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

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ABSTRACT

An investigation was carried out to evaluate the effect of selected plants (*Ziziphus jujube*) supplemented feed on the effect parameters and specific effect rate of *Oreochromis mossambicous.* Two experimental feeds were prepared by adding 10 ml of leaf extract to the basal diet and one control feed without plant extract. The fishes were supplemented with these feeds for 30days and the results were obtained for every 15 days once. The data were analyzed using standard deviation and the means were separated using least significant differences. The fishes fed with *Ziziphus jujube* supplemented feed showed maximum increase in gill congestion and specific growth rate. The maximum increase in length was observed in the fishes that were supplemented feed .This study indicated that inclusion of plant ingredients in fish feed resulted in superior effect performance and the formulation of plant based diet for fish will provide new opportunities.

KEY WORDS: *Ziziphus jujube,Oreochromis mossambicous.*

INTRODUCTION:

Aquaculture has become a key component of the animal health industry, due to the continued expansion of cultured fish and shellfish species Aquaculture is the fastest growing industry around the world with about 80 million tones being produced annually (Kolkovski and Kolkovaski., 2011). Aquaculture is therefore an emerging industrial sector which requires continued research with scientific, technical developments, and innovations (Alicia *et al*., 2005).

Medicinal plants have successfully been used to induce fertility in laboratory animals (Gary and Gary, 1971; Bodharker *et al*., 1974; Das, 1980). Jujube contains very rich folate, participate in the natural blood cells, promote the growth of fetus nervous system red date is nutritious tonic it besides contains rich vitamin and minerals, carbohydrate, protein, the pregnancy women and the health of the fetus is highly beneficial. The common jujube *(Ziziphus jujube)* is a plant that is native to Asia and Southern Europe. Own investigation shows that all Lebanese people asked knew the plant and have eaten the fruit while all Syrians asked about the plant did not know or eaten its fruit. It is a thorny *Rhamnaceous* plant that is widely distributed worldwide. It is a deciduous tree that grows very well in Mediterranean climate and can tolerate heat and acidity. It bears fruits that are size and delicious and of various shapes and size and great nutritional and medicinal value but has not been exploited commercially on the proper scale. The fruits can be eaten fresh, dried like dates or processed (jam, loaf, cakes, jelly etc.,). Different parts of *Ziziphus jujube* possess multiple medicinal properties. Although *Ziziphus* extract is considered of low toxicity towards non-target aquatic life (Martinez and Souza., 2002), aquaes leaf extracts of the bark of the jujube plant caused respiratory problems in Tilapia (Omoregie and okpanachi,1997), while long exposure to low concentrations of the leaf extract of *ziziphus jujube* delayed the growth of this *Oreochromis mossambicus* fish (Omoregie and okpanachi ,1992). Such results indicate that *Ziziphus jujube* extract added to water may cause disturbances on fish consequently it is important to recognize the effects that the employ of these products used to prevent the appearance of effects may have in different parameters of fish *(Rabaco Castro et al* .,2006).

Oreochromis mossambicous (Peters,1852) displays many favorable attributes as culture species; on the

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basis of its general hardness, resistance to disease, high yield potential and ability to grow on a wide range of natural and cheap artificial foods, A additionally it can also withstand low oxygen concentration, overcrowding, tolerates difficult ecological conditions and a wide range of salinities and still produces a high acceptable flesh (El-Sayed,2006).

Histopathological investigation has long been recognized to be reliable biomarkers of stress in fish for several reasons (The *et al.,* 1997; van der Oost *et al.,* 2003). The gill surface is more than half of the entire body surface area. In fish the internal environment is separated from the external environment by only a few microns of delicate gill epithelium and thus the bronchial function is very sensitive to environmental contamination. Gill are the first organs which come in contact with environmental pollutants, Paradoxically, they are highly vulnerable to toxic chemicals because firstly, their large surface area facilitates greater toxicant interaction and adsorption and secondly, their detoxification system is not as robust as that of liver (Mallatt,1985;Evans,1987). Additionally, absorption of toxic chemicals through gill is rapid and therefore toxic response in gills is also rapid. Gills have frequently been used in the assessment of impact of aquatic pollutants in marine as well as freshwater habitats (Haaparanta ,*et al.,* 1997; Athikesavan *et al*.,2006; Craig *et al.,* 2007; Fernandes *et al*., 2007; Jimenez -Tenorio *et al.,*2007). Therefore, lesions in gill tissues can be the star of imbalance of the physiological and metabolic processes of fish.

