WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 5, 884-909. Research Article ISSN 2277 – 7105

EFFICACY OF *CONVOLVULUS MICROPHYLLUS* ROOT EXTRACTS IN REVERSING CARBOFURAN INDUCED OVARIAN ANOMALIES IN *CLARIAS BATRACHUS* (Linn): AN ULTRA STRUCTURAL STUDY

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Article Received on
20 May 2014,

Revised on 14 June 2014,
Accepted on 08 July 2014

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ABSTRACT

The impact of aqueous extract of *Convolvulus microphyllus* root (CMR) was investigated against carbofuran induced ovarian atrophy in fresh water air breathing fish *Clarias batrachus*. Fishes were exposed to 1.5 mg L^{-1} of carbofuran for 1, 2 and 4 weeks respectively. These fishes were further treated with aqueous extract of CMR for a period of 30 days @ 50 mg kg^{-1} body weight daily orally by gastric intubation. The T_3 (Triiodothrionine), T_4 (Tetra iodo thrionine) and TSH (Thyroid stimulating hormone) in blood serum were assayed. The histopathological examination of ovarian tissues was done on light microscopy and transmission electron microscopy. The serum level of T_3, T_4 and TSH showed a significant elevation of carbofuran treated fish when compared with control. They showed a significant recovery after CMR treatment for 20 days. Concomitant treatment of carbofuran and CMR to the experimental group showed non significant (at $P<0.05$) changes in serum T_3,T_4 and TSH .The CMR treatment alone to control group didn't show any significant correction in elevated serum T_3,T_4 & TSH. The histopathological examination of ovarian tissues of corresponding groups showed a sign of retrieving towards normalcy. A perfect co-relation between hormone level and histopathology reveals the ameliorating activity of CMR against carbofuran induced ovarian anomalies in fish.

KEY WORDS: Carbofuran,T_3, T_4 ,TSH, Clarias batrachus, Convolvulus microphyllus.

1.0 INTRODUCTION

Increased reliance on fishes as prime source of food, employment and recreation has been witnessed in last two decades. The wide spread use of pesticides, as one of the implication of green revolution, has resulted in the pollution of many aquatic habitats worldwide. Pesticides

enter the aquatic system by different routes including direct applications, urban and industrial discharge, surface run-off from non point sources including agricultural soils, particulate deposition and rainfall etc. Among different pollutants, carbamate group of pesticides require special attention because of their high stability and toxicity to the aquatic organism[1].

The deleterious effects of carbofuran on the gonads of different group of fishes have been well elucidated[2][3][4][5]. Various hormonal factors are known to regulate gonad maturation, reproductive behavior and spawning in fishes[6][7][8][9].Environmental cues are translated into chemical messengers by several central mechanism which activate and maintain the reproductive organs[10].

Since ancient times the plant based formulations have been used as remedial measures against various human and animal ailments. But their wide spectrum use in aquatic organisms is still in its infancy stage. Some of medicinal plant extracts have been used as curative agents against various intrinsic diseases in aquatic organisms[11][12][13][14].

Shankhpushpi (*Convolvulus microphyllus, family*-Convolvulacae) is a fulvous, hairy herb and occurs in the plain of Northern India specially Nagwa Forest, Gwalior. The bioassay and phytochemistry of medicinal herb is well established by Central Drug Research Institute (CDRI), Lucknow. The plant root contains alkaloids like Convolvine, Convolamine, Phyllabine, Convolvidine, Scopolin & β-Setosterol[15]. The root extracts of *Convolvulus microphyllus* (CMR) have been shown to regulate serum T_3, T_4 and TSH. It is further associated in the regulation of testosterone and dihydroepiandosterone secretion from Leydig's cell in mice[16][17].Based upon its role in restoring gonad function in mice, in the present investigation, the aqueous CMR extract has been assessed against carbofuran induced ovarian anomalies in fish, on the basis of hormonal and ultra structural histopathological studies.

2.0 MATERIALS AND METHODS

2.1 Animal selected

Fresh water air breathing fish *Clarias batrachus* (Linn.), commonly known as "Mangur" of 60± 10 gm in weight and 12 ± 2 cm in length of same age group were collected from Nalanda Medical College fish Farm, which is situated at 25°23'N and 85°21'E and 5.3 meter above sea level, during spawning season (June-Aug). The average temperature fluctuations recorded were 23-26°C and relative humidity was 82-73%. The fish were brought to the laboratory,

disinfected with 0.01% $KMnO_4$ solution and kept in four large cylindrical metallic aquaria of 150 cm (diameter) X 180 cm. (height) having a constant flow of dechlorinated tap water. Fish were acclimated for 15 days in laboratory condition. A water temperature of 10°C and a constant photoperiod of (12 hrs light-12 hrs dark) were maintained .The aerated water was changed daily during morning hour.

2.2 Pesticide used

Carbofuran is a broad spectrum carbamate group of pesticides. According to Extoxnet, it is a restricted use pesticide (RUP). In most of the state of India including Bihar, the analytical grade carbofuran (2,3 dihydro,2,2 dimethyl, 7 benzo furanyl methyl carbamate) EC 3%, manufactured by Tata Rallis India Ltd. is most widely used pesticide against soil foliar dwelling pests viz. wire worm , white grub, millipeds, symphylides, fruit flies, been seed flies, root flies, flea beetles, weevil, sciaenid flies, aphids, thrips and nematodes in vegetables and other crops. Through agricultural run -off these pesticides assimilate to surface ground water of major aquatic bodies supporting fisheries industries. Fish get easily victimized to the deleterious impact of the pesticide. So the pesticide is selected for study.The 96 hrs. LC_{50} of carbofuran for fish was calculated by standard method [18]. The upper and lower limit of pesticide at which 100% and 0% fish mortality could be seen, were calculated by pilot test using different concentration of pesticides . The logarithmic intermediate concentrations were selected as for 48 hrs and 96 hrs respectively. It was calculated as 2.5 mg L^{-1}. The fishes were exposed to 1.5 mg L^{-1} of carbofuran for 1 week, 2 weeks and 4 weeks respectively, since four weeks are ideal for studying the deleterious impact of pesticides on any animal[19].

2.3 Biochemical kits

All the reagents and kits were of analytical grade. The thyroid kits (3rd generation micro well ELISA kit- MTY T_3 096, Total thyroxin MTY T_4 096 and Thyrophin MTY-TSH 048 manufactured by mono-bind Inc. California, USA) were purchased from Lilliac medicare Pvt.Ltd.,Mumbai. The sensitivity range of the kits was calculated as two times the SD from 5-10 pg for T_3, T_4 and TSH, the sensitivity was 0.5µIU/ml.

2.4 Medicinal plant used

In the experimental protocol, aqueous extract of *Convolvulus microphyllus* root (CMR) have been selected for evaluating their ameliorating impact against pesticides. The CMR were procured from Sanjay Gandhi Botanical Garden, Patna. The plant was identified and

authenticated by Late Prof. M.O.Siddiqui, Head, Dept. of Botany, P.U. and a voucher specimen was preserved in our laboratory.

2.5 Preparation of plant extract

Lyophilized aqueous extract of CMR was prepared as per standard method[20]. The freshly collected roots were weighed, washed and thoroughly grinded to a paste in mortar and then homogenized in Potter Elvehjem Homogenizer. It is dried in an incubator for 40°C for 2 days. It is further dissolved in hot distilled water. The suspension was filtered under suction and the filtrate was freeze dried using Labcono freeze drier Model 75018 yielding brown residue.

2.6 Administration of plant extract

The NOEL (No Observed Effective Level) and MPD (Maximum Permissible Dose) were calculated as 10 mg kg^{-1}b.w and 60 mg kg^{-1}b.w respectively by probate analysis and confirmed by pilot test. The determination was based on behavioral, serological and histopathological examinations of the fishes. A dose of 50 mg kg $^{-1}$b.w was selected for 15 days and 30 days treatment, 50 mg of lyophilized CMR powder were dissolved in 10 ml distilled water. For making stock solution of 40 ml 200 mg of lyophilized CMR powder were dissolved in 40 ml distilled water. 0.1 ml of the stock solution, equivalent to 50 mg kg $^{-1}$ b.wt. was administered to the fish @ 10 gm $^{-1}$ b.w. orally through gastric intubation technique daily for 15 days and 30 days respectively.

2.7 Collection of the sample

On the termination of exposure day, blood sample of both control as well as experimental group were collected in a heparinized glass culture tube syringe from caudal vein. The serum was separated by centrifuging blood at 5000 rev./min for 10 min at 4°C and stored at -20°C for further hormonal assessment. Fishes were anaesthetized with MS222 and the ovarian tissues were carefully removed. For Electron Microscopic examination tissues were fixed in 2.5% gluteraldehyde in chilled (4°C) 0.2M phosphate buffer for 2-24 hrs. Post fixation was done in 1% OSO_4 in 0.1M phosphate buffer .They were dehydrated through graded series of alcohol, cleaned in toluene and embedded in araldite mixture and the Ultrathin sections of 60-90nm were cut on Leica ultracut microtome of Richert Jung super nova. The section were stained in uranyl acetate and lead citrate and viewed under 'Morgaginy' TEM and electron micrographs were taken at SAIF EM Unit, Dept. of Anatomy, AIIMS, New Delhi.

2.8 Hormonal assessment

The hormonal assessment of T_3, T_4 and TSH were done on Merck 'mini mios' ELISA reader, as per standard protocol of WHO (2002). The animals were categorized into five groups – Group (I)- Control group (C), Group II- CMR treated alone, Group III- carbofuran treated (CF), Group IV- CMR treated to pre carbofuran treated group CF+CMR) and Group V- Self healing group (SHG). In all the five group serum hormonal analysis for T_3, T_4 & TSH was done and histopathological studies of ovarian tissues were done at TEM level.

2.9 Statistical Analysis

For each blood serum T_3, T_4 and TSH assay, six observations at random were taken. The arithmetic mean was calculated and subjected to statistical analysis. The evaluation of each parameter was performed by its mean ±SD. The obtained data were subjected to paired't' test .Values at $P<0.05$, $P<0.01$ and $P<0.001$ were considered to be significant. All statistical analysis were done using Sigma Plot 8.0 version.

