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Effect of fluoxetine on the growth and stress response of juveniles of golden mahseer, *Tor putitora*

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Abstract

Tor putitora is commonly known as golden mahsheer due to its attractive golden colour. *Tor* is commonly confined in the Himalyan River foothills of Indian subcontinents. The present study was analysed to determine the effect of fluoxetine on the growth and stress responses in juveniles of mahseer. Fluoxetine is a selective serotonin reuptake inhibitor. Serotonergic system of brain affects the anxiety behaviour of fishes. Whole body cortisol assay was measured in order to quantification of stress response. The average body mass of control was higher ($0.172 \pm 0.01g$) as compared to fluoxetine exposed group ($0.137 \pm 0.01g$) with a coefficient of variation 16.79% and 20.58% respectively. The body mass of experimental group shows a significant difference at ($t=5.542$ $df=4$), $P < 0.005$. The cortisol value of control was smaller $14.18 \pm 6.52ng/ml$ as compared to fluoxetine exposed group $16.90 \pm 6.38ng/ml$.

Keywords: *Tor putitora*, Fluoxetine, Serotonergic System

Introduction

Neuroendocrine system plays an important role in the physiological governing of animal. Serotonin is a neurotransmitter which plays an important role in stress. *Tor putitora* is commonly known as golden mahseer. Golden mahseer is an important cyprinid fish endemic to Asia^[1]. *Tor putitora* is found in temperate regions basically in trans himalyan region. In recent years its population is decreasing due to anthropogenic reason and pollution in natural water bodies^[2]. Many workers worked on the effect of antidepressant such as fluoxetine and its role in zebrafish^[3]. Jan A. Mennigen *et al.* (2010)^[4] worked on the Waterborne fluoxetine disrupts the reproductive axis in sexually mature male goldfish *Carassius auratus*. Neuroendocrine control of stress, reproduction are also correlated to each other in relation to physiological functioning. The monoamine serotonin has been implicated as an important neurotransmitter in the mediation of a variety of behaviours. In particular, a growing body of research has indicated that serotonin plays an important role in associative learning processes related to appetitive and aversive stimuli, impulsivity, as well as the onset and maintenance of aggressive behaviours^[5, 6, 7]. In addition, serotonin's relationship with affective disorders, such as depression and anxiety, has been well characterized; indicating that serotonin may play a role in mediating affective states^[8].

Akhtar *et al.* (2013)^[9] worked on the effects of temperature on the juveniles of *Tor putitora* in relation to oxygen consumption and haematological variables. Gupta *et al.* (2014)^[10] considered it as an intelligent fish species. Large head, body and scales are important morphological characteristic features of mahseer. The head length of the fish exceeds that of the body depth^[11, 12]. Significantly declining population of fish has been categorised as an endangered species in the IUCN red list^[13]. Joel Weinberger II, Rebecca Klaper (2014)^[19] worked on the impacts of fluoxetine on specific behaviours such as reproduction, feeding and predator avoidance in the fish *Pimephales promelas* (fathead minnow). Jan A. Mennigen *et al.* (2009)^[14] also worked on the affect of fluoxetine on the weight gain and expression of feeding peptides in the female goldfish Brain. In the present time the population of *Tor* declining with a fast rate. This declining population of fish raises a lot of questions in the biologists such as whether the stress affects its growth performance. There are no records of neuroendocrine study of *Tor putitora* juveniles in relation of antidepressant drugs. In this study our hypothesis is based on the above pertinent questions whether the exposure of fluoxetine affect the growth and stress in the juveniles of *Tor putitora*.

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Materials and Methods

Juveniles of *Tor putitora* were purchased from the Directorate of Coldwater fisheries Bhimtal and then transported to the laboratory of L. S. M. Govt. P.G. College Pithoragarh. Juveniles were acclimatised in plastic pools for 40 days. The liver paste of goat was given them as a food resource. The juveniles were grouped in two spate plaxiglass aquaria (60 ×30× 30cm) groups each group contains 12 juveniles as control and experimental. Fluoxetine at the rate of 250µl/litre was dissolved in the experimental tank before the exposure of fishes. The initial weight of each fish was taken in a digital electronic balance with precision of 0.001gm(Roy Electronics India).Each fish was weighed in order to study their growth performance for a period of 40 days. At the end of experiments whole body of juveniles were killed and stored in -20°C until extraction of cortisol. The stored body samples were immersed in 1X PBS buffer and homogenised by a homogeniser (Remi model no.RQT-127AD). The homogenate were mixed with diethyl ether and votexed for the proper mixing. The mixture was three times centrifuged at 3500 rpm

