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Cytogenetic, Fertility and Pathological Studies Organophosphorus Insecticide of (Profenofose) on Male Rats.

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Abstract

Oral administration of profenofose for 65 days in a dose of 1/10 and 1/100 LD_{50}, respectively, significantly increase in the percentage of micronucleated polychromatic erythrocytes (MPCE), ratio of polychromatic erythroctes to normal chromatic erythrocytes (PCE/NCE). The PCE/NCE ratio used as a measure for red blood cell proliferation which gave a sign of toxicity or damage of some organs of the body. Also, a significant decrease in weight of testis than the control except at high dose which cause an increase in testis weigh. Although accessory glands (S.V., prostate and epididymis) showed variationi in weights than the control group. The sperm concentration and percentage of motility were decreased, although sperm abnormalities was increased which characterized by coiled tail and headless. The tested pesticide significantly increased the activities of serum AST, ALT and AP and the level of cholesterol, testosterone, bilirubin, while total protein, testosterone and creatinine significantly decreased.The treated rats with profenofos showed congestion, haemorrhage focal area of necrosis and lymphocytic mononuclear cell infiltration in liver and kidney. The spleen was hyperplastic with degeneration of thewall of some blood vessels. Severe degeneration and necrosis of the spermatic cells of the semineferous tubules in testis. Histopathological changes in different organs appeared to be dose dependent, with damage increase in the high doses.

Introduction

The use of pesticides has come in for increasing criticism in recent years, with the aspects of public concern including both the possible accumulation of pesticides residues in food and crops. Therefore, they could be a source of many biochemical and physiological disturbance in animals and humans **Shalin et al (2006), Awasthi and Parkash,(2007)and Szeto and P rice (1991)**.

Numerous in- vivo and in-vitro studies of possible consequences of treating animals, enzyme systems with these substances have been conducted. It could induce adverse effects on the immunity, liver, kidney and reproductive systems moreover, it

increase incidance of micronuclei in bone marrow cells **Joshi et al (2003) and Behera and Bhnnya(1987)**.

The study regarding the effect of profenofos on cytogenrcity, fertility and pathological changes are rare. Therefore, the present work was designed to elucidate the effect of the commonly used pesticide profenofos on the fertility of male rats, cytogenicity and some biochemical analysis and pathological changes.

Materials and Methods

I-Profenofose: 4-bromo-2-chlorophenyl-(o-ethyl-propyl) phospho-rothioate. Was obtained from Ciba-Geigy of Egypt

II-Animals: Sixty mature male rats were used. Rats were fed on ordinary ration and water *ad libitum*.

A-Effect on sexual organs weight and epididymal sperm characters:

Thirty mature male rats (150-180g) were divided into 3 groups. The first group was kept as a control, wherease the second and third groups were administered orally profenofos in doses of 0.36 and 3.6 mg/kg.b.wt. which is equal to 1/100 and 1/10 of LD_{50} respectively daily for 65 successive days to cover a complete spermatogenic cycle **Hershberger et al, (1969).**

The sexual organs weight and the epididymal sperm characters were determined according to **Bearden and Fluquary,(1980).**

Blood samples :

was obtained from each rat , left to clot and the serum was separated for biochemical analysis. The activities of AST, ALT and AP were determined according to the method of **Reitman and Frankel (1957) and Roy (1970)**.

Bilirubin, cholesterol, total protein, creatinine and testosterone levels in serum were estimated as explained by **Michaelsson (1961), Watson (1960), King and Watton (1956), Henry(1974) Tietz(1970)** respectively.

B-Cytogenetic effect:

In order to assess the possible mutagenic effects of profenofos, the micronucleus test was performed to detect chromosomal damage associated with the treatment.

For the micronucleus investigation, 3 groups of 10 mature male rats, each were used and first was kept as a control, the second and the thirdwere orally administered 0.36 and 3.6 mg/kg.b.wt. daily for 30 successive days.

Following the protocol established by **Salamon et al (1980)**, bone marrow cells of rats were extruded with a pin into a clean dry glass slide and homogenized with two drops of fetal calf serum. Cells were smeared on the slide, air dried fixed in absolute methanol and stained with Giemsa stain in phosphate buffer pH 6.8.

The polychromatic erythrocytes (PCE, 1000 Per animal) were screened for micronuclei and the changes in the mitotic activity **(Hart and Engberg-Pederson, 1983 and AL-Bekairi et al, 1991)** were assessed on the basis of ratio poly chromatic to normochromatic erythrocytes (PCE/NCE ratio)..