Table –1 Showing fluctuation in serum T_3, T_4 & TSH in different group of fishes

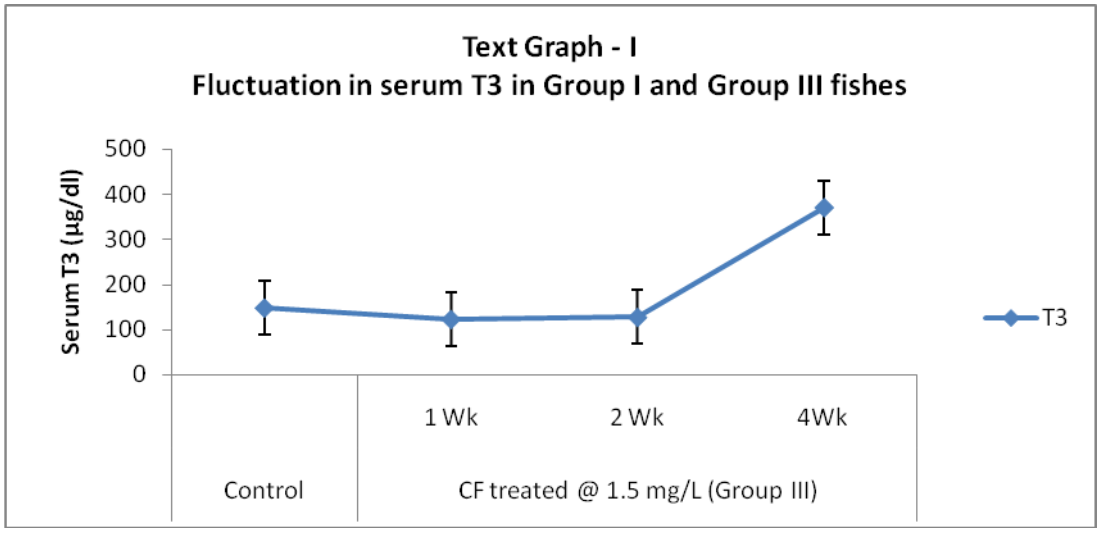
Serial No.	Parameters done	Control (Group I)	Control + CMR treated (Group II)		CF treated @ 1.5 mgL^{-1} (Group III)			CF and CMR treated (Group IV)						SHG (Group V)					
								CF one wk + CMR treated		CF two wk + CMR treated		CF 4 wk + CMR treated		one wk CF treated		2 wk CF treated		4 wk CF treated	
			I5Days	30Days	1 week	2 week	4 week	15Days	30Days	15Days	30Days	15Days	30Days	15Days	30Days	15Days	30Days	15D	30D
1	Serum T3 µg/dl	148.0± 0.201	147.5± 0.01	150.5± 0.664	122.7± 0.320***	127.24± 1.51**	370.065± 2.791***	130.16 ± 2.85**	158.33 ± 2.261***	131.64 ± 2.993*	134.64 ± 2.993**	358.73 ± 3.020*	354.73 ± 2.594**	123.6 ± 2.092	125.2 ± 1.026	118 ± 1.091	124.04 ± 1.019	345.65 ±2.012	325.02 ± 1.292*
2	Serum T4 µg/dl	0.25± 0.019	0.268± 0.018	0.283± 0.019	0.328± 0.004**	0.42± 0.068***	0.618± 0.169***	0.303 ± 0.073	0.286 ± 0.032	0.358 ± 0.032**	0.258 ± 0.009***	0.355 ± 0.02***	0.26 ± 0.027***	0.316 ± 1.620	0.312 ± 1.026	0.40 ± 1.22	0.38 ± 1.042*	0.602 ± 1.029	0.595 ± 2.32
3	Serum TSH µIU/ml	0.916 ± 0.194	0.936 ± 0.194	1.025 ± 0.142	1.05 ± 0.258	1.118 ± 0.2363*	1.87 ± 0.9310***	1.06333 ± 1.1899	0.996 ± 0.157	1.46 ± 0.298	1.638 ± 0.462	1.643 ± 0.582*	1.26 ± 0.587*	1.02 ± 0.921	1.011 ± 1.026	1.14 ± 2.036	1.78 ± 1.023***	1.84 ± 2.332	1.81 ± 0.239

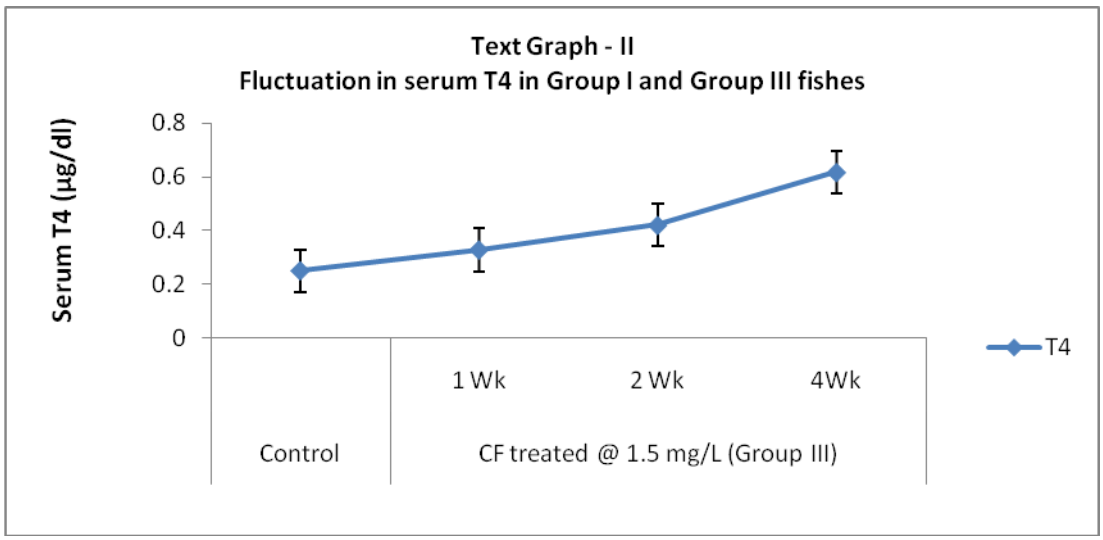
The values are expressed in Mean ± SD of six replicate (n=6) in each case. Paired 't' test have been applied between Group I & Group II, Group III & Group I, Group IV & Group III and Group V & Group III.

