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Influence of organophosphorus insecticide, Phoxim, on hematological, biochemical, histopathological, genotoxicity and fertility in male rats

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Abstract

The aim of the present study was to investigate the toxic effects of organophosphorus insecticide, Phoxim, on hematological, biochemical, histopathological, genotoxicity and fertility in male rats. 35 rats were used for determination of the acute oral LD_{50} of Phoxim. Twenty male rats were divided into two equal groups (10 rats each). Control group was kept without treatment. The treated group was administered Phoxim orally at a dose of 50mg/kg bw (equivalent to 1/40 LD_{50}) three times/ week for 65 days. Blood samples were collected at the end of the first and second month of Phoxim exposure for hematological and biochemical examination. At the end of the experiment, rats of each group were sacrificed and subjected to spermatozoal examination, cytogenetic analysis and pathological examination. The results of the present study revealed that hemogram showed significant decrease in RBCs count and PCV% in Phoxim treated group. Leukogram showed significant increase in WBCs count and neutrophil percent. Lymphocyte percent was significantly decreased in Phoxim exposed group after the two intervals of examination. The biochemical results indicated significant increase in ALT and AST activities and creatinine level in Phoxim group compared to control group. Serum total proteins, albumin and globulin revealed non-significant changes. Spermatozoa examination showed significant decrease in sperm count and life percent. Increase in abnormal sperm percent was also detected in Phoxim administered rats. Histopathological examination revealed pathological alterations in the liver, kidneys and testes of treated rats. Cytogenetic analysis showed significant increase of chromosomal aberration in male spermatocyte cells in Phoxim treated rats in comparison to control specially for autosomal univalents, breaks and hypo-ploidy. Micronucleus assay (MN) revealed clastogenic effect of Phoxim group compared to control. In conclusion, this study revealed that treatment with the organophosphorus insecticide Phoxim induced hematological, biochemical, histopathological changes as well as clastogenicity and infertility in male rats.

Keywords: *Phoxim, LD_{50}, biochemical, hematology, spermatozoal examination, clastogenicity, rats.*

Introduction

Pesticides are being widely used to control agricultural pests and insects causing public health hazards. Problems and/or outbreaks are reported to occur among animals and human from insecticides' toxicity. Toxicity usually occurs either from direct exposure to insecticides or indirectly from contaminated feeds or water by such chemicals (**Hussein *et al.,* 2012).** Organophosphorus (OP) compounds are widely used in agriculture as insecticides and acaricides. They are also frequently employed in medicine and industry **(Uzuna *et al.,* 2009).** Moreover, due to the wide availability of or-

ganophosphorus compounds, poisonings are common **(Garcia *et al.,* 2003).** Organophosphorus pesticides are known to inhibit acetylcholinesterase and pseudocholinesterase activity in target tissues **(John *et al.,* 2001; Kalender *et al.,* 2006).** Other systems that may be affected by OP exposure are the immune system **(Handy *et al.,* 2002),** pancreas **(Gokalp *et al.,* 2005)**, liver **(Kalender *et al.,* 2005),** and hematological indices **(Kalender *et al.,* 2006).** Some OP pesticides have also been reported to affect the reproductive system **(Farag *et al.,* 2000; Uzuna *et al.,* 2009).**

Phoxim (O,O-diethyl phosphorothioate) is an effective organo-phosphate (OP) pesticide used widely throughout the world for agricultural and domestic purposes. In veterinary field, it is used to control external parasites in cattle, sheep, goats and pigs **(WHO, 2000)**. Moreover, Phoxim is also, used to control mite population in chickens (**Meyer-Kühling *et al.,* 2007**).

Therefore, this study aimed to evaluating the effects of Phoxim exposure on hemogram, some serum biochemical parameters, male fertility, germ and somatic cells and histopathological changes of male rats.

Materials and Methods

Materials:

1-Chemicals:

Sebacil® EC 50% (Phoxim) was obtained from Bayer Animal Health GmbH, D-51373 Leverkusen, Germany.

2-Experimental animals:

A total of 55 adult male rats (120-130gm) used in this study were obtained from the animal house of the Research Institute of Ophthalmology, Giza, Egypt. Food and water were provided *ad libitum*.

