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Research Article

VITAMIN-D_3 INDUCED CHANGES IN THE CORPUSCLES OF STANNIUS GLAND OF *LABEO ROHITA* (HAMILTON, 1822), REARED IN LOW, AND HIGH CALCIUM-RICH WATER

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ABSTRACT. Change in the level of calcium in water affects the Corpuscles of Stannius (CS) in *Labeo rohita* (Rohu) which was administered by vitamin D_3 intra-peritoneally. This study examined the corpuscles of Stannius (CS) gland to the responses of vitamin- D_3 intra-peritoneally injected to *Labeo rohita* @ of 0 IU/kg bw, 100 IU/Kg bw and 500 IU/Kg bw reared in indoor, plastic tubs with 20 and 40 ppm of Calcium (Ca) enriched water. Cellular changes in CS are examined for 15 days with the regular time interval. Rohu administered with 500 IU/kg bw vitamin D_3 and exposed to 40ppm Ca-rich water exhibited notable hyperplasia of CS when compared with their control groups which showed normal cell volume. The results demonstrated that the change in calcium level of water modulated the cellular structure of CS to release stanniocalcin and to maintain Ca homeostasis in *Labeo rohita* which is influenced by intra-peritoneal vitamin D_3 and the effect of one-time injection persist up to 15 days of rearing. In the present study, we have observed the cellular changes in CS due to the synergistic effects of calcium and vitamin D_3 which will open a future line of research to understand calcium regulation in vertebrates with special reference to fishes cultured in soft and hard water aquaculture and/ or in saline and sodic soil-water system.

Keywords: *Labeo rohita, corpuscle of Stannius, Vitamin D_3, Calcium enriched water*

INTRODUCTION

Calcium plays an important role in a number of vital biological processes; the most important is in the maintenance of ionic levels [1]. The activity of ions is seen in many target tissues, which may include the gills, skin, kidney, intestine and bone to maintain plasma levels of these and other ions [2]. A unique endocrine tissue referred to as the Corpuscles of Stannius (CS) which are specific to teleost and embedded in the kidneys of teleostean [3,4,5,6] and release its homeostatic hypocalcemic hormone, stanniocalcin [STC] [7] that helps in calcium homeostasis by lowering the calcium level.

Labeo rohita is one of the Indian Major Carps (IMC) selected for this study because it is commonly available in Indian freshwater system and also widely cultured species. The level of ionic concentration in Indian freshwater system may vary according to geographical area and flowing path. To regulate this change in ionic concentration, fishes undergo various physiological processes such as osmoregulation and other endocrinal hormone secretion to maintain homeostasis.

The regulation of calcium by corpuscles of Stannius (CS) and its cellular changes in *Labeo rohita* are not yet studied in Indian waters. In this study, the change in the cellular structure of CS is observed at the different time interval.

MATERIALS AND METHODS

Experimental Design

The experimental design consists of 18 uniform size plastic rectangular tanks (80 cm × 57 cm × 42 cm, 150 L capacity) with a 2×3 factorial design in triplicates. The calcium level in water is enriched using Calcium chloride and the experimental fish *Labeo rohita* (weight: 30±2g) were randomly and equally distributed into experimental tanks @12 fish per tank. The tanks were separated into 2 groups, namely group A- 20 ppm Ca, and group B - 40 ppm Ca enriched water.

Vitamin D_3 Administration

Fishes were injected intra-peritoneally with vitamin D_3 at different doses according to their designed groups respectively. By considering 0.2ml Vitamin D_3 injection for 25g size fish, the 3 Lakh IU Arachitriol (oil-based) was used under dilution with Arachis oil (Vehicle).