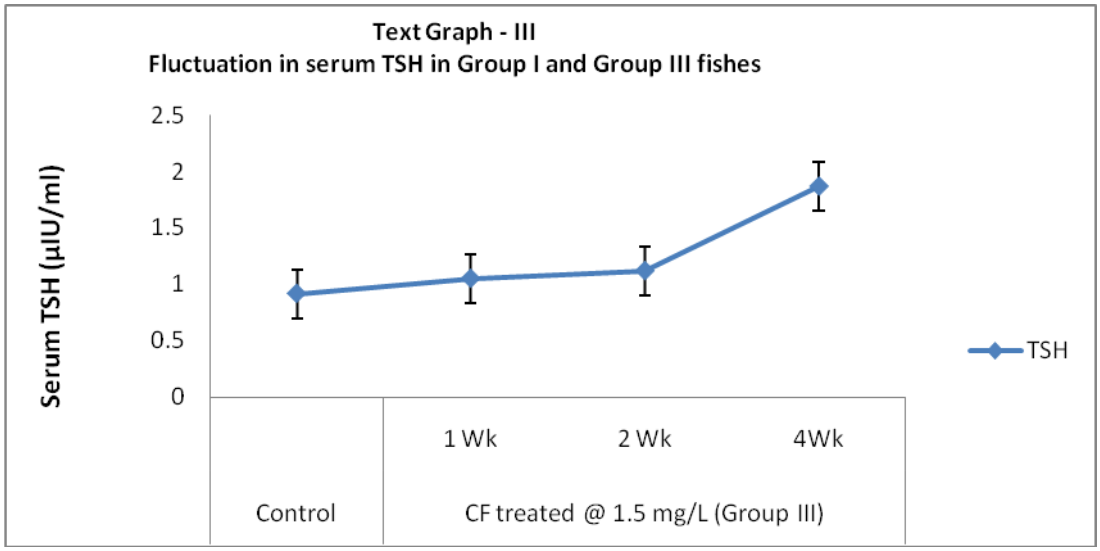
* = significant at P < 0.05

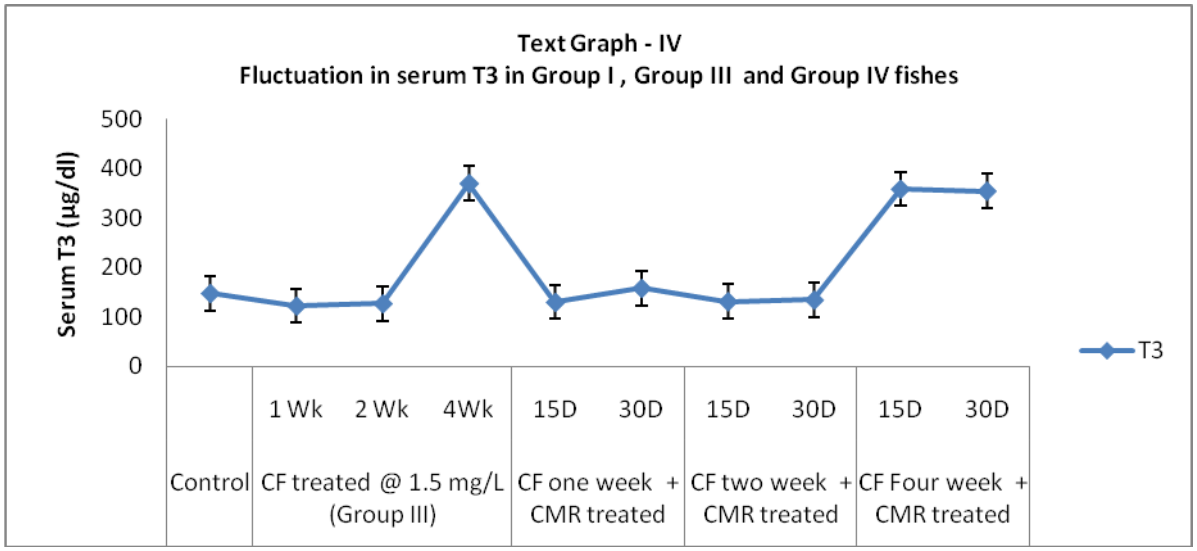
** = significant at P < 0.01

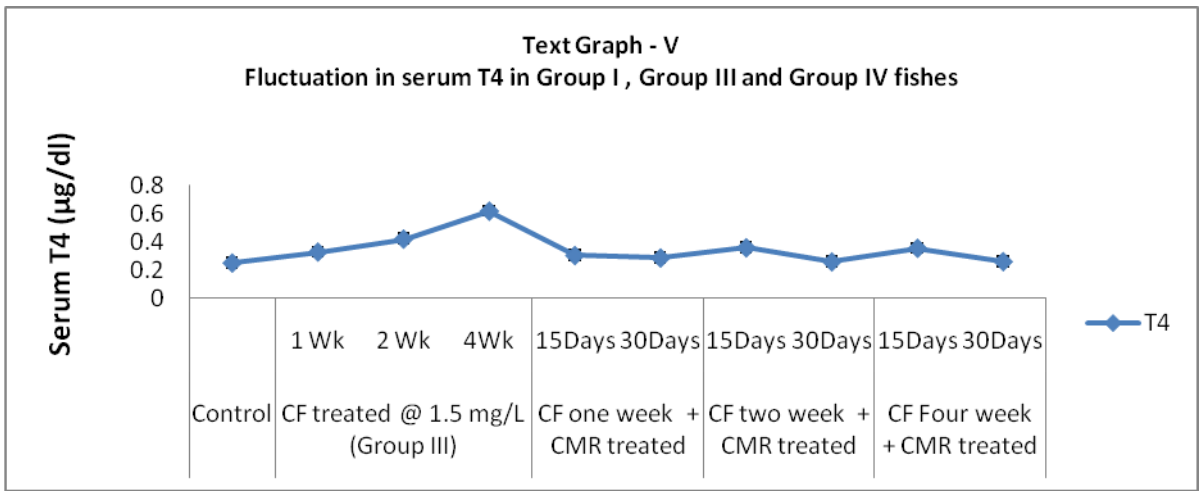
*** = significant at P < 0.001

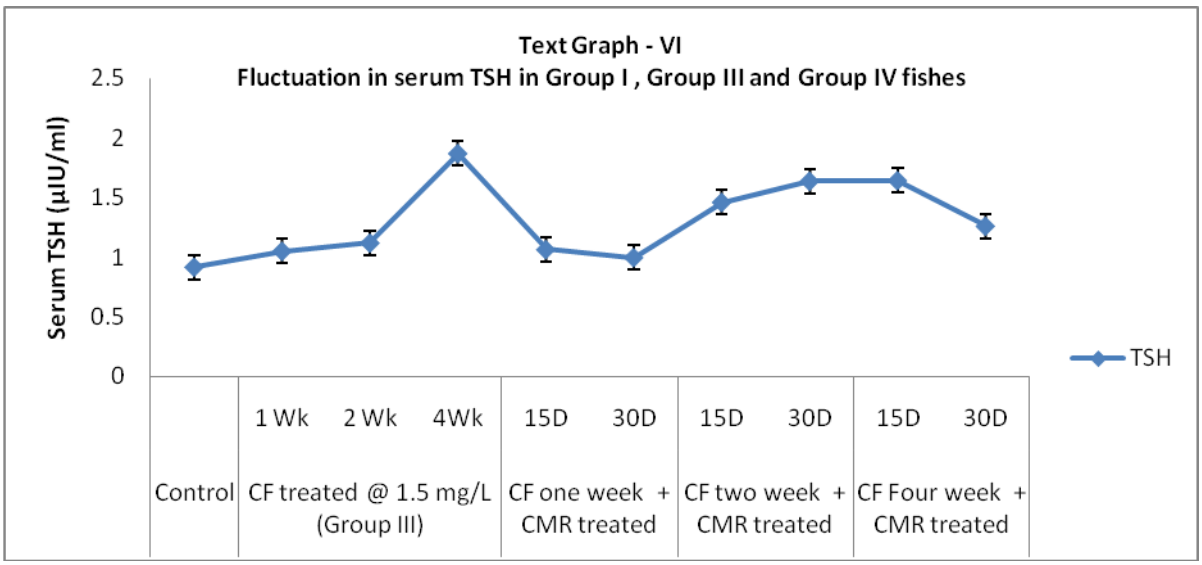

Text Graph - I
Fluctuation in serum T3 in Group I and Group III fishes
Serum T3 (µg/dl)
500
400
300
200
100
0
1 Wk
2 Wk
4Wk
Control
CF treated @ 1.5 mg/L (Group III)
T3

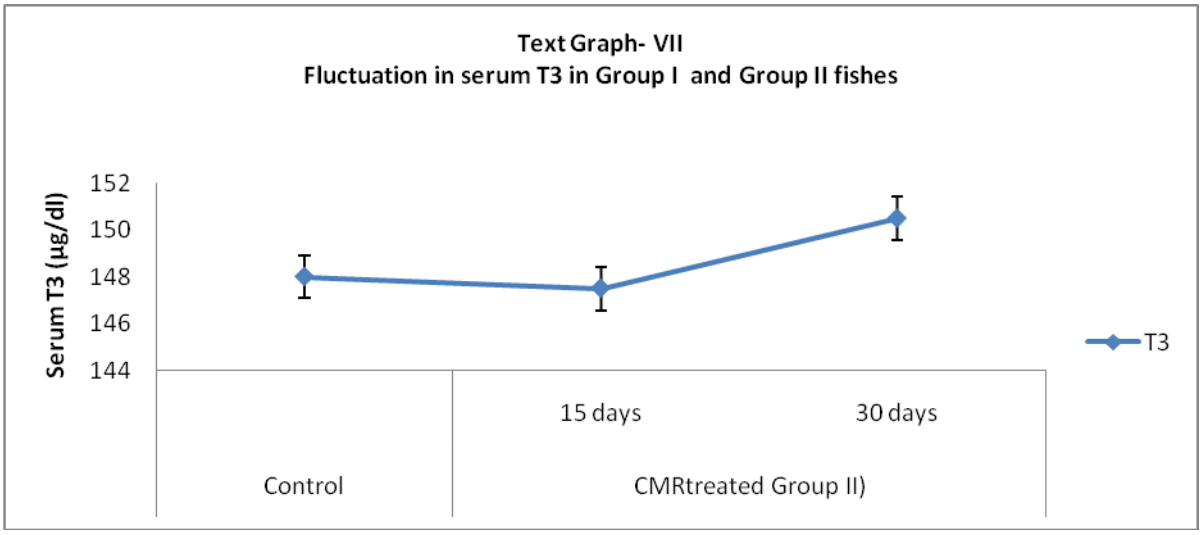

Text Graph - II
Fluctuation in serum T4 in Group I and Group III fishes
Serum T4 (µg/dl)
0.8
0.6
0.4
0.2
0
1 Wk
2 Wk
4Wk
Control
CF treated @ 1.5 mg/L (Group III)
T4

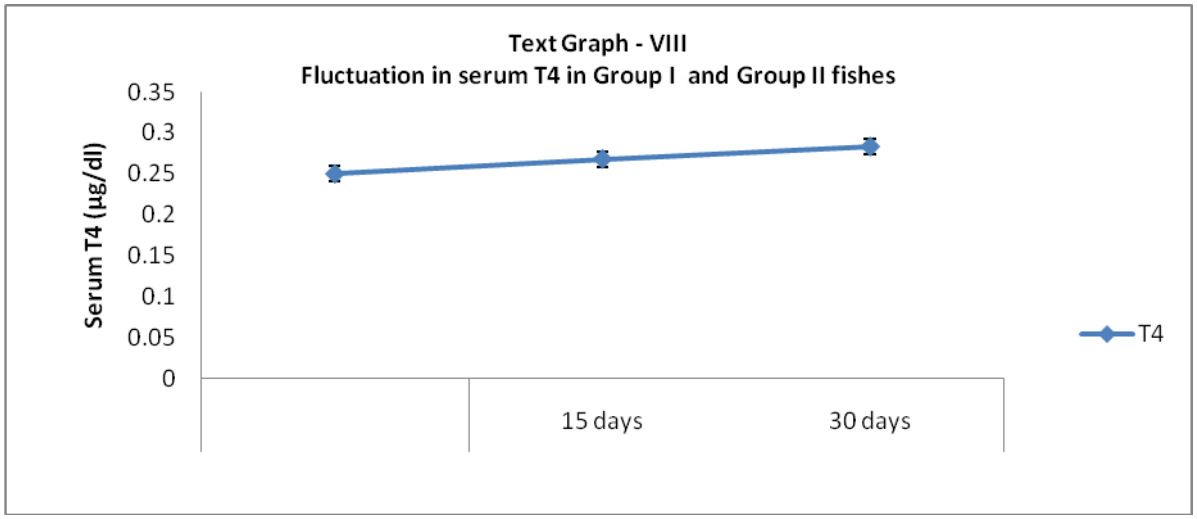

Text Graph - III
Fluctuation in serum TSH in Group I and Group III fishes
Serum TSH (µIU/ml)
2.5
2
1.5
1
0.5
0
1 Wk
2 Wk
4Wk
Control
CF treated @ 1.5 mg/L (Group III)
TSH

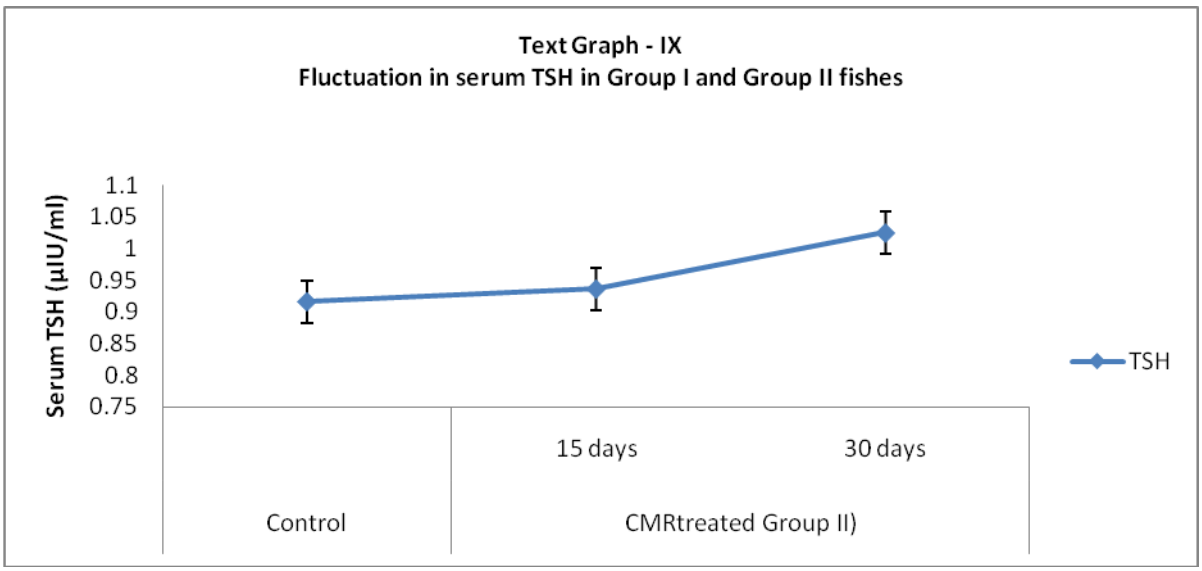

Text Graph - IV
Fluctuation in serum T3 in Group I , Group III and Group IV fishes
Serum T3 (µg/dl)
500
400
300
200
100
0
T3
1 Wk 2 Wk 4Wk 15D 30D 15D 30D 15D 30D
Control
CF treated @ 1.5 mg/L (Group III)
CF one week + CMR treated
CF two week + CMR treated
CF Four week + CMR treated

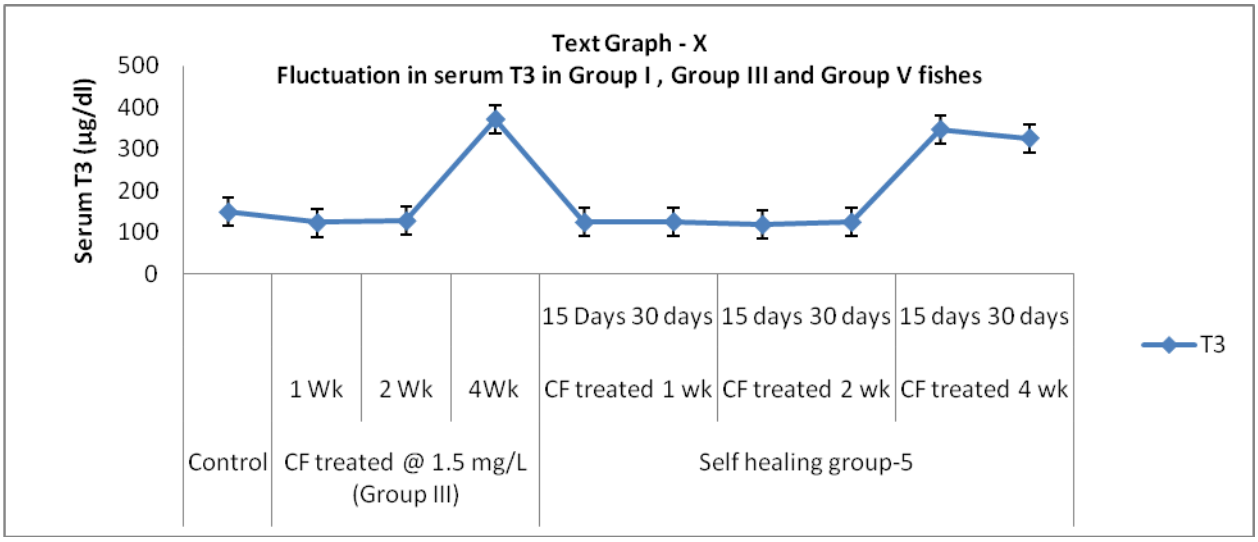

Text Graph - V
Fluctuation in serum T4 in Group I , Group III and Group IV fishes
Serum T4 (µg/dl)
0.8
0.6
0.4
0.2
0
T4
1 Wk 2 Wk 4Wk 15Days 30Days 15Days 30Days 15Days 30Days
Control
CF treated @ 1.5 mg/L (Group III)
CF one week + CMR treated
CF two week + CMR treated
CF Four week + CMR treated

