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In *vivo* biochemical alterations and associated nephrotoxicity in *Clarias batrachus (Linn.)* due to pesticide exposure.

Prakriti Verma[a]*, Zakia Khatoon[a] & Prabha Rani[a]
*[a]Department of Zoology, Patna University, Patna- 800 005, Bihar, India.

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Abstract : The present study was carried out in order to assess the synergistic or antagonistic action of rogor and endosulfan induced toxicity in air breathing fish. Fresh catfish *Clarias batrachus* were exposed to dimethoate (o,o-dimethyl,S-methyl-carbamoyl-methyl phosporodithioate) and organochlorine endosulfan pesticides commonly used in agricultural field with a dose of 0.004 µl/L endosulphan,1 µl/L rogor and a combination dose of {rogor(1 µl/L) + endosulfan (0.008 µl/L)} for 21 days . Every week blood was collected for serum biochemical analysis. Kidney tissues were dissected to see any histopathological changes viz. degeneration of epithelial cells uriniferous tubules, vacuolation, cytoplasmic shrinkage and shrinkage in glomerular network increases with the dose and duration of the pesticide exposure. Serum biochemical analysis also reveals increasing urea and decreasing protein level throughout the experimental period as compared to normal. Endosulfan was found more toxic than rogor as indicated by the biochemical analysis and histopathology of kidney cell. Administration of rogor and endosulfan in combined dose was found more toxic in comparison to individual treatment. It has been concluded that rogor and endosulfan sets a wave of biochemical imbalance and leading to nephrotoxicity.

Key words: Pesticide nephrotoxicity, Protein, Urea, Kidney, *C.batrachus.*

INTRODUCTION

Application of pesticides has contributed greatly in enhancing agricultural yields and also for the control of insect-borne diseases. Excessive use of broad spectrum or nonselective pesticides damages the ecosystem, sometimes irreversibly, contaminates soil surface and ground water as well as food chains and thus compromises the health and well being of the inhabitants of aquatic and terrestrial environment. These pesticides are carried into aquatic ecosystem by surface runoff from sites of application, where they enter the organisms through food webs and also through contact in water. Therefore, the health of aquatic ecosystem is being adversely affected because they serve as ultimate sink for these pesticides. These pesticides are found to be highly toxic not only to fish but also to other organisms which constitute food of the fishes. Many works has been done to assess the toxicity of pesticides on different organs of test animals. Arasta *et al.* (1996)[1] have studied the effect of Nuvan on some biochemical parameters of Indian catfish *Mystus vittatus*. Begum and Vijayaraghvan, (1996)[2] have reported on alteration in protein metabolism of muscle tissue in the fish *Clarias batrachus* (Linn) by commercial grade dimethoate. Begum and Vijayaraghvan, (1999)[3] have determined the effect of acute exposure of the Organophosphate insecticide Rogor on some biochemical aspect of *Clarias batrachus* (Linn). Ghosh, (1987)[4] have observed the toxic impact of three organophosphate pesticides on carbohydrate metabolism in a freshwater Indian catfish, *Clarias batrachus*. Therefore in present research work special emphasis has been given to the toxicity on kidney cell of freshwater catfish, *Clarias batrachus* (Linn) exposed to rogor and endosulfan alone and in combination.

*Correspondent author :
Phone : 009431877698
E-mail : drprakriti@gmail.com;

MATERIALS AND METHODS

Clarias batrachus is a seasonal breeder, tropical freshwater catfish, popularly known as "Mangur. They are hardy, restraining the most of the adverse ecological condition and pesticide toxicity, having fast growth rate and prolific breeding ability Different age groups of *Clarias batrachus*, ranging from 50-80 ±10 gms and size between 18-20±20 cms were collected from wetlands of north Bihar. The fishes were brought to the laboratory disinfected with 0.1% $KMnO_4$ solution and kept in large plexy glass aquarium of 80L to 120L water holding capacity respectively, having dechlorinated, aerated tap water at NTP. Fishes were acclimated for 15 days in the laboratory condition. During acclimated period they were fed with rice bran and mixture of eggs and chopped wheat (suji) with a pinch of starch as a binder @5% of their body weight daily. Fishes were also fed with sliced goat liver after every alternate day during morning hours. The acclimated fishes were categorized into following groups –Group I – Normal, Group II – Endosulfan treated, Group III – Rogor treated and Group IV – Synergistic (Endosulfan + Rogor) treated.

