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Effect of Neurotransmitters on the Reproductive Biomarker and Ovarian Development in Giant Freshwater Prawn *Macrobrachium rosenbergii* (De Man, 1879)

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Abstract

In crustaceans, one of necessary things for embryonic and larval development is an extensive quantity of yolk accumulation within the developing oocytes during maturation. This type of changes are by the deposition of yolk material in the oocytes, by this oocytes diameter increases rapidly and in each new maturation stages and the colour of oocytes also changes due to the presence of a specific components which is called carotenoid. In this case the starting of vitellogenesis during early maturation is hemolymph vitellogenin concentration act as a good indicator of rapidly increasing until maturation.In central nervous system, the distribution of dopamine and serotonin a recognized as a neurotransmitter in invertebrates. This neurotransmitter has been involved in the control of gonadal development in decapods crustacean. Serotonin (5-HT) stimulates, while dopamine inhibit gonadal development in *M. rosenbergii*. In fact 5-HT stimulating release of the gonad-stimulating hormone (GSH) that is present in the brain and thoracic ganglia. DA is also present in the hemolymph. The treatment of prawns with 5- HT showed observable histological changes resulted in shortening the period of the ovarian development as well as increased GSI and oocytes diameters and 5-HT also significantly increased whereas dopamine had the opposite effect.

Key Words: Dopamine, giant fresh water prawn, *Macrobrachium rosenbergii*, ovary, serotonin, vitellogenin.
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Introduction

Fresh water prawn culture demand increases day by day in world-wide because it is a good source of protein and find an alternative for fisheries, in this case giant fresh water prawn *Macrobrachium rosenbergii* is an important species for prawn production the major limited access in crustacean aquaculture industry is the inadequate accessibility of good quality of seed. Reproduction in crustacean is regulated by various neurohormones that are synthesized and released from the X-organ-sinus gland complex located in the eyestalks of the species. The regulatory significance of various neurohormones involved in the process of oocytes growth and maturation in different crustacean species has also been discussed. Among the neurohormones that have been studied in the past are vitellogenesis-inhibiting hormones (VIH, also called gonad-inhibiting hormone, GIH) from the x-organ sinus gland complex Quackenbush 1989. vitellogenin stimulating ovarian hormone (VSOH) from the follicular layers of oocytes, vitellogenesis stimulating hormone (VSH, also called gonad stimulating hormone GSH) from the brain and thoracic ganglia (Tkayanagi *et al*., 1986) and juvenoids (methyl farnasoate) from the mandibular organ .Several neurotransmitter have been shown to about the release of these reproductive hormones such as the biogenic amines (dopamine and 5-HT) have also been shown to play important role in the synthesis and release of neurohormones in crustacean (Kulkarni *et al*,1992, Sarojini *et al*, 1995, Fingerman,1997,Vaka and Alfaro,2000, Aktas and Kumlu,2005). Particularly 5-HT (Serotonin) and dopamine both are present in the CNS of the crustacean (Butler and Fingerman 1983, Lxmyr, 1984, Fingerman *et al* 1994). Many studies have reported the antagonistic effect of 5-HT (serotonin) and Dopamine on mostly decapods crustacean reproduction. In this case of giant fresh water prawn *M.rosenbergii* injected with sertonin significantly increase the vitellogenin level in the hemolymph at increasing maturing stages whereas DA plays the opposite role (Sarojini *et al*., 1995., Fingerman, 1997). This study was investigating the effect of 5 hydroxytryptophan and dopamine in the giant fresh water prawn *M.rosenbergi*i. In crustaceans, an extensive quantity of yolk accumulation is the basic requirement of embryonic and larval development (Adiyodi and Subramanian, 1983) within the developing oocytes during maturation. By the injection of serotonin the level of hemolymph Vitellogenin is increases but dopamine so the opposite role, this level of vitellogenin in hemolymph is quantify by the using ELISA techniques The ELISA (enzyme-linked immunosorbent assay) technique is considered as a sensitive and specific method to quantify lipoprotein compounds, such as vitellin and vitellogenin (Specker & Anderson 1994). Vitellogenin levels have been previously measured in several crustacean species, either in hemolymph and other tissues (Lee & Chang 1997, Pateraki & Stratakis 2000, Tsukimura 2001, Vazquez boucard *et al*. 2002, Chen *et al*.