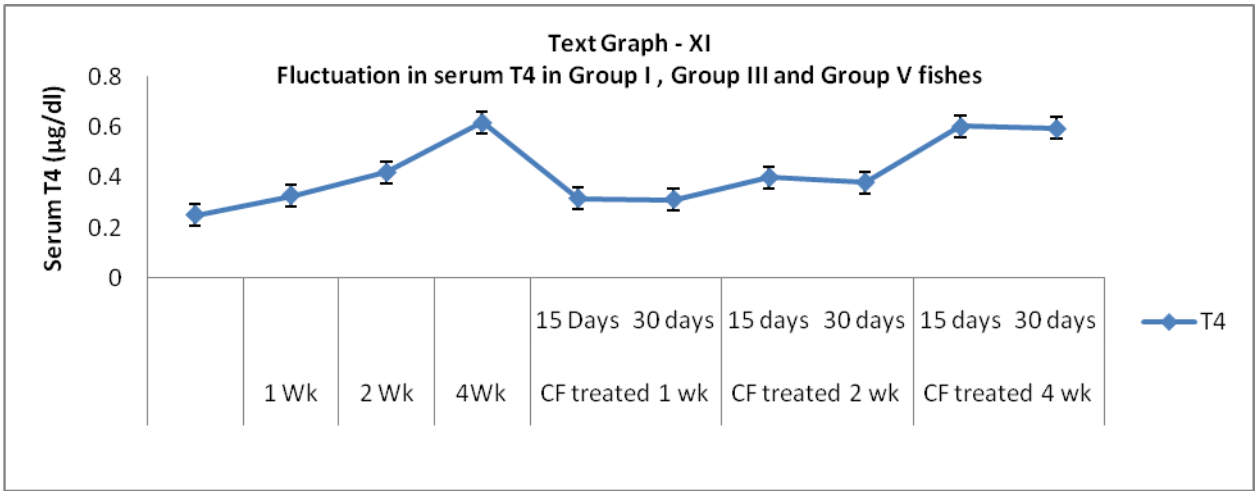

Text Graph - VI
Fluctuation in serum TSH in Group I , Group III and Group IV fishes
Serum TSH (µIU/ml)
2.5
2
1.5
1
0.5
0
TSH
1 Wk 2 Wk 4Wk 15D 30D 15D 30D 15D 30D
Control
CF treated @ 1.5 mg/L (Group III)
CF one week + CMR treated
CF two week + CMR treated
CF Four week + CMR treated

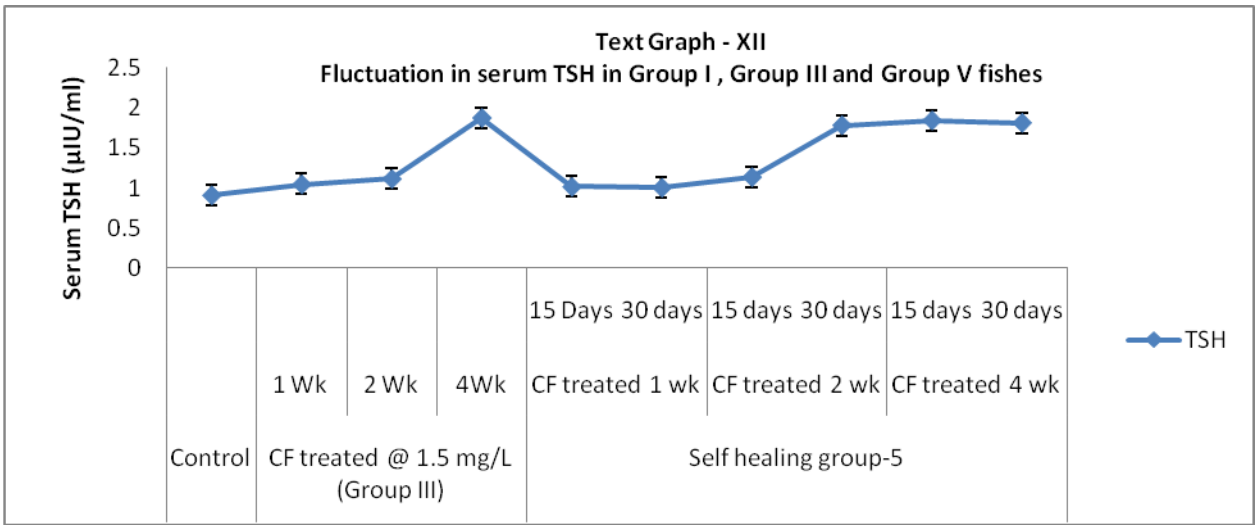

Text Graph- VII
Fluctuation in serum T3 in Group I and Group II fishes
Serum T3 (µg/dl)
152
150
148
146
144
15 days
30 days
Control
CMRtreated Group II)
T3


Text Graph - VIII
Fluctuation in serum T4 in Group I and Group II fishes
Serum T4 (µg/dl)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
15 days
30 days
T4


Text Graph - IX
Fluctuation in serum TSH in Group I and Group II fishes
Serum TSH (µIU/ml)
1.1
1.05
1
0.95
0.9
0.85
0.8
0.75
15 days
30 days
Control
CMRtreated Group II)
TSH


Text Graph - X
Fluctuation in serum T3 in Group I , Group III and Group V fishes
Serum T3 (µg/dl)
500
400
300
200
100
0
Control
1 Wk
2 Wk
4Wk
15 Days 30 days CF treated 1 wk
15 days 30 days CF treated 2 wk
15 days 30 days CF treated 4 wk
CF treated @ 1.5 mg/L (Group III)
Self healing group-5
T3


Text Graph - XI
Fluctuation in serum T4 in Group I , Group III and Group V fishes
Serum T4 (µg/dl)
0.8
0.6
0.4
0.2
0
1 Wk
2 Wk
4Wk
15 Days 30 days CF treated 1 wk
15 days 30 days CF treated 2 wk
15 days 30 days CF treated 4 wk
T4


Text Graph - XII
Fluctuation in serum TSH in Group I , Group III and Group V fishes
Serum TSH (µIU/ml)
2.5
2
1.5
1
0.5
0
Control
1 Wk
2 Wk
4Wk
15 Days 30 days CF treated 1 wk
15 days 30 days CF treated 2 wk
15 days 30 days CF treated 4 wk
CF treated @ 1.5 mg/L (Group III)
Self healing group-5
TSH

RESULTS

3.1 Effect of CMR extract on serumT_3 of different groups of fishes

The effect of CMR extract on serum T_3 of different group of fishes is shown in Table-1. carbofuran administration up to 2 week significantly decreased serum T_3 to 122.7±0.320 µg/dl from its normal value i.e. 148.0 ±0.201. But at 4 week exposure, carbofuran led to significant ($P<0.01$) abrupt increase in serum T_3 (370.065±2.799). Administration of CMR extract (50 mg kg^{-1}bw) for 30 days restored normalcy in otherwise reduced serum T_3 up to 2 week exposure of carbofuran significantly($P<0.05$), but failed to restore any normalcy in abruptly elevated serum T_3 after 4 weeks of carbofuran exposure. CMR extract treatment for both 15 days and 30 days to the control group showed non significant changes in serum T_3 i.e. 147.5±-0.01and 150.5±0.664 when compared to control. In SHG non-significant changes were noticed in serum T_3 for both 15 days & 30 days when compared to altered serum T_3 in Group III fishes.

3.2. Effect of CMR extract on serum T_4 of different groups of fishes:

The effect of CMR extract on serum T_4 of different group of fishes are shown in Table 1.Carbofuran administration at all the three durations i.e. one week, two week and four week showed a significant (at $P<0.05$) rise in serum T_4 (i.e. 0.328±0.004 µg/dl, 0.42±0.038 µg/dl and 0.618±0.169 µg/dl respectively) as compared to control (0.25 ± 0.019 µg/dl). Administration of CMR extract for 30 days to the carbofuran treated groups significantly lowered otherwise elevated serum T_4 i.e. 0.26±0.027 when compared to 0.618±0.169 µg/dl in 4 week carbofuran treated groups. CMR extract treatment for both 15 days and 30 days to the control group showed non-significant changes in serum T_4 i.e. 0.268±0.018 µg/dl and 0.283±0.019 µg/dl when compared to control. In SHG for both 15 days and 30 days non-significant changes were noticed in serum T_4, when compared to altered serum T_4 in different carbofuran administered groups.

3.3 Effect of CMR extract on serum TSH of different groups of fishes:

The effect of CMR extract on serum TSH of different groups of fishes are shown in Table – 1.Carbofuran administration at all the three durations i.e. one week, two week and four week exposure showed a significant ($P<0.05$) rise in serum TSH to 1.05±0.258, 1.118±0.236 and 1.87 ± 0.931 µIU/ml when compared to normal TSH value of 0.916±0.194 µIU/ml. Administration of CMR extract for 30 days to the different carbofuran treated groups showed

a general trend of significant ($P < 0.05$) decline in otherwise elevated serum TSH i.e. 0.996 ± 0.157 µIU/ml in contrast to 1.05±0.258 µIU/ml (at one week carbofuran exposure), 1.138±0.466 µIU/ml in contrast to 1.118 ± 0.236 µIU/ml (at 2 week carbofuran exposure) and 1.26±0.587 µIU/ml in contrast to 1.87 ± 0.9310 µIU/ml (at four week carbofuran exposure) respectively. In SHG, non-significant changes were noticed in serum TSH, when compared to different carbofuran administered groups.

3.4 Effect of CMR extract on cytoarchitecture of oocytes of different group of fishes:

While studying the Transmission Electron Micrographs of cytoarchitecture of oocyte of different group of fishes, interpretations were done for nearly ten micrographs in each case and after finding similar observations the best illustrated electron micrograph have been cited in the text plate. Transmission Electron micrographs of pre-vitellogenic oocytes of Group I fish showed uniform folding of plasma membrane well developed follicular epithelial cells and theca externa (Plate I, Fig.1). Besides abundant cytoplasmic inclusion, clusters of polyribosomes, smooth endoplasmic reticulum and mitochondria were present in ooplasm. Distinct nuclear membrane, homogeneous chromatin materials and nucleoli marked normal cytoarchitecture of oocytes (Plate-I, Fig.2).

Histopathological examination of transmission electron micrographs of oocytes of Group II (C+CMR treated) fishes showed almost normal cytoarchitecture of oocytes except appearance of numerous elliptical mitochondria and increased incidence of polysomes. (Plate II Fig.1, 2).

Transmission electron micrograph of oocytes of carbofuran treated fish (Group III) showed a clear cut correlation between dose, duration and severity of ovarian anomalies. Important changes in the oocytes of carbofuran treated fish were appearance of numerous vacuoles, dilation of mitochondrial cristae, wavy plasma membrane, conical and disrupted nuclear membrane and dilated nuclear pore (Plate III, Fig.1, 2, 3). Besides congregation of polyribosome, dense osmiophilic granules and abundant Golgi cisternae showed hyperactive condition of cellular organelles (plate III, Fig.4). At few places, oozing out of nuclear materials from nucleus, loosening and streaming movement of collagen fibres were prominent, marking typical apoptotic condition of oocytes (Plate III, Fig.2, 4). 30 days treatment of CMR extract in carbofuran treated fish showed restoration of nearly normal cyto-architecture of follicular epithelial cells of oocytes (Plate IV, Fig.1) Regeneration of

mitochondrial cristae, uniform nuclear membrane with homogenous chromatin material & flanking nucleoli, accumulation of smooth ER were the prominent sign of recoupment after CMR extract treatment in Group IV fishes (Plate IV, Fig.2, 3, 4). When pre carbofuran treated fishes were left untreated for 30 days as self healing group (Group V), their oocytes did not show any significant retrieval as marked by abundance of vacuole, wavy and fuzzy plasma membrane, dilated nuclear pore, disrupted nuclear membrane, dilated mitochondrial cristae, marking cellular necrosis (Plate V, Fig.2,3,4).

