Seasonal Changes in the Number of Dahlgren Cell in the Spinal Cords of Two Fish species and Their Cyclic Activity

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Abstract: Seasonal Changes in the number of Dahlgren cells in the spinal cords of two Freshwater fishes namely: *Barbus luteus* and *Capoeta trutta* were investigated along with their cyclic activity as detected using three different stain, Harris Haematoxyline –eosin (H-E), Aldehyde haematoxyline technique, (AF), Mallory triple stain, Amonical silver nitrate. These fishes were caught from River Tigris passing through Mosul city. After random counting of Dahlgren cells in the spinal cord of the 3 year old fish (*B. luteus*) three types of cells can be distinguished according to their sizes; small, medium and large. Small Dahlgren cells of *B. luteus* were most dominant in summer (average 81.6) then the number decreases in autumn, and then the minimum was in winter. The median sized cells were also large in number in summer (19.3), then the number was less than that in spring then winter and the lowest was in autumn. The large Dahlgren cells were more abundant also in summer (16), and less than that in spring, then in autumn, then the lowest was in winter. Dahlgren cells of *C. trutta* at age of 3 year, small cells were dominant in summer (79.67), and less than that in autumn, and lesser in spring. The medium sized cells were also dominant in summer (30.65), then spring, then autumn, while the large cells were dominant in spring (11.75). Anyhow, as seen above, the large number of Dahlgren cells were observed in summer especially small, then medium while large cells were more in spring. In *B. luteus* small Dahlgren cells were dominant in age 1 year, then 3 year, then 6 years, while medium sized cells were dominant in year 1, then 3, then 6 years. Generally number of Dahlgren cells decreases especially large once with increased age of *B. luteus* and *C. trutta* while Dahlgren cells of *V. trutta* was higher in number in age 1 year, then 3 year, then 6 year. Anyhow, the median and small size cells decreases in number as age of fish increases. It was revealed from the present study that Dahlgren cells possess secretory materials in the cytoplasm, their distribution and staining affinity varies from one type to other in both species of fishes investigated. The secretory cycle can be categorized into three stages: Stage I: mostly empty of few secretory material. Stage II: secretory material increase in cytoplasm far from nucleus. Stage III: discharge of secretory material from cell and its axon.

Introduction

Fishes possess neurosecretory system in the caudal spinal cord in addition to hypothalamo-hypophyseal system (5). Dahlgren cells were discovered by Dahlgren in 1914, then more identified and recognized in a number of skate species which then well identified in the Japanese eel, *Anguilla japonica* (5), these Dahlgren cells were categorized into three types according to their sizes(16). As concern the distribution of the Dahlgren cells in *Platicthyes flesus*, which adapted to the freshwater environment, the Dahlgren cells were located in last 6 or 8 vertebrate of spinal cord(3), others in the last three vertebrae (18).

It is known that these cells contain secretory granules in their axons with a diameter 800-2500 A Golgi apparatus also contribute in the synthesis of these granules and surrounding them with membranes (11).

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These cells play a role in caudal neurosecretory system (CNSS) as it produced at least three types of sensory neuropeptide namely, urotensin (UI), Urotensine II (UII), and corticotropin releasing factor (CRF). These peptides are known to have a major role in osmoregulation and ionic equilibrium and the activity of arterial pressure in many species of fishes (4, 19, 3). Noteworthy that Ichikawa *et al*. (13, 12) concluded that UI secreted in the common carp, *Cyprinus carpio* consists of a chain of amino acids similar to corticothrophin releasing factor (CRF) and sauvagin while UII is similar to somatostatin.

In a trial Chen and Mu (5) trace the seasonal morphological change in Dahlgren cells in the reproductive cycle of the golden fish, *C. auratus*, especially detecting the enzymes cytochemistry. They concluded that the protein content decreases in spring compared to summer and was estimated to be the least among the four seasons, while the protein increase in summer which is consider the highest in the four season. These subsequent change in Dahlgren Cells was synchronized with the development of ovary .These two authors found that the enzymatic activity of cytochrome oxidae synchronized with seasonal change in the protein level in these cells, in addition , achase activity is against cytochrome oxidae which is indicating the two types of enzymes playing a role in the reproductive cycle of fishes.

In the present investigation, a trial was made to trace the seasonal changes in the number of Dahlgren cells in the spinal cord of two freshwater fishes namely *Barbus luteus* and *Capoeta trutta* caught from River Tigris river passing through Mosul city. In addition to investigate the cyclic changes of the secretory materials inside these cells.