for the complete extraction of cortisol. After the centrifugation the slight yellowish supernatant was transferred in separate eppendorf tube and kept in a 4°C for the evaporation of ether. The evaporated samples dissolved in 1X PBS buffer and kept at 4°C. After this process the ELISA was performed as manufacturer’s instructions to quantify cortisol concentration using cortisol assay kit (Oxford Biomedical Research. The Elisa reactions intensity was measured in Elisa Plate Reader (Systronics) at 450nm.All the statistical analysis was done by Graph Pad Prism.

Results

The both groups of juvenile’s showed a zig-zag pattern of body mass in fig 1.All the data of different days were exposed to t test in order to find out a significant difference in the body weight of the juveniles of *Tor putitora*. The fluoxetine exposed group of juveniles showed a significant difference in the body weight at day 27($t=2.658$, $df=14$, $P<0.02$) and 40($t=2.431$ $df=14$, $P<0.03$) as compared to control group of juveniles of *Tor putitora*.

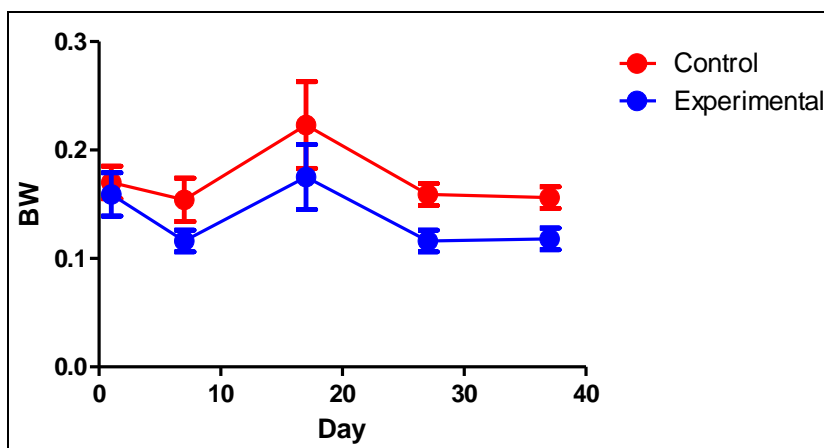


Fig 1: Body mass growth of the juveniles of Golden Mahsheer *Tor putitora*.

The average body mass of control was higher (0.172±0.01g) as compared to fluoxetine exposed group 0.137±0.01g) with a coefficient of variation 16.79% and 20.58% respectively. The overall body mass showed a highly significant difference

($t=5.542$ $df=4$, $P< 0.005$.) fig2. Wilcoxon signed rank test also showed a significant difference in median value of body mass at, $P<0.029$.

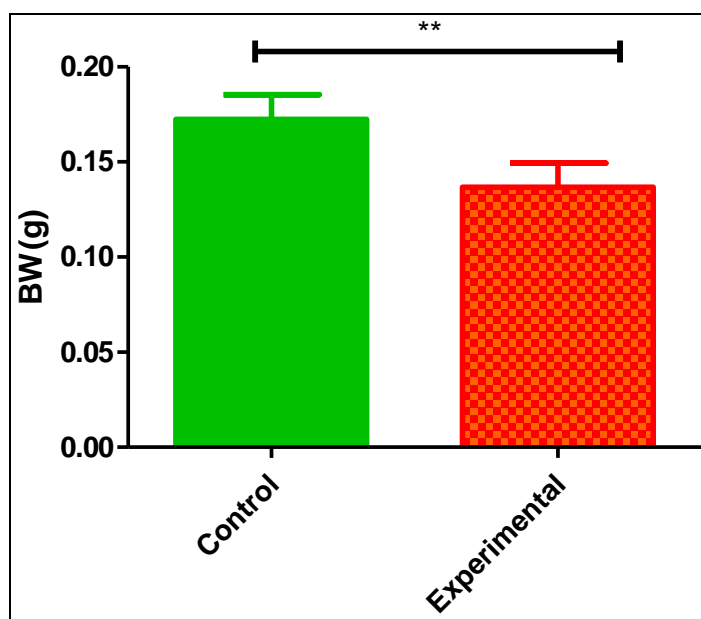


Fig 2: Average body mass of the juveniles of Golden Mahsheer *Tor putitora*.

