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IV. RESULTS

1- HISTOLOGY, HISTOCHEMISTRY AND CYTOPHOTOMETRIC ANALYSIS

1.1 Liver

1.1.1. Histology

1.1.1.1. Normal Liver :

On using Heidenhain's iron haematoxylin method , the liver appears to be composed of laminae (Fig. 1) that are separated by blood sinusoids . Each lamina is two cell thick .

The liver cells , hepatocytes are large , polygonal parenchymal cells with spherical nuclei , which have dispersed chromatin and central deeply stained nucleolous (Fig. 2) . The cytoplasm of the hepatocytes is ususally vacuolated. The vacuoles are of different size formed probably of lipid droplets , (Fig. 2) .

The blood vessels are surrounded by a thin layer of smooth muscle fibers and , the sinusoids contain two types of cells ; flattened endothelial cells and large fixed macrophages ,or kupffer cells (Fig. 2) . The endothelial cell contains a thin ellipsoid nucleus . The kupffer - like cells occur at various points along the sinusoidal lumen , which contain oval dense nuclei (Fig. 1 ,2) .

The liver of grass carp contains several hepatopancreatic ducts , which contain some pancreatic cells (Fig. 1) .

Fig. (1) :Normal grass carp liver showing; **S:** sinusoid, **En** :endothelial cells; **K:**Von kupffer cells **HPD:** hepatopancreatic duct , **Arows;** showing one of the laminae (H X & E x 500).

Fig. (2) : Normal grass carp liver showing; **H;** hepatocytes . **Va;** intracellular vacuole , **Bs:** blood sinusoid **En** : endothelial cells . **NEn** , nucleus of endothelial cells (H X &E x 1250).

1.1.1.2 . Liver of fish treated with 10 mg/ L Sevin.

After treatment with 10 mg /L Sevin , some differences in the general structure represented mainly in the collapse of blood vessels (Fig. 3) are noticed in comparison to control .The parenchymal hepatocytes were also structurally affected in treated fish (Fig. 4) . The nuclei of the treated liver cells became irregular with signs of pyknosis or karyolysis (Fig.4) .The chromatin material of cell nuclei undergoing karyolysis became diffused , the marginal chromatin became thinner , and the nuceleolei became less conspicuous than in the normal liver cells (Fig. 2) . Some hepatocytes appear in different stages of degeneration . While a well defined cytoplasmic vacuole is observed in control hepatocytes , a large number of small vacuoles appear in treated liver giving the cell a foamy appearance .

The kupffer cells decreased in number after the exposure of the fish to the insecticide (Fig. 4) .

1.1.1. 3 . Liver of fish treated with 13 mg/ L Sevin

In fishes treated with 13 mg/ L Sevin , the collapse of blood vessels is more evident . Swelling and vacuolation of cells appears in the form of widely separated nuclei and foamy cytoplasm (Fig. 5) . Irregularity and swelling of hepatocyte nuclei is also clear (Fig. 6) . Signs of hemolytic activity is indicated by the appearance of some deterred erythrocytes. Kupffer cell nuclei appear fainter and ellipsoid (Fig. 6) .

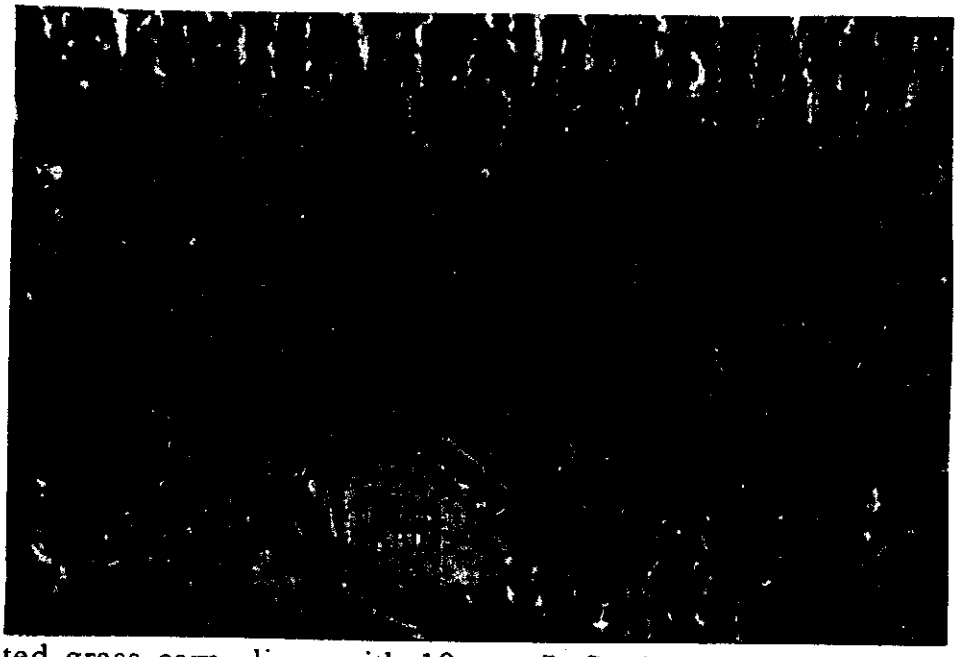

Fig. (3) :Treated grass carp liver with 10 mg /L Sevin showing; **HPD** : hepatopancreatic duct (H X &E x 250).