The toxic effects of herbal medicines on *Oreochromis mossambicus* fish. The aim of this study was determined the lethal concentration of the aqueous extract of *Ziziphus jujube* leaves exposed to *Oreochromis mossambicus* and to verify possible alterations in histopathological studies in response to an acute exposure to different concentration of this extract.

MATERIALS AND METHODS

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

Collection of Fish

Aliyar dam near pollachi and were acclimatized to fresh water condition 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.

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.

Preparation of Aquaous 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 50 grams of powdered leaves in 1 liter of distilled water and kept at room temperature for 24hrs, with intermittent shaking. After 24hrs the mixture was filtered and the extract was immediately in the experiment (Saravanan, *et al*., 2010).

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 15days.At the end of the experimental period, the fishes were sacrificed and samples were collected for further analysis. The Gill separated from the experimental fish were used for histopathological analysis.

Histopathological analysis of tissue sample

Gill 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 Gill of Oreochromis mossambicus in

normal condition and exposed to experimental conditions by long term exposure to leaf extract.

Toxicity Bioassay

The *Ziziphus jujube* tree is native in India and eco-friendly, The researched tree in the world. This leaves has water soluble extract of *Ziziphus jujube*, It is known as " Ber " in Iran and India and is widely used for medicine as a laxative and blood purifier. In china, it is used as taste enhances and if is recommended for treating fatigue, loss of appetite and diarrhea. It is believed the dried fruits of *Ziziphus jujube* are anodyne; anticancer, pectoral, sedative, stomachic, styptic and tonic and immune response enhances. Some of the compounds isolated from the seed of *Ziziphus jujube* exhibit significant pharmacological activities. In India it is as food, odder, nutrient, medicine, construction material and fuel. *Ziziphus jujube's* medicinal properties are attributed to a diverse group of secondary metabolites such as alkaloids flavonoids, terpenoids, saponin, pectin, triterpenoic acid lipids (i,e *Jujubiside* (saponin) isolated from jujube is reported to have hemolytic, sedative and sweetness inhibiting properties. Gill are the first organs which come in contact with environmental pollutants, Paradoxically, they are highly vulnerable to toxic chemicals because firstly, their large surface area facilitates greater toxicant interaction and adsorption and secondly, their detoxification system is not as robust as that of liver (Mallatt,1985;Evans,1987). Additionally, absorption of toxic chemicals through gill is rapid and therefore toxic response in gills is also rapid. Gills have frequently been used in the assessment of impact of aquatic pollutants in marine as well as freshwater habitats (Haaparanta ,*et al.,* 1997; Athikesavan *et al*.,2006; Craig *et al.,* 2007; Fernandes *et al*., 2007; Jimenez -Tenorio *et al.,*2007). Therefore, lesions in gill tissues can be the star of imbalance of the physiological and metabolic processes of fish.

The 96hrs LC50 values of freshwater fish, Oreochromis mossambicus was determined in the laboratory studies 1.1ml/lit LC50 value for 96hrs was presented in the Fig2. In this study, fish were exposed to0.11ml/lit and 0.33ml/lit sublethal concentration which corresponded to 10% and 30% of the 96hrs LC 50respectively.

Effect of Ziziphus jujube leaf extract on the Gill histology of the fish,

Oreochromis mossambicus

General Histology of Gill in control

Teleost fishes have five pairs of gill arches. In the front, four pairs the slender gill filaments from two lines facing towards the back and these two lines are joined to each other at the base by a gill septum. The last pair of gill arches generally transforms into the pharyngeal bone and does not play a role in respiration. Numerous semicircular secondary gill lamellae are lined up along both sides of the gill filament. The surface of the gill lamellae is covered with simple squamous epithelial cells and many capillaries separated by pillar cells run parallel along the surface. Numerous semicircular secondary gill lamellae consist of centrally placed rod like supporting axis with blood vessels on either side. The secondary lamellae, also termed as respiratory lamellae, are highly vascular zed and covered with a thin layer of epithelial cells, Blood vessels are extended into each of the secondary gill filaments. The blood cells of the secondary filaments.The blood cells of the secondary gill lamellae have a single nucleus, which are flattened in appearance. The region between the two adjacent secondary gill lamellae is known as inter lamellar region.