PLATE – I

Fig . 1&2 Transmission Electron Micrographs of contro1 (Group I) fish oocytes.

Fig.1. Surface of previtellogenic oocytes of adjoining follicular cells (FC), nucleus of follicular cells (FN), oocyte cytoplasm (OC) and intact mitochondria (M) are normal. The demarcation between follicular cell and ooplasm is distinct. X8500

Fig.2. The nucleus (N) of the ooplasm showing typical features of a normal cells with uniform nuclear membrane (NM)/,homogeneous chromatin material (CH) and distinct nucleoli (NL). Note the extrusion of some electron dense granules at the outer periphery of nucleus. X15500.

PLATE – II

Fig 1.2. Transmission Electron Micrographs of oocytes of only CMR treated fish. (GROUP III)

Fig.1. The oocyte showing wavy plasma membrane (PM), dense cored bodies (DCB), endoplasmic reticulum (ER) with heavy ionic deposition, ribosome(R) as polysomes in the lumen of ER and ooplasm and mitochondria (M) with dilated and disrupted cristae. X8500

Fig.2. The nucleus showing conical & disrupted nuclear membrane and dilated nuclear pore (NP) pointing towards hyperactive condition. X 21500.

PLATE – III

Fig 1-4. Transmission Electron Micrographs of carbofuran (@ 1.5 mgL^{-1}) treated fish

Fig.1 Amoeboid shaped oocytes showing highly disrupted ovigerous lamella, streaming movement in collagen fibres, amoeboid nucleus with distinct nucleoli, increased vacuolation in ooplasm and ionic deposition on Mitochondria and ER. X15500

Fig.2. Portion of nucleus (N) of other oocyte showing disrupted nuclear membrane (NM) at few places, condensed chromatin material (CH), arch shaped vacuity around nucleolus (NL)

Ooplasm (OC) is marked by increased lysosomal activities and multi vascular bodies. X 15500.

Fig.3. The portion of ooplasm (OC) showing abundant ribosomes (R), mitochondria (M) with tubular cristae. X 21500.

Fig.4. The portion of nucleus (N) showing oozing out of nuclear material, a typical sign of apoptotic condition of oocyte. X 21500.

PLATE – IV

Fig 1-4. Transmission Electron Micrographs of Group IV (carbofuran + CMR @ 50 kg^{-1} b.w.) treated fish oocyte.

Fig.1 Portion of follicular epithelial region showing normalcy in nucleus (FN) and ooplasmic content (OC). X8500.

Fig.2. Ooplasm near nuclear region (N) showing sign of recoupment in Mitochondria (M), ribosome (R) and lysosome (Ly). A distinct recovery is noticed in nuclear membrane. X 8500

Fig.3. The portion of nucleus showing flanking nucleoli (NL), normal chromatin material (CH) and some ooplasmic granules of nuclear origin (GN) at nuclear membrane (NM). X 3400

Fig.4. Nucleus of oocytes at higher magnification showing two nucleolus (NL) and normal chromatin material (CH) X 6500

PLATE-V

Fig 1-4. Transmission Electron Micrographs of Group IV (carbofuran + CMR @ 50 kg^{-1} b.w.) treated fish oocyte.

Fig 1 Showing portion of ruptured oocyte with abundance of vacuole, wavy and fuzzy plasma membrane, ionic deposition in mitochondria marking cellular necrosis. X21500

Fig 2 Portion of ooplasm showing dilated mitochondria with tubular cristae and abundance of poly ribosomes. X 21,500

Fig 3 Portion of ooplasm showing polymorphic mitochondria predominated by elliptical one. Note dissolution of mitochondrial cristae, congregation of polysomes & few secretory vesicles. X21500

Fig 4 Portion of oocytes showing wavy plasma membrane, heterochromatized nucleus, cleft formation in nucleus and reduction in cellular necrosis X21500

PLATE – VI

Fig 1-4. Transmission Electron Micrographs of Group v (SHG group) fish oocytes

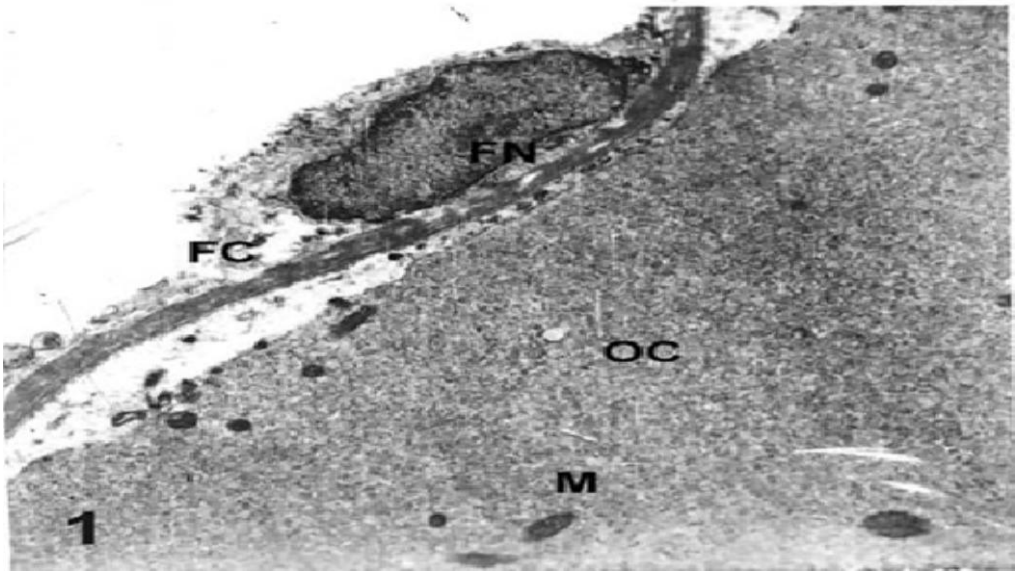
Fig.1 portion of ruptured oocytes with abundant vacuole, wavy and fuzzy plasma membrane, ionic deposition in mitochondria and cellular necrosis. X 21,500

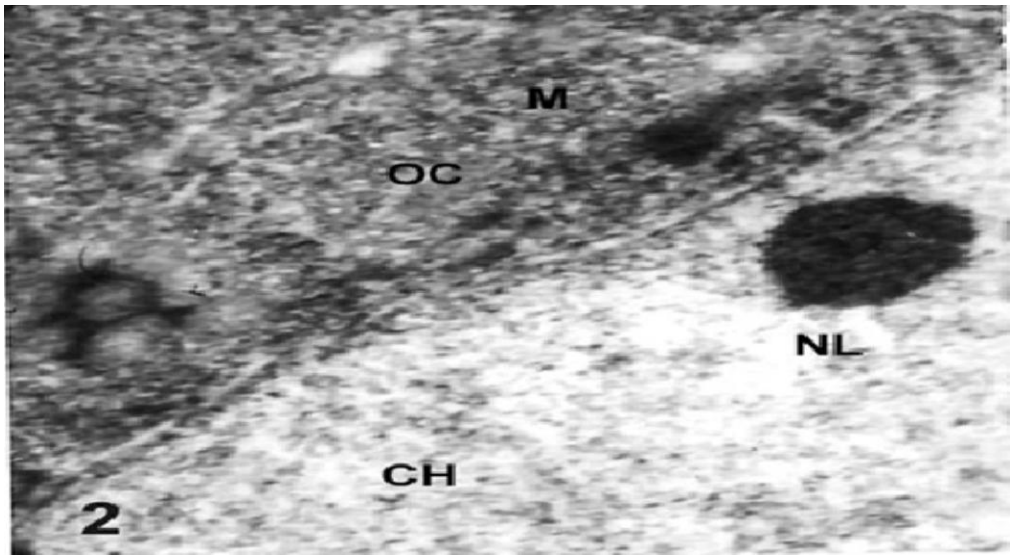
Fig.2. Portion of ooplasm showing dilated mitochondria with tubular cristae, and abundance of ribosomeX 21,500

Fig.3. Portion of ooplasm showing polymorphic mitochondria with abundance of elliptical mitochondria (EM), dissolved mitochondrial cristae, congregation of polysomes & few secretory vesicles X 21,500

Fig.4. Portion of oocyte showing wavy plasma membrane, heterochromatized nucleus, cleft formation in nucleus and sign of cellular necrosis. X 21, 500

PLATE I





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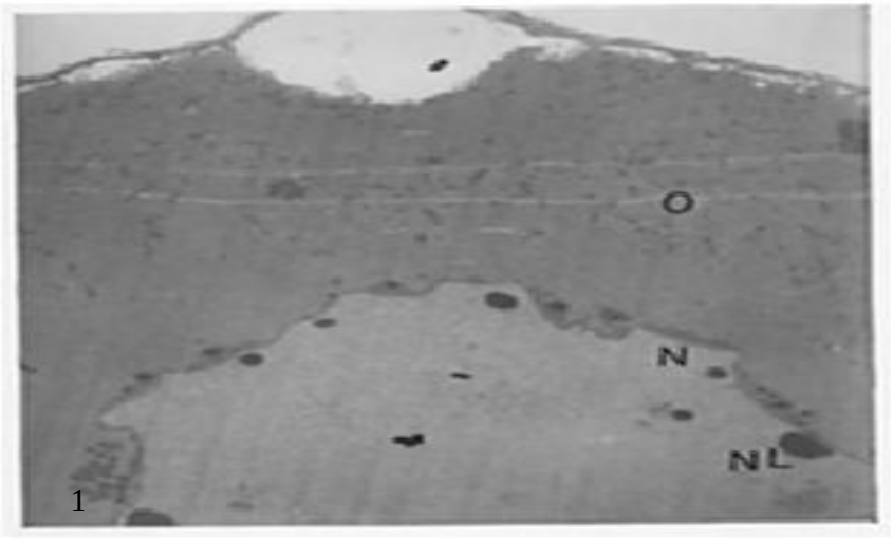

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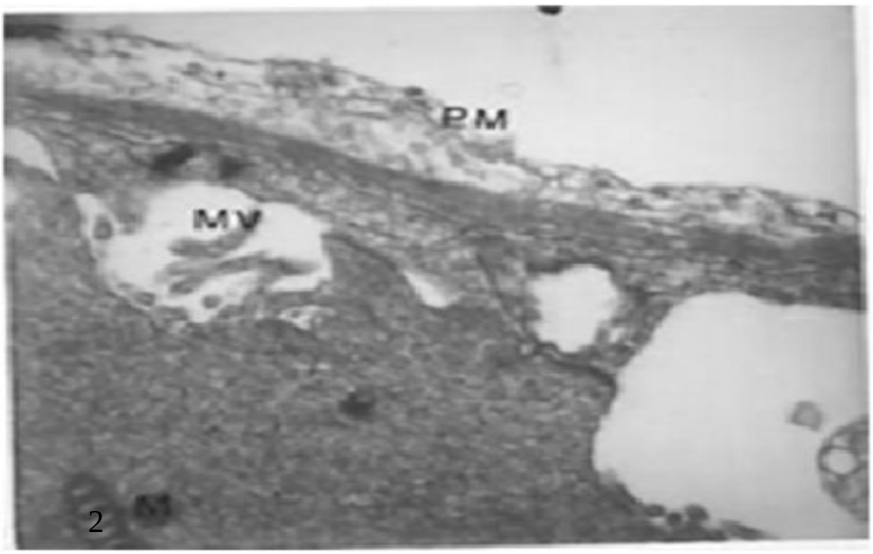