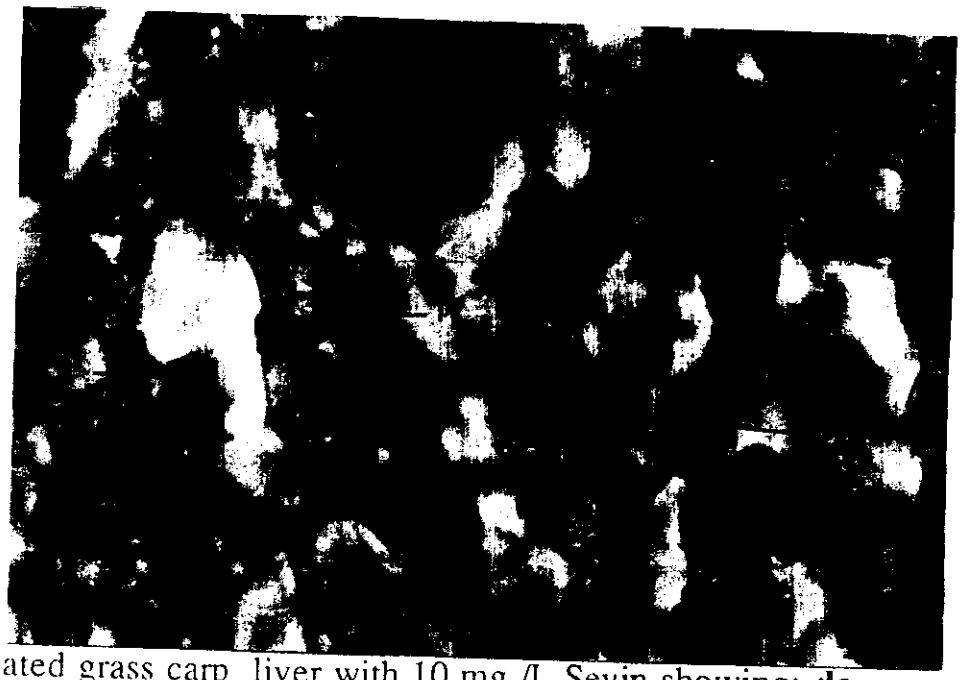

Fig.(4) :Treated grass carp liver with 10 mg /L Sevin showing; **de** :degenerated cells. **Kr:** karyolysis . **Py**: pyknotic nucleus .**Va**: Intracllular vacuole (H X &E x 1250).

Fig. (5) : Treated grass carp liver with 13 mg /L Sevin showing;The irregular shape of the rays of the liver cells (H X &E x 500).

Fig. (6) : Treated grass carp liver with 13 mg /L Sevin showing; **de** :degenerated cells .**Kr:** karyolysis .**Py**: pyknotic nucleus **De**:damaged erythrocyte **K:** kupffer cell (H X &E x 1250).

1.1.2. Histochemistry

1.1.2.1 Nuclear DNA in liver cells :

On using Feulgen , DNA of the nucleus of hepatocytes and kupffer cells , was purple (Fig. 7) .

1.1.2.1a, Nuclear DNA in liver of control fish .

The hepatocyte nuclei showed a moderate reaction with Feulgen , but an intense reaction was obnserved in the nuclei of the kupffer cells (Fig. 7,8) .

1.1.2.1b, Nuclear DNA in liver of the fish treated with 10 mg/ L Sevin

After treatment with 10 mg/ L Sevin , a weak reaction was noticed in the hepatocyte nuclei and also in the nuclei of the kupffer cells (Fig. 9 ,10) .

1.12.1c. Nulear DNA in liver of the fish treated with 13 mg/ L Sevin

The extent of the purple colour was less in cells of fish treated with 13 mg/ L. Sevin than those treated with 10 mg/ L (Fig. 11,12) .

1.1.2.2, Cytoplasmic RNA in liver cells

DNA and RNA were illustrated by the methyl green - pyronin method , where the methyl green stained the DNA of the nuclei greenish , and the pyronin stained RNA of the cytoplasm red to purple .

1.1.2.2a , Cytoplasmic RNA in liver of control fish .

The cytoplasm of the hepatocytes showed a moderate reaction with pyronin stain in the form of granules , scattered around the nucleus , but an intense reaction was observed in the cytoplasm of the hepatopancreatic cells (Fig. 13 ,14) .

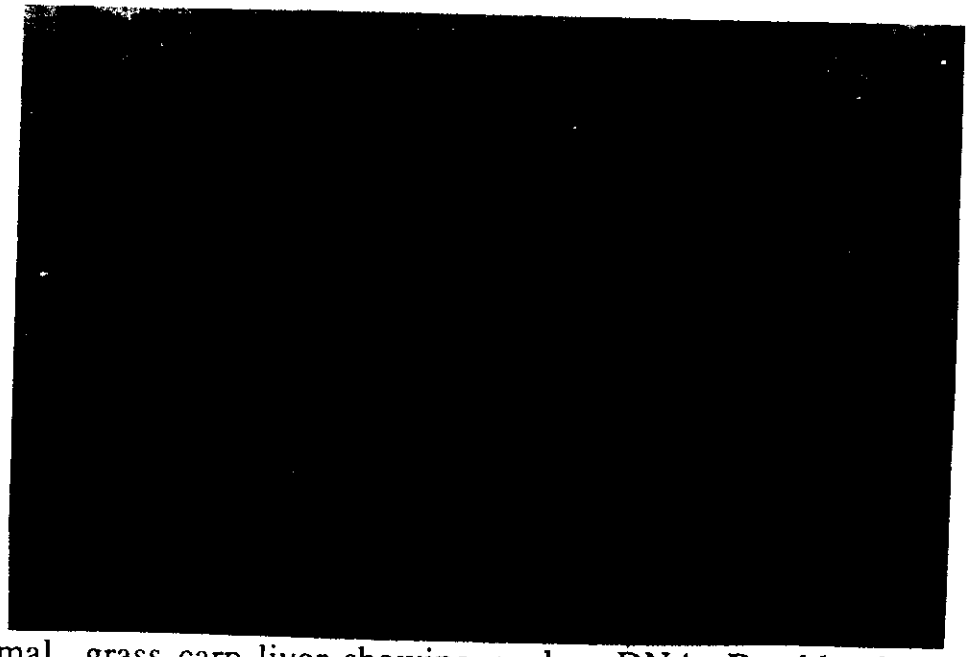

Fig.(7) : Normal grass carp liver showing nuclear DNA: **Bc.** blood cell nuclei ,**Kn** : kupffer cell nucleus (Feulgen x1250)..

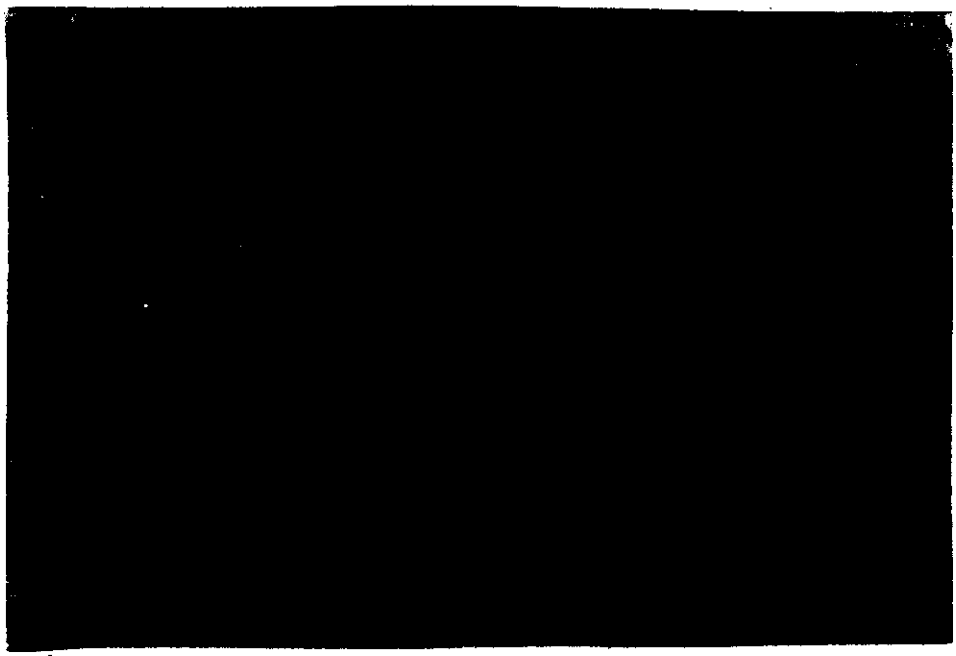

Fig.(8): Normal grass carp liver showing nuclear DNA: **K:** kupffer cell nuclei, **Hn**: hepatocyte nuclei **NEn**:endothelial cell nucleus . (Feulgen x1250).

