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ASSESSMENT OF TOXICITY OF 2-METHYLTETRAHYDROFURAN ON THE OVARIAN TISSUE OF AFRICAN CATFISH *CLARIAS GARIEPINUS* (BURCHELL, 1822) – A HISTOPATHOLOGICAL STUDY

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ABSTRACT:
Histopathological examination of ovary tissue of African catfish C. gariepinus exposed to 250 mg/lit.2-MeTHF for long term showed severe and variable effects. These includes degenerative change in ovary, vacuolization in the ooplasm, damaged follicular lining and deshped oocytes. Intramuscular exposure of 10 mg 2-MeTHF showed vacuolization in oocytes and degeneration of follicular wall in C. gariepinus. Exposure of 40 mg intramuscular dose of 2-MeTHF showed histological abnormalities like development of intrafollicular space in oocytes, broken wall in vitellogenic oocytes, decreased nucleoli in perinuclear stage, clumping of cytoplasm in mature oocytes in ovary of fish C. gariepinus was observed in the present study. It was observed that number of mature oocytes have been reduced with large follicular space due to the effect of 2-MeTHF. Similarly vacuoles in the vitelline follicules and broken albuginea was also observed in the ovary. The generation of follicular atresia was observed in the present study due to the effect of 2-MeTHF, which characterized by degeneration and necrosis such as dissolution and disappearance of the nucleus and changes in the mitochondria and other organelles were found. When the fish was exposed to 500 mg/lit concentration of 2-MeTHF and 20 mg intramuscular dose of 2-MeTHF cause degeneration of ovarian wall and breakdown of vitellogenic epithelium and degeneration of granulose cell in secondary oocytes.
Key words: - Toxicity, Ovarian tissue, Histopathology, fish.

INTRODUCTION:

The industrial effluents and urban sewage released into rivers directly after little or no treatment have added to the problem. Increasing pollution of rivers and other water bodies has become a matter of great concern in recent years. Further, fishes, though non-target organisms, are also affected and the accumulated toxicants may reach human being as they form a staple food for them. There have been few attempts to study the influence of aquatic toxicants on the fish reproduction. Endocrine disrupters or endocrine disrupting compounds (EDCs) include a diverse group of synthetic industrial and agricultural chemicals and even some naturally occurring compounds. They include industrial intermediates such as bisphenol-A and 4-nonylphen, PAHs, pesticides such as the insecticides endosulfan, carbofuran, DDT, and the herbicide atrazine, and a number of metals including lead and mercury. Many of the chemicals currently known to interact with the estrogen receptor can be found in the effluent from sewage treatment plants (STPs). The endocrine system in fish consists of various glands located throughout the body which synthesize and secrete hormones to regulate an array of biological processes. The major estrogen in female fish, 17β-estradiol (E2), is produced primarily in the ovary by the follicular cells. In addition to their importance in eliciting reproductive behavior and the development and maintenance of secondary sex characteristics, the estrogens and androgens are in the production of gametes. In oviparous or egg-laying fish,as in other egg-laying animals, the release of E2 from the ovary leads to the synthesis of large amounts of vitellogenin by the

hepatocytes (liver cells) (Figure 2). This high density lipoprotein, the precursor of egg yolk, is then transported from the liver via the circulatory system and incorporated into developing oocytes. Although estrogens are typically associated with females and androgens with males, that demarcation increased glycogen and lipid metabolism. Although most of what is currently known about the effects of EDCs involves reproduction and reproductive behavior, other areas of the endocrine system, such as the thyroid, may also be targets for EDCs.Diamanti-Kandarakis E. (2009). 2-methyltetrahydrofuran is a versatile aprotic solvent that is being used more often in industrial synthetic processes because of its favourable properties some of the useful properties of MeTHF are partial water miscibility (allows Me THF to function as a solvent for both reaction and product isolation) Aprotic polar solvent (useful for many organometallic reaction) Boiling point of 89ºC (convenient for reaction and heat removal but easy to remove from product. 2-MeTHF has been found to be a very effective solvent for biphasic reactions, especially for reactions with aqueous NaOH such as alkylation, amidation and nucleophilic substitution reactions. 2-MeTHF gives clean phase separations in these reactions and high reactivity.

