



RESEARCH ARTICLE

EFFECT OF *ZIZIPHUS JUJUBE* LEAF EXTRACT ON OVARY OF FRESH WATER FISH *OREOCHROMIS MOSSAMBICUS*

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ABSTRACT

In the present study an attempt was made to investigate the reproductive disturbance of the medicinal plant *Ziziphus jujube* leaves extract on edible fresh water fish *Oreochromis mossambicus* (*Tilapia mossambica*). Since, immemorial time the *Ziziphus jujube* commonly used various ways in human beings. The a adult fresh water fish were treated with the leaves extract of *Ziziphus jujube* 100mg/lit of water for a period of 15 days. At end of the experiment, the animals were sacrificed and dissected out the ovaries for his to pathological observation. The histopathology of ovary reveals that there were no changes in the structure of oogonia, yolk and tunica albugenea of control animals, but in 10% and 30% treated animal's ovaries shown a remarkable changes in the structure of oogonia, the content of yolk substance, structure of oocyte wall, arrangement of columnar cells and tunica albugenea. Therefore, it concludes that the aqueous extract of *Ziziphus jujube* has considerable impact on the reproductive organ of fresh water fish *Oreochromis mossambicus*.

Key words: *Ziziphus jujube*, Ovary, Tunica albugenea, Oogonia, *Oreochromis mossambicus*.

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INTRODUCTION

Ziziphus ziziphus is a herbaceous plant in family of Rhamnaceae, native to India and srilanka. It is wildly cultivated in South Asia, were it is used as antibiotics and antidotes to treat infection wounds and various ailments. Mostly the leaves and roots were used for medicinal purposes (Abhishek Mathur *et al.*, 2011). The plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compound that are used to perform important biological functions and to defend against attack from predators such as fungi, insect and herbivorous animals. Chemical compound in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compound in conventional drugs; thus herbal medicines do not either greatly from conventional drugs in term of how they work. This enables herbal medicines to be as effective as conventional medicines. But also gives them the same potential to cause harmful side effects (Tapsell *et al.*, 2004). Generally, the chemical substances differ from plant to plant, thus the plant kingdom provides which has been utilized in the treatment and cure of human and other animal diseases (Ogbunuga *et al.*, 2011).

This *ziziphus jujube* tree is eco-friendly, native in India, is perhaps most researched tree in the world. This water soluble extract of *ziziphus jujube* leaves was found to possess significant hypoglycemic, hypolipidemic, hepatoprotective, anti-fertility and hypertensive activity. The *ziziphus* genus is a widely distributed group of plants, which is wide variety of bioactive effects, have been reported. Mainly anxiolytics, sedatives, among others (Dhawan *et al.*, 2004; Ingale &Kasture,2014).The most studied species in this respect is *Ziziphus jujube* ,although research into its effects on reproduction is limited (Dhawan & Sharma, 2003). *Ziziphus jujube* leaf extract is known to possess a variety of pharmacological activities. The leaf extract have been the reproduction on the fertility agent in vertebrate animals,(Zhou *et al.* ,1998 ; Jiang *et al.*,1998;Tan *et al.* , 1983; Farnsworth and Res Front Fertil Regul ,1982;Tiwari, 1982). Histology is the most accurate method to determine the reproductive state of female fish (West, 1990). Basic study on histology of *Oreochromis mossambicus* is still limited especially in reproductive system. Some basic knowledge was mentioned that diploid ovaries from the fish of six to eight months of age contained oogenia and maturing previtellogatienic and vitellogenic oocytes with regular nuclei and vacuolated cytoplasm associated with endogenous and exogenous yolk formation (Hussain *et al.*, 1996). Reproductive development and reproductive histology in female fish are well understood by histological technique.

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Histopathology helps to identify target organs of toxicity and mechanism action (Wester *et al.*, 2002) As a tool for assessing endocrine disruption in the ovaries of *Oreochromis mossambicus* fish (Van der van *et al.*, 2005). However the reports on the effects of *Ziziphus jujube* leaf extract in the fresh water fish *Oreochromis mossambicus* are still scanty. In the light of above information and ideas, present investigation is aimed to study the 'Effects of *Ziziphus jujube* leaf extract on histology of ovary on fresh water fish, '*Oreochromis mossambicus*'.