Pesticide treatment:-

Commercially brand two pesticides were used. One was commercially brand "Endocel (EC 35%)" manufactured by Excel Industries Ltd., Ruvapari Road, Bhawanagar (Gujarat). Endosulfan is a chlorinated hydrocarbon insecticide and acaricide of the cyclodiene subgroup which acts as a poison to a wide variety of insects and mites on contact. Other was commercial brand "Rogor (EC 30%)"a Dimethoate (IUPAC Name- 0, o dimethyl S- (N methyl carbamoylmethyl)phosphoro-dithioate), CAS No. 60-51-5, is an organophosphate available in the market by the trade name of rogor Manufactured by ANU products, old Faridabad (Hariyana). Here in the experimental protocol, one dose was 0.008 µl/L of endosulfan and one dose of 1.5 µl/L of Rogor considered for 7, 14 and 21 days exposure. For combined treatment a very low concentration of 0.004µl/L of endosulfan with 1.00 µl/L of Rogor also have been considered. Accordingly, stock solutions were prepared by dissolving appropriate amount of endosulfan and Rogor to distilled water. After each schedule exposure fishes of all the four groups, blood sample were collected in heparinised tube by puncturing caudal vein and blood plasma were extracted and refrigerated at 20°C in sterilized paraffin covered tubes for further biochemical analysis. Serum was analyzed for quantitative estimation of total protein and urea respectively. Then fishes were anesthetized and kidney tissues were dissected out, ringed in NaCl (85%) to remove any adhering unwanted tissues. The tissues were cut into small pieces with sharp surgical blades, and were fixed and processed for Light Microscopy.

Biochemical analysis

For biochemical analysis was done in BT-260 plus Semi-Automatic Chemistry Analyzer, manufactured by Nanchang Biotech A & C Biotechnical Industry Co. China.All the biochemical assessments have been done for normal/ control, Rogor and Endosulfan treated fish *C. batrachus* independent. 6 observations have been taken in each groups**,** then Mean and Standard deviation was calculated by the formula. The 't' test have been applied through standard biostatistical formula by considering mean of normal *C. batrachus* as standard mean and comparing individual mean of different doses and duration of Endosulfan, Rogor and Synergistic exposures to their respective control mean. After applying 't' test the calculated values were referred to fisher's table to see level of significance at ($P<0.05$) and ($P<0.01$). The details, histopathological observations based on Light Microscopy (LM) as well as biochemical alteration in kidney (KFT) have been mentioned in observation.

Observations

Histomorphology of kidney

The kidney of the teleost fish is a mixed organ comprising haemopoietic, reticuloendothelial , endocrine and excretory element. The first three functions are all dealt with elsewhere and this section will be confined to consideration of the excretory component.

Light Microscope Observation

The kidney of control / normal *Clarius batrachus* shows normal glomerulus and distint Bowman's capsule which together constitute the renal corpuscles. The epithelium (visceral and paritetal layer) of Bowman's capsule is regular. Proximal and distal segment of the uriniferous tubule bounded by connective tissue is distinctly seen.(Plate I,Fig. 1,2).

Rogor treatment

Rogor treatment for seven days showing little aggregation of oedematous fluid around tubules which is increased after 14 days roger treatment. After 21 days some necrosed cell of the tubules is seen with some vacuolations and aggregation of macrophages around cytoplasm. Bowman's capsule show no structural alteration within 7th and 14th days treatment Aggregation of macrophages is seen after the 14th and 21st days treatment where as distal tubule shows longer lumen in then the proximal tubule after 21 days treatment. (Plate I, Fig. 3, 4)

Endosulfan treatment

Endosulfan exposure for 7 days shows no distinct changes in the glomerulus architecture. Bowman's capsule, proximal distal tubule sign of degeneration seen after the 14 days treatment. Moderate changes after 14 days showing extensive fibrosis of proximal convoluted tubules, distal convoluted tubule. Some region showing autolysis**.** Few glomerulus showing least tuft of glomerulus. But after 21days kidney show shrinkage and increase vacuolation in glomerulus, abnormal shaped glomerular tuft, complete acellularity (asterisk) in proximal and convoluted tubule. Swelling of renal and interstitial fibrosis pointing towards chronic glomerulonephritis. (Plate II, Fig. 1, 2, 3, 4).

