Dept. of Pathology,
Fac. Vet. Med., Assiut University,
Head of Dept. Prof. Dr. M.I. El-Sherry.

THE EFFECT OF AD_3 E VITAMIN MIXTURE ON EXPERIMENTALLY INDUCED MEPHOSFOLAN SHORT TERM TOXICITY IN MIC

(With 2 Tables and 13 Figures)

By

H.A. SALEM; S.S. EL-BALLAL and A.Sh.SEDDEK*

(Received at 28/9/1992)

تأثير خليط فيتامينات أ د هـ على السمية قصيرة الأجل المحدثة تجريبياً بمركب الميفسفولان في الجرذان البيضاء ٣

حمدي سالم ، صلاح البلال ، عبد اللطيف شاكر

نظراً للآثار المدمرة للمبيدات على الأجهزة المختلفة في جسم الانسان والحيوان والناتجة من التعرض لكميات قليلة منها على فترات زمنية مختلفة سواء عن طريق التلوث البيئي أو التلوث الغذائي اتجهنا الى القاء الضوء على سمية مركب الميفسفولان في الجرذان البيضاء كمثال لدراسة سمية المركبات الفسفورية العضوية ومدى استجابة هذه الجرذان للعلاج بخليط فيتامينات أ د٣هـ وتم اجراء الدراسة على ستون جرذ حيث قسمت الجرذان الى ثلاث مجموعات تجريبية ومجموعة ضابطة. تعرضت المجموعات التجريبية لعشر الجرعة نصف المميته من مركب الميفسفولان لمدة شهرين. ذُبحت المجموعة التجريبية الأولى لدراسة سمية مركب الميفسفولان بينما عولجت المجموعة الثانية بخليط الفيتامينات أ د٣هـ لمدة شهر وتركت المجموعة الثالثة بدون علاج. دلت نتائج المجموعة الأولى على أن مركب الميفسفولان قد أحدث تغيرات باثولوجية في كل من الكبد والكلية والخصيتين والرئتين والقلب والأثنى عشر ومكونات الدم وكانت هذه التغيرات في صورة تنكرز في خلايا هذه الأعضاء مع احتقان ونزيف في أوعيتها الدموية. كما أحدث هذا المركب انخفاض شديد في نشاط خميرة الكولين استريز. كما أدى العلاج بخليط فيتامينات أ د٣هـ الى استجابة الخصيتين فقط بصورة واضحة للعلاج، بينما لم تستجب بقية الأعضاء الأخرى ومكونات الدم. فيما يتعلق بنشاط خميرة الكولين استريز فقد حدثت أستعادة ذاتية في نشاط هذه الخميرة سواء في المجموعات المعالجة بالفيتامينات أو الغير معالجة. ومن نتائج هذه يمكن أن تنصح باستخدام مجموعة الفيتامينات سالفة الذكر لاستعادة الخصوبة في حالات التعرض للمركبات الفسفورية العضوية.

*: Dept. Forensic Medicine & Toxicology, Fac.Vet.Med.,Assiut Univ.

SUMMARY

In this study, sixty mice were used for induction of short term toxicity with organophosphorus compound "mephosfolan". They received tenth of the LD_{50} twice weekly for two months. Twenty mice were then treated with AD_3E vitamin mixture for further one month. Samples for histopathological and hematological investigations were taken. Histopathological changes after two months toxicity with mephosfolan were mainly degenerative and vascular. They were prominent in the liver, kidney, testis, lung heart, and intestine. Hematological examination revealed a significant decrease in total erythrocytic count, total leukocytic count, hemoglobin concentration, and packed cell volume. A highly significant decrease in true choline estrase activity was observed. Treatmemt with AD_3E vitamin mixture for one month resulted only in regeneration of the testis. The other organs as well as the hematological parameters did not respond. Spontaneous recpvery was observed in the true chroline estrase activiity without significant differences between vitamin mixture treated and non-treated groups. The significance of these results was discussed.

INTRODUCTION

Organophosphorus insecticides are of general importance because of their extensive use in combating the disease vectors and agricultural pests. Their serious effects on the animal health and reproduction were reported (DONALD and SHARMAN, 1971; LEIN et al., 1981). Mephosfolan is a member of this group, was used in this study as an example for organophosphorus compounds. Its toxicity to albino rat was documented (ABD EL-GHAFFAR, 1989). Previous studies revealed that simultaneous adminitration of organophosphorus compounds "phosfolan and mephosfolan" AD_3E vitamin mixture resulted in inhibition of their testicular toxicity (ABD EL-GHAFFAR, 1989). In this study, we reporded the therapeutic use of this vitamin mixture in the treatment of organophosphorus compound "mephosfolan" -induced toxicity in mouse.