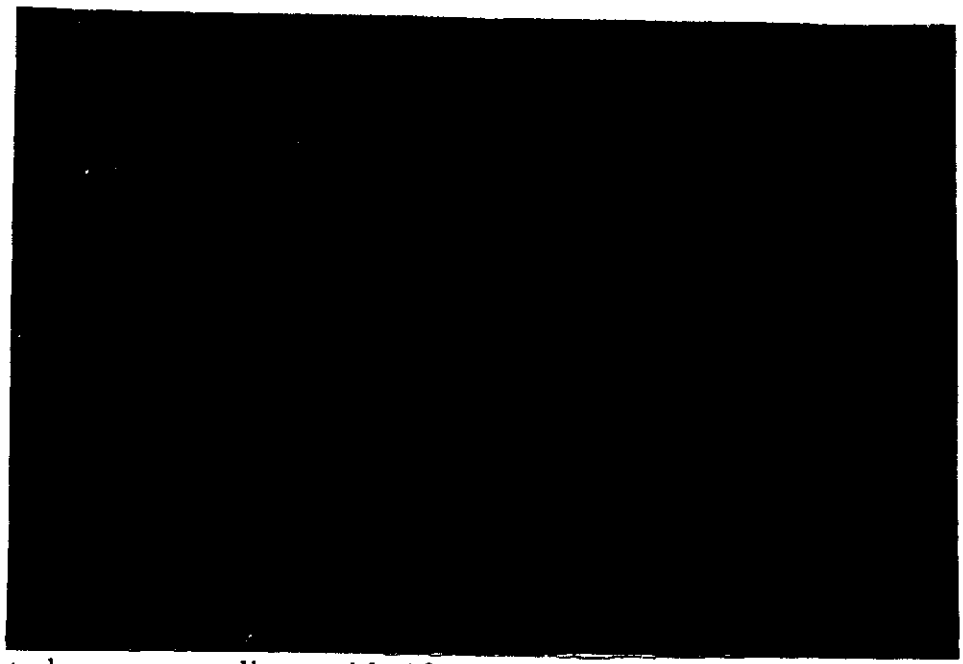

Fig.(9) : Treated grass carp liver with 10 mg /LSevin showing the nuclear DNA ;**Kn:** kupffer cell nuclei, **Hn:** hepatocyte nuclei.
(Feulgen x500).

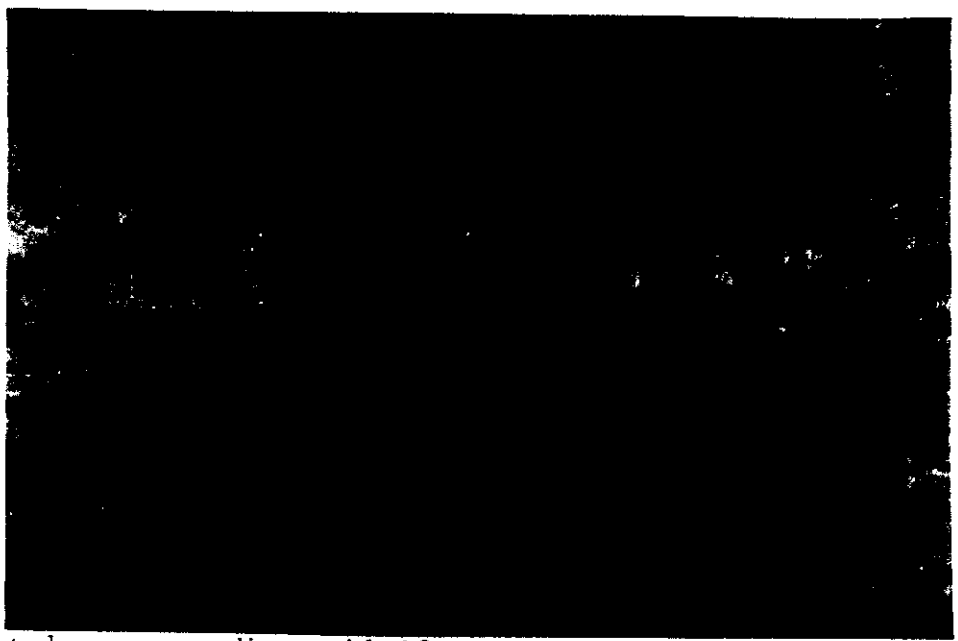

Fig. (10) :Treated grass carp liver with 10 mg /L Sevin showing the nuclear DNA ,**Kn:** kupffer cell nuclei .; **Hn:** hepatocyte nuclei.
(Feulgen x1250).