Rogor and Endoulfan treatment

Synergistic (rogor 1.00µl/L +endosulfan 0.004µl/L) exposure for 7 days show extensive vacuolation, loss of cellularity, increased autolysis of cellular organelles, fusion of bowmens capsule whereas,after 14 day patches of endosulfan clouds, intermingling of glomular tufts is seen. After 21 days exposure complete degeneration of renal tubules and infiltration of macrophages and increased infiltration of odeomatous fluid has been frequently observed.(Plate- III, Fig-1, 2, 3, 4)

Observations of biochemical analysis-

The observed serum urea and total protein of control, rogor, endosulfan and their mixture treated fish have been shown in the following next tables- Serum urea level just shoot up in the 7th days of 1.5µl/L rogor treatment ,it sustained increase level upto 14th days but its level decreases in 21st days below the normal. In 0.008µl/L endosulfan treatment urea level increase in 7th day but decrease in level is seen in both 14th and 21st days. treatment below the normal. In combined dose of rogor 1.00µl/L + 0.004µl/L endosulfan the urea level shoot up more than single doses whereas serum protein level showing decreasing tendency in all the doses than the normal level.

Table 1: Showing urea in blood of control, Endosulfan, Rogor and Synergistic endosulfan+Rogor) treated *C. batrachus*

Groups	Days of Exposure	Mean mg/l	SD	t-test
Control		5.03	1.001	-
1.5 µl/L Rogor treated	7	5.51	0.454	1.398
	14	5.71	0.589	1.845*
	21	6.54	0.481	4.291**
0.008 µl/L Endosulfan treated	7	5.7	0.581	1.845
	14	6.68	0.531	4.977*
	21	6.75	0.575	4.680**
0.004 µl/L Endosulfan + 1.0 µl/L Rogor treated	7	7.305	0.224	6.945
	14	7.11	0.368	7.227*
	21	7.5	0.369	7.23*

Table 2: Showing Total Protein in blood of control, Endosulfan, Rogor and Synergistic (Endosulfan+Rogor) treated *C. batrachus*.

Groups	Days of Exposure	Mean mg/l	SD	t-test
Control	-	5.64	0.280	
1.5 µl/L Rogor treated	7	5.1	0.196	4.715
	14	5.0	0.585	2.576*
	21	4.45	0.850	4.076**
0.008 µl/L Endosulfan treated	7	5.0	0.190	4.701
	14	5.1	0.196	4.715*
	21	3.9	0.871	5.815*
0.004 µl/L Endosulfan + 1.0 µl/L Rogor treated	7	4.1	1.317	3.360
	14	3.18	1.213	6.210*
	21	2.65	0.872	10.053**