C- Pathological examination:

Tissue samples were collected from different organs (liver, spleen, kidney and testis). They were fixed in 10% neutral formalin, dehydrated, cleared, embedded in paraffin, sectioned at 4-6 μ for H & E and stained by Haematoxylin and Eosin for the general histopathological studies **Harris (1989)**.

D-Statistical analysis:

The results were subsequently analyzed following the statistical methods established by **Snedecor (1982)** in order to determine whether a dose group was positive or negative

Result and Discussion

Effect of profenofos on sexual organs weight, epididymal sperm characters and biochemical analysis:

Oral administration of profenofos in doses of 0.36 and 3.6 mg/kg.b.wt., respectively daily for 65 days successive days showed a significant decreased in the weight of testis in dose of 0.36 mg/kg.b.wt., while at high dose (3.6mg/kg.b.wt.) showed significant increase, on other hand accessory glands (s.v., prostate & epididymis) showed variation in weight than the control but a significant decrease in sperm concentration and percentage of motility. The sperm abnormalities significantly increased and characterized by coiled tai and headless (Table 1) .

From the above results, it is clearly that the effect of tested pesticides on male fertility may be attributted to their direct effect on sexual organs (testis) or indirectly effect on sexual hormon (testosterone). These results in agreement with those obtained by **(Nashwa et al 2012, Amina et al 2012 and Faustin et al 2010)** who said that organophosphorus pesticides can cause various histopathological and cytopathological changes in male reproductive system.

The effect of profenofos significantly increased the level of cholesterol and bilirubin (Table 2). Hypercholesterolemia may be attributted to the generation of free radicals induced by profenofos that cause lipid peroxidation. Peroxidation of membrane phospholipids alters lipid milieu and increases the supply of non essential fattyacids which in turn increases cholesterol level. Our results inaccordance with **Fritz et al, 1999, Attion and Nasr, 2009 and Nashwa et al 2012.**

Pronofos intoxicated rats exhibited significant decreased level of testesterone hormone (Table 1). The previous findings reflect the adverse effect of pronofos on testicular function **(Okamnra et al 2009 and Uzunhisar cikli et al 2007).**

Organophosphorus pesticides have the ability to cross the blood testis barrier including oxiditive stress and lipid peroxidation that damage the biological membrane in the testis. This in turn may cause degeneration of spermatogenic and Leyding cells which disrupt spermatogenic cycle.

Significant decrease of protein and creatinine were observed in profenofos exposed rats comparable to control group (Table 2). This recorded reflect the hepatocellular injury and distributed amino acid metabolism induced by pronofos **(Yousef et al, 2006)** , these changes could be attributted to the adverse effect of pronofos on the absorption and assimilation of protein from gastrointestinal tract**(Nashwa et al 2012).**

In the present study, rats administered pronofos showed significant increase of serum activities of AST, ALT and AP (Table3). The elevated transferases enzymes denoted the adverse effect of pronofos on hepatic function. Our results are confirmed histopathologically as liver showed congestion of central veins, and hepatic sinuosoids as well as necrosis of hepatocytes (Fig.1, 2), testicular degeneration and intraluminal accumulation of necrosed germ cells as well as interstitial oedema were observed in the testes of profenofos intoxicated rats (Fig.3a,3b).

Histopathological finding of spleen which revealed sever haemosidrosis in the red pulp (Fig.4). The kidney lesions were detecting as sever degeneration of renal tubular epithelium and dilatation of some blood vessels induced by profenofos (Fig.5,6).

Cytogenic analysis in our study revealed that pronofos at a doses of (0.36 and 3.6 mg/kg.b.wt.) induced significant increase the percentage of MPCE s when compared with corresponding control values. The PCE /NCE ratio significantly increased if compared with control (Table 4). Profenofos is an alkylating agent that chemically altered polynucleated chains. This essentially inhibits DNA synthesis in the alkylating region including one or more type of chromosomal aberration in the cells, furthermore organophosphate toxicity may be attributted in part by the generation of reactive oxygen species and reactive oxygen free radicals can damage DNA through oxidation of DNA bases or through covalent binding to DNA resulting in strand breaks and cross linking **(Bender et al, 1974, Saulsbury et al 2009 and Mansour et al 2009).**

Table (1):- Effect of profenofos on fertility in male rats and testosterone hormone.