MATERIAL and METHODS

Experiment:

Group 1: In this group sixty mice were used for the induction of chronic toxicity with organophosphorus compound "mephosfolan". Mephosfolan (2-diethoxyphosphiny-limino-4 methyl-1,3 dithiolanel) was commercially named as cytrolan and was supploeded by the Amerocam Cyanpoid Company. This group received tenth of the LD_{50} of mephosfolan twice weekly by stomach tube for two months. By the end of this period, mephospholan was removed and ten mice were sacrified for collection of their blood and tissue specimens. The remaining animals were equally divided to represent group two and three.

Group II: This group was treated with AD_3E vitamin mixture (Produced by Pfizer Egypt) in adose of 1 ml/liter of dirnking water. They were sacrified two and four weeks post treatment for collection of their blood and tissue amples.

Group III: Members of this group remained without vitamin mixture treatment. They used as a control group for experimental group II. They were sacrified parallel to group II.

Group IV: This group included ten mice and represented the control group. They were sacrified at different intervals throughout the experiment.

Methods:

Histopathology: Specimens from the liver, kidney, lung, intestine, heart, and testis were fixed in 10% netural in 10% neutral buffered formalin. They were processed routinely, embedded in parablast, sectioned at 7 um and stained with hematoxylin and eosin.

Hematology: Blood samples were taken after sharp cutting of the third end of the tail. Total erythrocytic count, total leukocytic count, and hemoglobin concentration were determined by using electronic cell counter (Cell dyne 300, Sequoi-turner) according to COLES (1980). Packed cell volume was determined by means of microhematocrit method (SCHALM, 1979). Other blood parameters including mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were estimated after the method described by COLES (1980).

Choline estrase activity: Choline estrase activity was evaluated by VOSS and SACHSSE (1970). Statistical analysis was calculated according to KALTON (1967).

RESULTS

Histopathology:

Group I: Two months exposure to mephosfolan resulted in degenerative and vascular changes in the parenchymatous organs. In the liver, the hepatocytes suffered various degenerative changes in the form of cloudy swelling and hydropic degeneration. Focal areas of coagulative necrosis with lymphocytic infiltration were frequently observed (Fig. 1). Congestion and perivascular hemorrhage were evident in the central vein and the vessels of the portal area. Additionally, the portal area was heavily infiltrated with lymphocytes (Fig. 1). The kidney showed congestion of the glomeruli and interstitial blood vessels. Hemorrhage was prominent in the renal medulla. Degenerative changes were seen in the tubular epithelium (Fig. 2). There was focal interstitial and periglomerular lymphocytic infiltration.

The histopathological findings in the lungs were mainly vascular. These include congestion of the interstitial blood vessels and the alveoli, perivascular edema and hemorrhage, and alveolar hemorrhage (Fig. 3). There was perivascular and peribronchial lymphoid cellular infiltration. In the intestine necrosis and desquamation of the enterocytes on the upper third of the villi together with necrosis of its underlying lamina propria were the only findings. The heart showed capillary congestion, hemorrhage, and degenerative changes in the myocardium.

In the testis, testicular degeneration was observed where most of the seminiferous tubules appeared free from the spermatozoa and spermatides. The remaining layers suffered degenerative changes (Fig. 4). In some tubules there was loss of most of the seminiferous epithelium up to the germinal epithelium (Fig. 5). The interstitium showed congestion of its blood vessels, edema, Lydig cell proliferation (Fig. 5).

Group II: The vitamin mixture successeded only in regeneration of the testicular tissue. Two weeks post treatment, the seminiferous tubules were histologicallly normal, where most of their layers were regenerated (Fig. 6). Restablishment of spermatogenic activity with appearance of spermatozoa in their lumena was observed one month post treatment (Fig. 7).

Group III: In this group the histopathological changes were similar to those described in group I Further more, the kidney and testis showed progressive changes. There were atrophy of the renal tubules, some of them were dilated and contained hyaline casts. Interstitial mononuclear cellular

infiltration and atrophy of the glomerular tuft was also observed (Fig. 8). In the testis, the degenerative changes became more sever where the seminferous tubules contained acidophilic debris which represented the reminants of the degenerated seminiferous epithelium. In other tubules, the seminiferous epithelium had a foamy vacuolated appearance (Fig. 9). By the end of the experiment (one month post-exposure), the seminferous tubules were severely involved. The seminiferous epithelium had a foamy vacuolated appearance, other tubules were completely empty (Fig. 10, 11). Spermatid giant cells appeared in most tubules (Fig. 12). Focal interstitial lymphocytic infiltration was observed (Fig. 11).

Hematology: The hematological picture (table 1) showed a highly significant decrease in total erythrocytic count, packed cell volume, and hemoglobin concentration after two months exposure to mephospholan. These values raised two and four weeks post exposure in both vitamin mixture treated and non-treated groups. The total leukocytic count appeared to be significantly increased depending upon the lymphocytic and monocytic count inspite of a significanly decreased neutrophils.

Choline estrase activity: Administration of mephosfolan for two months resulted in a highly significant decrease in true choline estrase activity (table 2,Fig. 13). Two and four weeks after vitamin mixture treatment, choline estrase activity was increase without significant differences between treated and non-treated groups (table 2, Fig.13).Pseudocholine estrase showed mild changes.

