Effect Of Pinealectomy And Recovery Treatment Of Melatonin Dose On Vertebral Column Of Catfish (Heteropneustes Fossilis)

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Abstract: In order to study the effects of pineal removal on catfish (**Heteropneustes fossilis**) vertebral column, three groups of catfishes were formed.) Group-A was taken as control where normal fishes were placed. In Group-B, the surgery of pineal gland was performed i.e. pineal gland was removed and considered as pinealectomized group. Group-C fishes were pinealectominzed, administered with injections of 200mg melatonin dose/Fish. At the end of experimental period, growth abnormality in vertebral column and minerals contents in bones were measured. Results indicate that in pinealectomized group and melatonin received group fishes, the growth was reduced and hence lives weight gain values were obtained with negative sign. Results on vertebral column suggested that more curvature in spine was recorded in pinealectominzed fish. Whereas the fish which received melatonin dose the change in vertebral column was only slightly. Which indicates that melatonin is helpful to overcome the deficiency of mineral contents in bone.

Index Terms: catfish, melatonin, minerals contents, pineal removal, vertebral column

1 INTRODUCTION

The skeleton plays a major role in whole body physiology, metabolism and homeo- stasis (Karsenty and Ferron, 2012; Karsenty and Oury, 2012). Vertebral development is an active and complicated process, and a variety of influences cause its deterioration. One of the most important morphological deformity in spinal system has been reported in fish during the development (Kristen, et al, 2007). Melatonin is an important hormone mainly secreted by the pineal gland but there are some other organs like bone marrow, gastrointestinal tract and retina where its synthesis also be noticed. Further, Melatonin is also found suitable candidate to regulate physiological processes of bone (Cardinali, et al, 2003, Witt-Enderby, et al, 2006, Maldonado, et al, 2007, Fischer, et al, 2008, Peschke 2008, Jan, et al, 2009, Gitto, et al, 2009) and hence considered as important in skeleton system. At cell and tissue levels melatonin has been shown to promote osteogenesis and hence in this way bone deterioration in fishes can be prevented (Fjelldal, et al, 2004). Any Alterations in vertebral bodies related to week mechanical strength and deficiency in mineral content. Along with this it is also helpful to its easy diagnose and hence any malformations related to Phosphorous deficiency can be prevented in farmed fish like S. salar, Paul, et al, (2015). In Cyprinus carpio spinal deformities occurring at multiple sites which indicates that age of fish is also correlated with inter-vertebral osteophyte formation, vertebral compression and fusion (Chin, et al, 2016).

2 MATERIALS AND METHODS

2.1 Experimental design

Catfish (mean body weight 20-50gm) were randomly distributed @ 15 fish per aquarium with two replicates. All fish were fed daily @ 5% of the body weight twice a day. For this three groups were prepared: control, pinealectomized and a group of pinealectominzed fish received 200mg melatonin. At the termination of the experiment, the radiography of the vertebrae of all the fish was obtained from Apex Super Speciality Hospital in Model Town, Rohtak, Haryana. After 90 days final length and weight of the catfish were measured.

2.2 Pinealectomy

Pinealectomy (Px) of fishes was done by following procedure of (Francis, et al, 2004). After pinealectomy 90% survival rate were recorded in catfishes.

2.3 Melatonin solution

Melatonin solution was prepared by following the procedure of (Ghosh and Nath, 2005). The fresh solution of melatonin was prepared and preserved in dark bottles in a refrigerator at 4˚C every week.

2.4 Experimental Procedure

The I.P. injection of melatonin was given to the fishes by following the procedure of (De Vlaming, 1980).

2.5 Estimation of calcium and phosphorous

The values of calcium and phosphorus were calculated in the control, pinealectomized and pinealectomized H. fossilis treated with melatonin by using flame photometer following the procedure of (Shah, et al, 2011).

3 RESULTS AND DISCUSSION

After 90 days of the experiment the final weight and length of the catfishes were recorded which suggested that the body weight and length was accelerated in the catfishes of the control group as compared with that of the pinealectomized and pinealectomized catfishes which were administered with melatonin dose (Table 1). The results of the present study revealed that the calcium and phosphorous content was maximum in pinealectomized group treated with melatonin dose when compared with that of the control or pinealectomized group. The results suggested that after pineal removal the values of calcium and phosphorous were declined and these values were accelerated when the pinealectomized fishes were treated with the melatonin dose (200mg).