The cortisol value of control was smaller $14.18 \pm 6.52 \text{ ng/ml}$ as compared to fluoxetine exposed group $16.90 \pm 6.38 \text{ ng/ml}$. The paired t test showed no significant difference in the cortisol value ($t=0.6243$, $df=9$, $P<0.055$). Fig 3.

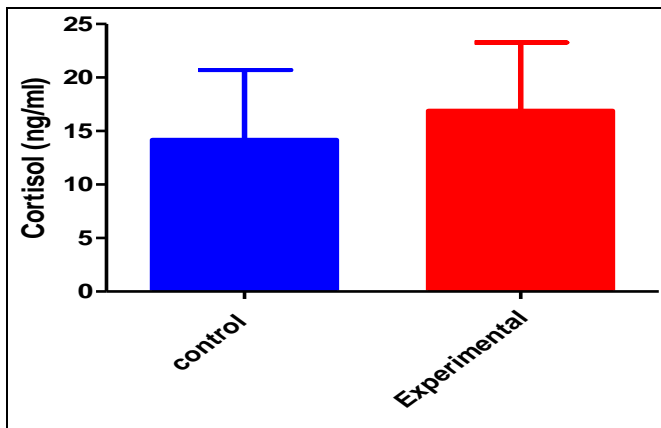


Fig 3: The cortisol value of the juveniles of Golden Mahsheer *Tor putitora*

Discussion

Fluoxetine is a selective serotonin reuptake inhibitor. Neurotransmitters play an important role in determining the neurophysiological traits of animal. Serotonin also acts in this series as a regulator of depression. The temporal expression of body mass shows a significant difference between the control and experimental. Anti depressant, fluoxetine plays an important role in decreasing the body mass of individuals as in the above study indicates. Jan *et al.* (2009) [14] also concluded that fluoxetine treated gold fish also showed a decreasing body mass as compared to its control. Augusto Barbosa Júnior *et al.*, (2012) [15], characterised anxiolytic activity of fluoxetine in piauçu fish. Our results indicates that the low concentration of antidepressant reduces the body weight without causing chronic stress as the value of cortisol indicates without any significant difference between the control and experimental group. Several studies have suggested that SSRIs may have a significant neuroendocrine effects, which would have an impact on behaviour, hormones and potential number of eggs laid [16, 17, 18] (Lister *et al.* 2009, Fernandes *et al.* 2011, Meningen *et al.* 2011). Joel Weinberger II and Rebecca Klaper (2014) [19] also explained about the environmentally relevant concentrations of fluoxetine, an SSRI antidepressant have an impact on specific behaviours important to reproduction and predator avoidance. Bogdan F. Kania *et al.* (2012) [20] studied the aggressive behaviour of male *Betta splendens* after the exposure of fluoxetine. Fluoxetine administered through aquarium water significantly inhibited the expression of aggressive behaviour in male *Betta splendens*. Pharmacological studies regarding the antidepressant effect of fluoxetine showed a potentiation of the 5-hydroxytryptophan (5HT) - induced suppression of operant response, probably due to its 5-HT [21]. Serotonin is an important neurotransmitter and or neuromodulator as a regulator of aggressive behaviour in vertebrates. Experimentally increased concentrations of 5-HT or serotonergic activity has also been shown to reduce aggression in birds, other fishes, reptiles and mammals [22, 23, 24, 25, 26]. Fluoxetine is antidepressant and called as selective serotonin reuptake inhibitor (SSRI) because it inhibits the neuronal reuptake pump for 5-HT at synapses in both, central and peripheral nervous system. Various workers

demonstrated its suppressive action against the aggressive behaviour [27, 28].

Conclusion

Antidepressant fluoxetine (SSRI) reduces the body mass of animals as compared to control groups. Animals showed a higher value of cortisol as compared to control but there is no significant difference. The increased level of cortisol indicates that animals are undergoing stress, without any significant increases in chronic stress.

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