DISCUSSION:

In this investigation, short term toxicity with organophosphorus compound "mephosfolan" was induced in mice. There after the animals were treated with AD_3E vitamin mixture for one month. Tissue and blood samples were taken throughout the experiment for histopathological and hematological studies. Hitopathological examination revealed that mephosfolan-induced toxicity resulted in vascular and degerative changes in the testis, liver, kidney, lung, heart, and intestine. The vascular changes were in the form of congestion, hemorrhage, and edema. These changes were attributed to the direct toxic effect of the insecticides on the vessel endothelium (KLAASSEN et al., 1986). The degenerative changes in the liver and other parenchymatous organs were attributed to the disturbances in lipid metabolism which accompained the toxicity with organophosphorus

compounds. These disturbances included the increase in the level of cholesterol which predisposed the cell membrane to degeneration (COPRA et al., 1979). The detgenerative changes in the liver observed in this study were similar to those described with various members of organophosphorus insecticides in guinea pigs, chickens, bulls, sheep, and rats (COPRA et al., 1979; MICHAIL et al., 1979; SAXINA & SARIN 1980 and ABD EL-GHAFFAR, 1989).

The nephrotoxic effect of mephosfolan was in the form of early degenerative and vascular changes and late chronic proliferative reaction. Similar conclusions were reported in rats following mephosfolan administration (ABD EL-GHAFFAR, 1989). Single oral dose of (C^{14}). phospholan resulted in its distribution throughout the body with the highest concentration in the liver and kidney (KAPOOR and BLINN, 1977). The renal toxicity might be caused by primary glomerular damage with subsequent involvement of the renal tubules (KLAASSEN et al., 1986). The primary toxic effect on the tubular epithelium was reported to induce toxic tubular nephrosis (JONES and HUNT, 1983).

Necrosis and desquamation of the enterocytes at the tips of the duodenal villi was the result of the direct toxic effect of mephosfolan. The enterocytes at this location was especially susceptible because of their age and their maximum contact with the toxin in comparison to those located at the base of the villi.

In the testis, the toxicity was manifested by testicular degeneration with the appearance of spermatid giant cells. Such cells were frequently observed in most cases of testicular degeneration (EL-SHERRY et al., 1980). Various members of organophosphorus compounds were reported to induce testicular degeneration (SMOKUTI et al., 1987; ABD EL-GHAFFAR, 1989). The testicular toxicity was attributed to the direct cytotoxic effect of organophosphorus compounds and/or indirect effect through their estrogenic action resulted from their primary hepatic damage (WAYLAND, 1975 and LOFTS & MURTAN, 1973).

Treatment with AD_3E vitamin mixture was successful on the testes while the other organs did not respond. Moreover, in the non-treated group, the testicular changes appeared progressed inspite of withdrawal of the toxin. This may reflect the influence of vitamin mixture in testicular regeneration. On the contrary, such vitamin mixture had no obvious role for the regeneration of other organs. This contraversy may neeed further investigation.

True choline estrase activity was markedly inhibited during the exposure period and this inhibition was an index for the toxicity with organophosphorus compounds (ABOU-DONIA et al., 1986). Reversible recovery took place in choline estrse activity two and four weeks post exposure in both vitamin mixture treated and nontreated groups. This recovery of choline estrase activity following exposure to different members of organophosphorus compounds was reported in rodents (WALLACE and HERZBERG, 1988), pigs (VOJCIK et al., 1979), and steers (KHAN et al., 1990). These trends in acetyl choline and choline estrase activities indicated that initial recovery was principally attributable to spontaneous reactivation of both enzymes, however, a portion of both enzymes did not react by this process and was gradually replenished by de novo synthetic and replacment processes. In most instances, the enzyme inhibitor complex is unstable and the enzyme is reactivated by way of either spontaneous or chemicalty induced hydrolytic dephosphorylation (ALDRIDGE, 1953 and DAVISON, 1955). Alternatively, depending on the electronegativty of the alkoxy substituents of the phosphate ester, the complex may be dealkylated rendering the enzyme irriversibly inactivated or aged (HOBBIGER, 1955 and BERENDS et al., 1959).

ACKNOWLEDGEMENT

We would like to express our thanks to Mr. ABD-IL GHAFFAR, Ass. Lecturer department of Pathology, Assiut University, for his effort in this study.

REFERENCES

Abe El-Ghaffar, S. K. (1989): Pathological studies on the effect of some organophosphorus compounds "phosfolan and mephosfolan on Albino rats". M.V.Sc. Thesis, Assiut University.

Abou-Donia, M.R.; Abdo, K.M.; Timmons, P.R. and Proctor, J.E. (1986): Brain acetylcholinesterase, acid phosphatase, and 2,3 cyclic nucleotide-3-phosphohydrolase and plasma butrylcholinesterase activities in hens treated with a single dermal neurotoxic dose of S,S,S-tri-n-butyl phosphorotrithioate. Toxicol. Appl. Pharmacol. 82: 461-473.