Designed Groups

Group A (Water with 20 ppm Ca)

I. Group AC – Control group of dose 0.0 IU D_3 (Vehicle) / kg bw
II. Group AL – Low dose group of 100 IU Vitamin D_3 / kg bw
III. Group AH – High dose group of 500 IU Vitamin D_3/ kg bw

Group B (Water with 40 ppm Ca)

I. Group BC – Control group of dose 0.0 IU D_3 (Vehicle) / kg bw
II. Group BL – Low dose group of 100 IU Vitamin D_3 / kg bw
III. Group BH – High dose group of 500 IU Vitamin D_3/ kg bw

Fish sampling and histological studies

Sampling was done on Day 1, Day 3, Day 5, Day 7, and Day 15 and in each sampling, one fish from each replicate was sacrificed and the Corpuscle of Stannius (CS) along with adjoining portion of the kidney was removed and kept in 70% alcohol for histological study [8]. The procedure includes dehydration, clearing and paraffin wax infiltration.

Dehydration

Stored tissue in 70% alcohol was transferred to a container containing 80% alcohol and was kept immersed for 1 hour and 30 minutes. Immerse the tissues serially in 90% alcohol and 100% alcohol solutions for 1 hour and 30 minutes. End the process with immersion in 100% alcohol for 1 hour 30 minutes.

Clearing

Tissue gets immersed in xylene for 1 hour 30 minutes followed by immersion in xylene for 1 hour 30 minutes, which was given before transferring the tissues into molten liquid paraffin wax (embedding medium) in order to enable infiltration of the embedding media.

Paraffin wax infiltration

Tissue gets immersed in molten paraffin wax for 3 hours and 30 minutes and then embedded in paraffin melted at 65°C using embedding rings. Then the blocks were solidified by placing them over the cooling plate at -11°C for 15 minutes.

Sectioning, staining and analysis

The tissue blocks were cut into sections using Leica rotary microtome. The Corpuscles of Stannius gland were sectioned into 3 to 5 um thick sections and were allowed to expand by making them float over the hot water maintained at temperature 40°C to 50°C. The cut sections after expansion were collected with egg albumen coated slides. The slides were then dried at room temperature overnight. Then again immersed for overnight in xylene solution to remove wax and then stained using Mayer's Hematoxylin and Eosin-Phloxin solution. The stained sections were observed microscopically using an inverted light microscope.

RESULTS AND DISCUSSION

Histology of corpuscles of Stannius

In *Labeo rohita*, CS is of two in numbers and embedded in the dorsal portion of an anterior part of the posterior kidney (Fig 1 & 2). The cells are polygonal in shape with the nucleus at center and they are closely packed. The nucleus is positive for hematoxylin and cytoplasm with eosin stain. Due to the effect of Vitamin D_3 and calcium level in water shown major changes in the cellular structure of CS in group B cultured fish than that of group A fishes.