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2004, Tahara *et al*. 2005, García *et al*. 2006, Santhoshi *et al*.2009). In mature females of *C. quadricariantus,* both the ovary and hepatopancreas have been reported as the main sites for vitellogenin synthesis (Serrano Pinto *et al*.2003, 2004, 2005). As well, the presence of vitellogenin in hemolymph has been related to the secondary vitellogenesis that takes place in the ovary (Yehezkel *et al*. 2000, Abdu *et al*. 2002). Vitellogenin levels have been previously quantified in *C. quadricariantus* by ELISA at the beginning of secondary vitellogenesis (Sagi *et al*.1999). Studies related to ovarian cycle associated with vitellogenesis as well as biochemical changes in freshwater female prawn *Macrobrachium rosenbergii* is limited. Hence, this study was undertaken to find the effects of neurotransmitters (5-HT and DA) in the ovarian development of giant freshwater prawn *M. rosenbergii*.

Materials and methods

Collection and maintenance of prawns

Giant fresh water prawn *Macrobrachium rosebergii* were collected from National Bureau of Fish Genetic Resources, Chinhat, Lucknow. The prawns were maintained in a Glass aquarium tank with continuous aeration. Prawns were acclimatized in the laboratory for 3 Days before the start of the experiment. The In vivo experimental prawns were tagged and individually housed in separate cages for the entire experimental period to avoid any mortality from the cannibalistic behavior.

Reagent preparation

Serotonin (5-hydroxytryptaminacreatine sulfate) was purchased from Sigma (St Louis, MO, USA) and dissolved in phosphate buffer saline (450 mM NaCl, 15 mM $CaCl_2$, 10 mM $MgCl_2$, and 10 mM KCl) and dopamine (Domin) was purchased from Neon laboratory (Mumbai) dissolve in distilled water.

Experimental procedure

Test substance and exposure condition, duplicate tank and glass aquarium (150 dph), prawn were injected to a dose of 2.5 $x10^{-7}$ moles/prawn of serotonin and 2.5 $x10^{-7}$ moles/prawn of dopamine (10 µL) was injected into first abdominal somites of *M.rosenbergii* using a micro-syringe. Saline served as the control. Hemoymph samples were collected from prawns at the beginning of the experiment (day 0) and every 7^{th} day during the experimental periods. Half of the water was replaced on alternate day. The experiment was carried out for 21 days.

Biochemical analysis

Enzyme linked immunosorbent assay

Enzyme linked immunosorbent assay Hundred milliliter of hemolymph samples were taken individually from control, serotonin and dopamine treated groups. Samples were individually homogenized with phosphate buffer and centrifuged at 13,000 × g for 10min at 10ºC, to remove cellular debris. The supernatant was collected in separate vials and stored at -80ºC until assay. Microlitre plates were filled with 100µl (six replicates) of different samples separately, diluted with coating buffer and incubated over night at 4ºC. After three washings with buffer, the wells were blocked with 200µl of blocking buffer and incubated at 37ºC for 1h. Washing was followed by the addition of 100µl of primary antibody (anti Vg at 1: 2000), for 3h at 37ºC. The primary antibody was priory raised in rabbit using the purified Vg from *M. rosenbergii*. After three times washing, the wells were coated with 100µl secondary-antibody enzyme conjugated (anti rabbit IgG-Alkaline phosphates) at 1: 500 dilutions for 1h at 37ºC. Incubation was terminated by washing and wells were filled with 100µl of substrate solution (1mg pNPP - paranitrophenyl phosphate/ml of substrate buffer). The reaction was stopped with the stop buffer after the required color development was attained. Concentrations of Vg standard was ranged from 0.1 - 100µg/ml. Absorbance at 405 nm was measured in an automated ELISA plate reader (Revathi *et al.*, 2012).