DISCUSSION

Pesticides and heavy metals have been found to alter the histoarchitecture aspects of normal organs of animals, and various researches have been done in this field. Histoarchitectcure alteration in kidney due to pesticide toxicity causes degeneration of epithelial cells of uriniferous tubules, vacuolation, necrosis, cytoplasmic shrinkage and shrinkage in the glomerular network. Sharma, (1978)[5], Rankin (1982)[6]. The changes undergone by various parameters related to kidney of fish upon different doses and durations of rogor, endosufan and synergistic effect of both pesticides. Marked shrinkage in the diameters of Bowman's capsule and glomerulus is observed after exposing *Clarias batrachus* of 1.5µl/L rogor after 14th & 21st days. Shrinkage is moderate at 14th days but the necrosis of epithelium of glomerulus and Bowman's capsule can be seen even after 21 days treatment. This can be correlated with finding of Ghosh, (1990)[7] who observed damaged epithelial cells with deltamethrin treated fish kidney tubules. Sharma, (1978)[5], observed shrinked glomerular network and discontion of tubular cells in *C.punctatus* exposed to 0.01 ppm endrin for 30 days Appearance of unusual gaps inside the malpigian capsule was noticed. He also reported shrinkage of the glomeruli to various degrees. Proximal tubule is the most affected region of kidney due to endosulfan treatment 0.006 ppm for 8 days and 12 days Total protein constituting mainly albumin and globulin, the fraction vary independently and widely in diseases. Increased levels are found mainly in dehydration .Decreased levels are found mainly in malnutrition, impaired synthesis, protein losses as in hemorrhage on excessive protein catabolism. Anusha *et al* (1996)[8] also determined the total protein and lipid content depletion after exposure to sub lethal effects of organophosphorous pesticide quinalphos in *Cirrhinus mrigala.* Characteristic alterations in protein metabolism of muscle tissue in the fish *Clarius batracus* was observed after exposure to dimethoate Begum and Vijayaraghavan (1996)[2]. Urea is the end product of protein metabolism .It is synthesized in the liver from the ammonia produced by the catabolism of amino acids .It is transported by the blood to the kidneys from where it is excreted. Increased levels are found in renal diseases, urinary obstructions, shocked, congestive heart failure and burns. Decreased levels are found in liver failure and pregnancy Fawcet & Scott, (1960)[9].

In the present research work rogor and endosulfan both in single and in combination dose elicits a series of sequential histopathological anomalies due to their synergistic impact. Histological findings coincide at several places with biochemical alteration in different parameter of kidney function test. Thus in the present research work a perfect correlation among biochemical and histological findings have clearly shown that rogor and endosulfan toxicity causes a wave of biochemical imbalance followed by depletion and variation of protein and urea level and histopathological abnormalities in renal tissues of *Clarias batrachus* (Linn).

PLATE-I

Photomicrographs of transverse section of kidney of *Clarias batrachus* stained with Haematoxylin and Eosin

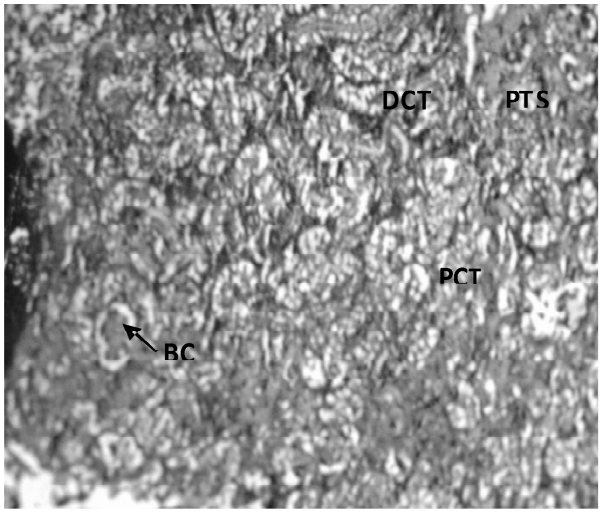

Fig.1 Normal kidney of fish showing Bowman's capsule(BC) and glomerulus (G), number of collecting tubule(CT), proximal convoluted tubules (PCT), distal convoluted tubule(DCT) and peritubular space (PTS). X 200

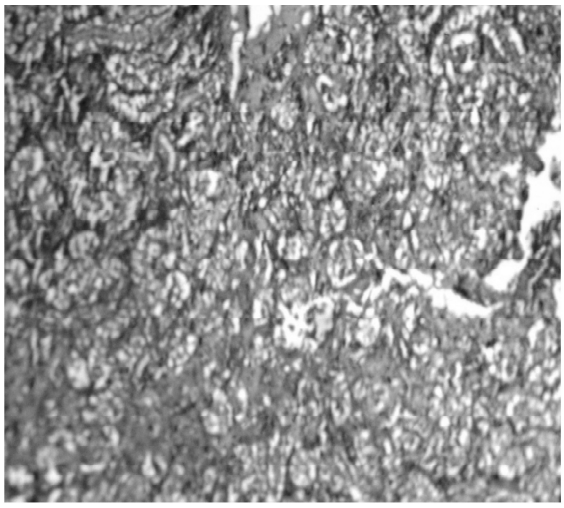

Fig.2. Rogor treated (7days) kidney of fish showing slight degeneration in renal tissue. X 200