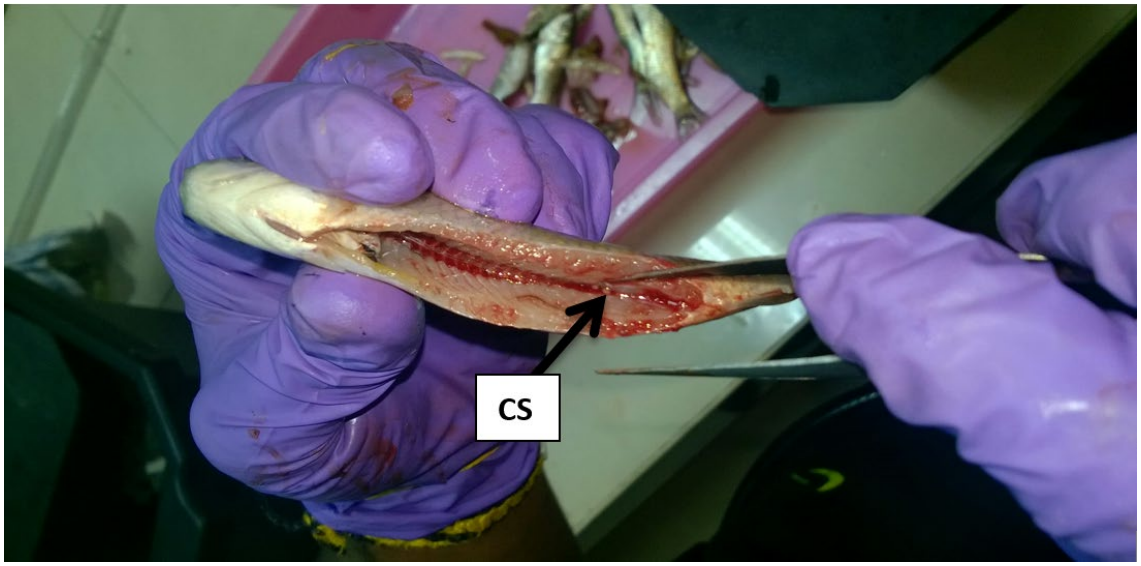

***Fig.1**. Location of corpuscles of Stannius in Labeo rohita embedded in kidney*

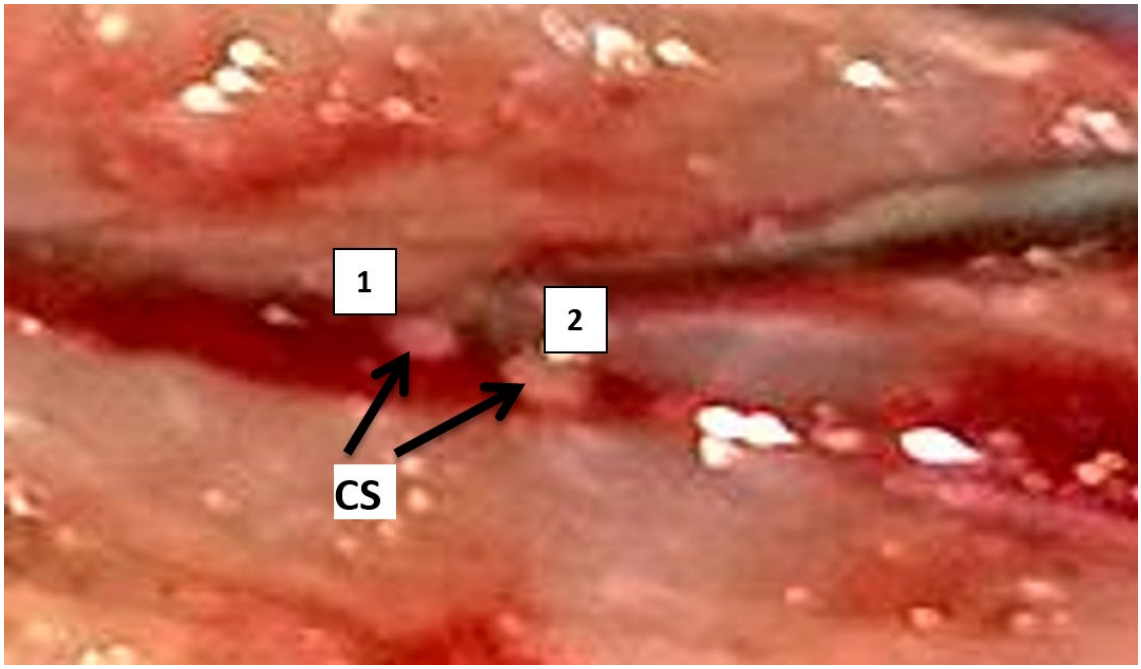

***Fig.2**. In Labeo rohita: Pair of CS of 0.5-1µm in diameter; Whitish yellow and round in shape; Located at ventral or below the posterior part of kidney; Loosely attached with kidney and vertebral column*

Group AC – Group with control dose 0.0 IU D_3 (Vehicle) / kg bw

The secretory cells of Stannius are arranged in follicles and they are characterized by oval or round nuclei exhibit several complete or incomplete lobules which contain epithelial cells. These cells possess oval or rounded nuclei. The cellular structure of CS was recorded to be similar from Day1 to Day 15 is shown in Fig. 3 & 4.