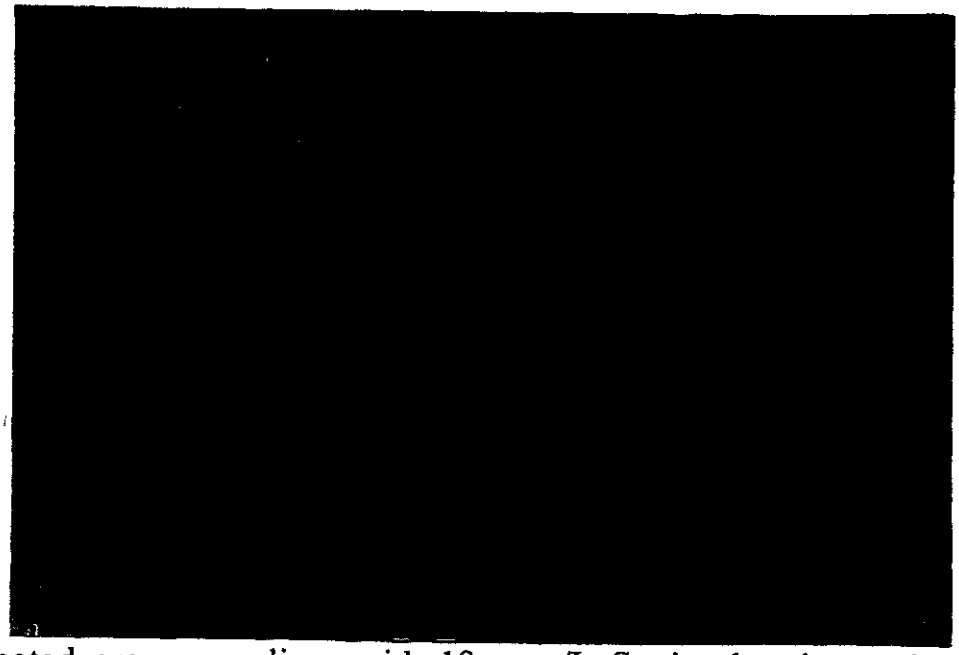

Fig. (11) : Treated grass carp liver with 13 mg /L Sevin showing the nuclear DNA ;**Kn:** kupffer cell nuclei ., **Hn:** hepatocyte nuclei. (Feulgen x1250).

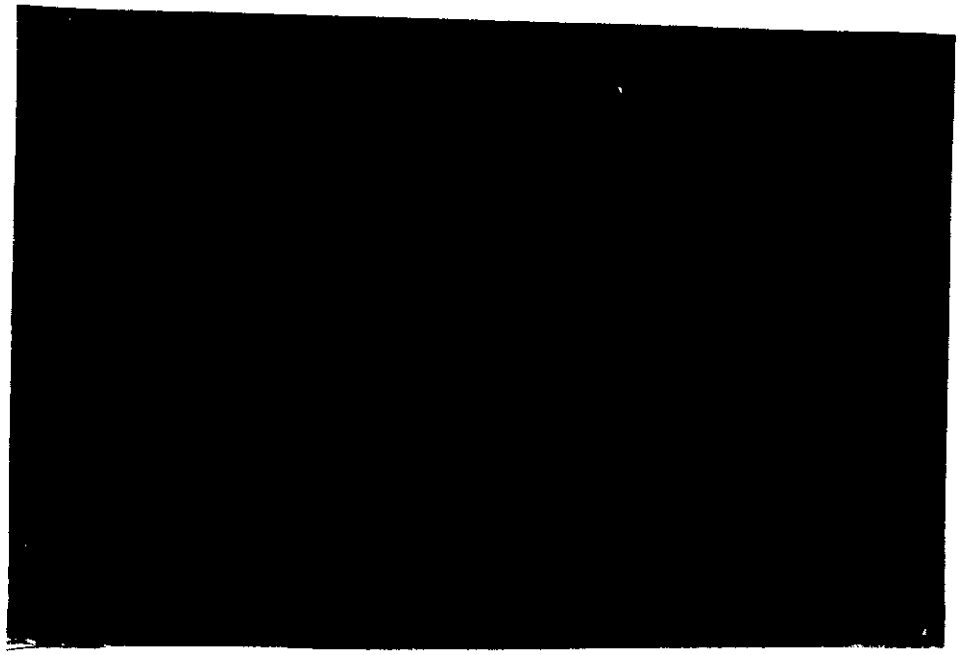

Fig. (12) : Treated grass carp liver with 10 mg /LSevin showing the nuclear DNA; **Kn:** kupffer cell nuclei ., **Hn:** hepatocyte nuclei. (Feulgen x1250).

1.12.2b , Cytoplasmic RNA in liver of the fish treated with 10 mg/L Sevin

After treatment with 10 mg /L Sevin the intensity of the colour increased in the cytoplasm of hepatocytes and kupffer cells (Fig. 15 ,16) .

1.12.2c, Cytoplasmic RNA in liver of the fish treated with 13 mg/ L Sevin

Even more intense reaction was noticed in liver cells of fish treated with 13 mg/ L Sevin (Fig. 17 ,18) .

1.1.2.3. Iron pigments in liver

The control ,10 and 13 mg/ L Sevin treated liver , gave almost negative reaction with Turnbull Blue method for iron (Fig. 19, 20 and 21) . The positive reaction appears as few blue spots associated with kupffer cells.

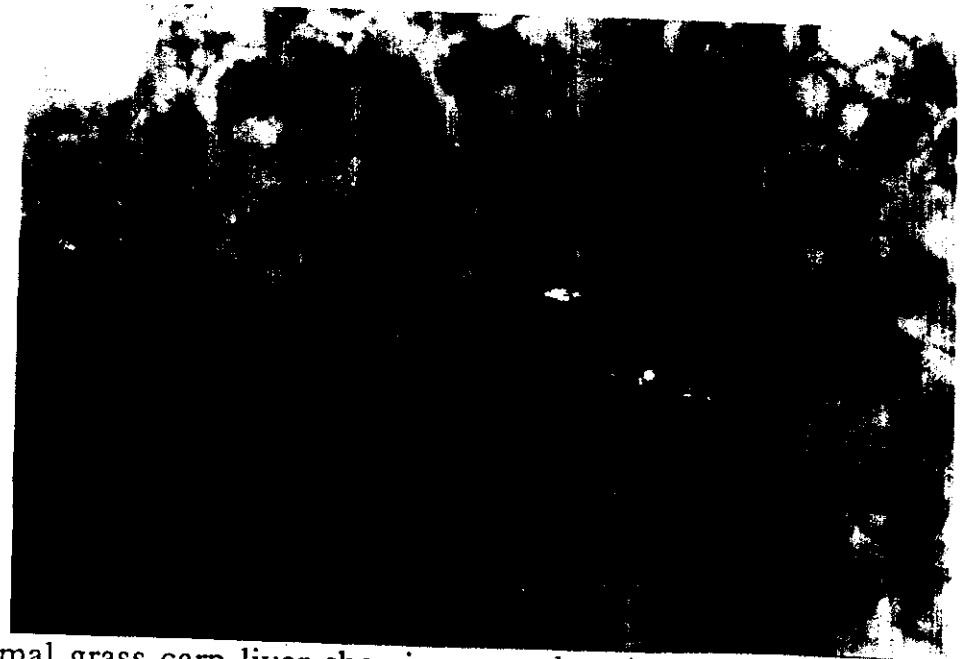

Fig.(13) :Normal grass carp liver showing cytoplasmic RNA **K:** kupffer cell . **Hn:** hepatocyte nuclei. **Hc.** hepatocyte cytoplasms (Methyl green - pyronin x1250).