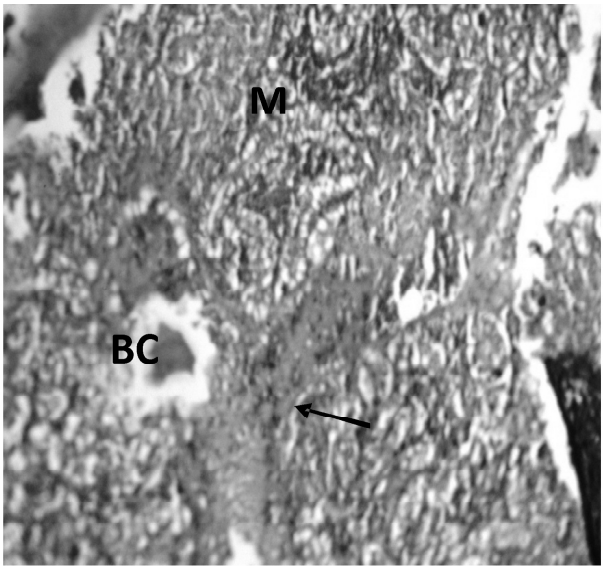

Fig.3. Rogor treated (14days) kidney of fish showing highly increased peritubular space (PTS), excessive vacuolation in distal convoluted tubule (DCT), collecting tubules (CT), inter tubular space shows infiltered macrophages (M),leucocytes (L), acidophils (A), ramnents of cellular debris(arrow) . X 200

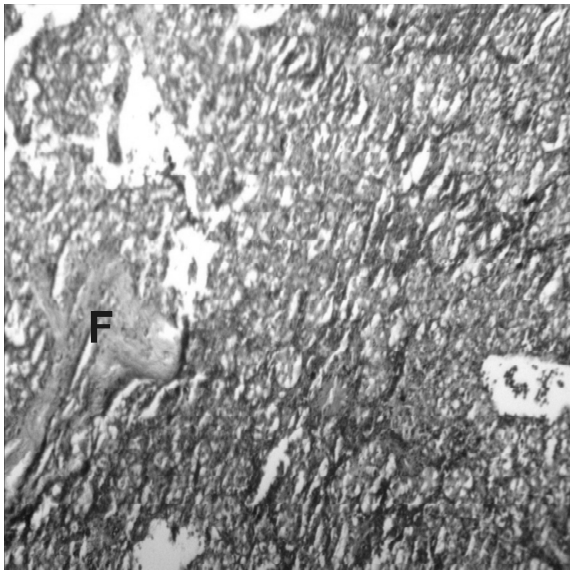

Fig.4 Rogor treated (21days) kidney showing increase fibrosis and hyalinization in renal portal areas.complete acellularity in renal cellsX 200

PLATE II

Photomicrographs of transverse section of 0.008µl/L endosulfan treated kidney of *Clarias batrachus* stained with Haematoxylin and Eosin:

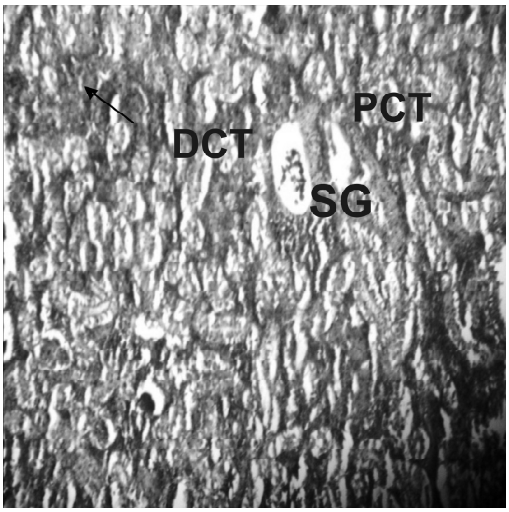

Fig.1. The transverse section of 0.008µl/L endosulfan treated (7days) kidney showing shrinkage in glomerulus(SG) forming clear space and starting of fibrosis(Arrow) at proximal convoluted tubules (PCT), distal convoluted tubule(DCT) . x 200.