Histology method

After dissection a small piece of ovary was fixed in bouins fixative for 24 hrs and then processed by standard histological methods. Histological changes in *Macrobrachium rosenbergii* ovaries were evaluated using H and E stained Sections briefly slides were deparaffinized in xylene and rehydrated through a descending ethanol series slides were stained in hematoxylin and processed through alcohol and stained in eosin slide dehydrated through an ethanol series and cleared in xylene and mounted in Canada balsam or DPX. Sections were examined under a compound microscope and phase contrast microscope. An advantage of Histopathology is the visualization of actual influence of a test compound on the gonad condition.

Statistical analysis

All data were calculated and presented as mean ± standard deviation. Student t-test was calculated for comparison between control and treated group. Data were analyzed by one way analysis of variance (ANOVA) using a statistical package for social sciences (SPSS) version 10.

Results

In the present study the effect of neurotransmitters on the ovarian development and vitellogenesis in the adult freshwater prawn *M. rosenbergii* was studied with the analysis of GSI and quantification of vitellogenin in hemolymph of both control and treated prawns. The treatment of prawn with 5-HT showed observable histological changes result in shortening the period of the ovarian development as Well as increase Gonado somatic index and oocyte diameter whereas dopamine had the opposite effect.

Gonado Somatic Index

GSI is the main sign of ovarian development. The GSI was gradually increased during experimental period by the injection of serotonin. The GSI value in control is (2.9± to 0.04) and GSI value increased to (3.88 ± 0.88) by the injection of serotonin. Whereas, GSI show no significant increscent by the injection of Dopamine (2± 0.03) in the comparison of control and serotonin injected prawn. On the other hand, GSI values differed significantly by the injection of these neurotransmitter ($P<0.05$).

Assessment of vitellogenin

After assessment of vitellogenin, its content in hemolymph varied during experimental period. The vitellogenin level in the hemolymph showed a gradual increase in control is initially on the day 7th (85.37±1.67ng/ml) and after the completion of experiment on the 21th days (181.49±1.48ng/ml) during the experiment. The vitellogenin content marginally decreased by the injection of Dopamine on the day 7th (84.7±0.62 ng/ml) and after the completion of experiment on the 21th days (102.7±1.32ng/ml) it is not significant incresment. On the other hand, vitellogenin level is increase on the day 7th (168.2±0.75ng/ml) and after the completion of experiment on the 21th days the hemolymph vitellogenin level is increases to (374.6±0.89ng/ml) by the injection of serotonin. The variations in the vitellogenin content in hemolymph differed significantly (P<0.05) see in **(Fig.2.)**

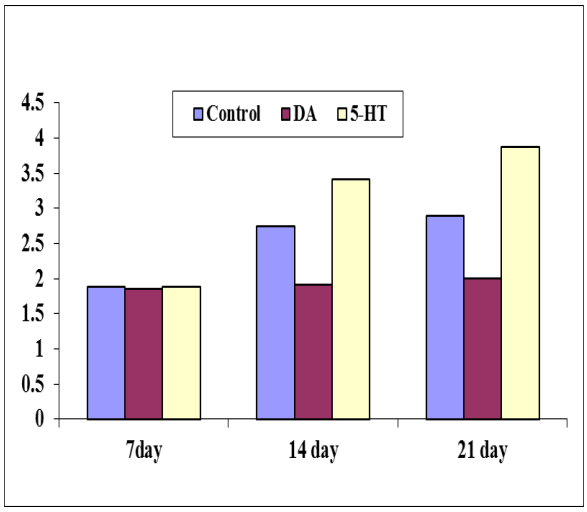

Figure 1. GSI of control and experimental *M. rosenbergii* treated with dopamine (DA) and serotonin (5-HT)

