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Influence of bilateral eyestalk ablation on gonads of fresh water prawn, *Macrobrachium dayanum*

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Abstract

The study was carried out in laboratory for one month to know the effect of bilateral eyestalk ablation on gonads of *Macrobrachium dayanum*. Healthy specimens of *Macrobrachium dayanum* in the size group of (4-6 cm in length) were taken for the experiment. The eyestalk ablation was done by cutting away the eyestalks from their bases with sterilized scissor. The results here obtained indicated positive effects of eyestalk ablation on ovary and testes of *Macrobrachium dayanum*. The histological details of the female prawns which were ablated showed post-vitellogenic oocytes; where as unablated females prawn never developed beyond pre-vitellogenic oocytes. Similarly in males, testes showed pronounced development of different cells as compared to unablated ones. Fully developed spermatozoa were seen in ablated ones. Gonadosomatic studies also showed that ovarian and testicular cells developed better as compared to control and these findings suggest the fact that the eyestalks of *M. dayanum* contain ovary and testis inhibiting factors.

Key words

Eyestalk ablation, Gonad, Histology, Prawn

Introduction

The technique of eyestalk ablation has been widely used for manipulating ovary development and maturation in captivity, and is commercially practiced in shrimp hatcheries, particularly with shrimp that do not spontaneously mature and spawn (Kuo *et al*., 2009). Reproduction in crustaceans is regulated by various neurohormones that are synthesized and released from the X-organ sinus gland complex located in the eyestalks of species (Sainz-Hernandez *et al*., 2008; Pervaiz *et al*., 2011; Nagaraju, 2011). In addition to reproduction, other physiological and metabolic processes are affected by removal of the X-organ sinus gland complex located in the eyestalk (Sainz-Hernandez *et al*., 2008; Nagaraju, 2011).

Recently, a neuropeptide that regulates methyl farnesoate synthesis by mandibular organs has been isolated from the sinus gland sequenced, and termed as mandibular organ inhibiting hormones (MO-IH), and is considered to be a member of the crustacean's hyperglycemic hormone (CHH) family (Liu *et al*., 1997). The occurrence of family of crustacean hyperglycemic hormone (CHH) neuropeptides is common in phylum Arthropod (Drexler *et al*., 2007). CHH in many crustacean species is associated with multiple functions and tissue specific structural isoforms (Chung and Webster, 2004). Eyestalk-CHH (ES-CHH), present in a moult stage-independent manner, is associated with hyperglycemia, osmoregulation and the inhibition of ecdysteroid and methyl farnesoate synthesis (Tsutsui *et al*., 2005). The presence of CHH in the brain (eyestalk) gut axis has also been reported in *Carcinus maenas* (Chung *et al*., 1999).

Eyestalk ablation is a frequently adopted procedure for induced maturation of gonads. The increased use of eyestalk ablation technique in the captive breeding of shrimps and lobsters has brought forth both positive and negative effects on the quality of spawning and seed production (Bray and Lawrence, 1992). In most penaeid shrimps, there is a natural inhibition of ovarian maturation and spawning under captive condition and this inhibition is removed by eyestalk ablation. Even in other species which develop ovary and spawn in captivity, the use of eyestalk

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ablation reduces the interbreeding time significantly (Subramoniam, 1999), thus augmenting total egg production in a given time. Prawn and shrimp tend to show rapid ovarian development and spawn two to four weeks after eyestalk ablation (Okumura, 2007). In shrimp, crayfish and lobsters, it has been documented that successful eyestalk ablation can result in spawning however, the larvae are often less viable than larvae from intact animals (Vaca and Alfaro, 2000).

Knowledge concerning the effect of eyestalk ablations is of importance with respect to understanding reproductive biology and growth. Hence, the primary aim of this paper was to study the gonadal activities under eyestalk ablation.

Materials and Methods

Effects of eyestalk ablation : Fresh water prawn *Macrobrachium dayanum* were collected from local Lake of Sagar in early morning hours (5:00-7:00AM). 80 specimens of *M. dayanum,* of both sexes looking apparently healthy, in the size group of 45-55 mm in total length, with uniform testes and ovarian conditions (immature) were only selected for experiment. The bilateral eyestalk ablation was done by cutting the eyestalks from their bases with the help of fine sterilized scissor. During ablations, the eyestalks were removed after holding the prawn in pre-cooled water (4-5°C) to reduce the heart beat rate and loss of hemolymph. The ablated prawns were then released in the fiber aquaria. The prawns were divided into four aquariums (female: I control, II: ablated; male: III control, IV. ablated), each having 20 specimens. All aquaria were aerated with oxygen by supplying air continuously through air-stones from an aerator. The total duration of experiment was 30 days and then terminated for all groups. The gonadosomatic indices were determined using standard formula.