MATERIALS AND METHODS :

The fresh water African catfish, Clarias gariepinus, was selected for the present study because of its availability from local tanks or market and for its convenient size. It could be safely transported and maintained easily under laboratory conditions because of its air breathing habit. Its hardy nature, moreover suit the experimental work. Burchell (1822) discovered the species C.gariepinus. The fish is locally called as “magur”.

COLLECTION AND MAINTENANCE OF FISH

All the fishes used in the present study were brought from local market. The body weight of fish ranged between 300-370 gm and their length varied between 30-40 cm. The fish were maintained in glass aquarium containing 20 lit. of tap water, normal condition of light and temperature. The fishes were fed with fingerling or minced goat liver every alternate day and water was changed at the interval of one day.

In the present study the chemical 2-methyltetrahydrofuran was purchased from Sisoresearch laboratories Pvt. Ltd (CAS number: 96-47-9). The threeaquarium were taken filled with 20 lit tap water, in each aquarium 2 female fishes were kept the fishes in the aquarium were grouped into control and experiment respectively and labeled aquarium accordingly.From the two experimental aquarium, first aquarium have been given 2-MeTHF (500mg/lit) in the water medium. The second aquarium have been given 2-MeTHF (250mg/lit) in the medium.

PREPARATION OF WORKING SOLUTION

As 1ml of 2-MeTHF solution contains 0.855g 2-MeTHF, On the basis of this concentration we have calculated the volume of 2-MeTHF for the preparation of the working solution for 250mg/L. We have taken 20 litres of water in each of the four aquaria.

HISTOLOGICAL STUDY FOR OVARY

- **Fixation:**

The fishes were anesthetized with paraldehyde to dissect out the tissues i.e, ovary. Then the tissues were cut into small pieces and fixed in alcoholic Bouin`s fluid for hrs.

- **Embedding and sectioning:**

Tissue fixed in Bounis fluid were transferred to 70% alcohol for 24hrs.Dehydration were carried out in 90 % alcohol, absolute alcohol clear in xylene an embedded in paraffin wax. Block were made and tissue were cut at 5micron thickness with the help of rotary microtome at 30ºc room temperature .The sections of ovary were spread

on slides and then the slides were stains by using HE double staining method.

- **Staining:**

The sections were deparaffinised in xylene and were passed through descending grades of alcohol. The sections were stained haematoxylene and were kept in running water for some time. 1% HCL were used to remove the excess stain if tissues get over stained with eosin for 2-3min. They were then differentiated in 90% alcohol and dehydrated in absolute alcohol. Then it was cleared in xylene and mounted in DPX. The slides were then observed under microscope (100x and 400x).

Observation and results:

In the present study, vaculation, artesia, architecture loss were observed in treated fish for long term exposure to 2-MeTHF. The occurrence and degree of alteration were positively related with the exposure of different concentration of 2-MeTHF.While the tissue taken form the control group does not show any change during the experiment.

HISTOPATHOLOGICAL CHANGES IN OVARY

The ovary of the control fish C. gariepinus showed normal structure which appeared more are less circular in outline. (Fig.1) In transverse section most of the section was packed by several large mature oocytes, each containing an irregular germinal vesicle surrounded by lot of yolk matter. Each mature oocyte was bound by a thick zonaradiata. Transverse section of control ovary showing primary, secondary oocytes, consist of nucleus, nucleolus and follicular cell.