Aldridge, W.N. (1953): The differentiation of true and pseudo cholinestrase by organophosphorus compounds. Biochem. J. 53: 62-67.

Berends, F.; Posthumus, C.H.; Sluys, I.V.D. and Deierkduf, F.A. (1959): The chemical of basis the aging process of DFP- inhibited pseudocholinestrase. Biochim. Biophys. Acta. 34: 576-578.

Coles, E.H. (1980): Veterinary clinical pathology, 3rd Ed. W.B> Saunder Company, Philadelphia, London, Toronto.

Copra, A.; Ahuja, S.D.; Gupta, P.P. and Nagpal, S.K. (1979): Histopathological and lipid changes in various organs of chicks (Gallus domesticus) caused by acute and chronic toxicity of metasytor-R and Labycid. Zbl. Vet. Med. (A) 26: 822-834.

Davison, A.N. (1955): Return of cholinestrase activity in the rat after inhibition by organophosphorus compounds. Biochem. J. 60: 339-346.

Donald, K.S. and Sharman, G.A.M. (1971): Ilness and death in calves induced by treatment with systemic insecticides for the control of cattle crubs. Ann. Vet. Med. Ass. 141.

El-Sherry, M.I.; El-Naggar, M.A. and Nassar, S.M. (1980): Experimental study of summer stress in rabbit. II- The quantitative and qualitative pathogenesis of spermatogenic cell cycle in rabbit. Assiut Vet. Med. J. 7(13,14): 17-26.

Hobbiger, F. (1955): Effect of nicotinhydroxamic acid methiodide on human plasma cholinestrase inhibited by organo phosphates. Brit. J. Pharmacol. 10: 356-362.

Hobbiger, F. (1957): Protection against the lethal effects of organophosphates by pyridine-2-aldoxime methiodide. Brit. J. Pharmacol. 12: 438-446.

Jones, T.C. and Hunt, R.D. (1983): Veterinary Pathology, 5th ed. Lea & Febiger Philadelphia.

Kalton, G. (1967): Introduction to statistical ideas from social scientist. 2nd Ed. Acad. Press. London.

Kapoor, P.I. and Blinn, C.R. (1977): Absorption, excreation and metabolism of cyolane (Phospholan) systemic insecticides in rats. J. Agric. food chem. 25: 314-418.

Khan, A.A.; Coppock, R.W.; Schuler, M.M. and Lillie, L.E. (1990): Effects of dichlorvos on blood cholinestrase activities of cattle. Am. J. Vet. Res. 51: 79-82.

Klaassen, C.D.; Amdur, M.O. and John Doull, M.D. (1986): Toxicology, the basic science of poisons. 3rd ed., Macmillan Publishing Company.

Lein, D.H.; Mglin, G.A.; Hillman, R.B. and RebHun, W.C. (1982): Chloropyrifos (Durspan 44r). Toxicity in Dairy bulls. Cornel Vet. 72 (supple 3): 58.

Lofts, B. and Murtan, R.K. (1973): Reproduction in birds in Avian Biology, Academic Press. New York.

Michail, T.H.: Aggour, N.; Awadallah, R.; Boulos, M.N.; El Dessouky, E.A. and Karima, A.I. (1979): Acute toxicity of organophosphorus and organochlorin insecticides in laboratory animals. Ernahrungiwiss, 18: 258-268.

Saxina, A.K. and Sarin, K. (1980): Histopathological and biochemical changes in the liver and testes of desert gerbil, after treated exposure of thiamet (phorate). Toxicol., 18: 133-144.

Schalm, O.W. (1979): Veterinary haematology. 4th ed. Lee & Feibeger, philadelphia, USA.

Somkuti, S.G.; Lapadula, D.M.; Chapin, R.E.; Lamb, J.C. and Abou-Donia, M.B. (1987): Testicular toxicity following oral adminstration of tri-o-cresyl phosphate (TOCP) in roosters. Toxicol. Lett. 37: 279-290.

Vojcik, S.; Majewski, T.; Podgarski, W.; Racskiewiez, J.; Saba, L.; Bialkowski, Z.; Polonis, A.; Rzaczynski, B. and Krupinski, A. (1979): Long-term action of DDVP (dichlorvos) on some physiological values in growing pigs. Polskie Archiwun Veterynaryjne 21; 343.

Voss, G. and Sachsse, K. (1970): Red cell and plasma choline estrase activities in micro samples of human and animal blood determined simultaneously by modified acetylcholine-DTNB procedure. Toxicol and applied pharmacol. 169: 764-770.

Wallace, K.B. and Herzberg, U. (1988): Reactivation and aging of phosphorylated brain acetylcholinestrase from Fish and Rodents. Toxicol. and Applied pharmacol. 92: 307-314.

Wayland, J. Hoys (1975): Toxicology of Pesticides. The Williams and Wilkans Company.

