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Study of variation of renal β- glucuronidase in the reproductive cycle of *Ophiocephalus striatus* (Bloch)

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Abstract :
β- glucuronidase is a lysosomal glycosidase. This enzyme is ubiquitous in animal lysosomes. The enzyme activity estimated by using the phenolphthalein mono-β-D- glucuronic acid has been described in the kidney in the different stages of reproductive cycle of female snakehead, *Ophiocephalus striatus.* The β-glucuronidase activity in kidney varies with the stages of reproductive cycle. The β-glucuronidase activity goes on increasing from preparatory phase up to the spawning phase. It decreases in the post spawning phase.
The possible significance of this enzyme variation is discussed briefly.
Keywords : β- glucuronidase. *Ophiocephalus striatus.* Reproductive phases.

Introduction

β- glucuronidase is an acid hydrolase expressed at variable levels by virtually every cell in the vertebrate body (Paigen, 1989). It contains sialic acid which contains glycoprotein. It is reported to carry out the hydrolysis of glucuronide moiety in the tissues such as liver, kidney, spleen, intestinal epithelium, endocrine and reproductive organs (Dutton, 1980).

Ophiocephalus striatus is a common fresh water fish of India. It is an annual breeder, heterosexual fish characterized by the snake like depressed head, covered by scales. Long spineless dorsal and anal fins, paired fins also spineless, eyes lateral and body elongated.

Kidneys of female *O. striatus* is divided into head kidney and trunk kidney. The head kidneys are triangular or pyramidal in shape and attached to the anterior sides of the kidney proper by short neck. Anteriorly, kidneys are separate and somewhat elongated. Posteriorly, the kidneys extend beyond the cloacal region. The ureters arise somewhat suddenly from the hind portion , which emerge on the ventral side to expand into a large sized bladder.

Histologically kidney does not show any variation in five different phases of female reproductive cycle. It consists of Bowman's capsule, first proximal tubules intermediate tubules, second proximal tubules, distal tubule and collecting duct with some blood vessels embedded in the interstitial cells or haematopoitic tissue.

β-glucuronidase activity is estimated quantitatively in kidney throughout the reproductive cycle of this fish and its possible co-relation is worked out.

Materials and Methods

Matured fish from nearby markets were brought to the laboratory. Females were identified from external genitalia. They were anaesthetized, perfused and dissected to remove ovaries and kidney.

For biochemical estimations, phenolphthalein mono-β-D-glucuronic acid (Sigma, USA) was used as substrate and estimation was carried out according to the method described by Fishman (1967) with little modifications. 10 ml tissue extract was prepared in ice cold acetate buffer and raised to 1% final concentration. To study enzyme activity, 1nM substrate prepared in acetate buffer (0.1M, pH-4.5) was used. 1% stock tissue extract was used for the estimation. After incubation, all the tubes were immersed in boiling water for one minute and to each tube 1.5 ml of distilled water was added and centrifuged at 2000 rpm for 5 min. 2 ml of supernatant was taken in clean test tube and to that added 2.5 ml of alkaline glycine (0.1 M, pH- 10.5) and 1.5 ml distilled water. It was read at 540 nm on spectrophotometer and Fishman units (F.U.) were calculated by using the formula-

$$\text{F.U.} = \frac{\mu\text{g phenolphthalein x 10 x 2.5}}{\text{Time x Tissue weight x 0.1 x 2}}$$

where-
10 = Dilution of substrate solution
2.5 = Volume of alkaline glycine in ml
0.1 x 2 = Volume of substrate and extract
The reproductive phases were determined by calculating gonadosomatic index of the fish.

Results and Discussion

Variations in the β-glucuronidase activity during different reproductive phases of the kidney are given below. The GSI and β-glucuronidase values are listed in Table 1. P values were determined to know the level of significance of variations if any.

1. Resting phase (November-January)

During this phase, GSI is low. The β-glucuronidase activity is also low in this phase as compared to other phases. It is 885.62 ± 38.95 F.U./gm wet weight of the kidney.

2. Preparatory phase (February-April)

In this phase, GSI increases slightly. The β-glucuronidase activity also increases to 1146.87 ± 24.22 F.U./ gm wet weight of the kidney.

3. Prespawning phase (May-June)

In this phase, GSI again increases considerably. β-glucuronidase activity is also increases to 1386.12 ± 22.60 F.U./ gm wet weight of the kidney.

4. Spawning phase (July-August)

GSI is highest in this phase. In kidney, enzyme activity increases to its highest 2046.18 ± 38.59
F.U./ gm wet weight of the tissue.

5. Postspawning phase (September-October)

GSI decreases abruptly because of discharge of eggs from the ovaries.. β-glucuronidase activity also decreases abruptly to 1251.62 ± 23.08 F.U./ gm wet weight of the kidney.

The reproductive cycle of *O. striatus* studied here is divided into five phases which are resting phase **(**November-January), preparatory phase **(**February-April), prespawning phase (May-June), spawning phase (July-August) and postspawning phase (September-October). Ovaries show histomorphological variations during different phases of the annual ovarian cycle. Number of enzymes and metabolites are involved in such profound changes which result in the maturation of the eggs.

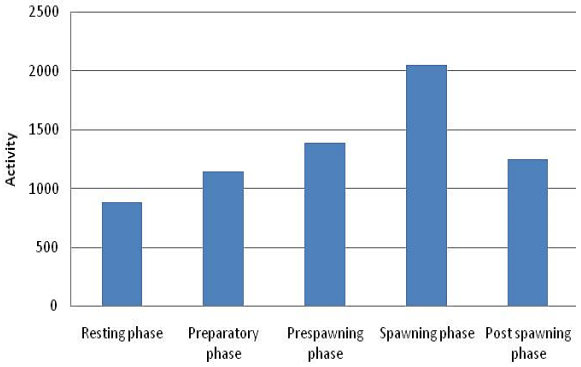
In kidney of female *O. striatus* , β-glucuronidase activity fluctuates with the changes in the reproductive cycle. These fluctuations in the female *O. striatus* indicate the hormone dependency of this enzyme. The enzyme activity in kidneys in resting phase is lowest among all the phases. It increases from preparatory phase to spawning phase. In spawning phase highest activity is observed. In postspawning phase, the enzyme content decreases. Varute, (1970a) studied β-glucuronidase activity both biochemically and histochemically in the kidney of Indian bull frog *Rana tigrina* and showed that the activity of this enzyme attained a peak during the breeding period of these animals, whereas in post-breeding period the activity showed a considerable decrease. Fishman (1964) presented evidence indicating that renal β-glucuronidase response is dependent on a DNA directed RNA synthesis.

Table 1: GSI and β-glucoronidase activity in kidney of female *O. striatus*

Phases	**GSI**	**Enzyme activity in kidney (F.U./gm wet weight of kidney)**
1. Resting (control)	0.203 ± 0.01	885.62 ± 38.95
2. Preparatory	0.991 ± 0.07 P‹ 0.01	1146.87 ± 24.22 P‹ 0.05
3. Prespawning	2.630 ± 0.26 P‹ 0.01	1386.12 ± 22.60 P‹ 0.001
4. Spawning	5.822 ± 0.32 P‹ 0.001	2046.18 ± 38.59 P‹ 0.0001
5. Postspawning	0.815 ± 0.16 NS	1251.62 ± 23.08 P ‹ 0.0001

NS- Non significant

Graph 1 showing β-glucuronidase activity in the Kidney of female *O. striatus*



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