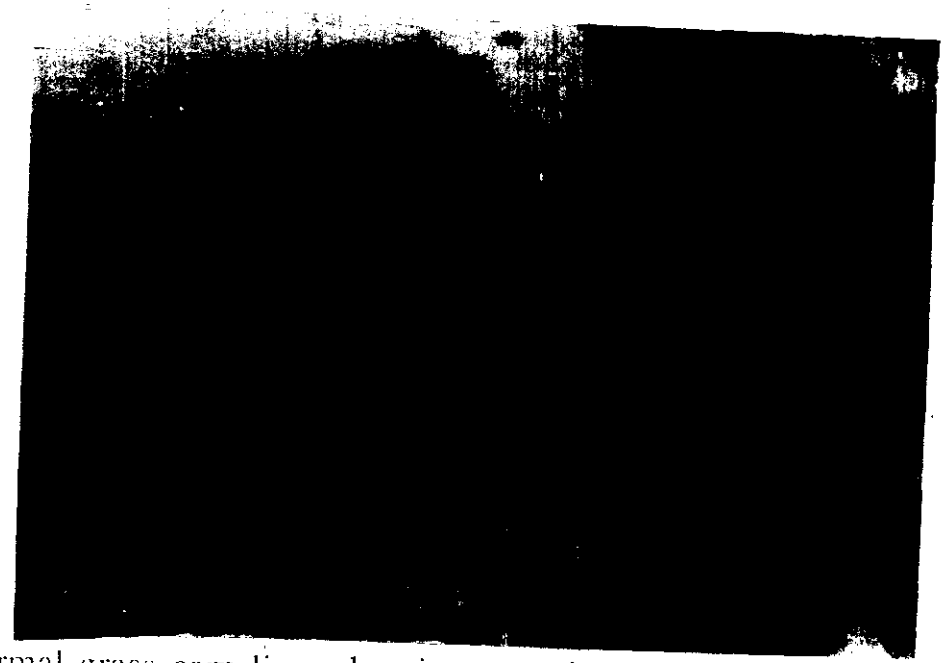

Fig. (14) : Normal grass carp liver showing cytoplasmic RNA **K:** kupffer cell nuclei **Hn:** hepatocyte nuclei. **Hcy.** hepatocyte cytoplasms : **HPD** ,hepatopancreatic duct (Methyl green - pyronin x1250).

Fig.(15) : Treated grass carp liver with 10 mg / L Sevin showing cytoplasmic RNA ; **K:** kupffer cell . **Hn:** hepatocyte nuclei.,**Hcy:** hepatocyte cytoplasms . (Methyl green - pyronin x1250)

Fig.(16): Treated grass carp liver with 10 mg / L Sevin showing cytoplasmic RNA ; **K:** kupffer cell . **Hn:** hepatocyte nuclei., **Hcy:** hepatocyte cytoplasms (Methyl green - pyronin x1250) .

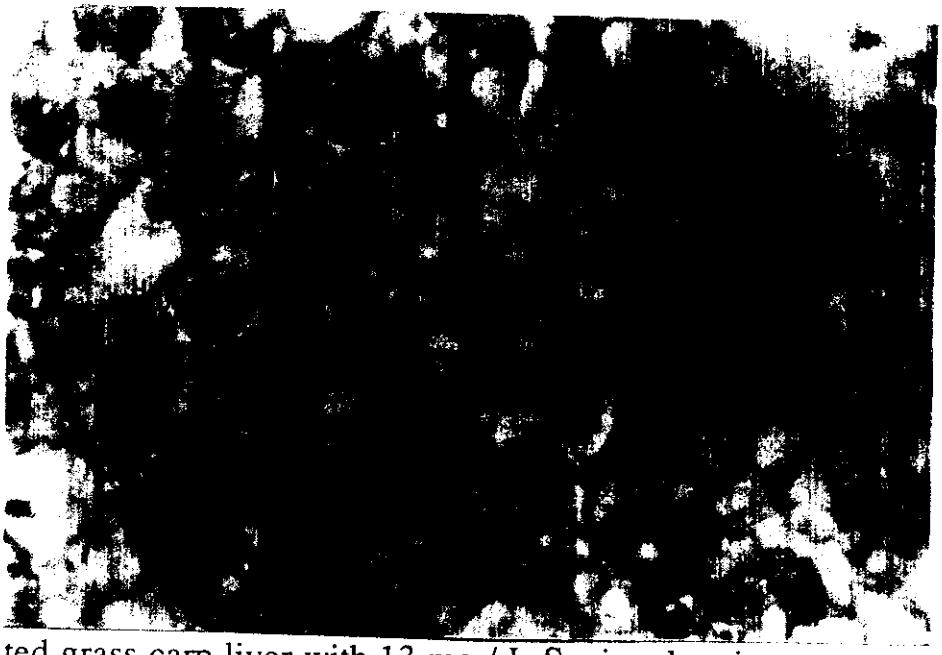

Fig. (17) : Treated grass carp liver with 13 mg / L Sevin showing cytoplasmic RNA ; **K:** kupffer cell . **Hn:** hepatocyte nuclei.; **Hcy:** hepatocyte cytoplasms (Methyl green - pyronin x1250)

Fig.(18) : Treated grass carp liver with 13 mg / L Sevin showing cytoplasmic RNA; **K:** kupffer cell . **Hn:** hepatocyte nuclei.,**Hcy.** hepatocyte cytoplasms **HPD** ,hepatopancreatic duct . (Methyl green - pyronin x1250).