LEGENDS OF FIGURES

Fig. 1: Liver showing degenerative changes in the hepatocytes, focal necrosis congestion of the portal blood vessels, infiltration of the portal area with lymphocytes (Hx. & E, X 16).

Fig. 2: Kidney showing degenerative changes in the proximal convoluted tubules, congestion of the interstitial blood capillaries (Hx. & E., X 40).

Fig. 3: Lung showing alveolar and perivascular hemorrhage (Hx. & E, X 10).

Fig. 4: Testis showing testicular degeneration (Hx. & E., X 10).

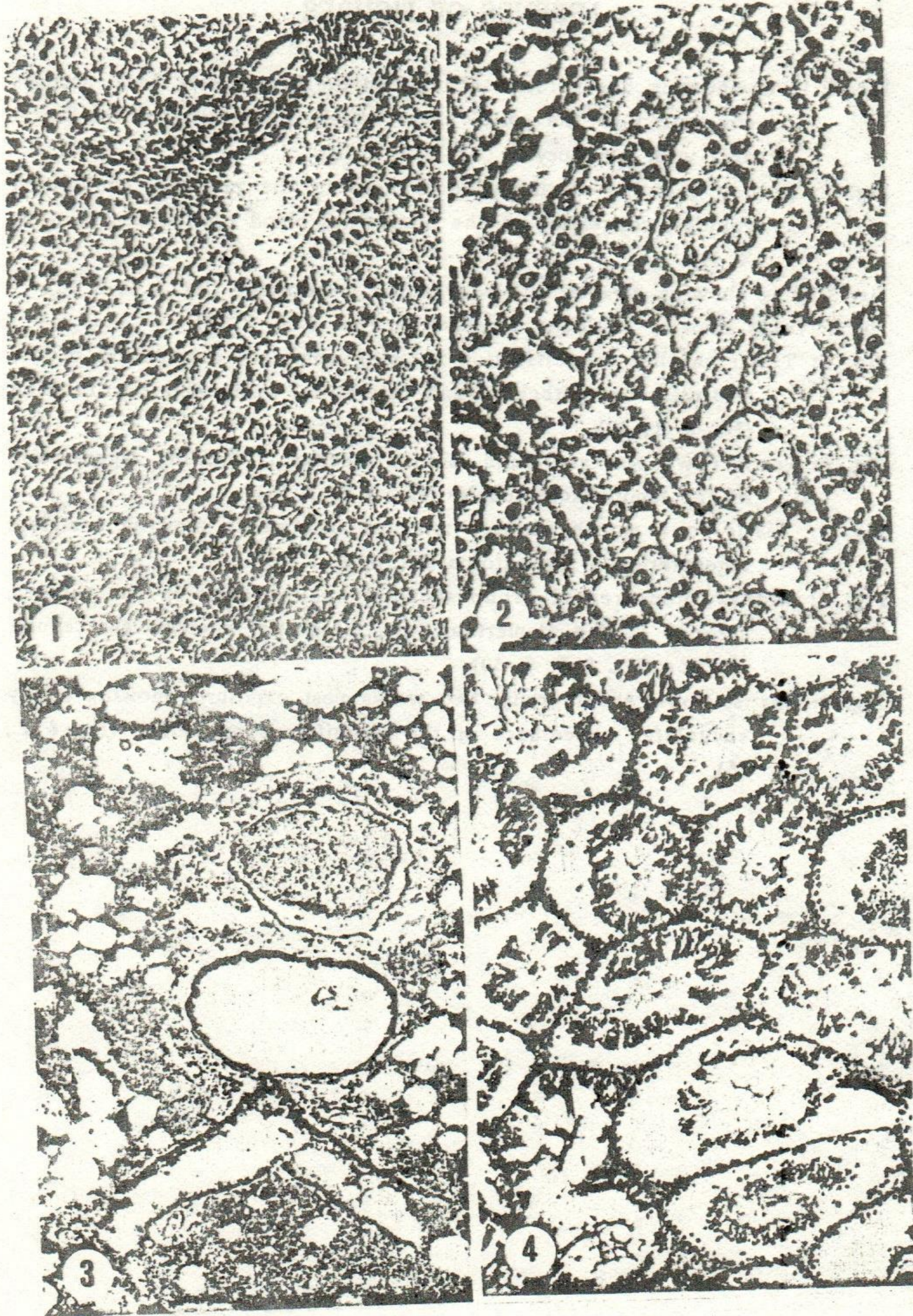
Fig. 5: Testis, higher magnification showing complete destruction of the seminiferous epithelium and interstitial lydig cell proliferation (Hx. & E., 40).