3-Experimental design:

Thirty five rats were used for determination of the acute oral LD_{50} of Phoxim. Fifteen rats of them were used in the preliminary pilot experiment to determine the range in which the LD_{50} of the Phoxim insecticide located. Twenty rats were used for the actual estimation of the LD_{50}. The other twenty rats were used for determination of the effect of Phoxim on hematological, biochemical, male fertility and germ cells. They were divided into two equal groups, each of 10 rats. Group (1) control group was kept without treatment. Group (2) OP exposed group was orally administered Phoxim at a dose of 50mg/kg bw (equivalent to 1/40 LD_{50}) three times/week for 65 days to cover all the spermatogenic cycle. At the end of the experiment rats of each group were sacrificed for revealing the reproductive toxicity studies of male (5 rats from each group) as well as the cytogenetic analysis of germ cells (spermatocytes) and somatic cells for micronucleus assay (5 rats from each group).

4-Methods:

Determination of acute LD_{50}: Determination of acute oral LD_{50} of Phoxim was performed according to the method described by **Finney (1964).**

Hematological examination: Blood samples were collected at the end of the first and second month from retro-orbital venous plexus under light ether anesthesia from the control and Phoxim exposed rats. Each blood sample was divided into two portions. The first was collected into EDTA containing tube for the hemogram adopting the techniques described by **Coles (1986).**The second portion was collected into plain centrifuge tube for serum separation for biochemical assays.

Serum biochemical examinations: Determination of serum total protein and albumin were carried out using the methods described by **Henry (1968)** and **Doumas** and **Biggs (1972)**, respectively. Serum globulin was calculated by subtracting the estimated serum albumin value from the determined serum protein of the same serum sample **Coles (1986)**. Serum albumin/ globulin ratio (A/G) was calculated by dividing the value of serum albumin by the value of globulin. Serum alanine amino transferase activity (ALT) and aspartate amino transferase activity (AST) were carried out according to the method of **Reitman and Frankel (1957).** Kinetic determination of serum creatinine was performed according to the method described by **Henry (1974).**

Spermatozoa examination: At the end of the experiment (65 days) sperm cells were obtained by cutting off the epididymis and vas deferentia and examined for sperm cell concentration (**Reddy and Bordekar, 1999).** Smears were also prepared for assessment of live and dead sperm percent and spermatozoal abnormalities as preceded by **Bearden and Fuquay (1997).**

Cytogenetic analysis: Spermatocytes were analyzed using standard protocols of **Russo (2000)**. The slides were stained with Giemsa stain and examined for structural and numerical chromosomal aberrations in germ calls (50 metaphases/rat). For micronucleus assay (MN)**,** bone marrow cells were subjected for MN analysis and the polychromatic erythrocytes (1000/animal) were screened for micronuclei frequency according to **Hayashi (2016).**

Histopathological examination: Samples from liver, kidneys and testes of scarified rats were fixed in 10% neutral buffered formalin solution, processed by the standard paraffin embedding technique and stained by hematoxylin and eosin **(Suvarna *et al.*, 2013).**

5- Statistical analysis:

The data are presented as means ±standard error (SE). Comparison between mean values of control and Phoxim exposed group was carried out using Student's t test according **Petrie and Watson (1999).**

Results

In the current study, the acute oral LD_{50} of Phoxim was calculated as 2007.13mg/kg bw in male albino rats (Table 1)**.** Hemogram revealed significant decrease in RBCs count and PCV percentage in phoxim group (Table 2). Leukogram showed significant increase in WBCs count and neutrophil percent while lymphocyte percent was significantly decreased in Phoxim exposed group at the two intervals of examination (Table 3). The biochemical results indicated non-significant change in serum total proteins, albumin and globulin (Table 4). There was significant increase in activity of ALT and AST and creatinine level in Phoxim treated group (Table 5). Spermatozoa examination revealed significant decrease in sperm count and life percent. Increase in abnormal sperm percent was also detected in phoxim administered rats (Table 6).

Cytogenetic analysis showed that chromosomal aberration in male spermatocyte cells in Phoxim treated rats was more than 2 fold compared to control specially for autosomal univalents, breaks and hypo- ploidy (Table 7). Micronucleus assay revealed a clastogenic effect of Phoxim (Table 8), where the micronucleus frequency was increased significantly in the Phoxim treated rats by about 3 fold after 1 month and 4 fold after 2 months exposure (3.18 vs. 8.82 and 3.4 vs. 12.58, for control and Phoxim treated groups after 1 and 2 months, respectively).