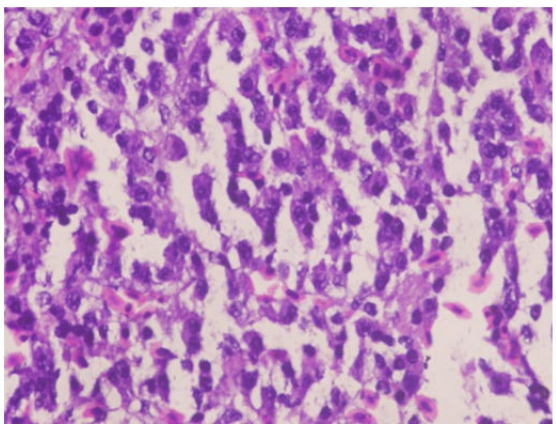

***Fig.3**.Section of corpuscles of Stannius on Day-1 of group AC. H&E × 40X*

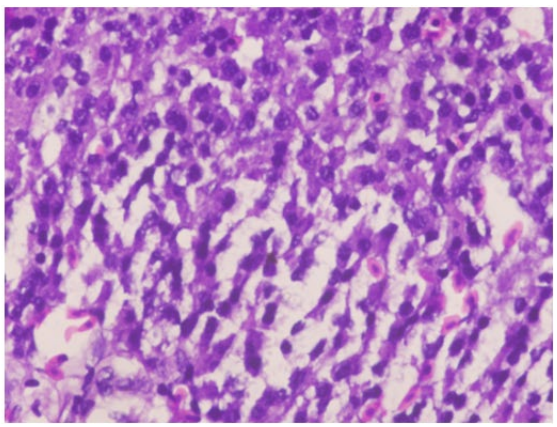

***Fig.4**. Section of corpuscles of Stannius on Day-15 of group AC. H & E × 40X*

Group AL – Group with low dose 100 IU Vitamin D_3 / kg bw

The cellular structures are shown in Fig.5 on day-7 showing the nuclear volume of cells records an increase with vacuolated cytoplasm and they slowly turned to normal on Day-15 (Fig. 6). This shows the activity of CS secretary cells and also there is an increased dilatation of sinusoids. The results of cellular activities are demonstrating that there was hypocalcemic response of CS gland.

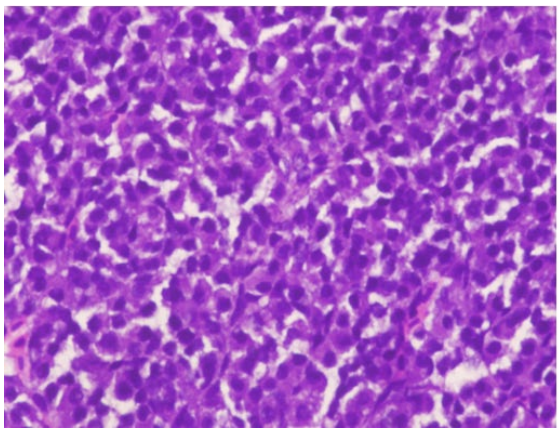

Fig.5*. Section of corpuscles of Stannius on Day-7 of group AL. H & E × 40X*

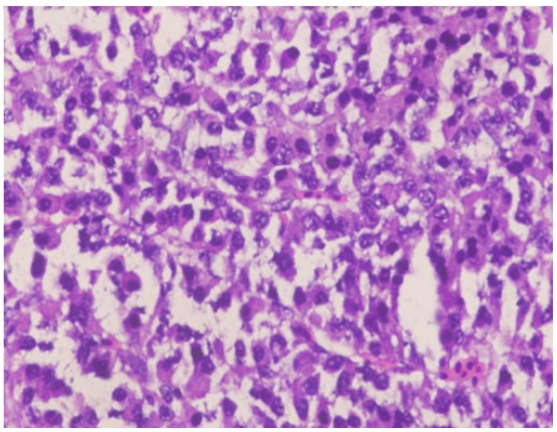

Fig.6*. Section of corpuscles of Stannius on Day-15 of group AL. H & E × 40X*

Group AH – Group with high dose 500 IU Vitamin D_3/ kg bw

The CS exhibits several complete lobules which contain epithelial cells. The cellular structures are shown in Fig. 7 on Day – 5 showing the nuclear volume of cells increases due to hypocalcemic activity and they become partially de-granulated as is evident by their weak staining response. However, the gland action was more severe to make normocalcemia (Fig. 8).