Table 6.4 *Effect of pinealectomy and melatonin dose 200mg on live weight gain, calcium and phosphorous content in catfish*

Parameter	1 (NP) (control)	2 (P)	3 (P) 200mg (M)
Initial weight (g)	27.29 ±0.02	27.50 ±0.30	28.55 ±0.10
Initial length (cm)	19.2 ±0.20	19.4 ±0.20	20.6 ±0.20
Final weight	28.44 ±0.03	25.93 ±0.05	25.95 ±0.03
Final length	20.3 ±0.20	18.5 ±0.30	18.3 ±0.20
Live weight gain	1.15 ±2.93	-1.57 ±2.35	-2.60 ±6.89
Length gain	1.1 ±0.40	-0.9 ±0.50	-2.3 ±0.01
Calcium	170.90 ±0.06	157.50 ±0.25	197.50 ±0.04
Phosphorous	0.028 ±0.003	0.026 ±0.002	0.027 ±0.004

All the values are mean ± S.E. of mean
(NP) non-pinealectomized, (P) pinealectomized, (M) melatonin

The significance value for initial weight gain was 0.055, C.V. 0.821, final weight 0.0009, C.V. 0.231, live weight gain 0.889, phosphorous 0.074, C.V. value 1.39.

After termination of the experiment (90 days) the radiographs of the H. fossilis were observed and analyzed which indicated that the vertebral column of the control group (Figure 2A) was straight while the vertebral column of the pinealectomized (Figure 2B) catfish was curved and slightly curved vertebral column (Figure 2C) was recorded in the pinealectomized catfish treated with the melatonin dose (200mg). The results of the present investigation revealed that the pinealectomy caused the abnormality (curved) in the vertebral column and melatonin administration (200mg) in the pinealectomized fish decreased the curve of the vertebral column. Circadian rhythm which is very important process in animals is regulated by melatonin. Recently, studies have shown that melatonin may have a positive effect on the skeleton (Amstrup, et al, 2015). Melatonin may affect bone metabolism through bone anabolic as well as antiresorptive effects this can proved by many researchers with their work. In animals where pineal is removed, bone mineral density is found to be significantly decreased supporting the importance of sufficient melatonin levels (Amstrup, et al, 2013) The pineal gland releases melatonin hormone which affect the growth and development of bone (Cardinali, et al, 2003). The melatonin treatment in mice accelerated the bone mass (Koyama et al., 2002). In Atlantic salmon (Salmo salar) the pinealectomy caused 82% of the fish showed lateral and dorso-ventral curvatures of the spine (Fjelldal, et al, 2004). The bone mineral density was declined in the pinealectomized chicken (Turgut, et al, 2005) and resulted in scoliosis (Beuerlein, et al, 2001). Pinealectomy is also responsible for scoliosis deformity resulting melatonin-deficiency (Man, et al, 2014) In Atlantic salmon pineal removal caused the deformation of the spine resulted in the decreased amount of calcium and phosphorous of the vertebral bodies (Fjelldal, et al, 2004). In chicken pineal removal also lead to the development of spinal curvature (Machida, et al, 2001). The melatonin administration 5mg/kg/day resulted in the accelerated bone mineral density and bone mass. The melatonin treatment decreased the bone resorption parameters and osteoclast number (Cardinali, et al, 2003). In mice, melatonin administration, increased the bone mass. In chicken, pinealectomy has been showed to development of spinal deformity (Beuelien, et al, 2001). Melatonin hormone was involved in the calcium metabolism (Hakanson and Bergstrom, 1981). The non-pinealectomized chickerns in the control group showed normal spinal column but a prominent deformity was found in pinealectomized chickens (Turgut, et al, 2005). In Salmon, the pinealectomy was resulted in the development of abnormal spinal curvatures (Inoh, et al, 2001, Machida, et al, 2001). In very recent study carried on humun by