Histopathological examination of liver showed dilation of hepatic sinusoids with marked distortion of hepatic cords (photo 1). Marked dilation of hepatic sinusoids lead to loss of hepatic cords architecture with adenoid arrangement (photo 2). Pathological alteration of the Kidneys revealed coualgulative necrosis of tubular lining epithelia, the glomeruli were enlarged in size with marked hypercellularity (photo 3) and had franking appearance (photo 4). Testes examination revealed marked detachment and loss of spermatogenic series with marked reduction of spermatids (photo 5).

Discussion

Organophosphate compounds are widely used as insecticides. The toxicity of an organophosphate is related to its leaving group, the double bonded atom, usually O or S and the phosphorous ligands, the groups surrounding the phosphate in the compound. The metabolic replacement of sulphur by oxygen in the liver or other detoxicification organ activates the sulphur containing organophosphate into a much more potent form. The extreme toxicity of these compounds is due to their ability to bind to the amino acid serine, rendering it incapable of participating in a catalytic reaction within enzyme and the further blocking of the active site

by the organophosphate residue **(Mahananda and Mohanty, 2012).**

The current study was planned to estimate the acute oral LD_{50} of Phoxim and screen the effects of exposure to 1/40 LD_{50} three times/ week for 65 days on hemogram, certain serum biochemical parameters, sperm cells, histopathological alterations and genotoxic effects in male rats**.**

The acute oral LD_{50} of phoxim was calculated as 2007.13 mg / kg bw. in male rats (Table 1). This result was in accordance with **WHO (2000)** which mentioned that the LD_{50} of Phoxim in rats was 2000 mg/kg bw .

Hemogrram examination revealed significant decrease in RBCs count after one and two months of Phoxim exposure. PCV showed significant decrease in the 2nd month in phoxim exposed rats when compared to control group (Table 2). In accordance to these findings, some investigators reported decrease in RBCs count, PCV and Hb content in rats exposed to other organophosphates (**Mossa and Abbassy, 2012; Holy *et al.*, 2015)**. These findings revealed a state of anemia that might be attributed to excessive destruction of the red blood cells due to the toxic effect of insecticide used and/or inhibition of erythrocyte formation in bone marrow (**Mossa and Abbassy, 2012** and **Holy *et al.*, 2015)**.

Evaluation of the leukogram of rats administered Phoxim showed that the number of leukocytes as well as neutrophil percentage significantly increased while lymphocyte percent was significantly decreased in Phoxim group compared to control (Table 3). The recorded leukocytosis coincide with the results reported by **Al-Sahhaf (2006)**; **Holy *et al.* (2015)** using other organophosphorus insecticides. On the other hand, **Mossa and Abbassy (2012**) reported a decrease in leukocytic count in chlropyrifos treated rats. The observed increase in the total leukocytic count in the peripheral blood of rats exposed to Phoxim could be attributed to the redistribution of white blood cells from marginal granulocytic pool (MGP) to the circulating granulocytes pool (CGP) under the effect of high concentration of epinephrine **(Coles, 1986).** In addition**, Latimer and Prasse (2003)** reported that leukocyte changes, especially the increases in neutrophils and decreases in lymphocytes, are typical of those observed in stressed animals of many species.

Protein profile revealed non-significant change in serum total proteins, albumin and globulin in Phoxim exposed rats when compared to control group (Table 4). This finding is in agreement with **Reena *et al.* (1989)** who reported that serum total proteins were unchanged in rats after treatment with dimethoate. On the other hand, **Al-Sahhaf (2006)** found a decrease in serum total protein in rats orally given sumithiom. In addition, **Abo El-Soud *et al.* (2015)** stated that serum total protein significantly decreased in rats orally administered profenofos for 65 days**.**

Concerning the liver function tests, significant elevation in ALT and AST activities were recorded in the sera of rats after the 1st and 2nd months of Phoxim exposure (Table, 5). Since transaminases (AST and ALT) are considered as indicators for adverse effect on the liver. Also, they are important enzymes in amino acids biosynthesis and catabolism. In addition, they are responsible for detoxification processes, metabolism and biosynthesis of energetic macromolecules for different essential functions **(Aly *et al.*, 1997).** This elevation of ALT and AST may be indicative of initial cell injury occurring in advance of gross hepatic pathology. Not only distinct cellular damage but also any condition leading to changes in membrane permeability causes a generalized release of these enzymes from the liver cells **(Mossa and Abbassy, 2012**).The increase in ALT and AST activities is in agreement with the results obtained by **Abo El-Soud et al. (2015)** who reported increase of serum AST and ALT activities in rats orally administered pronofos for 65 days. In addition, several studies have

shown that the activities of transaminases were increased after exposure to pesticides (**Mossa, 2004; Mansour and Mossa, 2011; Mossa *et al.,* 2011; Mossa and Abbassy, 2012**).