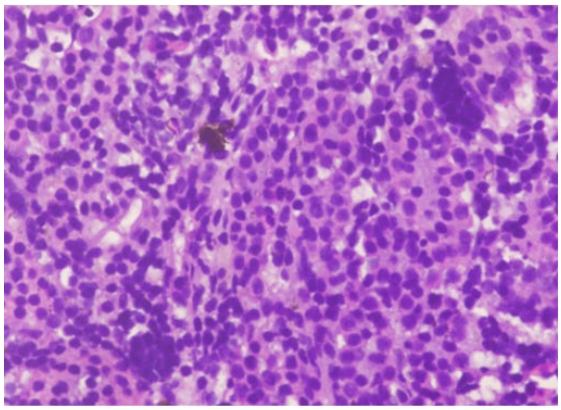

Fig.7*. Section of corpuscles of Stannius on Day-5 of group AH. H & E × 40X*

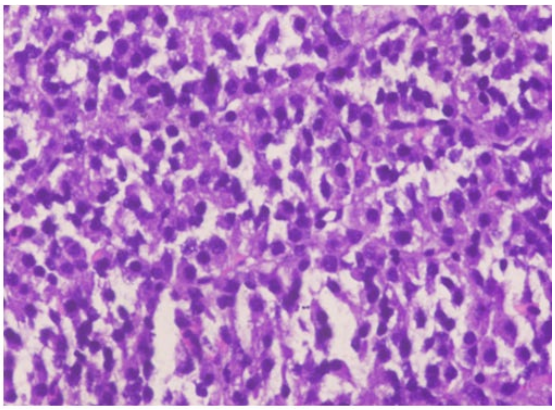

Fig.8*. Section of corpuscles of Stannius on Day-15 of group AH. H & E × 40X*

Group BC – Group with control dose 0.0 IU D_3 (Vehicle) / kg bw

The CS cells exhibits minor cellular changes on Day 1(Fig. 9) and then turned normal on Day -3 (Fig. 10).

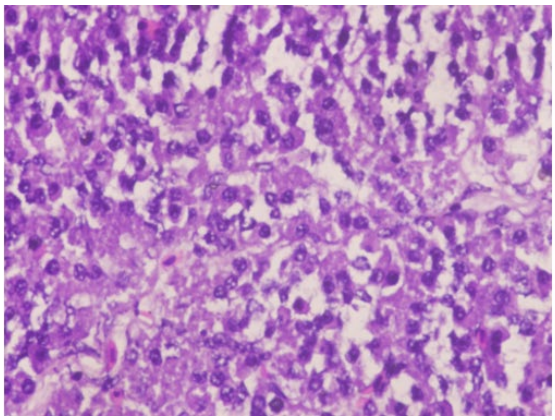

Fig.9*. Section of corpuscles of Stannius on Day-1 of group BC. H & E × 40X*

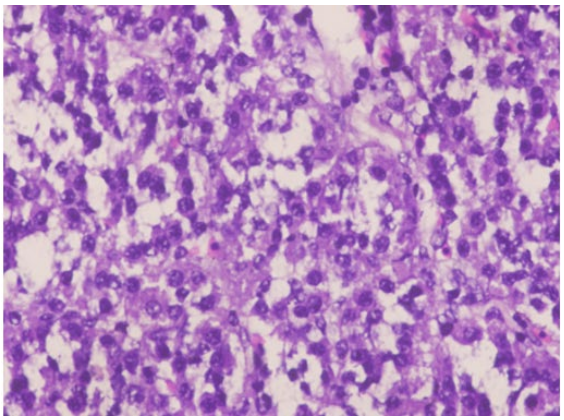

Fig.10*.Section of corpuscles of Stannius on Day-3 of group BC. H & E × 40X*

Group BL – Group with low dose 100 IU Vitamin D_3 / kg bw

The CS cellular structures are shown in Fig. 11 on Day-5 showing the maximum increase in nuclear volume which is seen by vacuolization of cytoplasm. The cellular change in CS recorded for prolong period of up to Day-9 (Fig. 12).