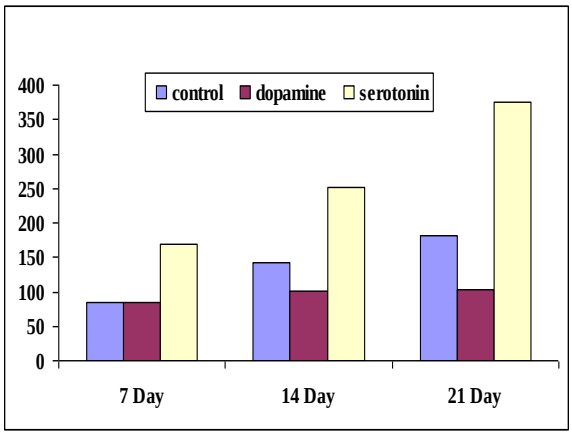

Figure 2. Vitellogenin and vitellin content in control and experimental prawn, *M .rosenbergii* treated with dopamine (DA) and serotonin (5-HT) (* F test P<0.05)

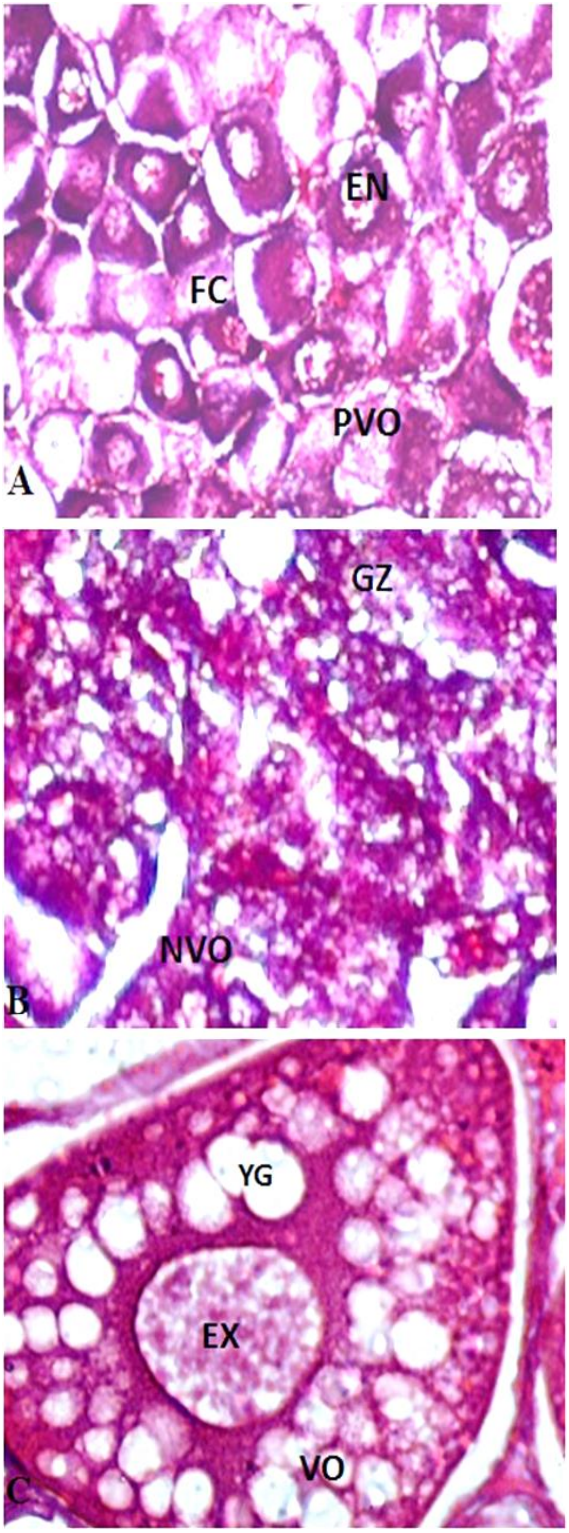

Figure 3. Transverse sections of Ovary of experimental control females *M.rosenbergii* (A), DA(B) ,5-HT(C) injected prawn in 21 Days experimental period in the *M.rosenbergii* PVO: Previtellogenic oocytes ,FC: follicular cells ;NVO: no vitellogenic oocyte, GZ: germinal zones;VO:Vitellogenic oocytes ,YG: yolk globules. Mallory triple x 200

Histology

After the histological observations of the ovary, in the case of control the ova diameter was (31.1.±0.23µm to 57.1 ±0.13 µm) and no significant changes were observed neither in gonado somatic index and nor in the oocytes diameter in control in a 21 days experimental period.

Administration of DA did not result in any significant changes in gonado somatic index and in the oocytes diameter (30.8.±0.23µm to 46.8 ±0.11 µm) in the comparison of control during the experimental duration. On the other hand, injection of serotonin significantly increases the gonad somatic index and oocytes diameter (45.7±0.15 µm to 247.4 ±0.15 µm) and see in **Fig 1 and 3**. The histological observations of the ovary from DA injected prawn indicated that the ovaries were at immature stages, whereas the ovary of experimental prawn treated with serotonin was in vitellogenic stages which could be evidenced by the appearance of yolk globules in the oocytes of the fresh water prawn *M. rosenbergii.*

Discussion

Record after regular observation the changes in hemolymph vitellogenin concentration that occurred later than the administration of DA and 5-HT, we determined the influence that these biogenic amines have on the ovarian development of the fresh water giant prawn, *M.rosenbergii*, without the administration of either DA or 5-HT a marked increase in hemolymph vitellogenin level was seen in intact prawns over a 21 days experimental period. The major findings of our study were:

1. Specimen treated with 5 hydroxyptyptophan shows significantly ($p<0.05$) higher Gonado somatic index, oocytes diameter and hemolymph vitellogenin level than untreated control
2. Specimen treated with Dopamine shows significantly ($P<0.05$) lower Gonado somatic index, oocyte diameter and hemolymph vitellogenin level than untreated control.