Histopathological examination of the liver of Phoxim treated rats confirmed the observed increase in ALT and AST activities. Hepatic parenchyma showed severe dilation of hepatic sinusoids with marked distortion of hepatic cords. Marked dilation of hepatic sinusoids lead to loss of hepatic cords architecture with adenoid arrangement. These changes declare the adverse effect of Phoxim on the liver as it is responsible for facing any foreign molecule which is carried through portal circulation (**Mossa and Abbassy, 2012**). This change partially agreement with **Holy *et al.* (2015)** who found sinusoidal dilatation, congestion, fatty changes, steatosis, centrilobular necrosis and mononuclear cells infiltration in dichlorvos -treated rats. **Abo El-Soud *et al.* (2015)** recorded congestion of central veins and hepatic sinuosoids and necrosis of hepatocytes in profenofose exposed rats. **El-Maghraby and Farghaly (2012)** observed aggregation of inflammatory cells in the portal area and abnormal shape of the liver cells in mice.

Regarding renal function, significant increase in serum creatinine level was observed after the 1st and 2nd month of Phoxim administration. This coincide with the result obtained by **Al-Sahhaf (2006)** who mentioned an increase in creatinine level in rats orally administered sumithion at a dose level of 60 mg kg/ b. wt (1/4 LD_{50}) daily for 12 days. Also, **Mossa and Abbassy (2012**) found an elevation in creatinine of male rats treated with organophophorus insecticides. Creatinine is a metabolite of creatine and is excreted completely through urine via glomerular filtration. An elevation of its level in the blood is thus an indication of decrease glomerular filtration rate **(Klaassen, 1996)**.

Histopathological examination of the kidneys of rat group treated with Phoxim showed coualgulative necrosis of tubular lining epithelia, the glomeruli are enlarged in size with marked hypercellularity and had franking appearance. **Abdeen *et al.* (1994)** observed an enlargement of the glomerulei, hydropic degeneration, damage of the epithelial lining of the renal tubule and rupture of the distal tubules in fenvalerate-treated mice. **Al-Sahhaf (2006)** found severe damage of the renal tubules**,** eroded Bowman's capsule with atrophied glomeruli in rats exposed to sumithion for 12 days. **Abo El-Soud *et al.* (2015)** detected severe degenerative changes in the epithelium lining the renal tubules and dilatation of some blood vessels in rats administered profenofos for 65 days.

Concerning the effect of Phoxim on male fertility, spermatozoa examination of rats exposed to 50mg/kg bw three times/week for 65 days revealed significant decrease in sperm count, life percent and increase in the percentage of morphologically abnormal spermatozoa in the examined rats . These changes may be attributed to the ability of Phoxim as an OP insecticide to cross the blood testicular barrier where it induce oxidative stress and lipid peroxidation that damage the biological membranes of the testes. This in turn may result in degenerative changes in the spermatogenic and Leydig cells, which disrupt the process of sperm production and consequently decrease the sperm counts **(Uzunhisarcikli *et al.,* 2007)**. Moreover, reactive oxygen species may lead to infertility as a result of defective sperm function. The oxidative effects of OP insecticides affect the activities of mitochondrial enzymes and structure of the microtubules in the sperm which result in decrease of the sperm motility **(Latchoumycandane *et al.,* 2002).** The obtained results agreed with those of **Atef *et al.* (1995)** who found an increased percentage of the dead and morphologically abnormal spermatozoa in Phoxim treated rats. **Xu *et al.* (2004)** also reported significant decreases of the daily sperm production in rats treated with Phoxim (8.2 mg/kg, 5 days a week for 60 days) in comparison with the controls**.** In addition, **Uzuna *et al.* (2009)** mentioned that male rats given malathion, an OP pesticide, (27 mg/kg; 1/50 of the LD_{50} as an oral dose) significantly

lower sperm counts and sperm motility, and significantly higher abnormal sperm numbers, than that of the untreated control rats. **Abo El-Soud *et al.* (2015)** stated that male rats orally administered profenofos for 65 days showed significant decrease in sperm count, percentage of motility accompanied with significant increase in the sperm abnormalities.