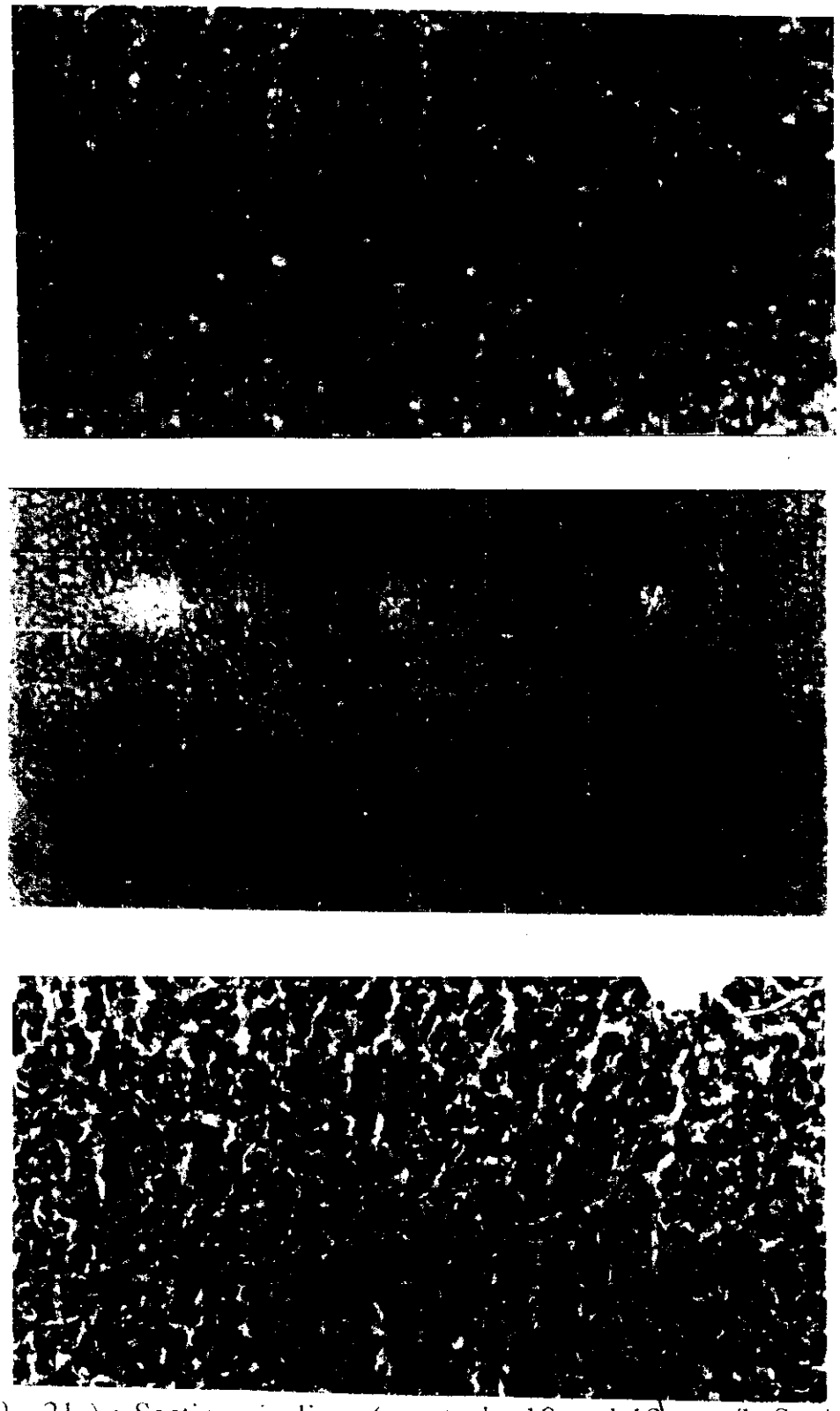

Fig. (19 , 20 , 21) : Sections in liver (control , 10 and 13 mg /L Sevin) showing negative reaction with Turnbull Blue method for iron (Turnbull Blue x 250) .

1.1.3.Cytophotometric analysis :

1.1.3.1 , Nuclear DNA content of liver cells in control and treated fish .

The mean optical density value indicative of nuclear DNA content of hepatocytes shows a statistically significant higher value for the control (0.202) as compared to 10 mg/L Sevin (0.133) and 13 mg/L Sevin (0.132) groups (Table .1) . There is ,however, no statistically significant difference between 10 and 13 mg/ L Sevin treatments(Table , 1 and Fig. 22) .

Frequency distribution of cells containing different amount of DNA suggests an effect of Sevin on liver cell DNA synthesis (Fig. 23) . In treated fish hepatocytes , a large number of cells appear in the area probably occupied by diploid nuclei . S. phase nuclei , however, are fewer in both treated groups than in control . While very few nuclei appear in the zone occupied by G_2 - phase in 10 mg/ L treated hepatocytes , non are formed in 13 mg/L treated .

1.1.3.2, Cytoplasmic RNA of liver cells in control and treated fish

The mean optical density values for RNA control in the cytoplasm of control hepatocyte (0.540) is significantly lower than that of 10 mg/L Sevin (0.610) and 13 mg/ L Sevin treated fish cells (0.668) (Table 2 and Fig. 24) .

The increase in the optical density of the stain relative to RNA content was dose dependent showing statistically significant difference .

Table (1) : Mean values for the amount of DNA expressed as O.D.units cytophotometrically measured from slides of liver cells stained with Feulgen .

Dose mg Sevin/L	mean O.D.	statistical analysis					
		SD.	SE	t- test treated x control		t -test treated x treated	
				value	P	value	P
control	0.202	0.030	3.159×10^{-3}				
10 mg/L	0.133	0.027	2.882×10^{-3}	16.136	≤ 0.001		
13mg/L	0.132	0.032	3.464×10^{-3}	14.931	≤ 0.001	0.220	N.S*

* N.S. = non significantly .
Statistically significant using student (t) test at P ≤ 0.001

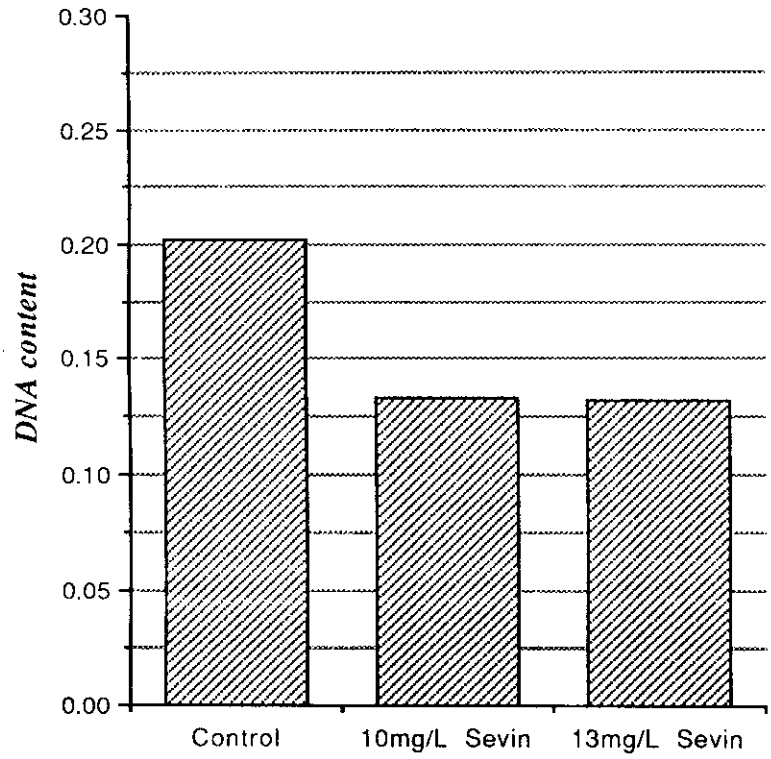

Fig.(22) : DNA content of liver cells (Control & Treatred).