When 5-HT or DA was injected in to prawns both showed a dose dependent effect on ovarian development in the dose 2.5 $x10^{-7}$ moles/prawn used in this study. while 5-HT demonstrated a stimulatory effect on vitellogenesis, Dopamine appeared to inhibit ovarian development in *M .rosenbergii* Our results clearly indicated that the level of vitellogenin content in control and dopamine injected prawns showed low level compare to 5-HT injected. Our result indicating the possible stimulatory role of 5-HT on ovarian maturation. After 21 days of administration of 5-HT may be concluded to be to its stimulatory effect on central nervous system, triggering vitellogenin synthesis and its release in to the serum. After assessment of vitellogenin, its content in hemolymph varied during experimental period. The vitellogenin level in the hemolymph showed a gradual increase in control is initially on the day 7^{th} (85.37±1.67ng/ml) and after the completion of experiment on the 21^{th} days (181.49±1.48ng/ml) during the experiment. The vitellogenin content marginally decreased by the injection of Dopamine on the day 7^{th} (84.7±0.62 ng/ml) and after the completion of experiment on the 21^{th} days (102.7±1.32ng/ml) it is not significantly increases. On the other hand, vitellogenin level is increase on the day 7^{th} (168.2±0.75ng/ml) and after the completion of experiment on the 21th days the hemolymph vitellogenin level is increases to (374.6±0.89ng/ml) by the injection of serotonin. A similar pattern has been reported in *M. nipponense* (Vg range 1-9 mg/ml) Okumura *et al.*, 1993 and *H. americanus* (0-12 mg/ml) Byard and David, 1984. In both vivo and vitro studies of *P.clerkii*, Dopamine inhibitory and 5-HT act as a stimulatory effect on ovarian maturation Srojini *et al*,1996,1997 Similarly various report on many crustacean species, with a substantial increase of vitellogenin content in the hemolymph during vitellogenesis (Lee, 1991; Okumura *et al*., 1993; Quackenbush, 1989; Vafopoulou and Steel, 1995). Vitellogenesis act as a biomarker of female reproductive activity, which indicate that the vitellin accumulation gradually increased in oocytes during ovarian development (Paulus and Laufer; 1987& Quackenbush, 1989). The opposing effects of 5-HT and DA on vitellogenesis in *M.rosenbergii* were shown in our study and similar opposing effects of 5-HT and DA have been shown on different physiologic processes in other species. In the fiddler crabs,5-HT Produces red pigment dispersion while DA leads to red pigment concentration (Fingerman and Fingerman,1977).Previous studies in decapods crustaceans, including *P.clerkii, U.pugilator, Litopenaeus stylirostris, L.vannamei and P. monodon,* have shown that 5-HT is stimulatory and Dopamine is inhibitory to gonadal development in both males and females (Alfaro *et al*.,2004) and (Wongprasert *et al*., 2006). Administration of 5-HT resulted in shortening the period of the ovarian development, as well as increased gonado-somatic index and oocyte diameters (Meeratana *et al*., 2006) and (Tinikul *et al*., 2009).the histological examination of ovary shows that in dopamine injected prawn mostly follicular cells are in the germinal stages and the yolk globules are not present these condition indicate that ovary was in immature stage. In serotonin treated prawn the histological structural design of ovary indicated accumulation of yolk globules, it's a characteristics feature of vitellogenesis. 5-HT also significantly increased hemolymph vitellogenin (Vg) level, whereas DA had the opposite effect .it was, therefore, suggested that these two biogenic amines play opposite roles in controlling ovarian development and oocyte maturation in this prawn. The conclusion of the present study is give a alternative to eyestalk ablation to induce spawning in commercially important crustacean and thereby to expand sustainable crustacean aquaculture industry, however further research is required to established the applicable value of Neurotransmitter like 5 –HT to produce superior seed in captive breeding and this will help understand the mechanism of neuroendocrine which regulating gonadal maturation of commercially important giant freshwater prawn, *M. rosenbergii,* which has been identified as an important candidate species for diversification of fresh water aquaculture in India well as south Asian countries. This investigation will also provide an opportunity to explore the possibility of hormonal manipulations for advancing gonadal maturity for better gamete output/round the year quality seed production. So the conclusion of this experiment is like neurotransmitters 5-HT not Dopamine treatment may be used as a better alternate in promoting aquaculture by inducing the ovarian maturation without stressing the commercial important this crustacean species.

Conclusions

After 21 days of administration of 5-HT may be concluded to be to its stimulatory effect on central nervous system, triggering vitellogenin synthesis and its release in to the hemolymph. The level of vitellogenin content increased by

the injection of serotonin, the level of hemolymph is decreases by the injection of dopamine. Similar results are reported from several crustacean species, with a substantial increase of vitellogenin content in the hemolymph during vitellogenesis (Lee, 1991; Okumura et al., 1993; Quackenbush, 1989; Vafopoulou and Steel, 1995). Hormonal manipulation of crustacean reproduction is limited to eyestalk ablation for the induction Gonads collected from the control as well as experimental prawn were also subjected for development of ovarian maturation along with weight length and Ganado somatic index is presented in Female *Macrobrachium rosenbergii*. This type of work seems to be a practical substitute to eyestalk ablation to induce spawning in commercially important crustacean there by to expand Sustainable crustacean aquaculture industry and these hormonal treatments like neurotransmitter indicted the possibility of introducing new strategies in induce ovarian maturation in aquaculture.

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