Spermatozoa examination results were greatly supported by the histopathological changes of the testes of Phoxim administered group that showed marked detachment and loss of spermatogenic series and reduction of spermatids. These findings coincide with **Abo El-Soud *et al.* (2015)** who observed necrosis in the spematogenic cells of the testis of rats exposed to profenofos for 65 days. In addition, **Farag *et al.* (2000) and Khan *et al.* (2001)** reported that OP insecticides decrease the number of spermatogenic cells in the testes and inhibit spermatogenesis.

The genotoxic damage caused by Phoxim using spermatocyte analysis and micronucleus assay as a biomarker of genotoxicity. These assays are more sensitive indicators of genotoxic effects **(Luzhna *et al.*, 2013**). Chromosomal aberration in male spermatocyte cells in phoxim treated rats were more than 2 fold compared to control specially for autosomal univalents, breaks and hypoploidy. Micronucleus assay revealed a clastogenic effect of Phoxim, where the MN frequency was increased significantly in the treated rats for the 2 periods of treatment compared to control rats. This coincide with **Joshi *et al*. (2003)** who found that organophosphorus insecticide Parathion, increase incidence of micronuclei in bone marrow and chromosomal aberrations in germ cells of male rats.

A variety of genotoxic agents such as the organophosphorus insecticide Phoxim in our study may induce micronucleus (MN) formation leading to cell death, genomic instability, or cancer development, which have been classified into two classes according to the mechanism of action: clastogens which cause breaks in chromosomes of spermatocyte cells (10% of total aberrations, represented in 6% autosomal univalents and 4% breaks), and aneugens (10% hypoploidy as a numerical variation) which affect cell division and mitotic spindle apparatus leading to aneuploidy. Accordingly, MN significantly formed as a result of clastogenic effect of Phoxim treatment in somatic cells which coincide with **Terradas *et al.* (2010)** and is clear in our study concerning the effects of Phoxim insecticide for both somatic and germ cells. The genotoxic effects of organophosphate toxicity may be attributed to the generation of reactive oxygen species and reactive oxygen free radicals which can damage DNA through oxidation of DNA bases or through covalent binding to DNA resulting in strand breaks and cross linking **(Abo El-Soud *et al.*, 2015**).

In conclusion, this study revealed that treatment with the organophosphorus insesticide, Phoxim, induced hematological, biochemical, histopathological changes as well as clastogenicity and infertility in male rats. Thus, these results encourage us to do more investigation with a protective agent in combination with the treatment of these toxic insecticides to mitigate their toxicities.

Table (1). Determination of acute oral LD_{50} of Phoxim in male rats.

Group	Dose (mg/kg)	Number of rats in each group	Number of dead animals	Mortality ٪
1	500	5	-	-
2	1000	5	1	20
3	2000	5	3	60
4	4000	5	5	100

Calculation of LD_{50} was as follows:M = x1+1/2d-dr1/N
Log LD_{50} =Log 4000+1/2 Log 2-9/20 = 3.60206+0.150515-0.45 = 3.302575
Therefore, the LD_{50} = 2007.13 mg Phoxim /kg bw.

Table (2). Hemogram of control and Phoxim treated groups

Parameter	Frist Month		Second Month	
	Control	phoxim	control	Phoxim
RBCsX10^6/ µl	6.60 ±0.17	4.96** ±0.17	6.53 ±0.42	3.87** ±0.28
Hb(g/dl)	12.93 ±0.40	12.96 ±0.41	14.2 ±0.55	13.98 ±0.74
PCV %	40.50 ±0.87	39.00 ±0.77	40.00 ±0.83	34.60** ±0.92

Values represent means ± standard errors (n=5).
** Significantly at P< 0.01 using t- student test.