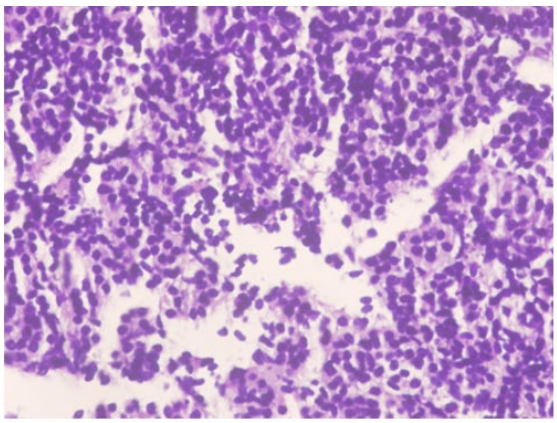

Fig.11*.Section of corpuscles of Stannius on Day-5 of group BL. H & E × 40X*

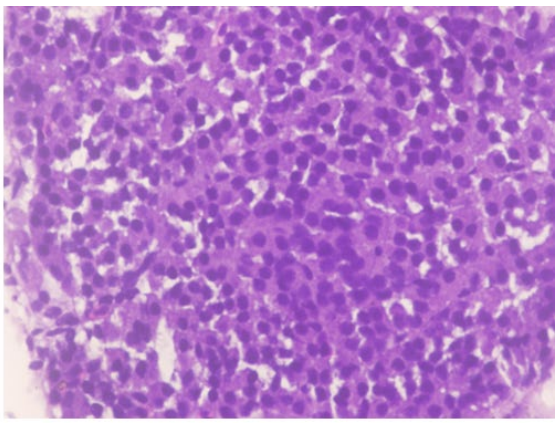

Fig.12*.Section of corpuscles of Stannius on Day-9 of group BL. H & E × 40X*

Group BH – Group with high dose 500 IU Vitamin D_3/ kg bw

The CS cellular structures showing the maximal increase in nuclear volume of cells on Day-3 (Fig.13) and they are recorded by different staining response. In this high dose, the hypocalcemic activity of CS got activated and maintained for long period of time (Fig.14).

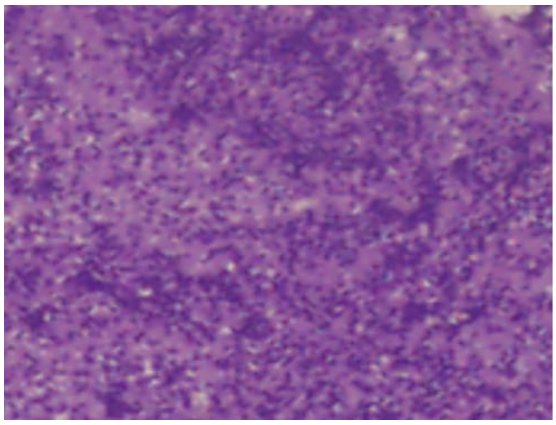

***Fig.13**. Section of corpuscles of Stannius on Day-3 of group BH. H & E × 40X*

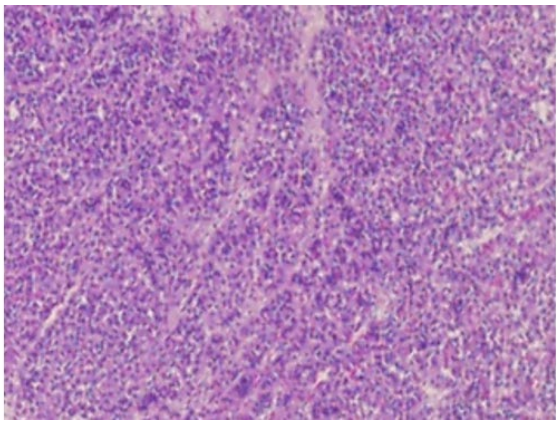

***Fig.14**. Section of corpuscles of Stannius on Day-15 of group BH. H & E × 40X*

Histology of corpuscles of Stannius (CS)