Treatment of 2-MeTHF (250 mg and 500 mg in water medium)

In the present study, fish C. gariepinus treated with 250 mg/lit (Fig.2) and 500mg/lit (Fig.3) showed disorganization, architecture loss, degeneration of oocyte generation or development of intrafollicular space, vaculation and atresia. Degeneration of ovarian wall and breakdown of vitelline epithellium was observed in 500 mg /lit concentration of 2-methyltetrahydrofuran.

RESULT AND DISCUSSION :

In the present study, the histopathological examination of ovary tissue of C. gariepinus exposed to 250mg/ lit 2-MeTHF for long term showed severe and variable effects. These includes degenerative change in ovary, vacuolization in the ooplasm, damaged follicular lining and deshaped oocytes. These findings are in accordance with the study of Kumar and Pant (1984); Dutta and Ghosh (1997) and Saxena and Garg (1978), who reported a significant atresia in the ovary with major damage to effect of mature oocytes and increase in atretic follicles in fish treated with 2-MeTHF.

Intramuscular exposure of 10mg 2-MeTHF showed vaculization in oocytes and degeneration of follicular wall in C. gariepinus. These findings are supported by the findings of Jalila Masaratet al. (2014) who reported the degeneration in follicular wall due to effect of mercuric chloride in catfish, Clarias gariepinus.

Exposure of 40 mg intramuscular dose of 2-MeTHF showed histological abnormalities like development of intrafollicular space in oocytes, broken wall in vitellogenic oocytes, decreased nucleoli in perinuclear stage, clumping of cytoplasm in mature oocytes in ovary of fish C. gariepinus was observed in the present study. Similar abnormalities were reported in the study of Kling (1985) and Sukumar and Karpagagnpathy (1992) in the freshwater fish exposed to aquatic pollutant.

During the present work it was observed that number of mature oocytes have been reduced with large follicular space due to the effect of 2-MeTHF.These observations coincide with the findings of Mazrouch and Deshmukh and Kulkarni (2005) and Olfat and EI-Greisy (2007) who observed exposure of cadmium chloride on

gonads of the fish Channaorientalis affects cytoplasmic clumping in mature oocytes.

In the present study, vacuoles in the vitelline follicules and broken albuginea was observed in the ovary of fish C. gariepinus exposed to 2-MeTHF. Similar findings are reported by Hatikakoty (2002), Nermin E-Morshedi and Nadeem A. kizilbash (2014) in labax ovaries and in gonads of Sea Bass fish as consequence of water pollution.

The generation of follicular atresia was observed in the present study due to the effect of 2-MeTHF, which characterized by degeneration and necrosis such as dissolution and disappearance of the nucleus and changes in the mitochondria and other organelles were found. These findings suggest that the death of oocyte is involved in the process of atresia. Palumbo &Yeh (1994), shows apoptosis, a type of physiological cell death, isconnected with the process of follicular atresia in birds and mammals and Joblinget al. (2002) recorded atresia in roach living in rivers that receive treated sewage effluents. These findings supports the findings of present study.

The fish C. gariepinuswhen exposed to 500mg/lit concentration of 2-MeTHF and 20mg\mlintramuscular dose of 2-MeTHF cause degeneration of ovarian wall and breakdown of vitellogenic epithelium and degeneration of granulose cell in secondary oocyte. Similar observations were reported by GaneshwaderajuMarutirao (2013) who studied the effect of dimethoate on fish Puntiustictoand found that the granulose layer get separated and complete or partial rupture in maturing oocytes.

Summary and conclusion:

The industrial effluents and urban sewage released into rivers directly after little or no treatment have added to the problem. Increasing pollution of rivers and other water bodies has become a matter of great concern in recent years. Further, fishes, though non-target organisms, are also affected and the accumulated toxicants may reach human being as they form a staple food for them. There have been few attempts to study the influence of aquatic toxicants on the fish reproduction. Fish reproduction is affected by the direct or indirect exposure to aquatic pollution.