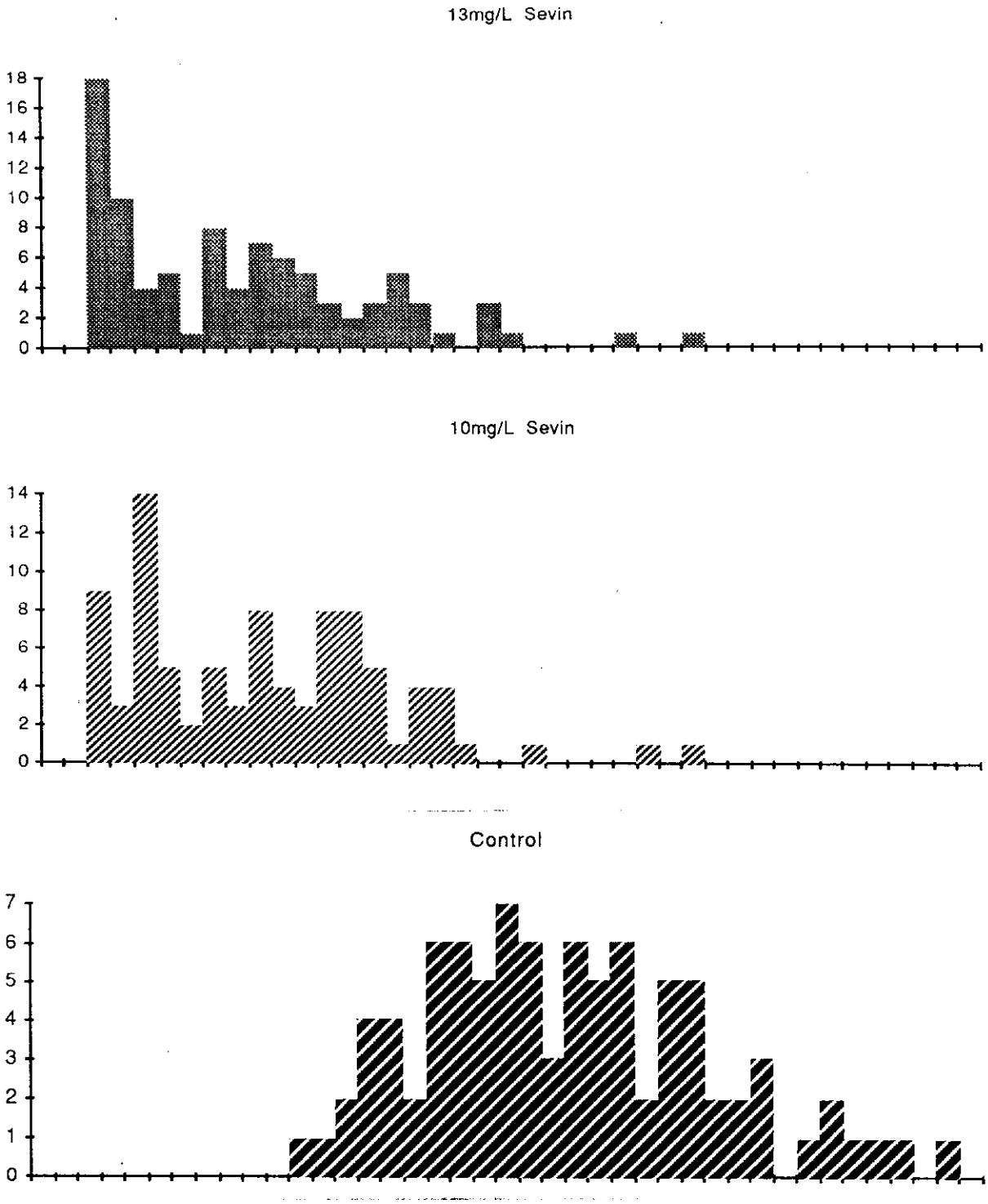

Fig. (23) : Frequency distribution of liver cells containing different amounts of DNA expressed as O.D. units cytophotometrically measured from slides stained with Feulgen.

Table (2) : Mean O.D.units cytophotometrically measured from slides stained with pyronin to represent the amount of cellular RNA in control and Seven treated fish hepatocytes.

Dose mg Sevin/L	mean O.D.	statistical analysis					
		SD.	SE	t- test treated x control		t -test treated x treated	
				value	P	value	P
control	0.540	0.063	6.597×10^{-3}				
10 mg/L	0.610	0.064	6.780×10^{-3}	7.400	≤ 0.001		
13mg/L	0.668	0.066	6.977×10^{-3}	13.331	≤ 0.001	5.962	≤ 0.001

Statistically significant using student (t) test at P ≤ 0.001

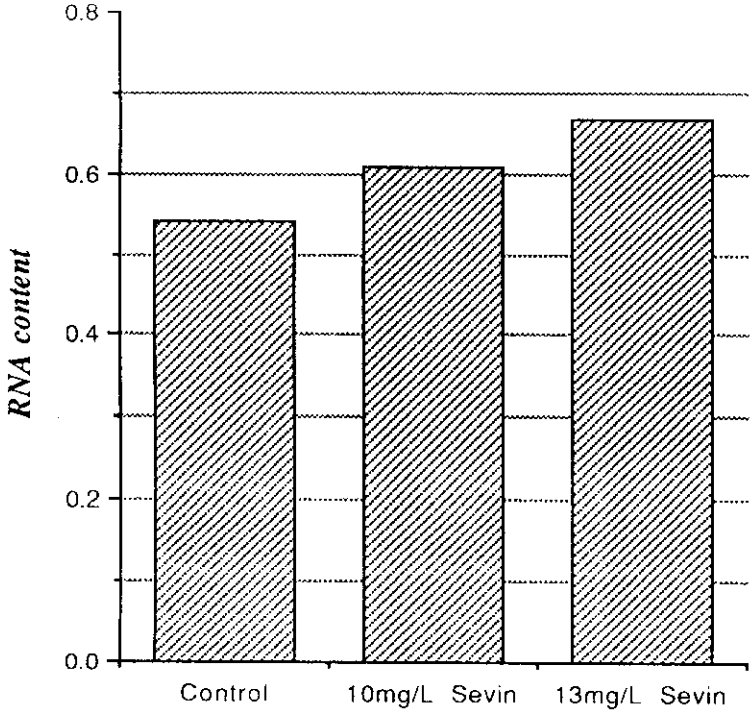

Fig.(24) : RNA content of liver cells (Control & Treatred).