Group	Dose mg/100g.b.wt.	Weight of sexual organs (g/100gm.b.wt.)Epidid. Sperm Character						Testosterone ng/ml
		Testis	S.V.	Prostate	Epidid.	Sperm conc.10^6/ml	Motility %	
Control	-	1.877+0.0897	0.188+0.014	0.164+0.014	0.744+0.06	523+5.79	90.5+0.898	1.18+0.02
profenofos	0.36	1.97+0.065	0.362+0.02**	0.213+0.03***	0.721+0.05	275+7.93***	76+1.29***	0.99+0.08***
	3.6	1.75+0.09	0.189+0.06	0.153+0.011	0.628+0.04	204+5.82***	70+1.45***	0.75+0.05***

P values represented the means ± SE

** P < 0.00 *** P < 0.00

Table (2):- Effect of profenofos on some serum constituents.

Group	Dose mg/100g.b.wt.	Bilirubin µ mol/l	Cholesterol mg/100ml	Creatinine mg/l	Total protein mg/100ml
Control	**-**	**100.7+1.29**	**194.53+14.68**	**24.92+0.76**	**9.78+0.23**
Profenofos	**0.36**	**46.75+0.83*****	**266.54+6.59*****	**22.41+0.35****	**9.05+0.26**
	3.6	**108.91+2.11*****	**229.44+10.04**	**21.21+0.32****	**8.39+0.24*****

P values represented the means ± SE
** P < 0.005
*** P < 0.001

Table (3):- Effect of profenofos on serum enzymatic activities.

Group	Dose mg/100g.b.wt.	AST U/L	ALT U/L	AP U/100ml
Control	**-**	**125.87+3.89**	**98.67+0.68**	**88.54+2.95**
profenofos	**0.36**	**137.1+1.42***	**116.48+6.45**	**110.81+1.24*****
	3.6	**147.17+0.35*****	**183.1+9.5*****	**140.49+0.98*****

P values represented the means ± SE
** P < 0.005
*** P < 0.001

Table (4):- Effect of profenofos on the incidence of micronucleated PCE on the relation of PCE to NCE

Group	Dose mg/100g.b.wt.	PCE	MPCE/100 PCE+S.E.	%	NCE Screened	PCE/NCE+S.E. ratio
Control	**-**	**5000**	**5.6+0.3**	**0.56**	**2183**	**2.33+0.4**
Profenofos	**0.36**	**5000**	**13.44+0.36***	**1.34**	**952**	**5.25+0.36*****
	3.6	**5000**	**15.6+0.43****	**1.56**	**661.3**	**7.561+0.23*****

P values represented the means ± SE
* P < 0.01
** P < 0.005
*** P < 0.001

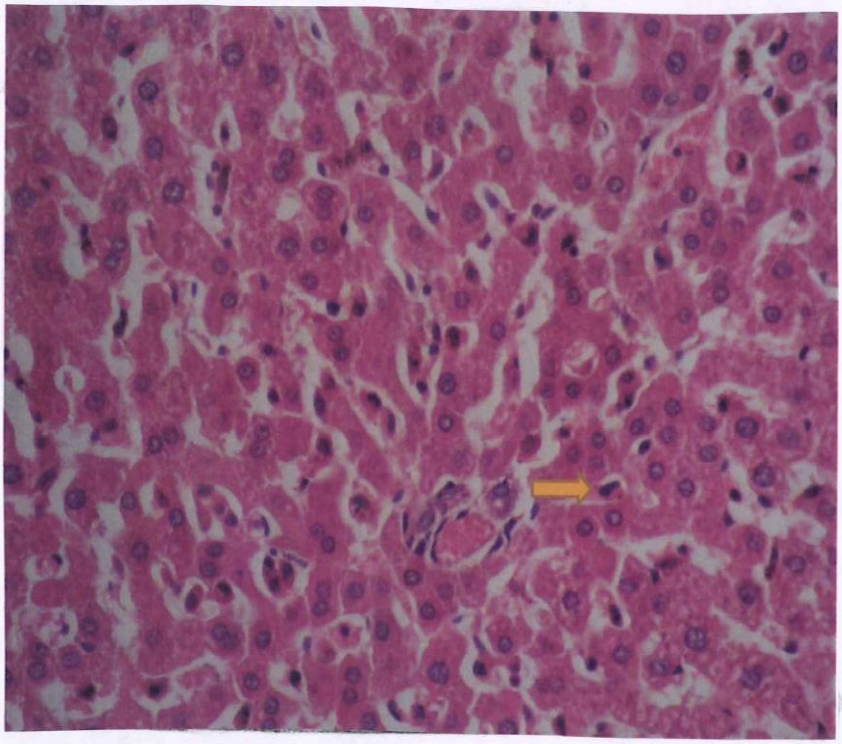


Fig (1) : Liver from rats received 1/100 LD_{50} of profenofos shows congestion of portal blood vesels, portal fibrosis and newly formed.

