

EFFECT OF ZIZIPHUS JUJUBE LEAF EXTRACT ON LIVER OF FRESH WATER FISH, OREOCHROMIS MOSSAMBICUS

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ABSTRACT : An investigation on the toxicity of biopesticidal plant, *Ziziphus jujube* aqueous leaf extracts to *Oreochromis mossambicus*. The LC_{50} value of *Ziziphus jujube* aqueous leaf extracts was 1.1ml/lit respectively. The sub lethal concentration for *Ziziphus jujube* was 0.11ml/lit and 0.33 ml/lit respectively. The fishes were exposed to the sub lethal concentration for a period of 15 days. At the end of 15th day the fishes were dissected and the liver were removed from exposed and control group of fishes. The tissues were processed and sectioned at 4 μ m and then were stained with Haematoxylin- Eosin. The observation of the slides was done under light microscope at 40x magnification and photographed. The exposed group showed histopathological changes in the tissues, such as: the parenchymal architecture of the liver is distributed and hepatocyte show dissociation, the hepatocyte appears swollen and cytoplasm appears granular. The hepatocyte nucleic become pyknotic. This result indicated that the aqueous extract of *Ziziphus jujube* has considerable impact on the liver of fresh water fish, *Oreochromis mossambicus*.

Key words: *Oreochromis mossambicus*, *Ziziphus jujube*, columnar epithelial

I. INTRODUCTION

The Mozambique tilapia (*Oreochromis mossambicus*) native to southern Africa. It is a popular fish for aquaculture. Dull coloured, the *Mozambique tilapia* often lives up to a decade in its native habitats. Due to human introductions, it is now found in many tropical and subtropical habitats around the globe, where it can become an invasive species because of its robust nature.

Recently the application of medicinal plants from different families in the management of aquaculture ponds is gaining momentum because they are safe, effective, widely available and inexpensive. *Ziziphus jujube* is one of the most promising medicinal plant, having a wide spectrum of biological activity, well known for its medicinal properties (ICAR, 1993).

Ziziphus jujube has been used successfully in an aquaculture system to control fish predators (Dunkel and Ricilards, 1998). Although leaf extract is considered of low toxicity towards non target aquatic life, water extracts of the bark of the *ziziphus* leaf caused respiratory problems in *Oreochromis mossambicus* (Omorieg and Okpanachi, 1997) such results indicated that *ziziphus jujube* extracts added to water have been expressed toxicity in the natural feed (Dabrowski 1984; Dave 1989, Roopavathy and Mary Fabiola (2018).

The aim of this study evaluated the toxicity of *Ziziphus jujube* leaf extracts on fresh water fish *Oreochromis mossambicus*. The effects of leaf extracts toxicants in living organisms can be studied by biomarkers which in a histological system reflect the defined toxicity mechanisms such as overproduction of reactive fish species and affect. The effects of exposure to sub lethal levels of toxicants to fish can be measured in terms of histological responses (Mondon *et al.*, 2001).

II. MATERIALS AND METHODS

The present study was made to investigate the acute toxicity and the effect of leaf extract (*Ziziphus jujube*) on histology fresh water fish, *Oreochromis mossambicus*.

2.1 Collection Of Fish

Aliyar dam near pollachi and were acclimatized to fresh water conditions for 2-3 weeks in laboratory, Care was taken to avoid contamination. The polythene bags carrying the fish were floated in the pond water for 1 h. Then, they were allowed to enter into the pond voluntarily by opening the bags. These fry were grown in the pond

till they attained fingerling size with artificial feeding.

2.2 Acclimatization Of Fish

Fishes were in large plastic tubs, which were washed thoroughly prior 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.

2.3 Preparation Of Aqueous Leaf Extract

Fresh leaves of *Ziziphus jujube* plant fresh leaf were collected and washed in tap water and dried in shade for ten days. After complete drying, the leaves were pulverized to fine powder in electric blender. 50% of aqueous leaf extract was prepared by dissolving 100 grams of powder leaves in 1 liter of distilled water and kept at room temperature for 24 hours, With intermittent shaking. After 24 hrs the mixture was filtered and the extract was separated for this experiment (Saravanan, *et al*, 2010).

2.4 Experimental Set Up

1gm of leaf extract is mixed with 1000ml of distilled water for preparing stock solution. Appropriate narrow range of concentration (0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8 and 2.0) was used to find the median lethal concentration using a minimum of 10 fishes for 96 hours. It was found as 1.1 ml /lit for 96 hours using probit analysis method (Finney 1964). Two groups of fishes were exposed to 10% and 30% of LC₅₀ values (0.11 and 0.33 mg/lit) concentration of leaf extract for 15 days. Another group was maintained as control. For each experimental study tissue samples were collected from the fish for the analysis of histology of liver.