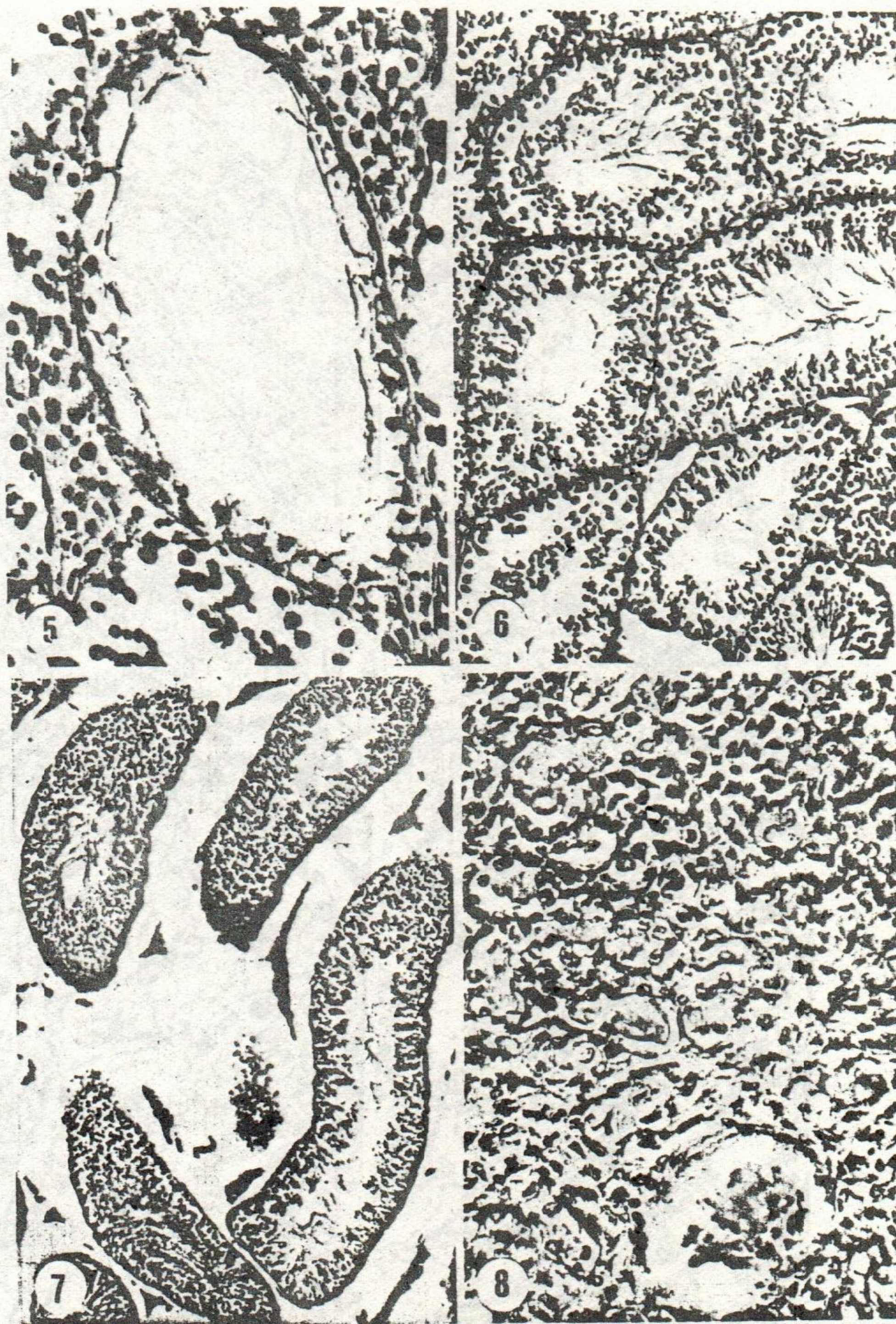
Fig. 6: Testis (Two weeks vitamin mixture treatment) showing regeneration of the seminiferous tubules (Hx. & E., X 16).