MATERIALS AND METHODS

In the present study, fishes were exposed to different concentration of leaf extract on the histological studies of the freshwater fish, *Oreochromis mossambicus*.

Collection of Fish

The fishes were brought to the laboratory in well packed polytene bags containing oxygenated water. Fishes were procured from Azhiyar dam, Pollachi. The fishes with an average weight of 15-20 cm long were taken irrespective of sex.

Acclimatization of Fish

Fishes were collected and stocked in the large tubes, which were washed thoroughly prior to introduction of fish to prevent functional infection. Fishes were acclimatized to laboratory condition for about 15 days before the commencement of the experiment. Water was changed frequently to avoid fungal growth and contamination by metabolites. Normal fish feed were provided for the fishes once a day.

Experimental Set Up

Ziziphus jujube leaf extract was dissolved 100mg/lit of water. The fishes were grouped into three groups such as group I, II and III. The group I fishes were maintained as control. Group II and III were exposed to the extract of 10% and 30% concentrations of *Ziziphus jujube* for 15 days. At the end of the experimental period, the fishes were sacrificed and samples were collected for further analysis. The ovary separated from the experimental fish were used for histopathological analysis.

Histopathological analysis of tissue sample

Ovary was excised from the fish of control and experimental groups were fixed with 10% formalin solution. After proper dehydration of graded alcohols paraffin blocks were prepared and 4-5µm thick ribbons were cut in rotator microtome and were stained with Eosin and Hematoxylin. The histological changes observed were photographed. (Humason, 1972).

RESULTS

The present study, investigated the histological changes on ovary of *Oreochromis mossambicus* in normal condition and exposed to experimental conditions by long term exposure to leaf extract.

Toxicity Bioassay

The 96hrs LC₅₀ values of freshwater fish, *Oreochromis mossambicus* was determined in the laboratory studies 1.1ml/lit LC₅₀ value for 96 hrs was presented in the Fig 2. In this study, fish were exposed to 0.11ml/lit and 0.33ml/lit sublethal concentration which corresponded to 10% and 30% of the 96 hrs LC₅₀ respectively.

Effect of *Ziziphus jujube* leaf extract on the Ovary histology of the fish, *Oreochromis mossambicus*

General Histology of Ovary in control

In the control fishes, the mature ovaries contain fully grown yolky oocytes. The ovaries are surrounded by zona pellucida and a single layer of follicle cells. In yolky oocytes there is a clear demarcation between animal and vegetal poles. The nucleus of oocyte has large prominent nucleolus and lobulated nuclear membrane. Several small healthy previtellogenic oocytes and large early vitellogenic oocytes are also found. Besides follicular atresia is noticed in a few large vitellogenic and early vitellogenic oocytes. The ovary of *Oreochromis mossambicus* consists of series of ovarian lamelle, radially oriented towards the lumen and containing oocytes at different stages of development, in agreement with the maturation stage of development, with the maturation stage of the ovary. The control group of ovary also revealed well developed stage IV oocytes with properly distributed provitelline nucleoli. Control group showing mature oocytes, nucleus and nucleolus in the oocytes. Oocyte filled with yolk granules.

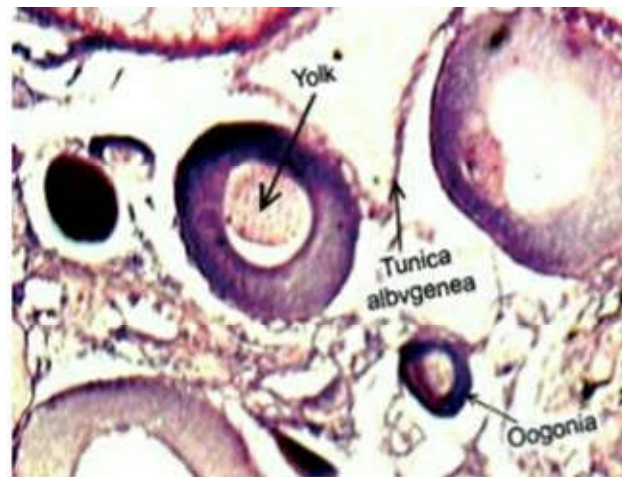


Fig. 1. Shows the normal structure of Ovary of *Oreochromis mossambicus*

Experimental group

10% Concentration of *Ziziphus jujube* leaf extract treated on ovary of fresh water fish *Oreochromis mossambicus*

