائه على عناصر علاجية كثيرة تهدف هذه الدراسة الى تقييم الاثر الوقائى لنبات الانشيسيا بربورا ضد التأثير السام للدايمثويت على مناعة ذكور الفئران البيضاء. و قد استخدم فى هذا البحث أربعون من ذكور الفئران البيضاء و قسمت الفئران فى هذه التجربة الى اربع مجاميع متساوية كل مجموعة تحتوى على عشرة فئران. ظلت المجموعة الاولى كمجموعة ضابطة بينما تم تجريع الفئران فى المجموعة الثانية بمركب الدايمثويت بجرعة ٣مجم / كجم مساوية لعشر الجرعة نصف المميتة و فى المجموعة الثالثة تم تجريع الفئران مستخلص نبات الانسيشيا بربورا فى صورة اميولانت بجرعة ٢.٥ مجم لكل كجم من وزن الجسم بينما تم تجريع المجموعة الرابعة بمركب الدايمثويت و الاميولانت معا و نفس الجرعات السابقة . تم تجريع الفئران الجرعات السابقة لمدة سبعة ايام متتالية تم بعدها حقن الفئران فى المجاميع المختلفة بخلايا الدم الحمراء للاغنام تم ذبح خمس فئران من كل مجموعة بعد ٧ و ١٤ يوم من الحقن. و قد أسفرت النتائج عن وجود نقص معنوى فى كل خلايا الدم البيضاء و الخلايا الليمفاوية مع زيادة فى خلايا النتروفيل . أوضحت النتائج ان مركب الدايمثويت له اثر مثبط للاستجاة المناعية للفئران ضد خلايا الدم الجمراء للاغنام متمثلة فى الحصول على اجسام مناعية أقل و أقل معدل صد بأختبار التحدى و وجود نقص معنوى فى الامينوجلوبيولن ام و كذلك البروتين و الزلال و جاما جلوبين . و قد لوحظ وجود تغيرات باثولوجية واضحة فى كل من الكبد و الطحال و غدة الثايمس ومن ناحية اخرى اسفرت النتائج ان مستخلص نبات الانسيشيا بربورا له دور فعال للوقاية من اثر مركب الدايميثويت المثبط للمناعة حيث احدث تحسن ملحوظ فى القياسات السابقة عند مقارنتها بالمجموعة الضابطة.