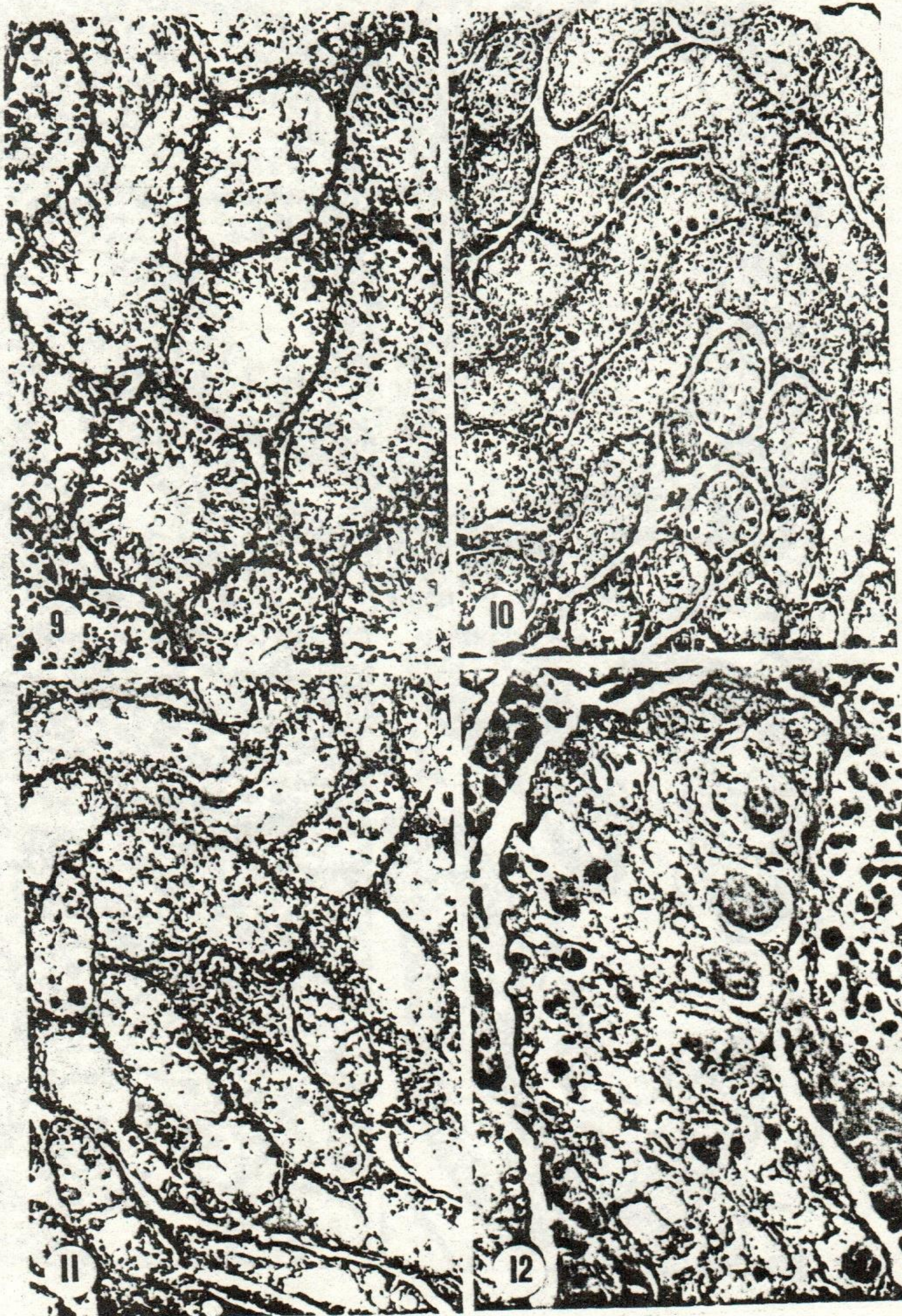
Fig. 7: Testis (one month vitamin mixture treatment) showing complete regeneration & spermatozoa (Hx. & E., X 10).

Fig. 8: Kidney, non-treated group showing hyaline casts in the tubules, atrophy of the glomerular tuft, interstitial mononuclear cellular reaction (Hx. & E., X 40).

Fig. 9: Testis, non-treated group, two weeks post exposure, showing foamy vacuolated appearance of the seminiferous epithelium (Hx. & E., 16)