Materials and Methods

Two freshwater fishes namely, *Barbus luteus* (45 specimens) and *Capoeta trutta* (32 specimens) were caught by gill net from Tigris river passing through Mosul City, Iraq. These fishes brought to the laboratory, their tails were cut, spinal cords removed after cutting the vertebral neural arches, then fixed in the following fixatives:

Duboscq-Brasil or Alcoholic Bouins fluid (10), or Buffered neutral fornmalin solution (14), Formalin saline solution (14). Spinal cords washed after fixation by proper solution, mounted in Paraffin wax, sectioned 5-8 microns in thickness and stained. The following stains were used: Harris Haematoxyline-eosin (HE) stain (14); Aldehyde –Fuchsin (AF) technique (8). Mallory triple stain (MTS) (7); and Ammonical silver nitrate (7). Stained sections were examined, photographed using Olympus compound Microscope. Fish age was determined by counting the number of annuli in the scales removed dorsal to the lateral line and in the middle of the body. Classification of fishes were performed depending on the books available such as Al-Daham (1) and Coad (6).

Results and Discussion

After a random counting of Dahlgren cells in the spinal cord of a 3 year old fish, *B. luteus* three types of cells can be distinguished according to their sizes: small, medium and large.

As indicated in Table (1) the small Dahlgren cells of *B. luteus* were the most dominant in summer (average number 81.6), then the number decrease in autumn and winter. Furthermore the median sized cells were also large in number in summer (19.3) then decreases in spring then in winter and the lowest value was in autumn.

Table (2) showing counting of Dahlgren cells in the spinal cord of *C. trutta* in the age of 3 years, the small cells were dominant in summer, then autumn, then spring. The medium sized cells were also dominant in summer, then spring, then autumn, while the large cells were more dominant in spring. The large number of Dahlgren cells were observed in summer especially the small, then medium while the large cells were more in spring.

After a comparison was made with the number of Dahlgren cells reported in *B. luteus*, in spring and in the three ages (1, 2 , 3 years) as illustrated in Table (3), it is revealed that the small cells were more dominant in age 1 year, then less than that in the 3 years, then lees in the 6 years, while the medium sized cells was dominant in 1 year and less than that in 6 years then lesser in 3 years. Larger Dahlgren Cells were dominant in 1 year, less than that in 3 year, then lesser in 6 year. Generally number of Dahlgren ss cells decrease especially the large once as the age of *B. luteus* increases.

As shown in Table (4), the number of Dahlgren cells in the spinal cord of *C. trutta* in spring was higher in age 1 year, then less than that in 3 year, then lesser in 6 year, and it decreases especially the medium and small sized with the increased age.

Table (1). Random counting of the three types of Dahlgren cells in the spinal cord of *B. luteus* in the age 3 years and the average number of cells in four seasons.

Size of Dahlgren cells	Average number in winter	Average number in spring	Average number in summer	Average number in autumn
Small	47.18	42.5	81.6	58.6
Medium	14.8	17	19.3	8.6
Large	6.36	14.4	16	8.6

Table (2). random counting of three types of Dahlgren cells in the spinal cord of C. *trutta*, age 3 years.

Size of Dahlgren cells	Average number in winter	Average number in spring	Average number in summer	Average number in autumn
Small	--	46.25	79.67	68.6
Medium	--	22.6	30.65	16.6
Large	--	11.75	10.3	9.32

Table (3). Random counting of the three type of Dahlgren cells in the spinal cord of *B. luteus* in age: 1, 3, and 6 year in spring.

Size of Dahlgren cells	1 year	3 year	6 year
Small	47	42.25	39.60
Medium	25.4	17	25.1
Large	27.3	14.4	2.8

Table (4). Random counting of three types of Dahlgren cells in spinal cord of *C. trutta* in age 1, 3 and 6 in spring, 40X.

Size of Dahlgren cells	1 year	3 year	6 year
Small	58.6	46.36	45
Medium	23.25	22.6	20.7
Large	12.5	11.75	4.3

It was revealed from the present study that Dahlgren cells possess secretory materials in the cytoplasm of these cells, their distribution in the cytoplasm and staining affinities varies from one type to another. These DC cells showed secretory cycle which can be categorized into three stages:

Stage I: cells in this stage is mostly empty or with few secretory materials accumulated around nuclei as belt, nuclear membrane is active, as it is deeply stained, this stage may represent the start of synthesis of secretory material in *B. luteus* (Fig. 1). Cytoplasm appear with vacuoles, staining ability differ in the three types of vacuoles but was more strongly or intensively stained in small cells compared to the medium- or large sized cells.