Table (3). Leukogram of control and Phoxim treated groups

Parameter	Frist Month		Second Month	
	Control	phoxim	control	phoxim
WBCsx10^3/µl	10.38 ±0.68	18.10** ±0.78	10.75 ±0.75	13.91* ±0.96
Neutrophil %	15.00 ±0.71	24.60** ±1.29	17.00 ±0.55	20.80* ±1.24
Lymphocyte%	82.40 ±0.60	72.60** ±1.12	81.40 ±0.92	77.60* ±1.29
Monocyte %	1.20 ±0.37	0.40 ±0.24	1.00 ±0.32	0.60 ±0.24
Eosinophil %	1.40 ±0.40	2.40 ±0.40	0.60 ±0.24	1.00 ±0.44

Values represent means ± standard errors (n=5).
* Significantly at P< 0.05 using t- student test.
** Significantly at P< 0.01 using t- student test.

Table (4). Protein profile of control and Phoxim treated groups

Parameter	Frist Month		Second Month	
	Control	phoxim	control	phoxim
Total protein(g/dl)	5.23±0.23	4.84±0.09	5.19±0.25	4.99±0.03
Albumin (g/dl)	3.40±0.12	3.27±0.16	3.38±0.05	3.17±0.10
Globulin (g/dl)	1.83±0.11	1.57±0.12	1.81±0.30	1.82±0.07
A/G ratio	1.85±0.05	2.08±0.23	1.86±0.43	1.74±0.12

Values represent means ± standard errors (n=5).

Table (5). Serum biochemical parameters of control and Phoxim treated groups

Parameter	Frist Month		Second Month	
	Control	phoxim	control	phoxim
ALT(u/ml)	30.63 ±1.46	40.77** ±2.03	33.33 ±0.82	42.23** ±1.75
AST (u/ml)	32.73 ±0.92	39.38** ±1.75	33.20 ±1.30	38.71** ±0.88
Creatinine (mg/dl)	1.04 ±0.13	2.20** ±0.10	0.78 ±0.16	1.90** ±0.13

Values represent means ± standard errors (n=5).
** Significantly at P< 0.01 using t- student test.

Table (6). Spermatozoa examination of control and Phoxim exposed groups.

Groups	Countx10^6/ml	Life %	Abnormalities %		
			Head %	Tail %	Total %
Control	14.25 ±0.63	88.66 ±0.88	1.50 ±0.29	4.75 ± 0.63	6.25 ±0.85
Phoxim	3.48** ±0.57	61.20** ±1.96	6.00** ±0.55	7.50** ±0.29	13.50** ±0.39

Values represent means ± standard errors (n=5).
** Significantly at P< 0.01 using t- student test.

Table (7). Percentages of chromosomal aberration in male spermatocyte cells of control and Phoxim exposed groups

Treatment	No. of examined cells	Structural aberrations				Total aberrations		Numerical variation			Total numerical variation	
		Autosomal univalent		Break				Hypo-ploidy		hyper ploidy		
		No.	%	No.	%	No.	%	No.	%	0	No.	%
Control	250	5.0	2.0	5.0	2.0	10	4	10	4	0	10	4
Phoxim	250	15	6	10	4.0	25	10	25	10	0	25	10

Table (8). Micronucleus frequency in control and Phoxim exposed male rats.

Parameter	Frist Month		Second Month	
	Control	phoxim	control	phoxim
No. of examined cells/ group	5000	5000	5000	5000
Percentage of cells with MN (%)	15.9	44.1	17	62.9
Mean ±SE	3.18 ±0.13	8.82** ±0.20	3.40 ±0.07	12.58** ±0.17

Values represent means ± standard errors (n=5). ** Significantly at P< 0.01 using t- student test.

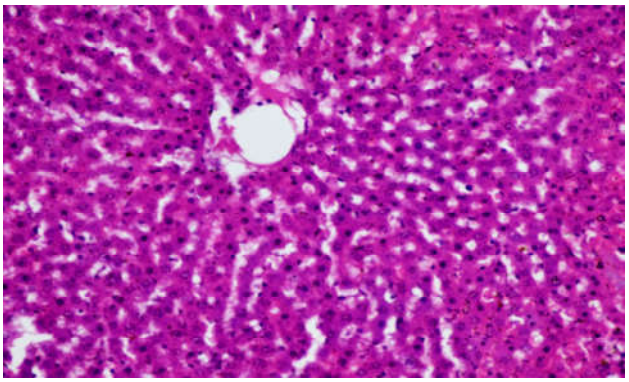

Fig. (1): Hepatic parenchyma showing dilation of hepatic sinusoids with marked distortion of hepatic cords. (H&E X 200).