2.5 Histopathological Analysis Of Tissue Sample

The histological sections of the liver of the control and experiment fish were taken by adopting the procedure as described. The tissues were isolated from control and the treated fish and rinsed with physiological saline solution (0.9% NaCl) to remove blood, mucus and debris adhering to the tissues. They were fixed in Bouins fluid for 24 hours and the fixative was removed by washing through running tap water overnight. The tissues were processed for dehydration using ethyl alcohol as the dehydration agent and passed through a graded series of alcohol, cleaned in methyl benzoate and embedded in paraffin wax sections were cut at 5 μ thickness and stained with hematoxyline and counter stained with eosin (dissolved in 95% alcohol) by Humason (1972). Then the sections were mounted in canada balsam after dehydration and cleaning and photomicrographs were taken using the mangassnous photomicrographing equipment.

III. RESULTS AND DISCUSSION

The present study investigated the histological changes of liver of *Oreochromis mossambicus* in normal condition and exposed to experimental condition by long term exposure to effect of leaf extract. Various changes observed in the liver of *Nile tilapia* exposed to *Azardiracta indica* plant extract included cytoplasmic degeneration, focal inflammation, fatty infiltration, karyolysis, pyknosis etc. Higher concentrations of *Ziziphus jujube* caused hypertrophy of both hepatic cells and nuclei, accompanied by the gradual reduction of cytoplasmic materials and vacuolation. The dislocation of nucleus and hepatitis reported by Kaplan *et al.*, 1967, and Mcfarland and Lacy (1968). *Ziziphus jujube* exposed to *B. melanostictus*, the damage to the hepatic cell body progressed gradually and the cytoplasm became less and less dense and ultimately disappeared completely. (Grandall and Goodnight, 1963; Dubale and Punitshah, 1979). Histopathological lesions in the liver of fish *Channa punctatus* induced by *M. olifera*. The similar observations have been reported by this study (Bane jee and Bhattacharya, 1997). The liver made up of hepatocytes that is arranged in branched lamellae, separated by sinusoids. We have observed the same result, the highest activity recorded 30 days at high concentration as well as duration. At the cellular level, it has been indicated that metal ions may compete with the required metabolic cofactors at the binding site affecting enzymatic activity. After entering the liver, the heavy metal might have undergone metabolism in cells to release reactive fish species which contributed to leaf extract activation (Roopavathy and Geetha 2019).

After 15 days of exposure to 0.33 ml/lit leaf extract affect the liver cell. Acute and extensive necrosis of liver cells was observed particularly focal necrosis of liver cells was observed. Hepatic tissue of treated specimens showed varied degree of hepatic cirrhosis as evident in the density of fibrous connective tissue within and around the hepatic paranchyma. Changes the occurred is also reflected in the treatment and consisted of damage to the biliary columnar epithelial cells which are separated from the connective tissue (Roopavathy and Geetha 2019)..

Azadiracta indica leaf extract exposed to difference concentration on liver of fish. It exhibited toxic responses which eventually lead to severe damage and it lead to death. Liver is the first organ to face any foreign molecule through portal circulation and is subjected to more damage (Roopavathy and Geetha, 2019) (Fig:

1,2,and 3).

The present study showed that histology is a useful biomarker for environmental contamination. The plant extract being used as bio pesticides are toxic to the fresh water fishes which constitute the non- target organism, causing large number of changes in liver structure. The observed necrosis and complete damage are direct response induced by the action of *Ziziphus jujube* leaf extract.

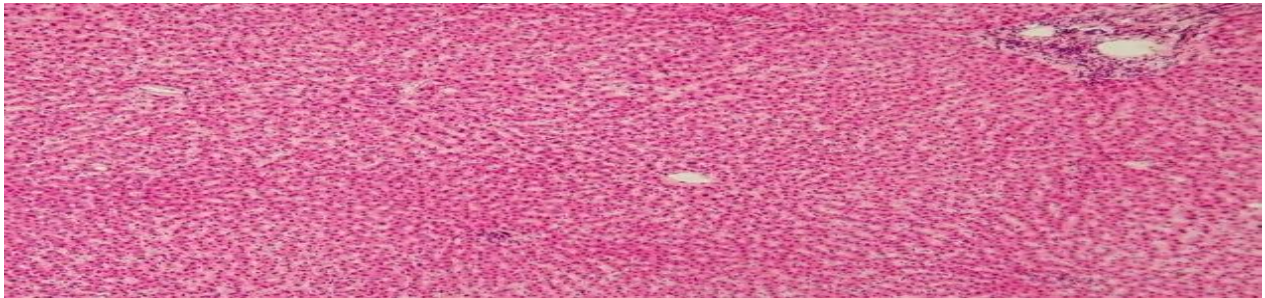


Fig 1. *Ziziphus Jujube* Leaves Extract Exposed On Liver Of Fresh Water Fish *Oreochromis Mossambicus*

