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Effect of melatonin and pinealectomy on gonadal activity during prespawning period of catfish (*Heteropneustes fossilis*)

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Abstract

Aim : The effects of intraperitoneal administration of melatonin dose (25, 50, 100, 200 and 400 mg per fish) on gonadal activities were evaluated during the prespawning period of catfish, *Heteropneustes fossilis* for 60 days.

Methodology : In this experiment, fourteen aquariums were used because the experiment was performed in replicate and fifteen fishes were suspended in each glass aquarium. One group of male and female catfishes were considered as control group and the catfishes in the second group were pinealectomized whereas the remaining five treatments were administered with low (25, 50 mg per fish) and high (100, 200, 400 mg per fish) doses of melatonin.

Results : During prespawning period of reproductive cycle, the gonado-somatic index of ovary of pinealectomized and melatonin treated fish decreased as compared to the control group. The number of yolky oocytes significantly decreased in the melatonin treated group as compared to the control group. After 60 days of pinealectomy, it showed inhibitory effects on the ovarian activity. The GSI of testes of pinealectomized fish decreased as compared to the control group. The number of spermatozoa cells increased with the increase in the melatonin dose as compared to control group. The GSI of testes was found maximum in the melatonin treated group as compared to the control group.

Interpretation : This study supports the concept that melatonin hormone administration helps in maturation of testes and increases the number of spermatozoa. This was just contrary to the results obtained in female catfish where melatonin treatment showed inhibitory effects on the ovarian activity during prespawning period.

Key words: Catfish, Gonads, Melatonin, Pinealectomy, Prespawning period

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Introduction

World's greatest challenge in near future is to feed a population of 9.6 billion and in this fisheries/aquaculture plays a significant role as they are considered as protein manufacturing industry (FAO, 2014). *Heteropneustes fossilis* is commonly cultured species all over India. It has the ability to survive in oxygen depleted waters compared with major carp species (Khan, 2014). *H. fossilis* feed on artificial diets at increased temperature and are able to withstand shifts in salinity (Thakur, 1991). Its flesh has high therapeutic and nutritional value and has high fecundity (Khan, 2014) and low production cost. *H. fossilis* is locally known as "Shingi" and can survive for maximum time when placed in captivity even in less amount of water (Narejo *et al.*, 2005). Further, in these fishes a number of physiological activities are carried out by pineal organ. Pineal gland is formed as an evagination from the embryonic features which consist of pineal stalk and end vesicle. Pineal gland releases an important hormone, melatonin that plays an important role via functioning of hypothalamo-hypophysial gonadal axis to establish seasonal reproduction in fishes (Reiter, 1986; Mazurais *et al.*, 2000). In fishes, secretion of melatonin is regulated by photoperiod. In pinealectomized fishes, the amount of melatonin is reduced.

During prespawning period, the hepatic synthesis of vitellogenin and its incorporation into the developing oocytes occurs, which results in the increase in ovarian weight with the development of yolky oocytes. Reproductive organs have melatonin receptors, thus melatonin affects the function of testis and ovary (Falcon *et al.*, 2007). Pineal gland and/or melatonin regulates the seasonal development of gonads in fish (Bromage *et al.*, 2001; Maitra *et al.*, 2001, 2006; Bayarri *et al.*, 2004). It also has inhibitory effect on ovarian vitellogenesis, plasma GtH II levels and GSI in *Clarias batrachus* (Ghosh and Nath, 2005), and affect the oocyte development in carps (Chattoraj *et al.*, 2005). The main source of melatonin hormone is pineal gland but several studies have reported that gastrointestinal tract and retina also release small amount of melatonin in fish (Mayer, 2000; Fernandez-Duran *et al.*, 2007) after pinealectomy. Fish seasonality has been demonstrated in fish species, but only some direct evidences exist that show how melatonin hormone influence brain-pituitary-gonadal axis to control reproduction, feed intake and growth. The complex effect of melatonin hormone on fish reproduction still awaits a more precise and complete knowledge. The objective of this study was to investigate the effect of pinealectomy and melatonin administration on the gonadal activity in *H. fossilis*, during prespawning period, when vitellogenic activity is at peak.