Testicular developments were testicular index (TI), number of follicles, their diameter and number of mature spermatocytes. Similarly, ovarian developments were ovarian index (OI), oocytes diameter and the color of the ovary. The oocyte and follicle diameter were determined by occular micrometer under phase contrast microscope used in eyepiece.

Reproductive functions were assessed by gonadosomatic and histological techniques. After completion of the experiment prawns were dissected. Body weight and gonadal weight were recorded immediately after sacrifice. After weighing, ovary and testes were fixed and processed. Paraffin blocks were prepared and sections were cut at 5µm in the thickness and stained by Harris-Hematoxylin and Eosin stain. All slides of gonadal sections were examined under Zeis binocular phase contrast microscope for maturation and density of different types of cells.

Statistical analysis : All the series of experiments were done in hextuple. The significance was calculated using analysis of variance (ANOVA) followed by Tukey's multiple comparison test of columns of Graph pad instat 3 Demo statistical software for windows. A value of $P<0.05$ was taken as statistically significant. The results were calculated as mean with standard deviation (±SD) values for the experimental data.

Results and Discussion

The histological details showed that the follicular cells (FC) in the ovarian epithelium were scattered and unrecognized (Fig. 1a). The non-ablated group showed predominance of oogonia (OG) and previtellogenic oocytes (PO) near germinal zone. Immature oocytes could be seen with high density. Reproductive centers were embedded among the lobes and consisted of oogonia near the previtellogenic oocytes (primary oocytes).

Table 1 (a) : Showing the effects of eyestalk ablation on the ovarian development and various oogonial cells of *M. dayanum*

Animal group	Oocyte diameter	Ovarian indices	Oogonia	Pre-vitellogenic oocytes	Vitellogenic oocytes	Post-vitellogenic oocytes
Control	86.12 ±0.078	0.910 ±0.23	++	++++	-	-
Eyestalk ablated	319.46± 4.098***	2.856± 1.11***	+	++	-	++++

Data has been represented as mean ± standard deviation (n=6); *** indicates values are highly significant $P<0.001$ compared to control; +to+++++ indicates the degree of abundance: -, means not found.

Table 1(b) : Showing the effects of eyestalk ablation on development of testes and various spermatogonial cells in testes of *M. dayanum*

Animal group	Testicular indices	No. of follicle	Follicle diameter	No. of mature follicles	Spermat ogonia	Spermat ocytes	Sperma tids	Spermatozoa
Control	0.750±0.042	6.89±0.023	149.22±3.005	71.25 ±1.068	++++	+++	+++	+
Eyestalk ablated	1.912±0.056**	17.34±0.032**	234.05±4.054**	137.04±1.098**	+	+	+++	++++

Data has been represented as mean ± standard deviation (n=6); ** indicates values are significant $P<0.01$ compared to control; +to+++++ indicates the degree of abundance: -, means not found.

The histological details of eyestalk ablated ovaries were mainly composed of postvitellogenic oocytes. The developing ovary increased in size, occupying most of the abdominal cavity. The developing oocytes were in radically disposed strings with the immature ones towards the germinal zone and the mature ones towards the peripheral zone. A large amount of basophilic cytoplasm was acquired by postvitellogenic oocytes. The nucleus was large and had nucleoli which had irregular external surface. There were follicular cells round the oocytes. Microscopically ripe cells were larger and present cortical rods (CR), a structural modification that indicates final maturation. Follicular cells with single layered were also found encircling developed oocytes. This stage was distinguished by the appearance of developed oocytes with spherical or rod-like bodies at the peripheral cytoplasm. The nucleus of these oocytes lost their rounded form. The postvitellogenic oocytes were of irregular shape with large nuclei and many nucleoli towards the periphery of the nucleus. The oocyte diameter and ovarian indices were significantly higher in eyestalk ablated group (319.46 and 2.856) as compared to control (Table 1a and Fig. 1b).