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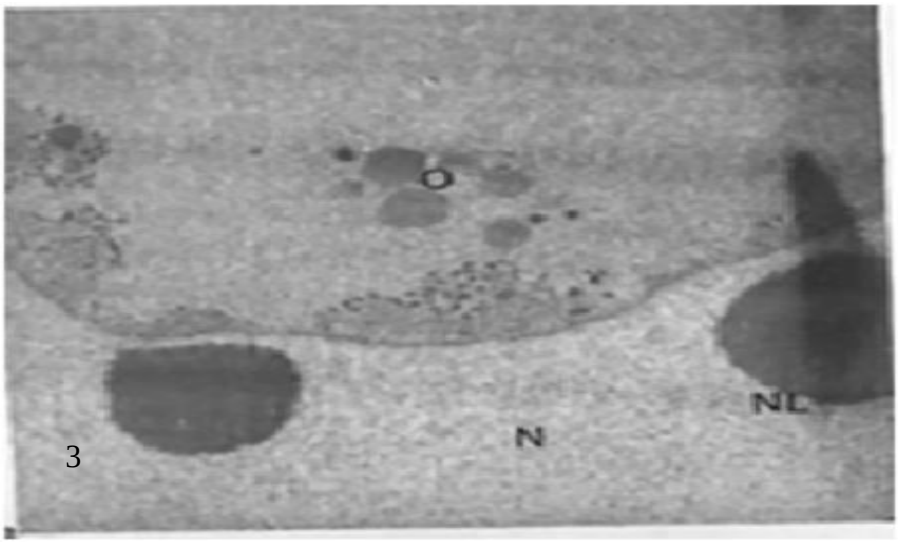

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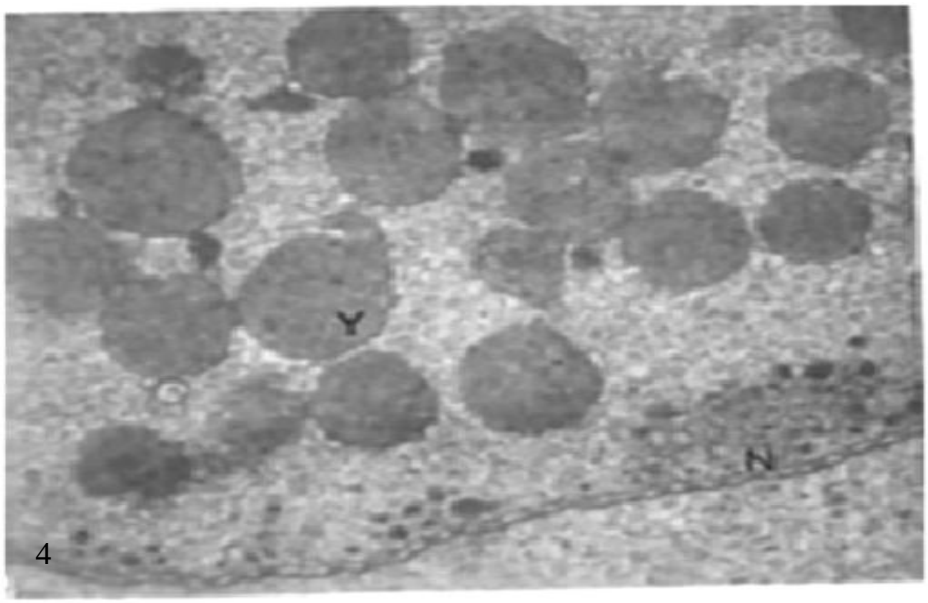

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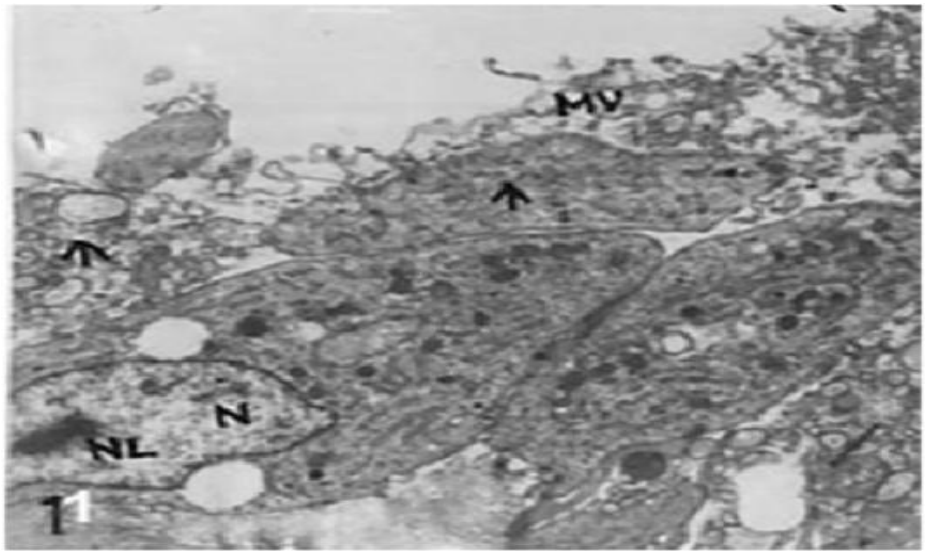

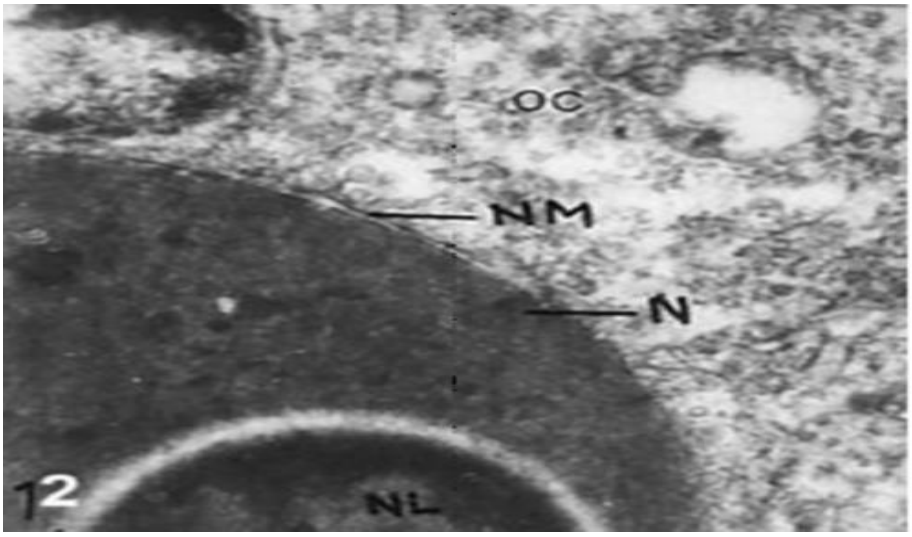

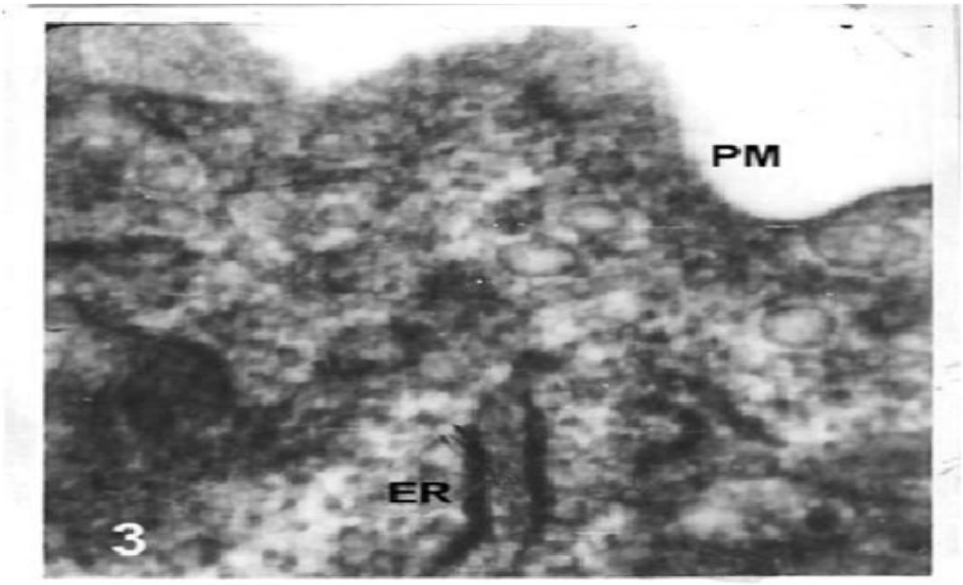

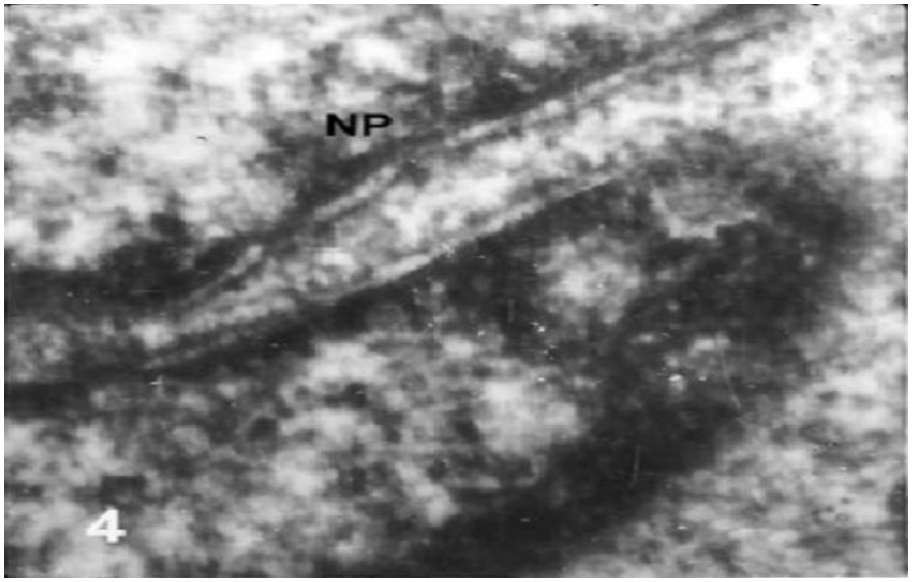

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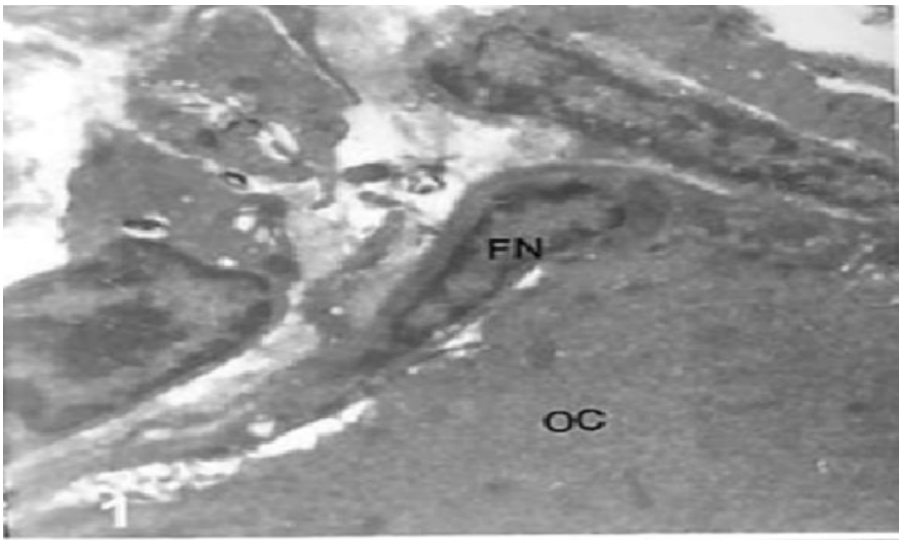