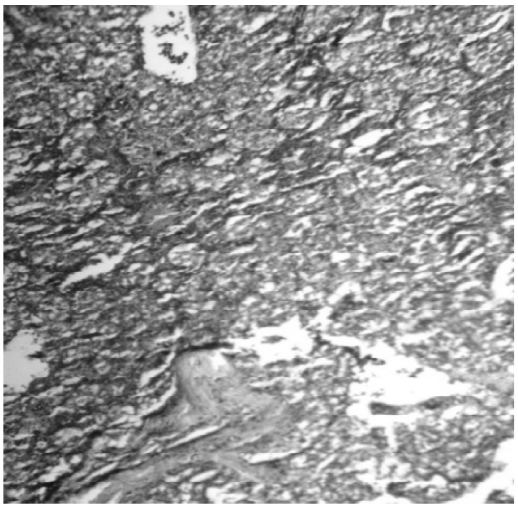

Fig.2. After 14days kidney showing extensive fibrosis of proximal convoluted tubules (PCT), distal convoluted tubule(DCT) . Some region showing autolysis (arrow head).X 200

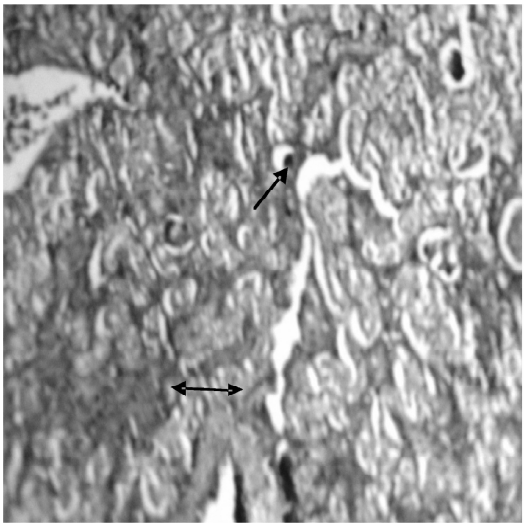

Fig.3 After 21days kidney showing shrinkage and increase vacuolation in glomerulus(arrow), abnormal shaped glomerular tuft, complete acellularity in proximal and convoluted tubule. Swelling of renal and interstitial fibrosis (IF) pointing towards chronic glomerulonephritis. X 200

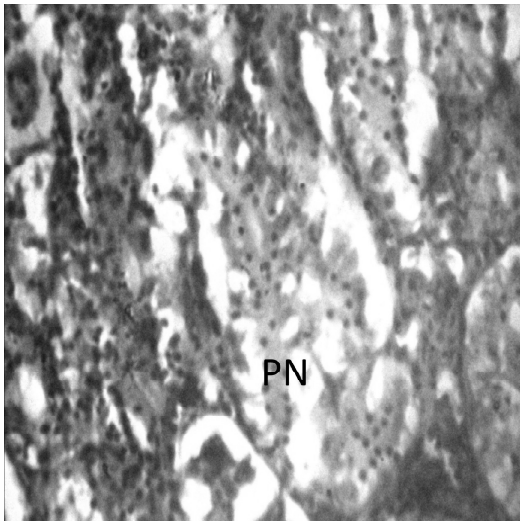

Fig.4. Magnified view showing increase vacuolation and picnotic nuclei (PN). Increase Accellularity of proximal convoluted tubules (PCT), distal convoluted tubule(DCT) . X 400

PLATE III

Photomicrographs of transverse section of (1.00µl/L rogor &0.004µl/L endosulfan) 21 days treated kidney of *Clarias batrachus* stained with Haematoxylin and Eosin :