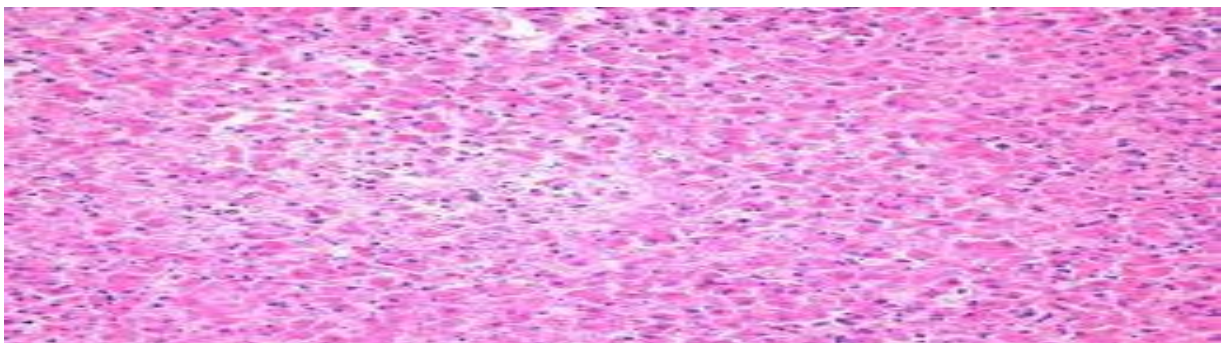


Fig 2. 10% Concentration Of *Ziziphus Jujube* Leaf Extract Treated On Liver Of Fresh Water Fish, *Oreochromis Mossambicus*

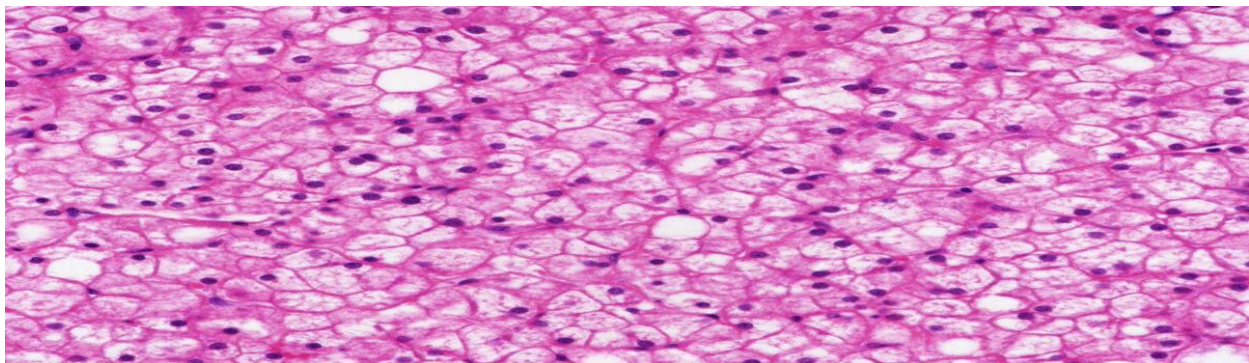


Fig 3. 30% Concentration Of *Ziziphus Jujube* Leaf Extract Treated On Liver Of Fresh Water Fish, *Oreochromis Mossambicus*

IV.CONCLUSION

The fish was collected from Palakad fish farm were acclimatized to fresh water condition for 2- 3 weeks in laboratory. Fresh leaves of *Ziziphus jujube* was collected from local area. Fresh leaves of plants then washed, shed dried and grinde into fine powder and used for test. Median Lethal concentration of leaf extract 1.1ml/lit for 96hrs, (24,48,72 and 96hrs) using probit anaylsis method (Finney 1961). After exposing *Oreochromis mossambicus* by *Ziziphus jujube* effect for a specific period, liver was dissected out from fish alive and were preserved in 10% and 30%leaf extract and processed for histological examination using standard histological technique. The present investigation has been recorded dissociation of liver tissues pyknosis, cirrohis. It exhibited toxic responses which eventually lead to severe damage and it lead to death.

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