An endocrine-disrupting compound was defined by the U.S. Environmental Protection Agency (EPA) as “an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are present in the body and are responsible for homeostasis, reproduction, and developmental process”.2-methyltetrahydrofuran is a versatile aprotic solvent that is being used more often in industrial synthetic processes.

In the present study, fish C. gariepinus treated with 2-MeTHF in concentration of 250mg/lit showed severe and variable effect these include degenerative change in ovary, vacuolization in the ooplasm, development of intrafollicular space in oocyte, broken wall of vitellogenic oocyte.

Fish C. gariepinus treated with 2-MeTHF in concentration of 500 mg/lit showed follicular atresia and necrosis, degeneration of ovarian wall and breakdown of vitelline epithelium.

The present study concludes that 2-MeTHF exerts adverse effect on the ovary of C. gariepinus. The ovarian cells are damaged by the action of various toxicant, example -heavy metals, pesticides, EDC’s. Ovary is reproductive organ which play an important role in reproduction. Hence the ovary is significantly damaged due to exposure of such toxicants as observed in the present study showing various histopathological changes caused by the destroyed fertility in female C. gariepinus.

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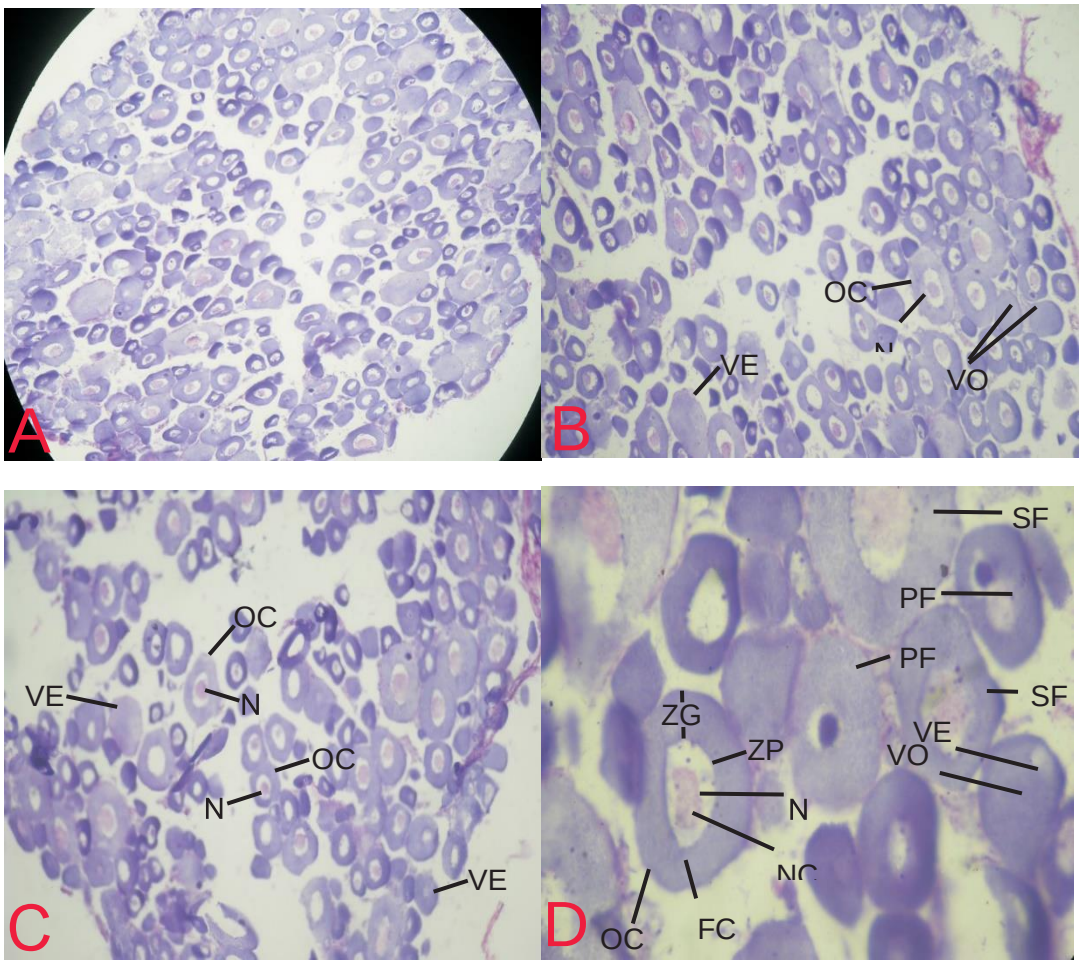