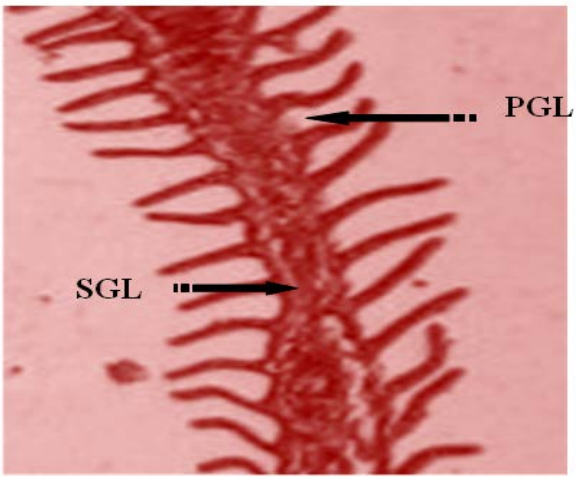

Fig.1: Control Gill of *Oreochromis mossambicus*; Primary gill lamellae (PGL), Secondary gill lamellae (SGL).

Experimental group

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

The histological analysis in the control fish showed normal structure but in the treated fishes the gill exhibits the abnormalities like hyperplasia of secondary lamellae, disruption of structure of mucous layer, shrinkage of secondary lamellae,

epithelial desquamation, blood lamellar congestion,, aneurism, lamellae clumping, hyperplasia of secondary lamellae, curling of secondary lamellae, and destruction of epithelium.

After 15 days of exposure to 0.11ml/lit leaf extract lamellar clumping, shrinkage of secondary lamellae, Hyperplasia of secondary lamellae were noted

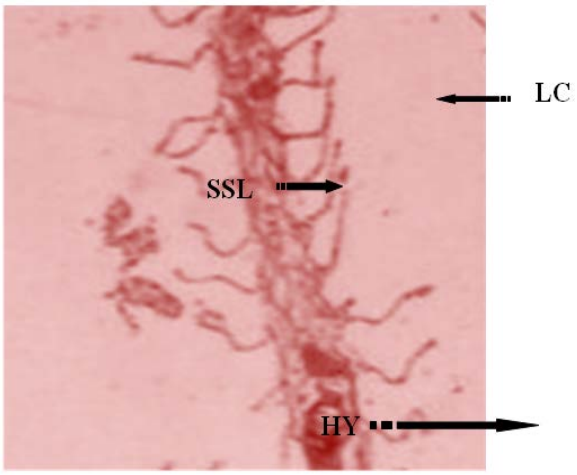

Fig.2: Gill of *Oreochromis mossambicus* after 15 days of exposure to 0.11ml/lit leaf extract; Lamellae clumping (LC), Shrinkage of secondary lamellae (SSL), and Hyperplasia of secondary lamellae (HY).

30%Concentration of *Ziziphus jujube* leaf extract treated on Gill of fresh water fish *Oreochromis mossambicus*

Histological features of the gills indicated from damage as there was Shrinkage of secondary lamellae, Disruption of structure of mucous layer, Hyperplasia of secondary lamellae, Blood lamellar congestion and aneurism were observed in the gill of the examined at a concentration of 0.33ml/lit after 15 days of exposure to leaf extract. Another important histopathological changes observed in the leaf extract treated group was hyperplasia. Morphologically, hyperplasia refers to an increase in the number of normal cells that constitute a given tissue. Gill alterations such as hyperplasia of the epithelial cells can be considered adaptive, since they increase the distance between the external environment and blood, serving as a barrier to the entrance of contaminants.