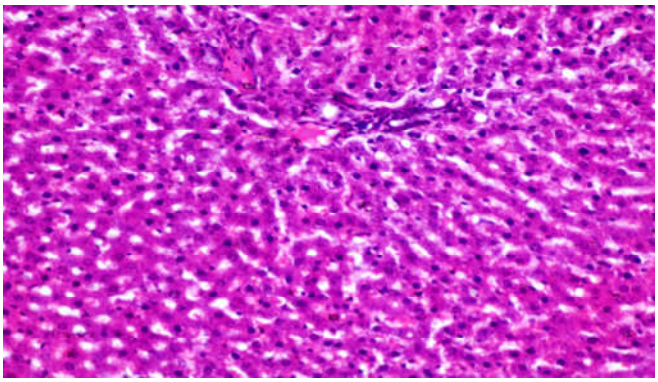

Fig. (2): Hepatic parenchyma showing marked dilation of hepatic sinusoids and loss of hepatic cords architecture with adenoid arrangement. (H&E X 200).

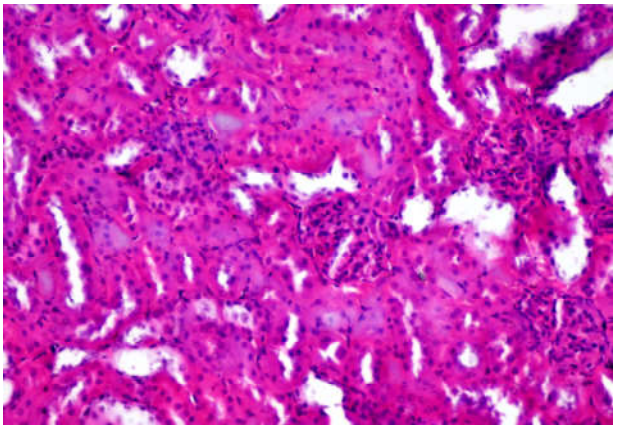

Fig. (3): Kidney showing coalgulative necrosis of tubular lining epithelia, the glomeruli are enlarged in size with marked hypercellularity. (H&E X 200).

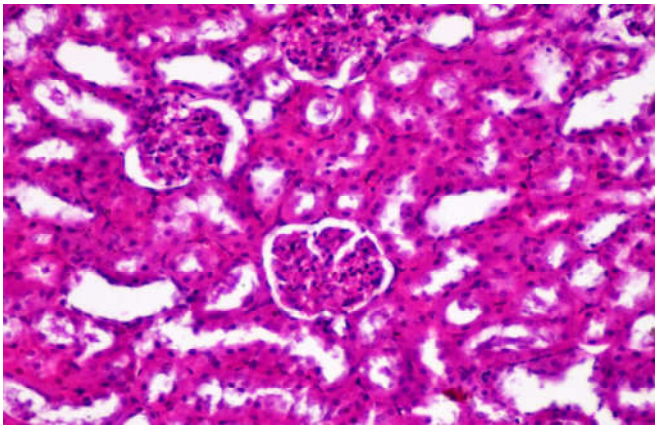

Fig. (4): Renal cortex showing coagulative necrosis of tubular lining, the glomeruli have franking appearance. (H&E X 200).

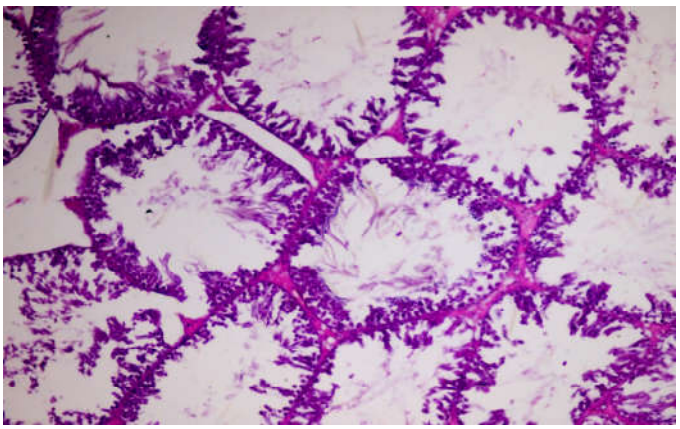

Fig. (5): Seminiferous tubules of phoxim group revealing marked detachment of spermatogenic series. H&EX 200

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