The testes of control group had tubules compactly packed with germ cells. However, spermatogenic cells *i.e.*, spermatogonia were dominant in this group. These cells stain

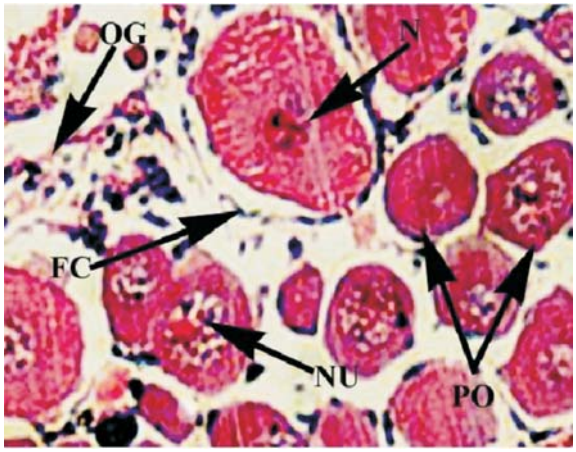

Fig. 1 (a) : Photomicrograph of longitudinal section of ovary of control group (non-ablated eyestalk) showing pre-vitellogenic (Primary oocytes). (X 100). H and E Stain

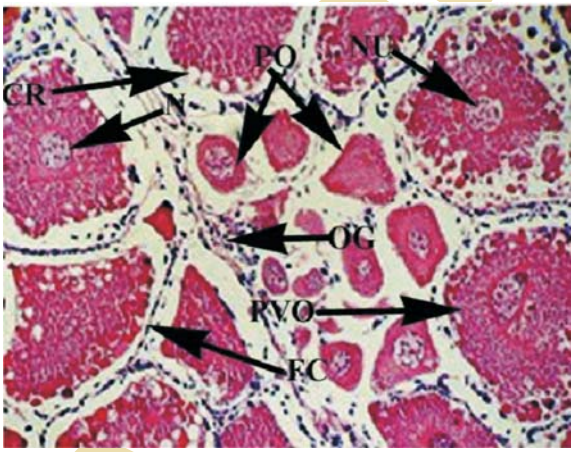

Fig. 1 (b) : Photomicrograph of longitudinal section of ovary of treated group (ablated eyestalk) showing post-vitellogenic oocytes (X 100). H and E Stain

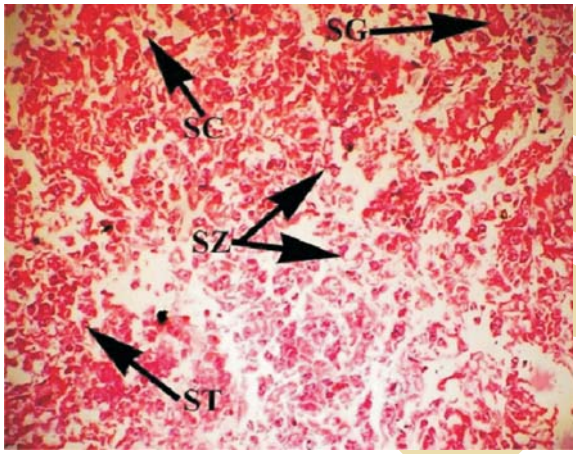

Fig. 2 (a) : Photomicrograph of longitudinal section of testes of control group (non-ablated eyestalk) showing developing spermatoza. (X 400). H and E Stain

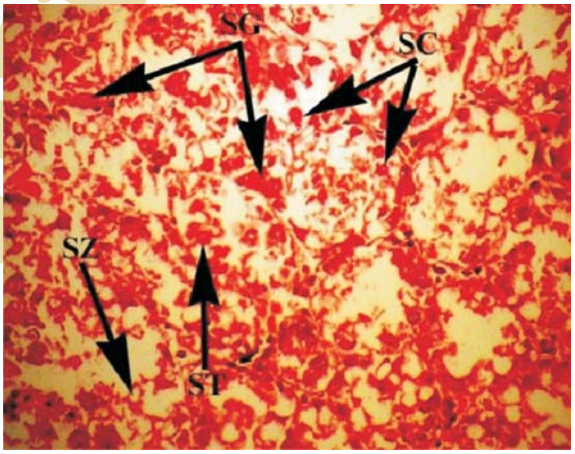

Fig. 2 (b) : Photomicrograph of longitudinal section of testes of treated group (ablated eyestalk) showing developed spermatozoa. (X 400). H and E Stain

