

Defensive Impact of *Linum Usitatissimum* (Flax Seed) On Dexamethasone Induced Osteoporosis in Zebra Fish

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I. INTRODUCTION

Osteoporosis being a systemic disease of the bone, affects millions of people worldwide. This disease not only burdens the affected individual but also the health care system worldwide (1). Osteoporosis is identified by low bone mass and micro-architectural destruction of the bony tissue leading to fragility of the bone and resulting in increased risk of fractures. The progress of the disease is asymptomatic meaning there is no warning sign until a fracture occurs (2). Widespread divergence in the event and dissimilarity of Osteoporosis is hard to survey because of the issues in diagnosis and treatment. An easy way to compare Osteoporosis prevalence in populations is by using the rate of fragility fractures in the aging population. Yearly a huge cost is being spent on Osteoporosis treatment especially on hip fractures (3). Approximately 1.66 million hip fractures occur every year throughout the world and it is predicted that it might increase by four times the year 2050 due to the aging population (4).

Treatment for Osteoporosis focuses on the change of pathogenic variables to decrease bone loss or construct another bone tissue. The cardinal parts of the treatment plan are nourishment, work out, fall prevention and medication treatment. The best or the suggested mediations or life style alterations in the prior phases of life (5). Despite the fact that there is no remedy for Osteoporosis there are prescriptions accessible for the palliative treatment of the disease which incorporates bisphosphonates Estrogen agonist or antagonist, calcitonin , PTH , Estrogen treatment , hormonal treatment and the as sanctioned RANK ligand inhibitor (6).

Choosing of a reasonable animal model is generally difficult. Animal models yield more predictable and consistent experimental information and considers enormous scope for

testing of possible treatments. A carefully picked relevant experimental animal model for the investigation of Osteoporosis reduced the risk and short comings which are related with concentrating on the disease in human beings. The significant expense and long time period of clinical testing is one of the reason why animal models play a pivotal part in Osteoporosis research (7). Even a model with a small representation of human functions may be used for some aspect of the human condition under examination (8).

Zebra fish develop a simple pattern of early larval cartilages and bones. It is this early basic skeletal pattern that is most highly conserved among all vertebrates. In recent years a variety of significant

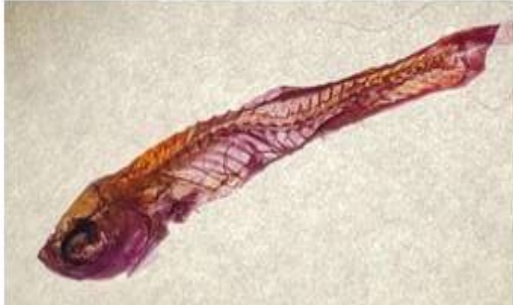
studies on molecular aspects of Zebra fish skeletal development has been published (9)(10). Morphological studies on skeleton of zebra fish has had a scattered coverage including studies on the development of the skull, paired fins, pharyngeal cartilages and developmental and adult axial anatomy (11). The aim of the present study was to evaluate the Osteoprotective effect of flax seed mixture on Dexamethasone induced Osteoporosis in Zebra Fish.

ZEBRA FISH SKELETON

Zebra fish physiology and genome association are in numerous angles like those of human's and the skeleton and mineralizing tissues are no exemption. *Danio rerio* stands apart as a great vertebrae model framework in which critical advancement in the space of improvement and development can be made. Regardless of its rising prominence little examination is being done on skeletal development. Lately methods have arisen that makes the investigation of tissue development conceivable in vivo. Likewise standard strategies are needed to evaluate mineralization of bone and ligament advancement including re-modelling and

regeneration(12).

SKELETON OF ZEBRA FISH STAINED WITH ALIZARIN RED S



II. MATERIALS AND METHODS

Animal Procurement

Wild type female Zebra fish was obtained from local aquarium. The procured fish was 2 months old when purchased. The purchased Zebra fish was raised in 28°C water. Fish were maintained in a closed flow through system under the standardized conditions of temperature (28±0.5°C) and a 14 hour light/ 10 hour dark rotation. Fish were fed twice daily with the prepared and the commercially available fish feed (13).

Grouping

Zebra Fish was randomly divided into the following groups as follows

Group 1 – Dexamethasone induced (control)

Group 2 - Dexamethasone + 17 beta estradiol (5µmol/L)

Group 3 - Dexamethasone + Commercially available holiday feed (10µmol/L)

Group 4 = Dexamethasone + Gelatin based flax seed holiday feed (10µmol/L)

Procedure for Making Gelatin Based Holiday Fish Feed

Weigh approximately 10g of shrimp, 4g of spinach, ½ table spoon of baby cereal (oats), 2g of grated carrot, 2-3 capsules of cod liver oil, 1-2 capsule of multivitamin tablet, 4g of unflavored gelatin powder, 2g of dry yeast (14).

- Blend shrimp, spinach, carrots, baby cereal, dry yeast and vitamins with few amounts of water
- Boil 15-20 ml of water in heating mantle
- Add boiling water to a gelatin in a bowl
- Cool the gelatin mixture until warm and not cold
- Add blended mixture to the warm gelatin mixture and mix thoroughly
- Lubricate the Petri dish with cod liver oil
- Pour the gelatin mixture to the Petri dish and refrigerate
- Cut the gelatinized mixture into cubes and store in a freezer

Treatment

The concentration of Dexamethasone solution utilized was 30µg per kg fish body weight. The technique for injection was chosen from the previous literature surveys. 0.9% w/v sodium chloride was utilized as a vehicle for dissolving the dexamethasone. The treatment with the medication as well as the feed was continued on for a time span of 5 days. Towards the end of the examination the fishes were anesthetized with ice cold water and stained to see the inner skeletal construction.