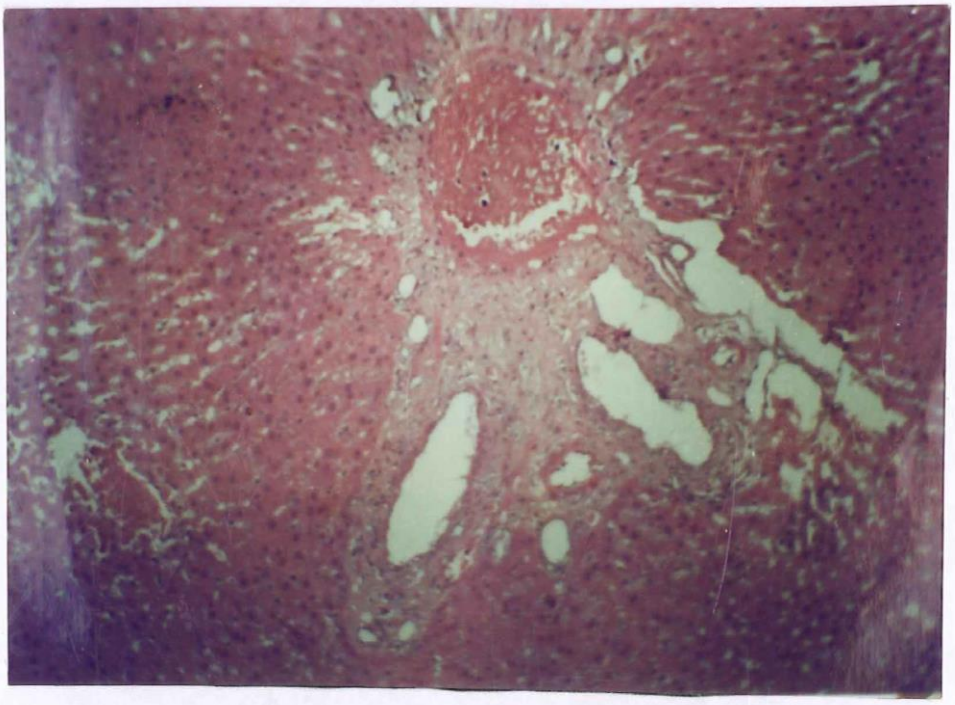
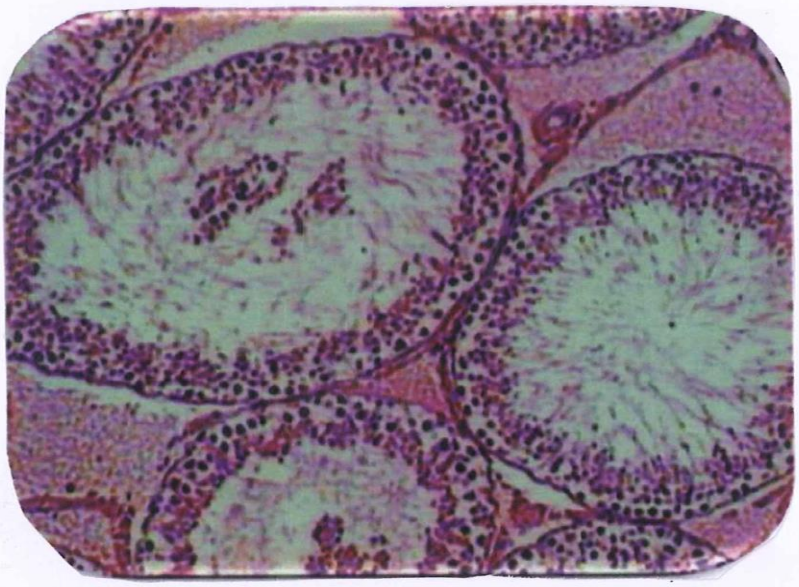
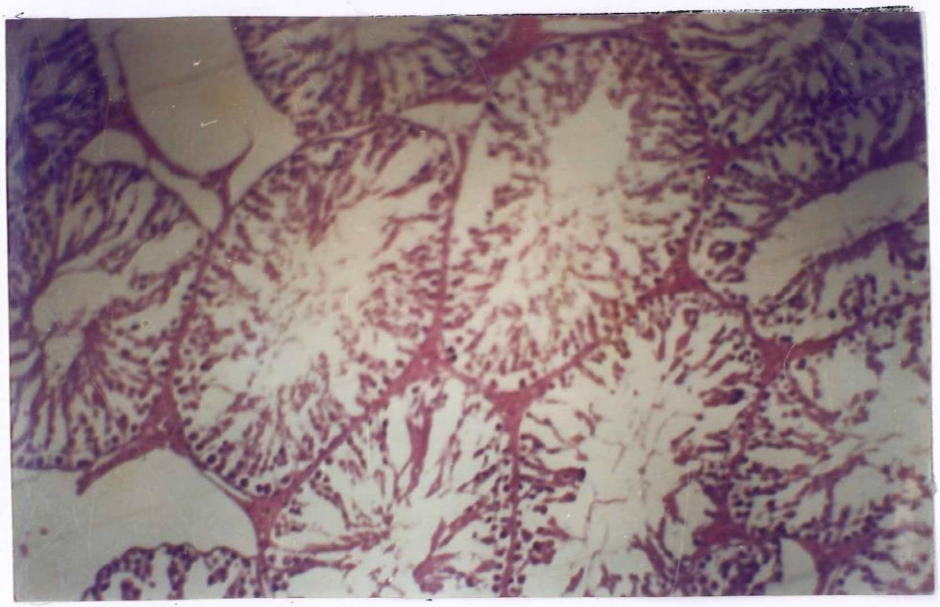