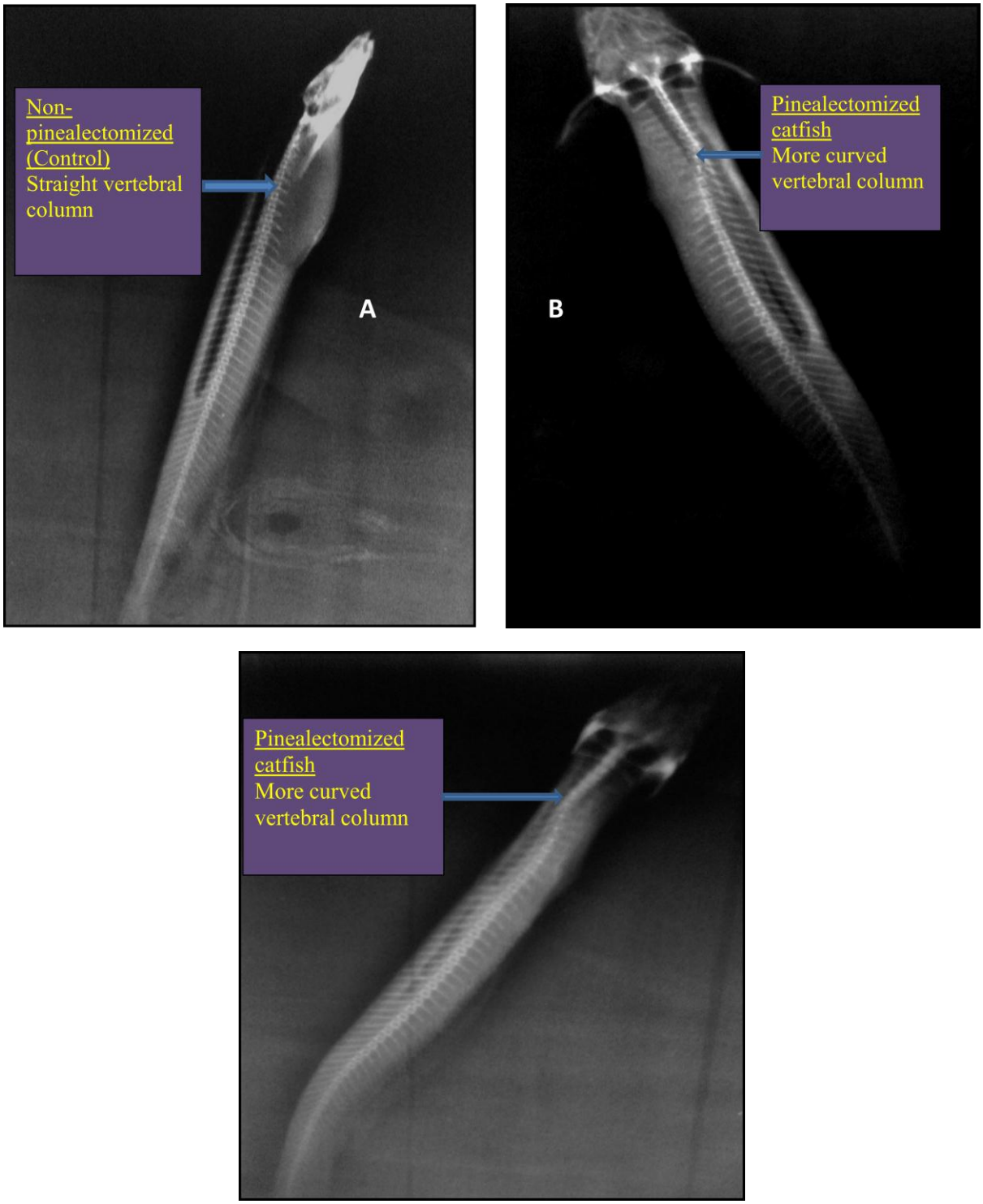

Fig. 2. *Radiograph of H. fossils (A) Control group (B) Pinealectomized group (C) pinealectomized group treated with melatonin dose (200mg)*

Bao, et al (2019) indicated that melatonin might be used as a safe nutritional supplement to improve bone density in perimenopausal and postmenopausal women and it can be consider as suitable candidate which Increases Bone Mass around the Prostheses of OVX Rats (Zhou, et al, 2019).

4 CONCLUSIONS

The present results indicate that melatonin deficiency in pinealectomized fish and because of this scoliosis may be arises. The reinstatement of melatonin with the help of injection enhances the low bone density and repairs the abnormality of bone.

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