Fig.1: (A, B, C, D):- Microphotograph showing ovary of control fish *Clarias gariepinus*

Fig. 1: Microphotograph showing normal histology in ovary (A, B, C & D)

Showing nucleus, nucleolus, oocyte, primary and secondary follicles, vitelline oocyte, epithelium zona pellucida and zona granulosa.

(A-HEx40X, B- HEx100X, C- HEx100X, D-HEx400X)

Abbreviations used

N – Nucleus, NC – Nucleolus, OC – Oocyte, FC – Follicular cell, VE – Vitelline epithelium, VO – Vitelline oocytes, ZP – zona pellucida, ZG – zona granulosa.

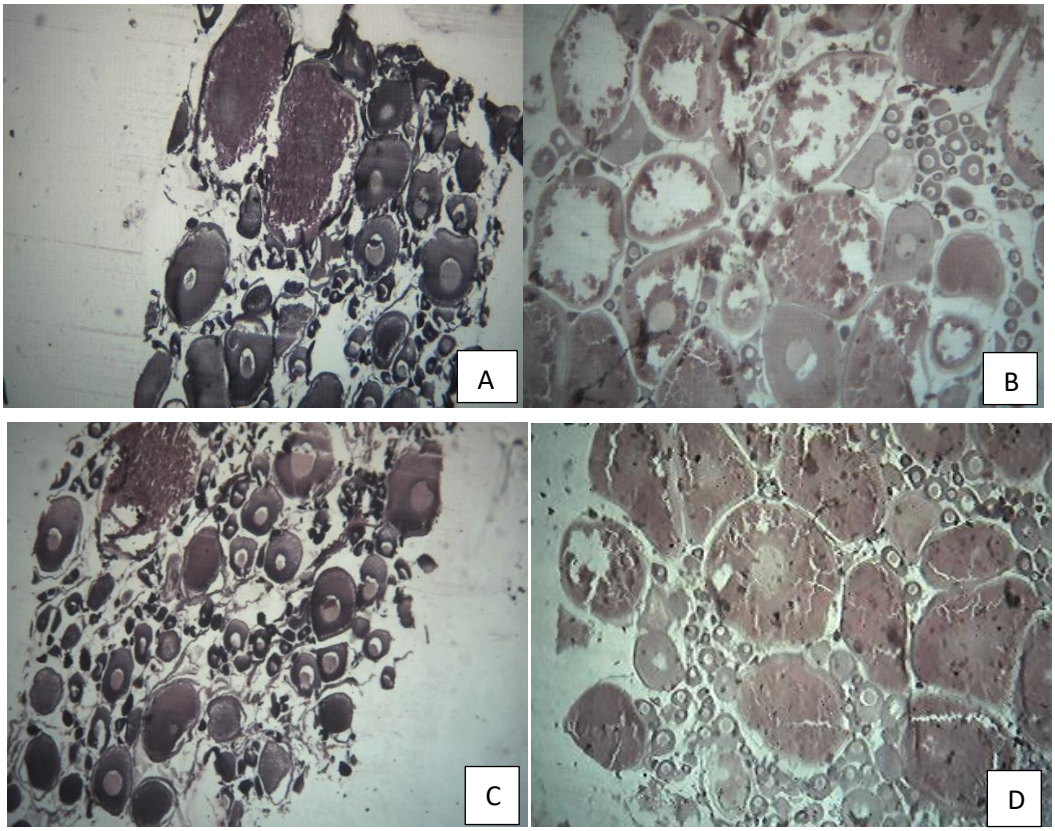
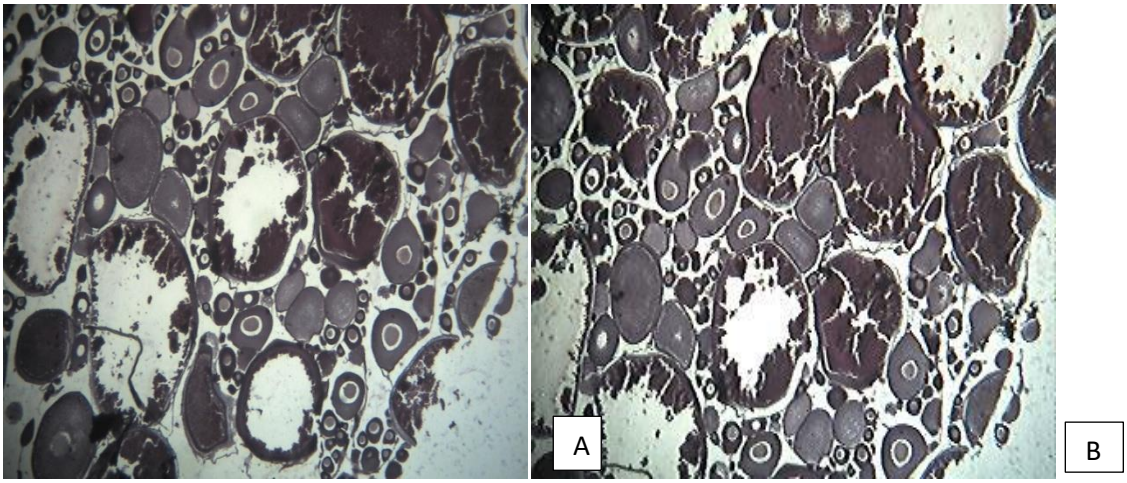