Fig (2) : Cross section of liver illustrated degeneration of hepatic enzyme cells in groups with profenofos (1/10LD_{50}).



Fig(3a)



Fig(3b)

Fig (3) : Testis from rats received 1/100 LD_{50} of profenofos (a) , 1/10 (b) reveals sever degeneration and necrosis of sperm and spermatids.

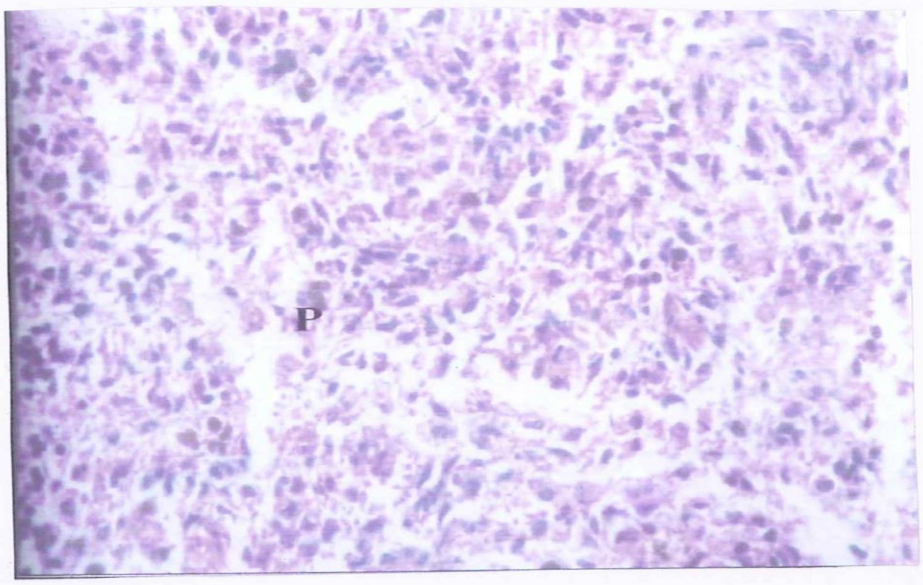


Fig (4) : Cross section of spleen illustrated the amount of haemosidrin pigments in groups received profenofos.