OG = Oogonia; N=Nucleus; NU=Nucleolus; FC = Follicular cells; PO = Pre-vitellogenic oocytes (Primary oocytes), PVO = Post-vitellogenic oocytes; CR = Cortical rods; SG = Spermatogoia; SC = Spermatocytes; ST = Spermatids and SZ = Spermotozoa

lighter with H/E and have a uniformly thick cell membrane. Spermatozoa were also observed but were in early stage of development. The formation of spermatozoids starts in a peripheral region of the testicular lobule, called the germinative zone, where the spermatogonia were located, while the cells in a more advanced stage of development were found in groups called testicular cysts. Testes of the early maturing stage showed tubules dominated by primary and secondary spermatocytes and few spermatids. The testicular indices, number of follicle, follicle diameter and number of mature follicles were found to be 0.750, 6.89, 149.22 and 71.25, respectively (Table 1b and Fig. 2a).

Thin walled tubule of eyestalk ablated in fully mature testes exhibited varying dimensions packed either with spermatozoa only or with both spermatids and spermatozoa. Spermatozoa were thinly scattered in lobules of the testes and never formed a dense cloud and were found in abundance as compared to other spermatogonial cells. Spermatophores were filled with spermatogonia, spermatocytes and spermatids. These haploid cells were larger than those of control prawns and lightly stained with H/E. In some cases, two seminiferous tubules were closely associated with each other showing a common lumen. The testicular indices, number of follicle, follicle diameter and number of mature follicles were found increasing in eyestalk ablated as compared to control (Table 1b and Fig. 2b).

The results of Table 1(a) clearly indicated that there were significant increase in the ovarian index and oocyte diameter in the prawns belonging to group II (eyestalk ablated) as compared to those belonging to group I (control) (... $P<0.001$) whereas, results of the data of Table 1(b) clearly indicated that there was a significant increase in the testicular index belonging to group IV as compared to those of group III (control) (.. $P<0.001$). In the present study, a significant increase in different parameters were observed after the eyestalk ablations in *M. dayanum*, which indicated increased reproductive activity due to the removal of gonad inhibiting gonadotropin present in the X-organ of eyestalk.

Chamberlain and Lawrence (2009) found that eyestalk ablation increased gonad size and doubled mating frequency in comparison to normal. This observation has led to the proposal that eyestalk contains a gonad-inhibiting hormone. Peixoto *et al*. (2002) predicted that the presence of advanced yolky oocytes (YO) in the ovaries of ablated females just few hours after spawning may indicate a relatively faster rate of ovarian maturation in these females. However, in contrast to present findings Khalaila *et al*. (1999) observed that on *Cherax quadricarinatus* eyestalk ablation did not cause significant differences in the male or female as components of the reproductive system compared to their control group.

Bilateral eyestalk ablation on *M. rosenbergii* presented positive results, such as higher rate of maturation, anticipation of first spawning, increase in the number of consecutive spawnings, reduction of periods between spawnings, high spawning by survival rate (Santos and Pinheiro, 2000). The result of the present study is in confirmation with the report of Nagaraju and Borst (2008) who observed that the duration of ovarian development was reduced on bilaterally ablated *M. rosenbergii* females, indicating the occurrence of inhibiting function of the eyestalk hormone on vitellogenesis. Eyestalk ablation in female crabs resulted in low hepatic index and high gonad index, which may be indicative of the utilization of organic reserves in tissue synthesis (Khazraeenia and Khazraiinia, 2009).

Reviewing all the findings of present study, it appears that best growth performance of the species was achieved in eyestalk ablated male and female animals as compared to non-ablated ones and this inturn was evident from microscopic as well as gonadosomatic observations.

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References

Bray, W.A. and A.L. Lawrence: Reproduction of *Penaeus* species in captivity. In: *Marine Shrimp Culture*: Principle and practices (Eds.: A.W. Fast and L.J. Lester). Elsevier Science Publishers, Netherlands, pp. 93-170 (1992).

Chamberlain, G.W. and A.L. Lawrence: Effect of light intensity and male and female eyestalk ablation on reproduction of *P. stylirostris* and *P. vannamei*. *J. World Maric. Soc.,* **12**, 357-372 (2009).