Fig.2:(A, B, C, D) : Microphotographs showing ovary of fish *Clarius gariepinus* treated with 250 mg/lit 2MeTHF

Fig. 2: Microphotograph showing histopathological changes in ovary of experimental fish C. gariepinus (A, B, C & D) treated with 250 mg/lit. 2-Methyltetrahydrofuran.

A. Disorganization, architecture loss and degeneration of vitelline epithelium. HEx100X.
B. Development of vaculation in vitelline oocytes and atresia of theca cell HEx100X.
C. Degeneration of vitelline epithelium, architecture loss of vitelliogine oocyte and degeneration of ovarian wall HEx100X.
D. Development of vaculation in vitelline oocyte and atresia of theca cell, HEx100X.

AL – architecture loss, VC – vaculation, DNG – degeneration of vitelline epithelium, TF – theca folliculi, AT – atresia



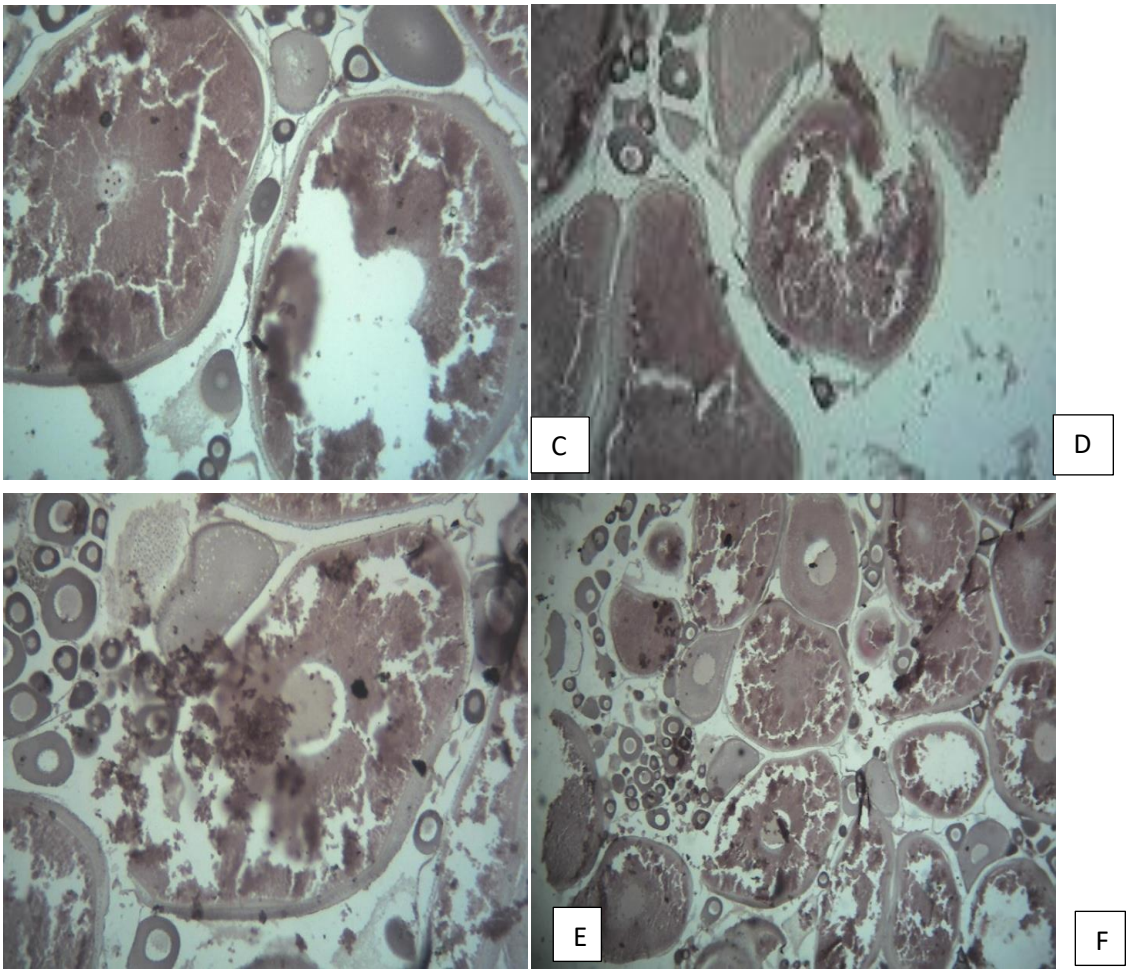


Fig.3: (A, B, C, D, E,F): Microphotographs showing ovary of fish *Clarius gariepinus* treated with 500 mg/lit 2MeTHF

Fig. 3: Microphotograph showing histopathological changes in ovary of experimental fish C. gariepinus (A, B, C, D, E & F) treated with 500 mg/lit. 2-MeTHF.

A. Development of vaculation in vitelline oocyte and atresia of theca cell HEx100X.
B. Development of vaculation in vitelline oocyte and atresia of theca cell and breakdown of vitelline epithelium and architecture loss. HEx100X.
C. Development of vaculation in vitelline oocyte and atresia of theca cell and necrosis HEx400X.
D. Disorganization, architecture loss and degeneration of vitelline epithelium. HEx400X.
E. Architecture loss, degeneration of vitelline epithelium, HEx400X.
F. Disorganization, architecture loss and development of vaculation. HEx100X.

Abbreviations used

AT – Atresia, VC – vacuolation, DNG – degeneration of vitelline epithelium, TC – theca cell, NC – necrosis, FC – follicular cell, FE – follicular epithelium