Materials and Methods

Experimental area : This experiment was carried out in the Fisheries/ Animal Biology and Behavior Laboratory, Department of Zoology, Maharshi Dayanand University, Rohtak, India.

Experiment set up : Male and female *H. fossilis* (mean body weight 20-50 gm) were procured from Sultan Aqua Integrated

Education and Research Foundation V.P.O. Nilokheri Karnal, Haryana. Before start of the experiment the fishes were acclimatized for 15 days under laboratory conditions, photoperiod (LD 12:12) and temperature $25 \pm 1^\circ\text{C}$. The experiment was carried out for 60 days. This experiment was performed in replicate, thus 14 aquariums were used and 15 fishes were suspended in each glass aquarium (60×30×30cm). Melatonin injections were given to 150 male and female fishes out of 210 fishes. Initial weight of the fishes were recorded before the experiment and the fishes were fed with formulated feed in the morning (at 8 a.m.) and in the evening (at 5 p.m.) @ 5% of their body weight. The water of each aquarium was restored with the stored tap water daily.

Pinealectomy : Pinealectomy (Px) of fishes was done by following the procedure of Francis *et al.* (2004). The fishes were anesthetized by tricaine methanesulfonate and posterior to the pineal window, a horizontal incision was made, which is clearly visible through the skin. The pineal gland is clearly visible when a flab of tissue was moved anteriorly. The pineal stalk was then removed from diencephalon and the pineal gland was extracted with the help of forceps and the area was cleaned with 70% ethanol. All procedure was done under binocular microscope.

Melatonin : Melatonin solution was prepared following the method of Ghosh and Nath (2005). Melatonin (25, 50, 100, 200 and 400 mg) was dissolved in (2, 4, 8, 16 and 32 ml) of 100% ethanol and diluted with teleost saline (20mg Na_2CO_3 per 100ml of 0.6% NaCl) to acquire the final desired concentration (25, 50, 100, 200 and 400 mg per 0.1ml). Every week fresh solution of melatonin was prepared and stored in dark bottles in at 4°C . Melatonin injections were given to fishes after 2 p.m.

Experimental procedure : Intra-peritoneal injections of 0.1ml melatonin were given to fishes thrice a week close to the ventral midline posterior to the pelvic fins following the procedure of De Vlaming (1980). Melatonin dose was given to the fishes according to Singh and Lal (1994). After 60 days of experiment, final weight of the fishes were calculated.

Live weight gain was calculated by the following formula :

$$\text{Live Weight Gain (g)} = W_2 - W_1$$

where, W_2 = final weight (g); W_1 = initial weight (g)

Gonadosomatic Index (GSI) : After 60 days, male and female *H. fossilis* were randomly selected from each treatment to calculate the GSI of each individual by the following formula :

$$\text{GSI} = \frac{\text{Gonad weight (g)}}{\text{Body weight (g)}} \times 100$$

Histological examination : Anesthetized catfish (male and female) were dissected and the gonads were fixed in 10% formalin for 24 hr. The gonads were then dehydrated in alcohol and then transferred from 100% alcohol to a mixture of 100%

Fig.1 : Light micrograph of pinealectomized and melatonin treated, *H. fossilis* ovaries during pre-spawning period showing different stages of development (H&E stain $\times 400$). T.S. of ovary (A) more SIII oocyte with increasing number. of acidophilic yolk granules incorporated in ooplasm; (B) large number of S I oocyte, large centrally placed nucleus bearing ring of nucleoli enveloped by follicular epithelium; (C) more S I and less S II (cortical alveoli at the periphery of oocyte); (D) S II and S III oocyte; (E) and (F) more S I, less S II, and SIII oocyte and (G) only S I oocyte. T.S.: transverse section, ZR : zona radiata, YG : yolk globules, OP : ooplasm, N : nucleus, ne : nucleoli, CA : Cortical alveoli.