The CS of *Labeo rohita* exhibits an increase in the volume of cells and sinusoidal dilatation due to hypertrophy of CS influenced by external Ca level and vitamin D_3 dosage. The same hypertrophy condition noted by Olivereau and Olivereau[9] and they indicated this act as the activity of CS in response to hypercalcemia. This suggested that the secretory activity of cells of CS may be directly affected by plasma ion levels, especially calcium [10]. This whole activity due to external calcium concentration leads to increase in volume and density of CS cells [11]. Singh and Srivastav [12] also noted the histological changes in the Corpuscles of Stannius of *Heteropneustes fossilis,* a freshwater catfish by injecting Vitamin D_3 and *kept* either in artificial freshwater, calcium-rich freshwater or calcium-deficient freshwater.

In seawater fishes, the activities of CS cells are high and it is noticed by Bonga et al. [13] and they have stated that the high activity of CS cells in seawater is apparently due to the high calcium concentration of seawater. But there is reduction of secretory activity of CS from fish transferred from freshwater to seawater have been reported by Bonga *et al.* [14] and Meats *et al*.[15]. The studies in *Clarias batrachus* [16, 17] under a hypercalcemic condition is shown sinusoidal dilations of CS that is similar to the present study in *Labeo rohita*.

In this, there is an increase in secretory cells or storage granules in CS of *Labeo rohita* for the secretion of hypocalcemic hormone and to reduce the serum calcium and inorganic phosphate levels. This principle seems to be similar to mammals [18, 19 ,20] in which secretory granules cells in response to hypocalcemia. In this study, when the *Labeo rohita* are treated for prolonged hypercalcemic condition the corpuscular cells started to degenerate which is similar to the reports stated by Hiroi [21] in *Oncorhynchus sp*.

CONCLUSION

In the present study, the histological section of Corpuscles of Stannius reveals the regulatory activity of CS in *Labeo rohita* reared at different levels of calcemic water for attaining the normocalcemic condition. Globally a large freshwater body with different ionic concentration according to its origin and geographical location may alter the ionic regulation in culturable species. This study helps in understanding the glandular regulatory mechanism, and endocrine function of CS in *Labeo rohita* which is one of the widely cultured Major Carps teleostean fish in many Asian countries including Indian sub-continent. The cellular changes in CS due to the synergistic effects of Ca and vitamin D_3 will open a future line of research in calcium regulation in vertebrates with special reference to fishes reared in low and/ or high calcium rich aquaculture in saline and sodic water system. Further, research should be required to look into hormones, gene expression, and other molecular work to know the detailed information regarding this endocrinal gland responsible for Calcium regulation.

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REFERENCES

[1] Aurbach, G.D. (1988): Calcium in human biology. Nordin B.E.C. *Spnnger-Verlag.* 15-42 pp.

[2] Feinblatt, J.D. (1982): The comparative physiology of calcium regulation in sub mammalian vertebrates. Advances in Comparative Physiology and Biochemistry 8:73-84.

[3] Bhattacharyya, T.K., Butler, D.G., You Son, J.H. (1982): Ultra structure of the corpuscles of Stannius in the garpike (*Lepisosteus platyrhynchus*). General Comparative Endocrinology 46: 29-41.

[4] Marra, L.E., Youson, J.H., Butler, D.G., Friesen, H.G.,Wagner, G.F. (1992): Stanniocalcin-like immunoreactivity in the corpuscles of Stannius of the bowfin, *Amia calva*. Cell Tissue Res, 267: 283-290.