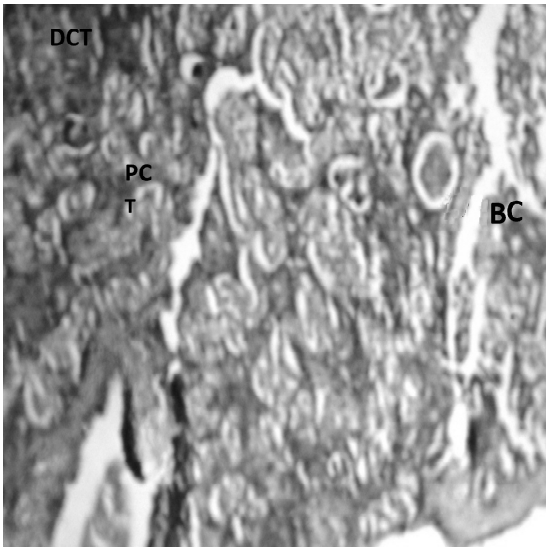

Fig.1. The transverse section of 1 µl/L rogor & 0.004µl/L endosulfan treated (7days) kidney showing extensive vaculation, loss of cellularity, increased autolysis of cellular organelles, fusion of bowman's capsule, no demarkation of proximal convoluted tubules (PCT), distal convoluted tubule(DCT) . 200

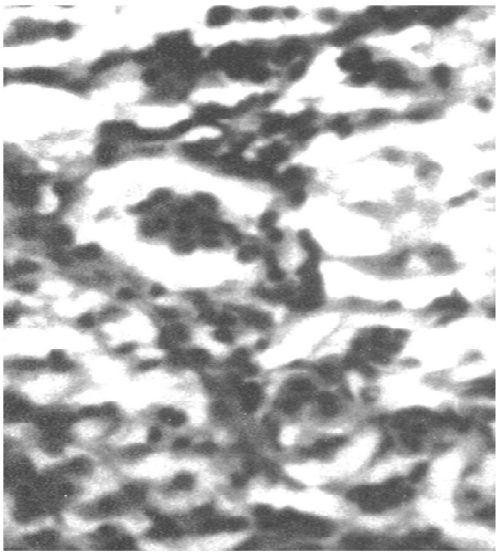

Fig.2.The transverse section of 1 µl/L rogor & 0.004µl/L endosulfan treated (7days) kidney showing extensive vaculation, loss of cellularity, increased autolysis of cellular organelles, fusion of bowman's capsule, no demarkation of proximal convoluted tubules (PCT), distal convoluted tubule(DCT).Magnified view .X 400

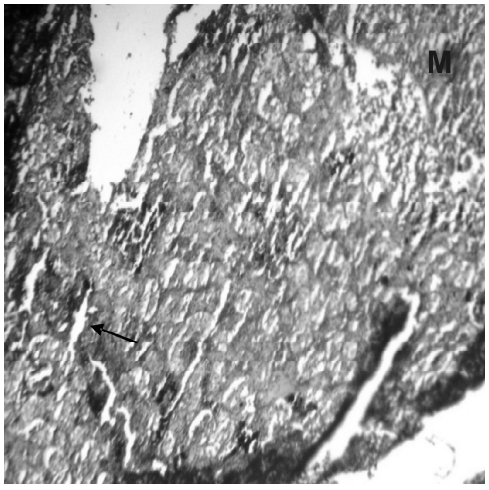

Fig.3. The transverse section of (rogor+endosulfan) treated (14days) kidney showing increase peritubular space(PTS) and complete disintegration of renal tubules, decrease in luminal diameter(LD), infiteration of several; macrophages(M). X200.

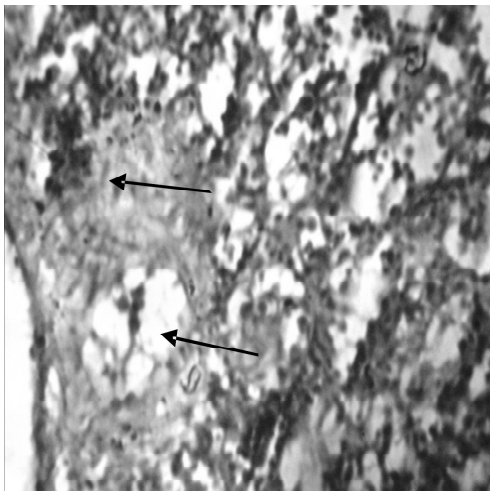

Fig.4. Magnified view showing of (rogor+endosulfan) treated(21 days) almost empty proximal convoluted tubule and interstitial fibrosis filled with odeomatous fluid is specific feature. X 400

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