Fig. 2 : Light micrograph of *H. fossilis* testis during pre- spawning period (H&E stain $\times 400$). (A-B) T.S. of testis shows increased interstitial tissue and intact spermatogonia; (C-G) T.S. of testis shows increased number of Leydig cells and spermatogenic cells and SG- spermatogonia, ST- seminiferous lobules, SZ- spermatozoa, IT- interstitial tissue.

alcohol and xylene (1:1) for 30 min. After dealcoholisation, the gonads were treated with a mixture of xylene and paraffin wax (1:1) for 30 min in oven at 58°C, just above the melting point of paraffin wax, and then the tissue was transferred to pure paraffin wax for 30 min. Metallic 'L' moulds were used for the block formation of the tissue and the tissues were sectioned with the help of microtome, which was adjusted to 5-7 micron. The sections of tissue or ribbon were then placed on the glass slide on which a small layer of egg albumen was applied on the surface of the slide and after that the slide was slightly heated.

Slide formation and mounting : The glass slide was then transferred to the coupling jar filled with xylene for 10 minutes and then the slides were submerged in 100% alcohol for 10 min. The slides were kept in coupling jar for 5 minute each in descending order of ethanol 100%, 70%, 50% and 30%. The slides were then placed in haematoxylin stain for 5 min and immediately transferred to acid water for 2 min. The slides were placed in ascending grades of alcohol 30%, 50%, 70% and then stained with eosin for 5 min. After staining with eosin the slides were transferred in 90% and 100% ethanol for 5 min. After dehydration, the slides were placed in xylene for 10 min. Canada balsam or DPX was applied on the glass slide and immediately the cover slip was placed on the slide (Singh *et al.*, 2012).

Statistical analyses : One-way ANOVA was applied on the data of body weight and GSI of male and female catfish to analyze the significance between the treated group and control group followed by Tukey's honest significance test.

Results and Discussion

Throughout the experiment, the fishes were found healthy and no mortality was recorded in the control, pinealectomized and melatonin treated groups. After 60 days of experiment, the final weight of male and female *H. fossilis* of control group was maximum as compared to pinealectomized

and melatonin treated groups (Table 1). Histological changes in the ovary and testes of treated and control group were observed in *H. fossilis* during the prespawning period (May-June) of reproductive growth. During prespawning period, the ovary of *H. fossilis* consist large number of yolky oocytes. In females, the GSI in control group increased during the prespawning period as compared to the melatonin treated and pinealectomized group. The results showed that in male *H. fossilis*, the GSI of control group and pinealectomized group decreased as compared to melatonin treated group. The values of significance between the groups for female and male GSI was $p \leq 0.05$ and the C.V. value for female was 5.07 and male 20.19, respectively.

The histopathological study revealed that the number of stage III oocytes increased in the control group. The stage I oocyte or previtellogenic oocytes showed a significant increase with the increase in melatonin dose (Fig.1. C,E,F and G) when compared with the control group (Fig.1. A). The number of stage II and stage III oocytes decreased when melatonin dose was increased. After 60 days of pinealectomy, the number of Stage I, Stage II and Stage III oocytes decreased significantly. In male *H. fossilis*, the melatonin treatment increased the number of spermatozoas (Fig.2. C-G) as compared to pinealectomized (Fig.2. B) and control (Fig.2. A) group. The histopathological study showed that the ovary of melatonin treated female catfish suppressed the developing stage of ovary (Fig.1) whereas melatonin treatment in male *H. fossilis* increased the GSI (Table 1) and number of spermatozoa (Fig. 2). These results favored the study carried on *Clarias macrocephalus* where melatonin act as an important reproduction regulating hormone in male catfish, and its dose increased the first puberty stage in male catfish (Aripin *et al.*, 2014).