1.2.Kidney

1.2.1. Histology

1.2.1.1. Normal Kidney

The kidney of the grass carp consists of nephrons and collecting tubules with haemopoietic tissue occupying the intertubular spaces . Peritubular spaces which represent blood sinusoidal spaces are prominant structures in histological sections .The nephron is composed of the glomerulus with Bowman's capsule , neck segment , proximal convoluted segment and distal convoluted segement (Fig. ,25, 26 ,27) .

The glomerular membrane is delicate and its tuft is heavily vascularized . Epithelial cells of the proximal convoluted segment are composed of high columnar cells and are intensely eosinophilic , with a brush border (Fig. 25) .

The convoluted tubules contain two types of cells in their walls which are described for the first time as far as it is presently known . A type with basal spherical large vesicular nucleus alternating with another with more apical oval smaller dense nucleus (Fig. 25, 26 ,43, 44) . The apical nuclei contain more DNA than the basal ones (Fig. 36 ,37) . Peritubular sinusoids are found around both types of tubules, which are connected to a network of blood sinusoids in the interstitial tissue in which the haemopoietic tissue appears in island - like organs (Fig. 25 , 26 , 36 ,37, 43 , 44 , 45 , 50) .

The glomerular space leads to a segment connected to the proximal

segment . The cells of distal convoluted tubules have less prominent brush border (Fig. 25) , the epitelium of which is cuboidal or columnar and distinctly less eosinophilic than that of the proximal convoluted segment . As in the proximal convoluted tubules , the wall of the distal convoluted tubules have two alternating types of cells , one with basal , large , rounded vesicular nuclei and another with apical , smaller , oval darkly stained nuclei .

The haemopoietic tissue is found inbetween the renal tubules in a variable shaped and sized cells and also the nuclei take irregular shape (Fig. 26, 27) . All over the tissue of the kidney , there is a network of sinusoids lined with flat epithelia .

1.2.1.2 , Kidney of fish treated with Sevin

When fish were transfered to an environment of, 10 and 13mg/L Sevin , the swimming activity was increased and the fish moved upwards and down - wards in the aquaria . Very characteristic changes were observed in the structure of the kidney . Both the glomerular capsule and its tuft shrinked after immersion in this concentration . The space between Bowman's capsule and tuft bacame larger (Fig. 28, 29) . The proximal convoluted segment was greatly affected after the treatment , which show a decrease in the diameter of the lumen signs of cytolysis appeared in some cells of the proximal and distal convoluted tubules especially in 13 mg/L Sevin treated fish (Fig. 31,32). The intertubular sinusoids appear highly dilated , while the haemopoietic cells were largely decreased in number occupying much less space (Fig. 37 , 38 , 39 ,40) as compared to control (Fig. 33 , 34) .

In the haemopoietic tissue , some differences in the general structure were observed represented in a smaller number of cells undergoing division (Fig. 30, 32) as compared to control (Fig. 27) .

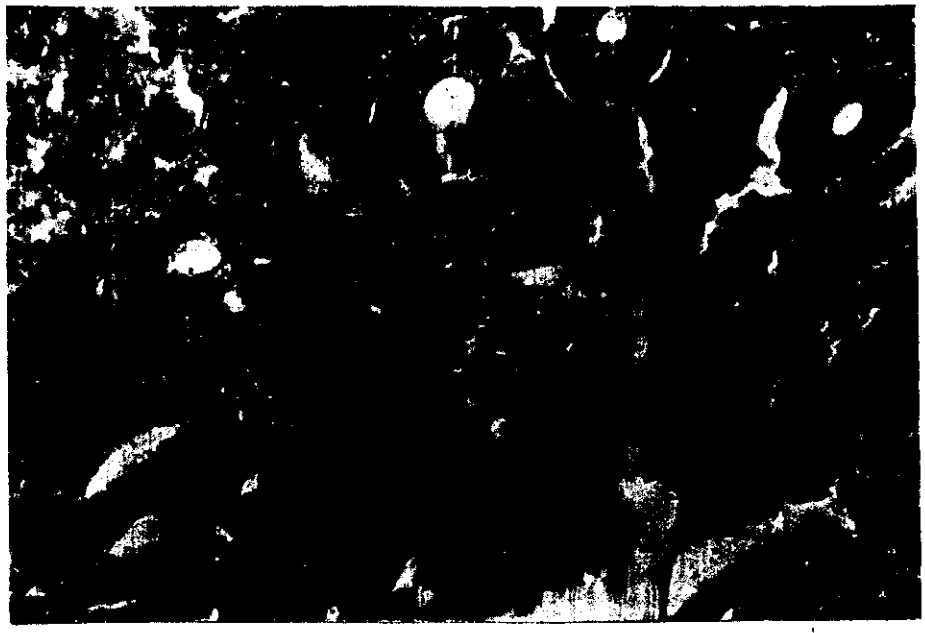

Fig. (25) : A Photomicrograph of T.S. of grass carp kidney showing the general structure.Note;**d:** distal convoluted segment . **P**.proximal convoluted segment with brush border (**b.b)** **G:** glomerular structure with Bowman's capsule **ht:** haemopoietic tissues . **Bs;** Blood sinusoid (H X &E x 500).

Fig. (26) :A photomicrograph of T.S. of normal grass carp kidney showing : **ht:** haemopoietic tissues **d:** dista convoluted segement **Pd** : peritubular duct . (H X &E x 1000).

APPROVAL SHEET

EFFECT OF POLLUTION ON HAEMOPOIETIC CELLS OF FRESH WATER FISHES IN QUALYOBIA PROVINCE

Thesis Advisors:	Approved
Prof. Dr. Fathy E. Mattar	.....
Prof. Dr.Nabil M. El-Nagar	.....
Assistant Prof. Dr. Sabry S. El- Serafy	.....
Dr.Mohammed E. M. Zowail	.....

Head of Zoology Department

.....

Assistant Prof. Dr. Sabry S. El- Serafy

DEDICATION

To
my
Family

The Systematic position of the GRASS CARP according to Berg*(1940).

Phylum	: Chordata
Subphylum	:Vertebrata
Superclass	: Agnatha
Class	: Osteichthyes
Subclass	: Actinopterygii
Order	: Cypriniformes (Ostariophysi)
Suborder	: Cyprinoidei
Family	: Cyprinidae

Ctenopharyngodon idella

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* *Berg , L.S. (1940)* :" Classification of fishes both recent and fossil". In "**Ichthyology**" 2nd edition (1977) " Ed.T Lagler , K.F.; Bardach, J.E. ; Miller , R.R. and Passino , D.R.M. p30 , 506 pages.(Jphn Wiley & Sons , Inc.)

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