As fish gills are critical organs for their respiratory and osmoregulatory functions, the injuries in gill tissues observed as a result of exposure to extract compounds may have reduced the oxygen consumption and resulted in the disruption of the osmoregulatory functions of the fish. As gills are the major site of osmotic and ionic regulation in fish also the histopathological alterations could be attributed to increased damage to gill membrane in fishes exposed to extract compounds. It is important to stress that lamellar fusion and disappearance of secondary lamellae

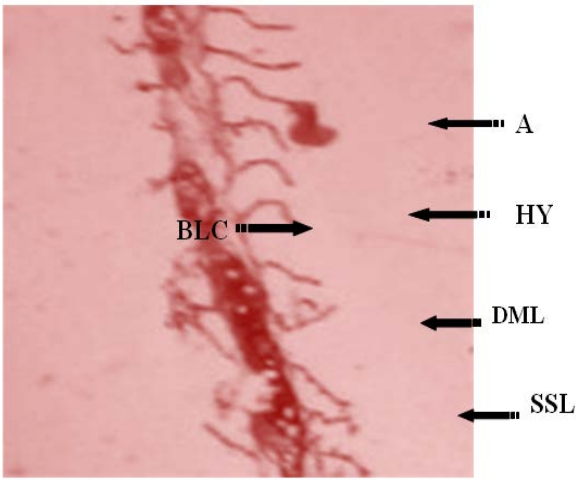

Fig.3: Gill of *Oreochromis mossambicus* after 15 days exposure of 0.33ml/l leaf extract; Shrinkage of secondary lamellae (SSL), Disruption of structure of mucous layer (DML), Hyperplasia of secondary lamellae (HY), Blood lamellar congestion (BLC), and Aneurism (A).

DISCUSSION

The gills perform many important functions in the fish, such as respiration, osmoregulation and excretion. They remain in close contact with the external environment and particularly sensitive to changes in the quality of the water and thus are considered the primary target of the toxicant (Chandra and Banerjee , 2003).

Chavin,1973 reported that the histopathological lesions observed in the organism are due to exposure to extract compounds. Histopathological characteristics of specific organs express condition and represent time- integrated endogenous and exogenous impacts on the organism stemming from alterations at lower level of biological organization (Chavin, 1973). The damage of gills of fish exposed to the sub- lethal concentrations of extract compounds was severe. Extensive architectural loss was observed in the gills of extract treated group. Richmonds and Dutta (1989) divided the commonly reported gill lesions into two groups: (1) The direct deleterious effects of the irritants and (2) The defense responses of the fish. The observed lamellar necrosis and desquamation of the gill epithelium are direct responses induced by the action of extract compounds.

Increased mucus production and fusion of lamellae were obvious on exposure to both the extract compounds. Mucus cells contain mucins,

polyanions composed of glycoproteins that can be effective in trapping toxicants and aid in the prevention of toxicant entry into the gill epithelium,(Perry and Laurent , 1993) . Extensive epithelial desquamation was also observed in the extract treated group. It is well known that changes in fish gill are among the most commonly recognized responses to environment (Mallatt , 1985, Laurent and Perry, 1991; Au , 2004) Similar results were observed in this study.

The gills of both extract treated group exhibited lamellar telangiectesis (localized dilation of blood vessel) This appearance of the secondary lamellae results from the collapse of the pillar cell system and breakdown of vascular integrity with a release of large quantities of blood that push the lamellar epithelium outward (Alazemi *et al.,* 1996) .Shortening and clubbing of ends of the secondary gill lamellae and clubbing of adjacent lamellae were well marked in the extract treated group. Complete lamellar fusion may have reduced the total surface area for gas exchange .Otherwise, they increase the distance of the water – blood barrier, which together with epithelial lifting and the increase in mucus secretion may drastically reduce the oxygen uptake.

Histological study of the gills shows a typical structural organization of the lamellae in the untreated fish. However fish exposed to extract shows several histological alterations, namely lamellae epithelium, shrinkage of secondary lamellae, Hyperplasia of secondary lamellae, Blood lamellae congestion, Fusion of lamellae and lamellae aneurisms. These similar results were observed by Marine Harsan, *et al.,* 2013, Poleksic and Mitrovic- Tutundzic, (1994).

Lamellae axis vasodilatation was also found in tilapia exposed to extract.(Shoeiba Tasneem *et al,* 2016) refer that this lesion can induce changes in pillar cell normal structure, with consequence loss of their support function and probably, and was responsible for the emergence of lamellae aneurism in fish exposed to *Jujube*. Similar result is observed by Thophon *et al.,* 2003. The present study was made to investigate the histological alterations of gill lamellar fusions and diffusion of secondary lamellae, shrinkage of secondary lamellae was observed. This study concluded *Ziziphus jujube* leaf extract severely damage the gill structure fresh water fish *O. mossambicus.*

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