[5] Marra, L.E., Youson, J.H., Bonga, S.E.W., Hanssen, R.J.G.M., Wagner, G.F. (1994): Immunoreactivity of the corpuscles of Stannius of the garpike, *Lepisosteus osseus*, to antisera against salmon and trout stanniocalcins. Cell Tissue Research 277: 511-518.

[6] Youson, J.H., Butler, D.G. (1976): Fine structure of the adrenocortical homologue and the corpuscles of Stannius of *Amia calva. L.* Acta Zoologica (Stockh) 57: 212-238.

[7] Nelson, J.S. (1994). Fishes of the World. Third edition. New York. John Wiley and Sons.

[8] Bell T.A., Lightner D.V. (1988): A handbook of normal penaeid shrimp histology. World Aquaculture Society, Baton Rouge, LA.

[9] Olivereau, M., Olivereau, J. (1978): Hypercalcemia and Corpuscles of Stannius in seawater eels. Cell Tissue Research 186 : 81-96.

[10] Aida, K., Nishioka, R.S., Bern, H.A. (1980): Degranulation of the Stannius corpuscles of coho salmon (*Oncorhynchus kisutch*) in response to ionic changes in vitro. General Comparative Endocrinology 41: 305-313.

[11] Urasa, F., Bonga, S.E.W. (1987): Effects of Ca and phosphate on the Corpuscles of Stannius of the teleost fish, *Oreochromis mossambicus*. Cell Tissue Research 249: 681–690.

[12] Singh, S., Srivastav, A.K. (1996): Vitamin D_3-induced histological changes in the corpuscles of stannius of a freshwater catfish, *Heteropneustes fossilis* kept either in artificial freshwater, calcium-rich freshwater or calcium-deficient freshwater. Okajimas Folia Anatomica Japonica 73(2-3):75-81.

[13] Bonga, S.E.W., Van der Meij, J.C.A., Pang, P.K.T. (1980): Evidence for two secretory cell types in the Stannius bodies of the teleosts *Fundulus heteroclitus* and *Carassius auratus*. Cell Tissue Research 212:295-306.

[14] Bonga, S.E.W., Greven, J.A.A., Veenhuis, M. (1976): The relationship between ionic composition of the environment and secretory activity of the endocrine cell types of Stannius in the teleost *Gasterosteus aculeatus*. Cell Tissue Research 175:297-312.

[15] Meats, M., Ingleton, P.M., Chester Jones, I., Garland, H.O., Kenyon, C.I. (1978): Fine structure of the Corpuscles of Stannius of the trout, Salmo *gairdneri*, and structural changes in response to increased environmental salinity and Ca ions. General Comparative Endocrinology 36:451-461.

[16] Srivastav, S.P., Swarup, K., Srivastav, A.K. (1985): Structure and behaviour of Stannius corpuscles in relation to vitamin D_3-induced hypercalcemia in male *Clarias batrachus*. Molecular Cellular Biology 31:1-5.

[17] Srivastav, A.K., Srivastav, S.P. (1988): Corpuscles of Stannius of *Clarias batrachus in* Response to 1, 25-Dihydroxyvitamin D_3 Administration. Zoological Science 5:197-200.

[18] Gittes, R.F., Toverud, S.V., Cooper, C.W. (1968): Effects of hypercalcemia and hypocalcemia on the thyrocalcitonin content of rat thyroid glands. Endocrinology 82:83-90.

[19] Srivastav, A.K., Swarup, K. (1982): Effects of calcitonin on calcitonin cells, parathyroid glands and serum electrolytes in the house shrew *Suncus murinus.* Acta Anatomica 114:81-87.

[20] Hirsch, P.F., Munson, P.L (1969): Thyrocalcitonin. Physiological Reviews 49:548-622.

[21] Hiroi (1970). Morphological changes in the Corpuscles of Stannius of the chum salmon, *Oncorynchus keta*, during the migration and maturation. Bulletin of the Faculty of Fisheries Hokkaido University, 21:179-192.