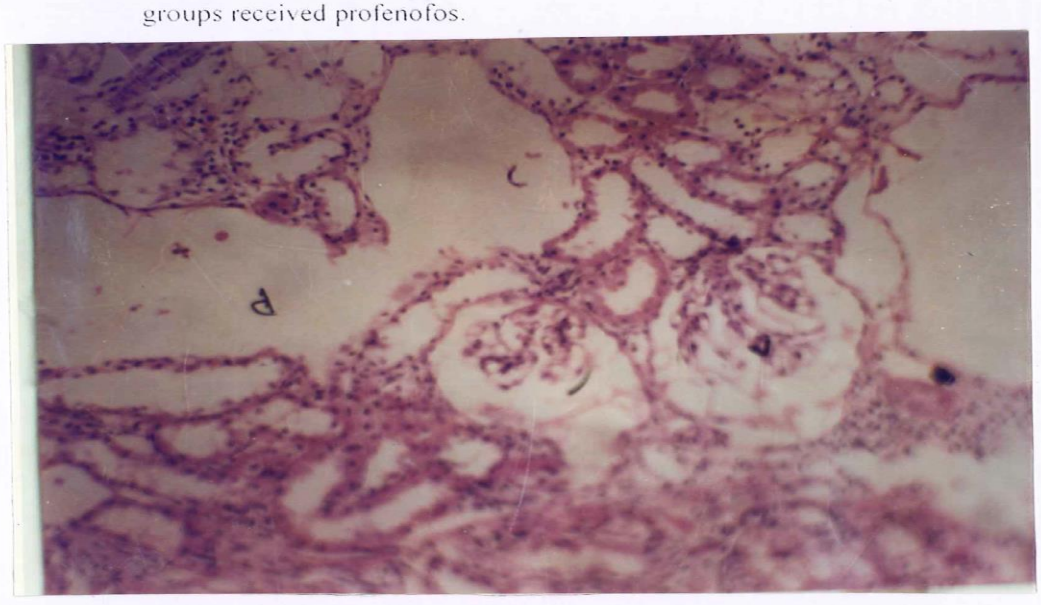


Fig (5) : Cross section of kidney illustrated degeneration of linning epithelium in group received profenofos 1/100.

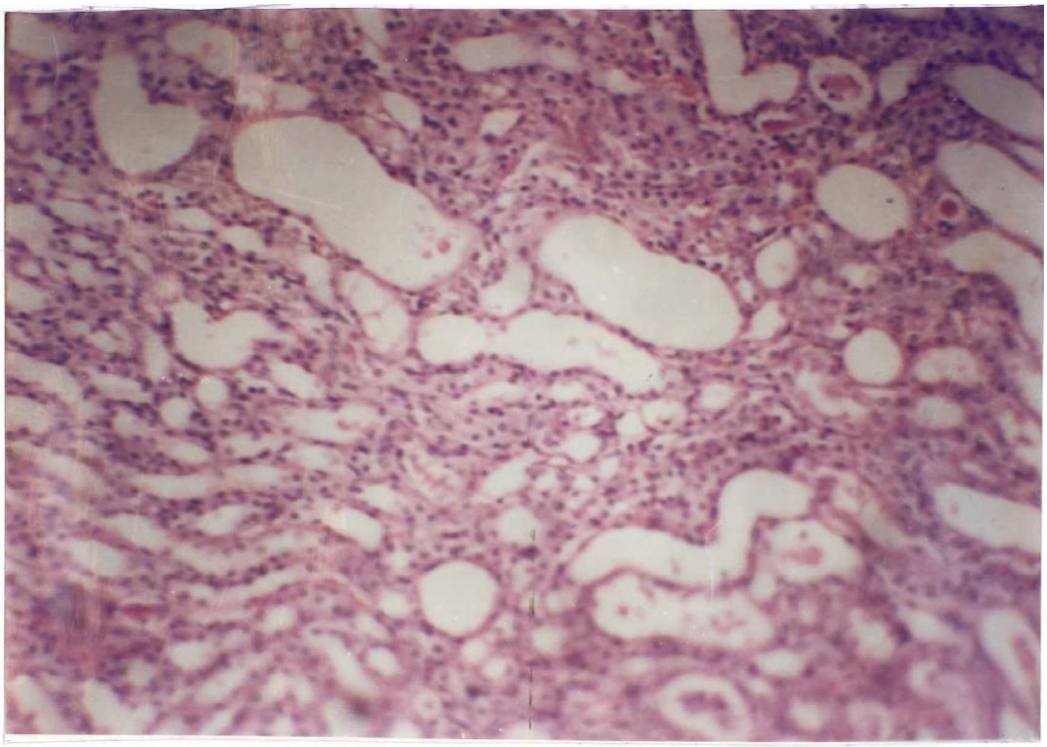


Fig (6) : Kidney from rats received 1/10 LD_{50} of profenofos showes sever degeneration of renal tubular epithelium & glomerular tubule capillaries witl dilatation of some renal blood vessels.

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