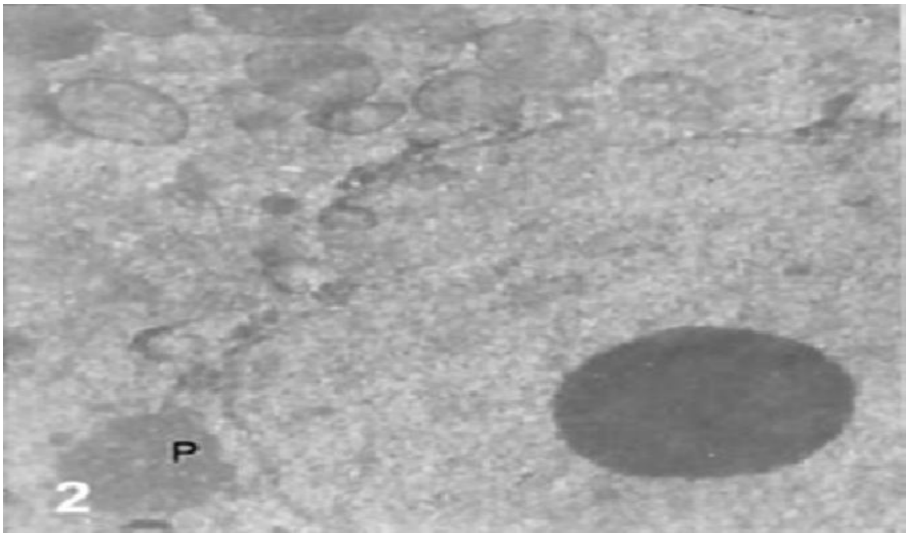
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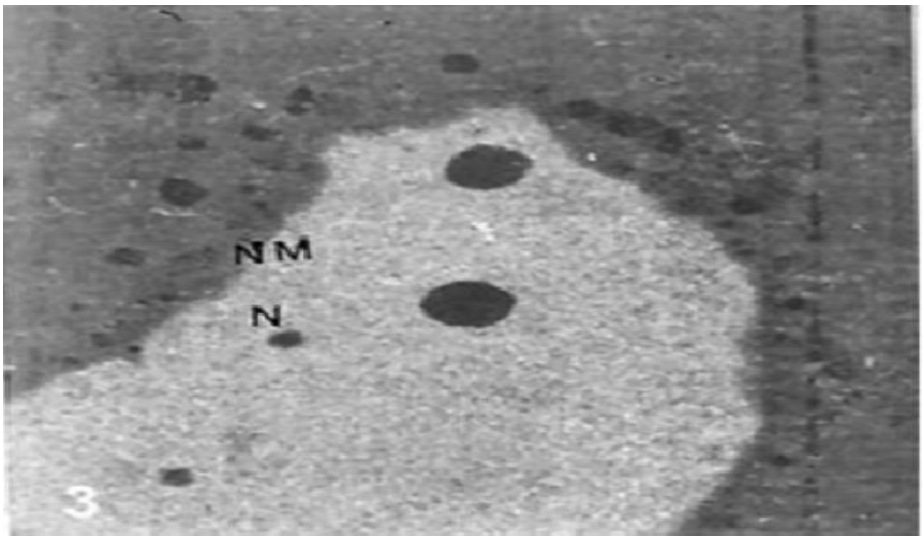
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N
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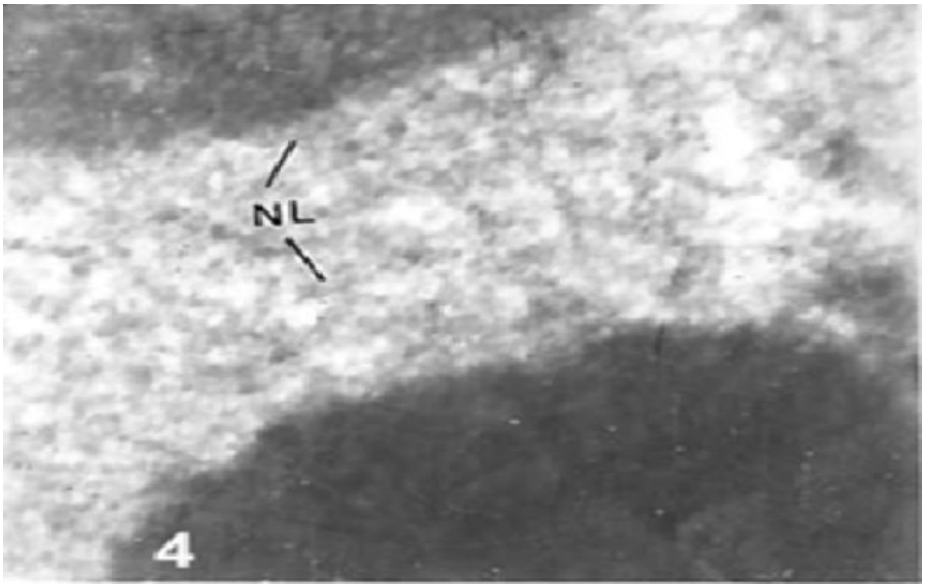
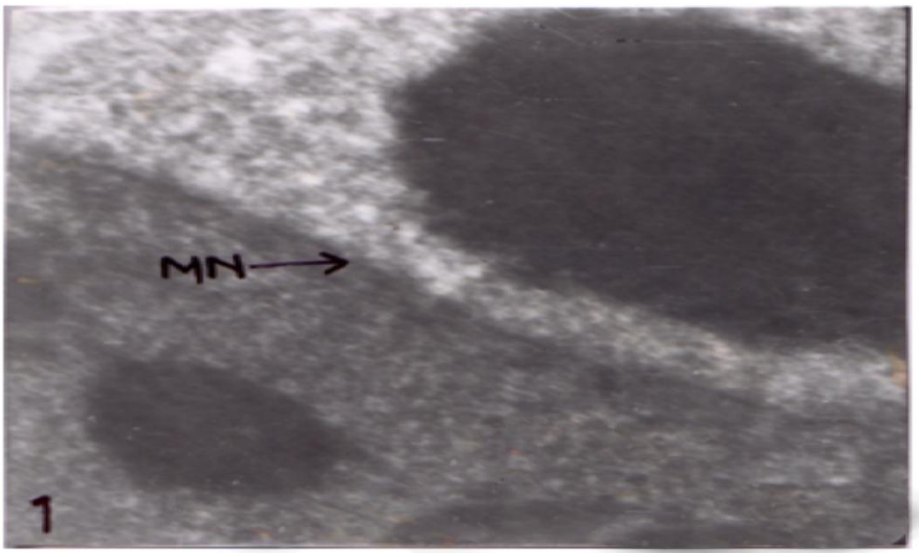
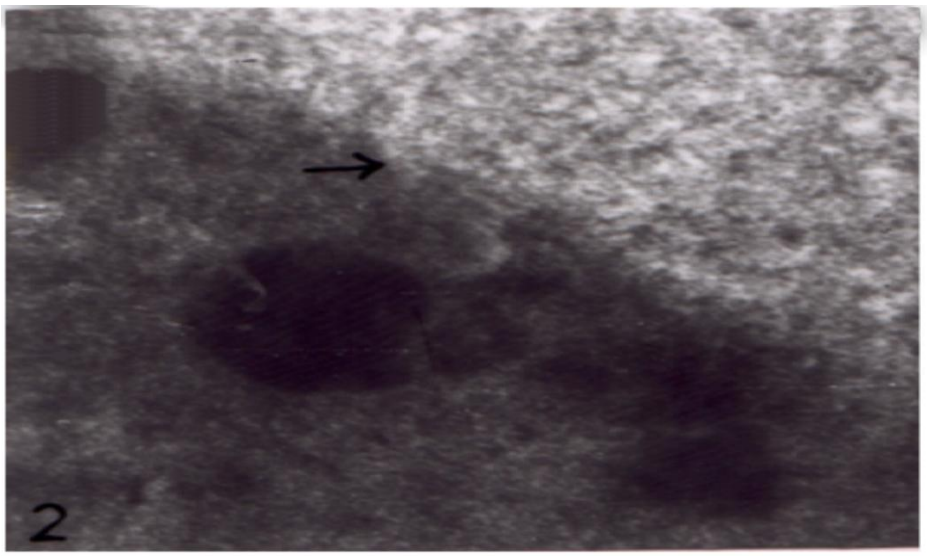
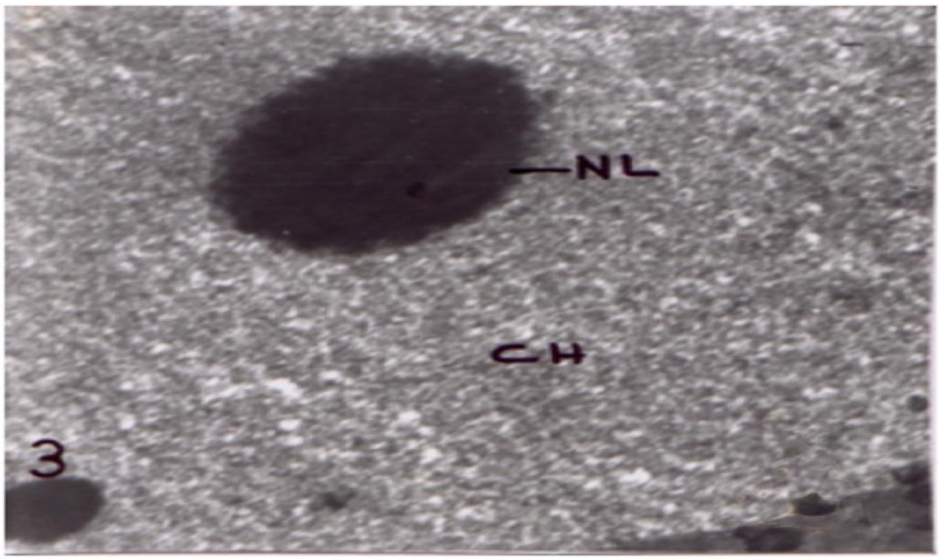