1
2
3
4


5
6
7
8


9
10
11
12

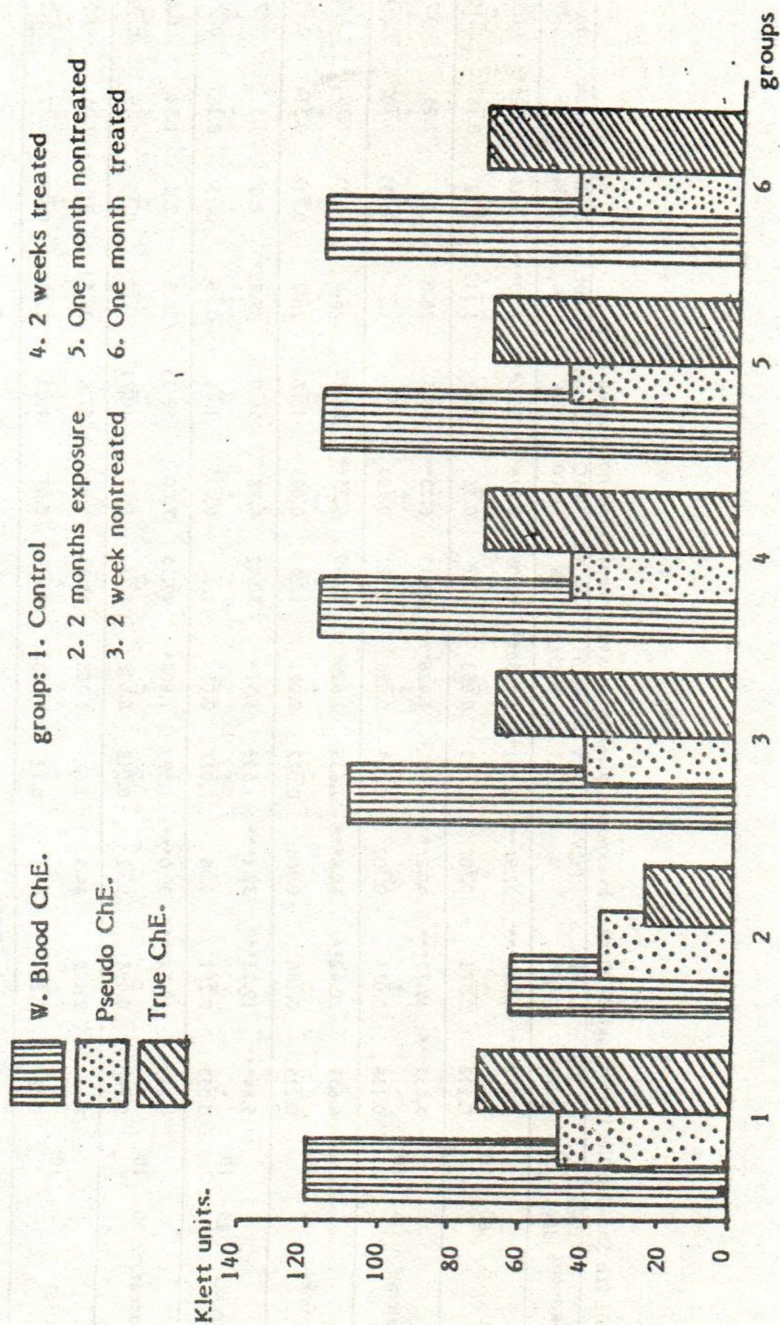


Fig. (13): ChE. activity (Klett units) in different groups of exposed mice.

Table (I): Showing the hematological changes in exposed, vitamine mixture treated and nontreated mice.

Treatment	Time days	No.	RBC Million	Hb g %	PCV %	MCV C/U	MCH N/Ug	MCHC %	WBC 1000	Lymph. %	Neut. %	Eosino %	Mono %	Baso %
O.P.	60	10	5.272*** ± 0.112	17.47*** ± 0.571	37.9*** ± 0.707	1.391*** ± 0.013	1.508*** ± 0.033	46.09* ± 1.02	8.216*** ± 0.38	66.6*** ± 0.83	25.5*** ± 1.113	3.16 ± 0.32	3.16*** ± 0.16	1.5 ± 0.109
Vitamine	15	10	5.635*** ± 0.136	19.75*** ± 0.071	40.2*** ± 0.702	1.401* ± 0.015	1.426*** ± 0.026	49.13 ± 1.12	6.13*** ± 0.101	66.5 ± 1.5	28.0 ± 1.2	1.5 ± 0.035	2.25 ± 0.707	0.75 ± 0.031
Vitamines	30	10	6.655 ± 0.215	20.42*** ± 0.186	40.6*** ± 0.301	1.639 ± 0.022	1.629*** ± 0.041	50.29 ± 1.24	6.25** ± 0.095	56.25 ± 1.47	38.0 ± 1.02	3.25 ± 0.718	1.50 ± 0.314	1.00 ± 0.239
No treat.	15	10	5.84*** ± 0.033	19.21*** ± 0.201	38.1*** ± 0.36	1.533 ± 0.017	1.519 ± 0.05	50.42 ± 1.12	6.88 ± 0.035	56.0 ± 1.41	39.75* ± 0.70	2.0 ± 0.35	1.5 ± 0.36	0.75 ± 0.35
No treat.	30	10	6.213* ± 0.290	19.325*** ± 0.098	39.0*** ± 0.41	1.593 ± 0.018	1.607* ± 0.038	49.55 ± 0.98	7.00 ± 0.23	59.33 ± 1.071	36.0 ± 1.22	2.0 ± 0.33	1.33 ± 0.19	1.33 ± 0.19
Control	-	10	7.43 ± 0.38	21.75 ± 0.27	44.5 ± 0.41	1.67 ± 0.12	1.708 ± 0.028	48.87 ± 0.78	6.95 ± 0.07	57.6 ± 1.03	36.5 ± 1.08	2.5 ± 0.40	1.75 ± 0.12	1.50 ± 1.2

*** Highly significant at / 0.001
** Highly significant at / 0.01
* Low significant at / 0.05

Table (2): ChE. activity in exposed, vitamine treated and nontreated mice.

Treatment	Time days	No.	Whole Blood CHE	Pseudo CHE	True CHE
O.P	60	10	63.66±0.971***	38.30±0.841***	25.33±1.311***
Vitamines	15	10	118.55±1.62	47.00±1.06	71.55±1.84
Vitamines	30	10	118.30±1.02	45.90±1.67	72.40±1.26
No treat.	15	10	110.21±2.159	42.66±1.50	67.55±1.62
No treat.	30	10	118.68±2.13	48.46±0.91	70.22±1.34
Control	0	10	120.80±2.36	48.50±0.907	72.30±0.813

*** Highly significant of ∠ 0.01