The concentration of extract resulted disappearing and degradation of yolk substance, and disintegrated oocyte wall. After 15 days of exposure of 0.1 ml/lit of *Ziziphus jujube* leaf extract, of 10% concentration, primary follicles began to show adhesion and as well as cytoplasmic reaction in oocyte occurred. Cytoplasmic degeneration and the number of atretic oocytes increased and damage to the oocyte. cytoplasmic reaction and clumping was more visible in oocyte. partial destruction of the lamelle and vitellogenic membrane occurred. So in 10% treated ovary, the ovaries of extract treated fish for 15 days revealed that the undeveloped post ovulatory follicle and suppressed maturation of younger follicle.

It would be due to the hormonal inhibitory effect of the extract. Over all deformed tissue was observed in the section of the ovary of *Oreochromis mossambicus*.

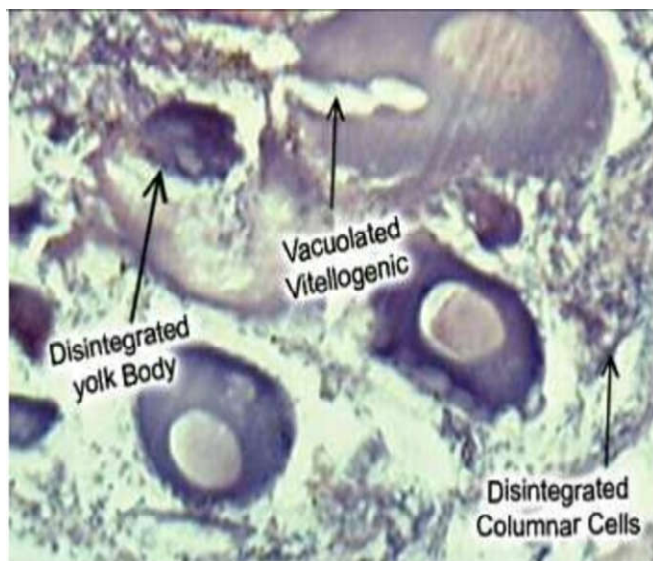


Fig. 2. Change in the structure of ovary treated with 10% concentration of leaf extract for 15 days

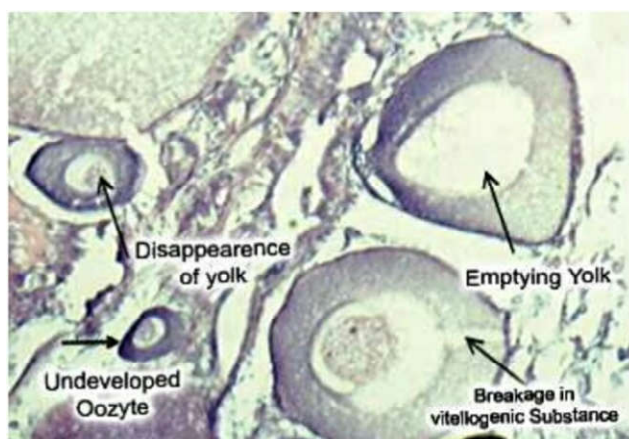


Fig. 4. Change in the structure of ovary treated with 30% concentration of leaf extract for 15 days

30% Concentraion of *Ziziphus ziziphus* leaf extract treated on ovary of fresh water fish *Oreochromis mossambicus*

Ziziphus jujube leaf extract of 30% concentration due to severe damage of the lamelle, increased alteration of follicular spaces, rupture of vitellogenic membrane, yolk reduction, reduction in size of follicle, disintegration and destruction of cytoplasm and vacuolated cytoplasm, extrusion of karyoplasms and necrosis in the cyrtoplasm were most evident following 15 days of exposure of 30% concentration. The ovarian wall became frayed and broken. Additionally, a remarkable changes of atretic follicles, shrinkage and embedded nucleoli onto the surrounding cytoplasm in oocyte was observed. This study revealed that oocytes at their different stages of maturation get affected differently at various exposures. Normally the oocyte nucleolus was large in size and located at centre of the nucleoplasm. The extract treated fish has small size nucleolus or absence of nucleolus development. The vitelline membrane appear commonly at the yolk substance, but the present result showed, that the disappearance of vitelline membrane in the extract treated fishes.