In *Oncorhynchus mykiss*, the melatonin administration decreased the body weight (Taylor *et al.*, 2005). The melatonin treatment in gold fish reduced the body weight which suggested that it may be due to less food intake caused due to melatonin dose (Pinillos *et al.*, 2001). In the present study, it was observed

Table 1 : Effect of pinealectomy (Px) and melatonin (MET) dose on body weight (g) and GSI of catfish, *Heteropneustes fossilis* under laboratory conditions

Parameters	Control	Px	25 mg	50 mg	100 mg	200 mg	400 mg
Initial weight	18.07 ±0.05	19.91 ±0.02	20.40 ±0.01	20.49 ±0.02	18.46 ±0.03	24.62 ±0.01	20.55 ±0.99
Final weight	21.12 ±0.02	19.16 ±0.01	18.50 ±0.03	17.96 ±0.03	15.67 ±0.02	21.15 ±0.05	16.47 ±0.02
Live weight gain (male and female)	3.05 ±0.03	-0.75 ±0.01	-1.91 ±0.04	-2.54 ±0.01	-2.79 ±0.05	-3.48 ±0.06	-4.09 ±0.96
GSI (Female)	16.05 ±0.07	15.06 ±0.19	12.48 ±0.70	11.76 ±0.68	10.83 ±0.19	8.79 ±0.21	8.58 ±0.35
GSI (Male)	0.94 ±0.03	0.73 ±0.04	0.84 ±0.04	0.95 ±0.01	1.43 ±0.46	1.60 ±0.08	1.71 ±0.04

Values are mean of two replicates ± S.E.

that the melatonin injection causes antigonadal effects by inhibiting the ovarian activity during prespawning period in catfish, *H. fossilis*. Exogenous administration of melatonin 100 mg/100g body weight/day showed accelerated oocyte growth in the preparatory period of reproductive cycle but the prespawning and spawning period was retarded in *Catla catla* (Mondal *et al.*, 2016). Melatonin administration (25, 50 and 100 µg) for 60 days inhibited the ovarian development in carp, *Catla catla* during prespawning season of reproduction (Maitra and Chatteraj, 2007). Melatonin dose (25, 50 or 100 µg per 100g b.wt.) decreased the number of stage II oocyte in *Catla catla* when compared to the control group during prespawning season, which indicated that melatonin dose suppressed ovarian maturation (Maitra *et al.*, 2005). During the prespawning phase, melatonin treatment decreased the GSI in female carp, *Catla catla* (Mondal *et al.*, 2016) where melatonin treatment in *H. fossilis* for 20 days during prespawning period inhibited vitellogenesis, induced follicular atresia and decreased the number of pituitary gonadotrophs. In a histological study carried out by Aripin *et al.* (2014) in male *C. macrocephalus* where the spermatogonia cells are maximum in control group and the spermatids and spermatozoa are in abundance in the melatonin treated group, which shows that melatonin administration increase the maturity in this fish. Melatonin treatment to fish inhibits the somatic and gonadal growth in *O. niloticus* (Singh *et al.*, 2012).

It also plays a significant role in the annual testicular regulation (Bhattacharya *et al.*, 2007). In *C. macrocephalus* melatonin feeding increased the maturation of sperm and testes (Aripin *et al.*, 2015), these results also resemble with the present study. This investigation showed that the Pinealectomy and melatonin dose causes inhibitory effects on the ovarian activity whereas melatonin treatment stimulates testes development during the prespawning period of catfish.

The information gathered in the present study collectively enhances the idea that exogenous melatonin administration and pinealectomy cause inhibitory effects on the ovarian development whereas exogenous melatonin treatment enhances the maturation of testes but pinealectomy retarded the testes development in *H. fossilis* during prespawning period of reproductive growth.

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