Chung, J.S., H. Dircksen and S.G. Webster: A remarkable precisely timed release of hyperglycemic hormone from endocrine cells in the gut is associated with ecdysis in the crab *Carcinus maenas*. *Proc. Natl. Acad. Sci*., **96**, 13103-13107 (1999).

Chung, J.S. and S.G. Webster: Expression and release patterns of neuropeptides during embryonic development and hatching of the green shore crab *Carcinus maenas*. *Development*, **131**, 4751-4761 (2004).

Drexler, A.L., C.C. Harris, M.G. Dela-Pena, M. Asuncion-Uchi, J.S. Chung, S. Webster and M. Fuse: Molecular characterization and cell specific expression of an ion transport peptide in the tobacco hornworm *Manduca sexta*. *Cell Tissue Res*., **319**, 391-408 (2007).

Khalaila, I., S. Weil and A. Sagi: Endocrine balance between male and female components of the reproductive system in intersex *Cherax quadricarinatus* (Decapoda: Parastacidae). *J. Exp. Zool.,* **283**, 286-294 (1999).

Khazraeenia, S. and P. Khazraiinia: Effects of bilateral eyestalk ablation on gonadal maturity, moulting and biochemical changes in the hemolymph of female crab *Potamon persicum* (Decapoda: Brachyura: Potamidae). *Int. J. Vet. Res*., **3**, 143-150 (2009).

Kuo, C.M., Y.N. Chen, H.F. Fan, H.S. Chuang and S.L. Hsieh: Enhancement of vitellogenin synthesis by serotonin in the giant fresh water prawn *Macrobrachium rosenbergii* (De Man). *Zoological Studies*, **48**, 597-606 (2009).

Liu, L., H. Laufer, Y.J. Wang and T.K. Hayes: A neurohormone regulating both methyl farnesoate synthesis and glucose metabolism in a crustacean. *Biochem. Biophys. Res. Commun*., **237**, 694-701 (1997).

Nagaraju, G.P.C. and D.W. Borst: Farnesoate couples environmental changes to testicular development in the crab *Carcinus maenas*. *J. Exp. Biol.*, **211**, 2773-2778 (2008).

Nagaraju, G.P.C. : Reproductive regulators in Decapod crustaceans an overview. *J. Exp. Biol.,* **214**, 3-16 (2011).

Okumura, T. : Effects of bilateral and unilateral eyestalk ablation on vitellogenin synthesis in immature female kuruma prawns, *Marsupenaeus japonicas*. *Zoolo. Sci.*, **24**, 233-240 (2007).

Peixoto, S., R.O. Cavalli, F. D'Incao, W. Wasielesky and A.M. Milach: A comparative study of the ovarian histology of eyestalk ablated and unablated *Farfantepenaeus paulensis* after spawning. *Boletim do Instituto do Pesica*, **28**, 71-76 (2002).

Pervaiz, P.A., S.M. Jhon, M. Sikdar-Bar, H.A. Khan and A.A. Wani: Studies on the effect of unilateral eyestalk ablation in maturation of gonads of fresh water prawn *Macrobrachium dayanum*. *World J. Zool.*, **6**, 159-163 (2011).

Sainz-Hernandez, J.C., I.S. Racotta, S. Dumas and J. Hernandez-Lopez: Effect of unilateral and bilateral eyestalk ablation in *Litopenaeus vannamei* male and female on several metabolic and immunologic variables. *Aquaculture*, **283**, 188-193 (2008).

Santos, M.J.M. and M.A.A. Pinheiro: Ablacao ocular no camarao *Macrobrachium rosenbergii* (De Man) (Crustacea: Decapoda: Palaemonidae). *Revi. Brasileira de Zoolo.*, **17**, 667-680 (2000).

Subramoniam, T.: Endocrine regulation of egg production in economically important crustaceans. *Curr. Sci.,* **76**, 350-360 (1999).

Tsutsui, N., H. Katayama, T. Ohira, H. Nagasawa, M.N. Wilder and K. Aida: The effects of crustacean hyperglycemic hormone family peptides on vitellogenin gene expression in the kuruma prawn, *Marsupenaeus japonicas*. *Gen. Comp. Endocrinol*., **144**, 232-239 (2005).

Vaca, A.A. and J. Alfaro: Ovarian maturation and spawning in the white shrimp *Penaeus vannamei* by serotonin injection. *Aquaculture*, **182**, 373-375 (2000).

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