NL
4

PLATE V







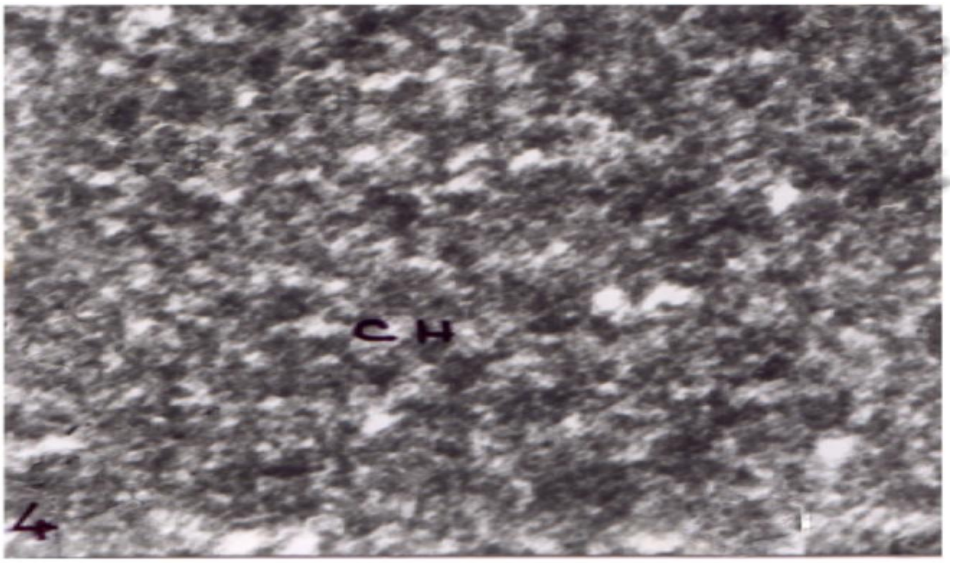
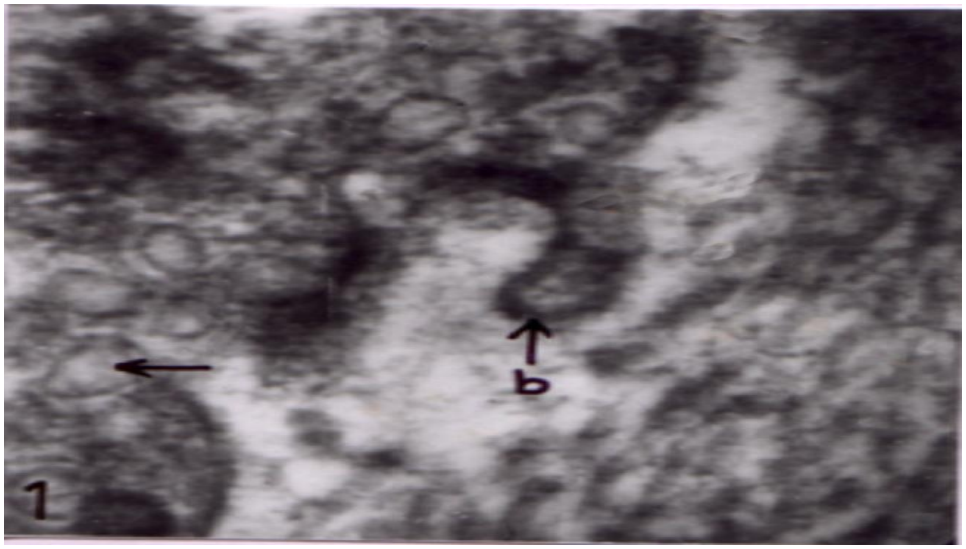
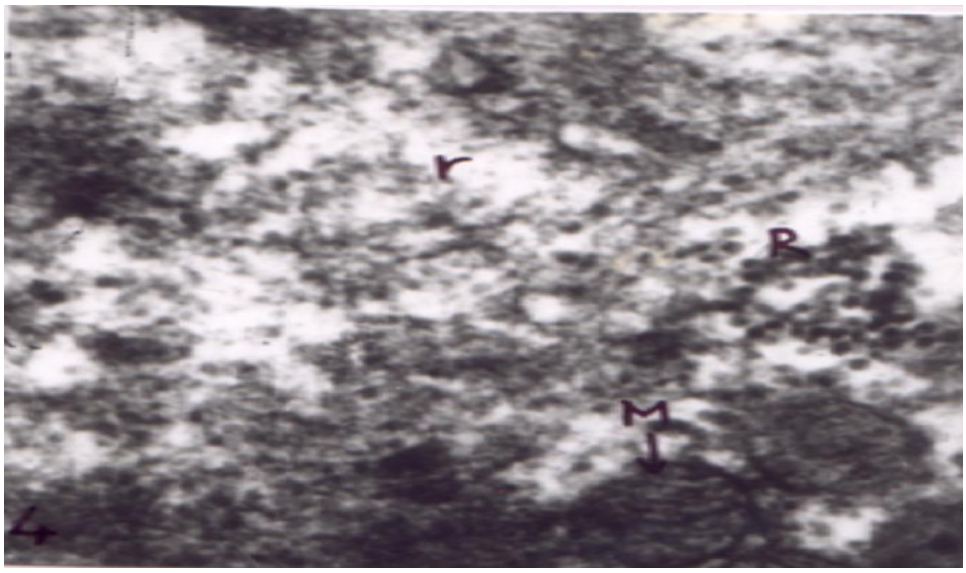
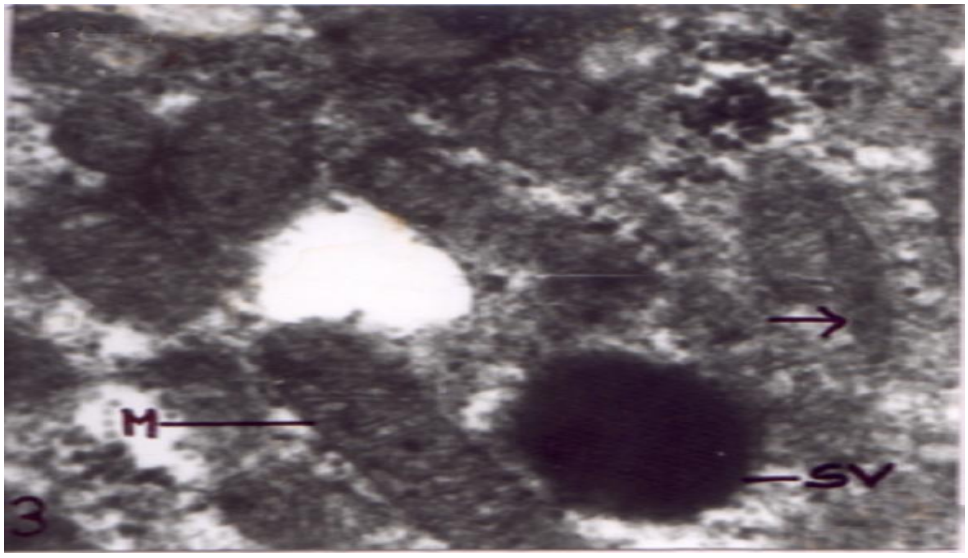
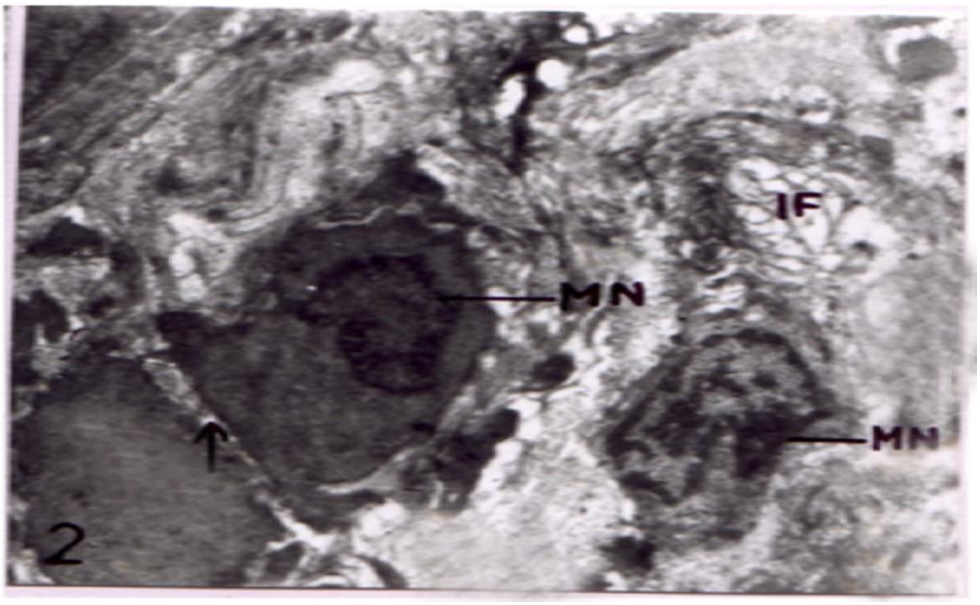


PLATE VI









DISCUSSIONS & CONCLUSION

The present study demonstrates the mitigating potential of aqueous extract of *convolvulus microphyllus* root (CMR) against carbofuran induced ovarian anomalies in fish. The growing oocytes of teleost are surrounded by concentric cellular or non-cellular layer in the ovarian follicle, zona granulosa and inner most zona radiata [(21) (22)]. The zona granulosa is a syncytial layer having deeply stained nuclei and their cells are actively involved in the protection of steroids regulating vitellogenesis and maturation of intra follicular oocytes [(23)]. Major hitopathological changes incurred in oocytes after carbofuran treatment at transmission electron microscopy were wavy plasma membrane, conical and disrupted nuclear membrane, dilated nuclear pore and mitochondrial cristae, congregation of polyribosomes, dense

osmiophilic granules and GERL material and increased lysosomal activities. Similar kinds of anomalies have been earlier reported [24].

Fish living in polluted areas are continuously exposed to toxic compounds; many of them exert cytotoxic effects by the production of reactive oxygen species (ROS)[25]. The ROS probably initiate peroxidation of lipid, which in turn stimulates a cascade of sequential reaction initiated by glycation of protein, inactivation of enzymes, alteration in the structure and function of collagen and basement membrane of other membrane bound organelles leading to disfiguration of oocytes.

Pesticides have been considered as reproductive biomarker and potential reproductive endocrine disrupter[26][27]. They are known to interfere with the basal metabolism of fish and suppress reproduction, steroidogenesis[28] and gonadotropin level in fish by altering the secertory activities of hypothalamo-hypophyseal - thyroid- gonadal axis[29][30]. In the present study carbofuran exposure causes a significant rise in serum T_3, T_4 and TSH in the experimental group of fishes. Similar kind of alteration in serum T_3, T_4 and TSH and other thyroid function due to industrial wastes and chemicals have been reported [31] [32]. A diminished T_3 and T_4 level due to synergistic impact of aldicarb, atrazine and nitrate have been reported [33].

Induced hypothyroidism causes delayed sexual maturation. The incubation of fish embryo with thyroid hormones is known to enhance the rate of embryonic development[34]. Treatment of CMR extract to Group IV fishes showed recoupment in cytoarchitecture of oocytes as evidenced by minimization in oocyte folding, restoration of double walled nuclear membrane and homogeneous distribution of chromatin granules. Restoration of nearly normal shape of oocyte might be associated with the synergistic action of CMR extract on the suppression of over expression of PTP-S2, accounting for leukemic cell proliferation and MAP essential for maintaining normal cytoskeleton of cell[35]. The accumulation of GERL material, increased no. of mitochondria and greater abundance of polysomes might be secondary response of fish to carbofuran induced stress. Aqueous CMR extract declines the elevated TSH at all the three doses of carbofuran. It clearly suggests the mitigating potential of CMR extract against pesticidal induced toxicity in fish. It brings about normalcy in serum T_3 & T_4 which would otherwise increased or decreased due to cabofuran exposure. Similar endocrinal finding has been reported [31]. Based on statistical analysis and numerical screening of the Electron micrographs of oocytes of different group of fishes, it can be concluded that administration of

lyophilized aqueous extract of CMR restores nearly 80% of normal cytoarchitecture of oocytes and its secretory activities in carbofuran treated fish. It restores normalcy in altered serum T_3 & T_4 . A perfect correlation between histopathological and endocrinal findings signifies the mitigating potential of CMR extract against carbofuran toxicity in fish.

ACKNOWLEDGEMENT

The authors are thankful to SAIF-EM Unit, Department of Anatomy, AIIMS, New Delhi for providing excellent facilities for Transmission Electron Microscopy.

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