The treated *Oreochromis mossambicus* resulted by gonadal maturation altered by the leaf extract, and its act as inhibitor and change the morphological characteristics of gonads and this might be due to the granulation of cytoplasm, and appearance of increase in number of vacuoles and irregularity in the shape of the oocyte. Based on this observations of the ovarian tissue compositional and structural changes in the overall histological structures of the fish, *Oreochromis mossambicus*. However, the developing oocytes had less yolk, follicle cells were deformed and necrosis was also observed at some places

DISCUSSION

The present study shown a effects, aqueous leaf extract on the fish and this having a mild Effect of the parental gonads. The use of *O. mossambicus* as a test of species will be provide specific information can be compared with observations of effects on other species. Direct observation of histological architecture is the most accurate method to let us know exactly at the ovary is undergoing. The aquatic organisms as fishes are particularly very much susceptible to pollutants in almost all stages of their life cycle. Fish are often used as sensitive organisms for eco-toxicological studies because they play a number of roles in the tropic structure, accumulate toxic substances and even respond to low concentration of the toxicants. Therefore, the use of fish reproductive parameters as indices of the effects of pollution are of increasing importance and can permit early detection of aquatic environmental problems.

Hussain *et al.*, (1996) studied the ovarian tissue of *Nile tilapia* consisted of various stage of oocyte development including the small size, chromatin nucleolar oocyte and perinuclear oocyte; the medium size, cortical alveolar oocyte and vitellogenic oocyte with yolk granules incorporation. The result correlated the histological study that the ovaries of *O.niloticus* (6-8months containing oogonia, maturing previtellogenic and matured vitellogenic oocytes. The histopathological impact of *Jujuphus jujube* leaf extract on the ovary of fresh water fish, *Oreochromis mossambicus*. Which shows the gonadotoxic impact of *Ziziphus jujube* induced deleterious on ovarian histology of *Oreochromis mossambicus* which showed effect of *Ziziphus jujube* on fertility and productivity of fresh water fish. Considerable degree of alteration in the ovary, reduction in size of mature oocytes along with vacuolation of cytoplasm were observed. In chronic exposure complete loss of normal configuration of ovary, necrosis, elongated ovarian follicles. Similar result was observed in this study.

The most important indication of the ovarian damage due to exposure to toxic chemical compound is the reduction in the weight of ovaries. In the male guppy *Poecilia reticulata* (Pikulkaew and Wongsathein (2006) Vasudeva and Sharma 2006) and (Tiwari, 1982) in fishes. Similar effects were also noticed in fishes such as *C. carpio* with *Azardiracta indica*, *O. mossambicus* with *Andrographis paniculata* with the fish *O.niloticus* with *M. olifera* leaf extracts. Similar work has been done on the avian ovary following the exposure of birds to various bio pesticide. Histopathological effects of *Moringa olifera* on the ovary of *O. mosambicus* were studied by (Adedapo *et al.*, 2009) and reported the total absence of mature vitellogenic and postovulatory follicles in its ovary which are characteristically seen in the abnormal Atretic follicles were also reported.

Among the fishes, such studies on the effect of pollutants are limited. In biopesticide treatment during the preovulatory phase caused atresia of yolky substance was increased percentage of atresia of yolky oocytes. (Dunkel and Ricilards 1998) suggested toxicological impact of biopesticide on ovary of air breathing cat fish *Heteropneustes fossilis*. They showed many structural changes in ovary of fish during 1ppm, 5ppm and 10ppm of biopesticide treatment alter normal ovary structure of *Heteropneustes fossilis*. Similar histopathological changes have been reported by many workers in birds and mammals following exposure to aqueous leaf extract of *Azardiracta india* and other plant extract of *Andrographis paniculata* (Wagh and Khalid Shareef, 1985). Concentration obtained the two suquin to two fresh water cyprinid fishes, *Rasbora daniconium* and *barbus tincto* (Ham). The results thus obtained clearly indicate that almost all stages of ovary are severely affected and this may result in impairment of reproduction of the fish. The present study was made to investigate that the ovary showed significant alterations in the treatment of aqueous leaf extract of *Ziziphus ziziphus*.

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