Gelatin Based Holiday Feed

The gelatin based fish food was prepared by utilizing carrot, spinach, oats, multi nutrient oil, cod liver oil, shrimp and flaxseeds. The prepared flaxseed mixture was refrigerated and fed twice a day after inducing the disease.

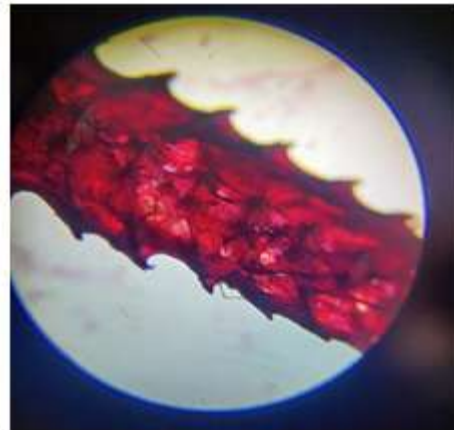


COMMERCIAL FISH FEED PREPARED USING FLAX SEED

III. RESULTS

The underneath figures show the alizarin red staining of the Zebra fish in the vertebrae. The prepared gelatine based flaxseed mixture was

viewed as powerful when compared with the control group. The mineralization of the gelatine based flaxseed was found to show better staining properties.



Group 1 – Dexamethasone Induced Group 2 – 17 beta Estradiol



Group 3 – Commercial feed Group 4 – Gelatin Based Flaxseed Feed
MICROSCOPIC EXAMINATION OF ZEBRA FISH SKELETON

IV. DISCUSSION

Osteoporosis is a systemic bone diseases bone diseases characterised by decreased bone mass and destruction of bone tissue microstructure, characterised by increased bone fragility and susceptibility to fracture(15). With the aging of our population and the increasing incidence of osteoporosis, it has seriously threatened human health. In this study, dexamethasone was used to establish a zebra fish osteoporosis model to evaluate the pharmacological and mechanism of gelatin based flax seed fish feed against Osteoporosis.

Osteopenia and Osteoporosis are bone disorders characterized by reduced bone mineral density

(BMD), altered bone microarchitecture and increased bone fragility. Because of global aging the incidence is rapidly increasing worldwide and novel treatment's that would be more efficient in preventing disease progression and at reducing the risk of bone fractures are needed. Pre-clinical studies are today a major bottle neck to the collection of new data and the discovery of new drugs, since they are commonly based on rodent in vivo systems that are time consuming and expensive, or in vitro systems that do not exactly recapture the complexity of low BMD disorder.

In this regard Zebra fish model mimic human Osteoporosis phenotypes has stimulated the interest of the academia and industry towards a

better understanding of the mechanism of pathogenesis but also towards the discovery of new bone anabolic or anti-resorptive compound's(10).

With the growth of the elderly population, osteoporosis has become a major public health problem worldwide. Since osteoporosis is a chronic disease, natural flavonoids have certain advantages because of their fewer side effects and adaptation for suitability for long term use. It has been reported that zebrafish is highly conserved in terms of bone architecture and bone metabolism regulation compared to human.

During experimental period, morphological and behavioral characteristics of fish were observed. Fishes were swimming actively throughout the entire tank ,not just hanging out or laying at the bottom They consume the fish feed regularly and swim to the surface quickly during feeding time .Fish do not show any white spot or blemishes on their body; fins were not torn, curved or ragged and eyes were not bulged. Gill movements were very normal and controlled Fish showed no stomach bulging or fin curving indicating that they were healthy and the feed was not toxic and can be used in aquaculture.

Alizarin red S is used in histopathology mainly to stain or locate calcium deposits in tissues(16). In the presence of calcium alizarin red s will bind to the calcium to form pigments that is orange to red in color. Whole specimens can be stained with alizarin red s to show the distribution of bone. Alcian blue is a by product of acid making process. The only thing the dyes have in common is their affinity for calcium where they encounter it and stick to it. This is because the bones are filled with calcium they uptake the dye and stand out red or blue in the translucent remains of the flesh.

Finally it can be reported that estrogen deficiency might lead to less deposition or nearly no deposition of calcium in the bones and cartilage. Previous studies have reported decreased calcium in dexamethasone induced animals. Calcium is one of the most important mineral in the formation of bones. Having low levels of calcium content might be a major risk for the formation of brittle bones and muscle weakness. Proper maintenance of calcium is not only important for bone growth but also to protect the bone strength. Estrogen supports this activity by aiding in intestinal calcium absorption. Having low estrogen levels negatively impacts the body's ability to make use of calcium. In course of time with this less or decreased deposition of the calcium the skeletal system tend to loss its strength and integrity leading to brittle

nature. So it can be concluded that estrogen deficiency might lead to decreased absorption.

The samples were so clear that the skeletal parts were clearly seen. The absorbed colors were stable and did not fade away in course of time. At the end of the staining process the other tissues and muscles did not absorb any color. Samples kept their rigidity throughout the staining process. The control animal showed good staining of the bones and the cartilage. While the induced animal showed less staining of the bones.

From this it can be concluded that the flax seed holiday mixture had bone protective effects on the Zebra Fish skeleton. Thus proving its efficacy in improving the deficiency.

V. CONCLUSION

It is clear from the study that feed prepared for fish are non-toxic and have good nutritive value of spinach, carrot and Flaxseed. There appeared no adverse changes morphologically. Fishes were very healthy and normal throughout the study period indicating no adverse effect on their health. No infection whatever was noted during experimental period. The Final Report was gelatin-based flaxseed fish food showed maximum staining along with mineralization.

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Nil

Conflict of interest

There are no conflicts of interest

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