Stage II: secretory materials increases in the cytoplasm of Dc far from nucleus, this accumulation continue in the cell body and axons, as it appear homogenous in distribution. Ability of Staining of cytoplasm varies between moderate and strong in B. luteus (Fig. 2&3) according to stages of synthesis in the types of cells, as the medium is stronger than the small, intense staining was obvious by using both H&E and AF (Aldehyde Fuchsin) stains as well as the cells are full with secretory materials which turn difficult to observe their nuclei its nucleus. Size of the secretory granules also varies as it is small, homogenous in the small cells and large in the medium sized Dahlgren cells.

Stage III: This is the stage of discharge of the secretory materials from the cell and its axon, as it is possible to observe secretory materials especially in the axons along axons especially the axial bulb, as pieces of bead (Figs, 4&5). Or in regions around cell body from extensions, secretory granules of these cells in the cytoplasm is homogenous (Fig. 6).

As concern the number of Dahlgren Cell types in the present study, three types were also categorized in other previous study as in the freshwater fish, *Chondrostoma regius* by Gorgees and Rahemo (9) and in three species of teleost and two species of elasmobranchs (20).

It is noteworthy that the above authors did not classify cells into three types as in the present study, but consider it as one type. Anyhow ultrastructural study of these cells using electron microscopy may reveal precise classification of these cells depending on the shape, plasma membrane boundaries, nuclear membrane boundaries, size, properties of these cells especially the cell organelles and secretory granules.

It seem from our results that Dahlgren Cells change their shape depending on change happen during formation of secretory materials, between subsequent changing is similar between type of cells, as the first change happen in nuclear membrane appear to be with strong staining ability by H&E and AF staining then activity increase to reach the area surrounding the nucleus, which may indicated the important role of nucleus in form of neurosecrtory materials. It is worthy to note that a method erected by Parmentier et al.(17) to distinguish between DC known as immunochemodetection for urotensin peptie as they can distinguish 2 type of DC according to their reaction with UI or UII.

Another recent method was erected by McCrohan *et al.* (15) by injecting of cells intracellularly with fluorescent Lucifer yellow dye as Dahlgren cells appear clearly with their dendrites and secretory materials which make easy to trace and thus to be compared with each other r and in same fishes or of different species fishes.

It is also suggested by Meselon and Stahl method [see Arm and Camp (2)] as cells can be distinguished according to the age of DNA molecules inside these cells using radioactive isotops.

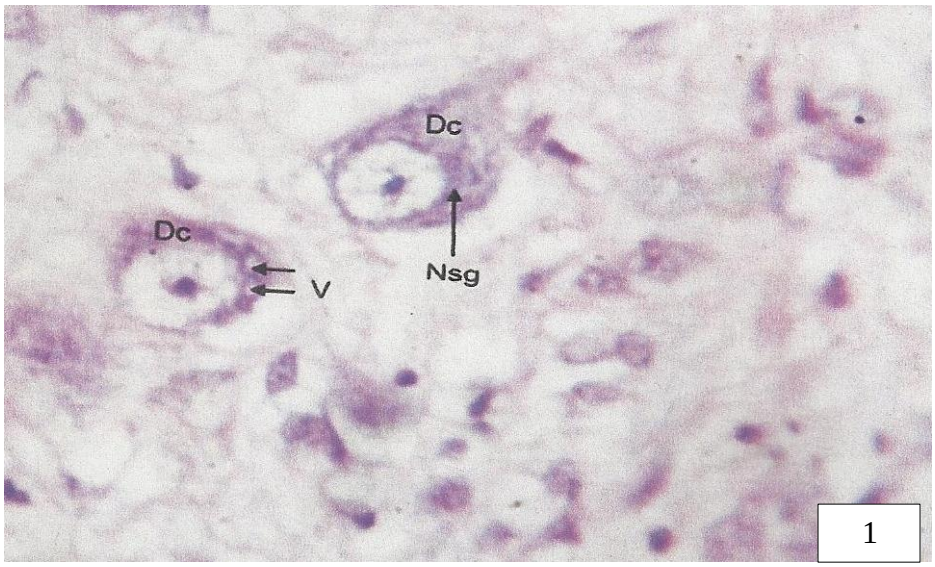

Fig. (1): Part of longitudinal section in the caudal neurosecretory system of *B. luteus* showing Dahlgren cells(DC) in the stage I of secretion, note the neurosecretory granules(Nsg) as bands around the nucleus with vacuoles (V). Haematoxyline-Eosin (H-E), 100 X.

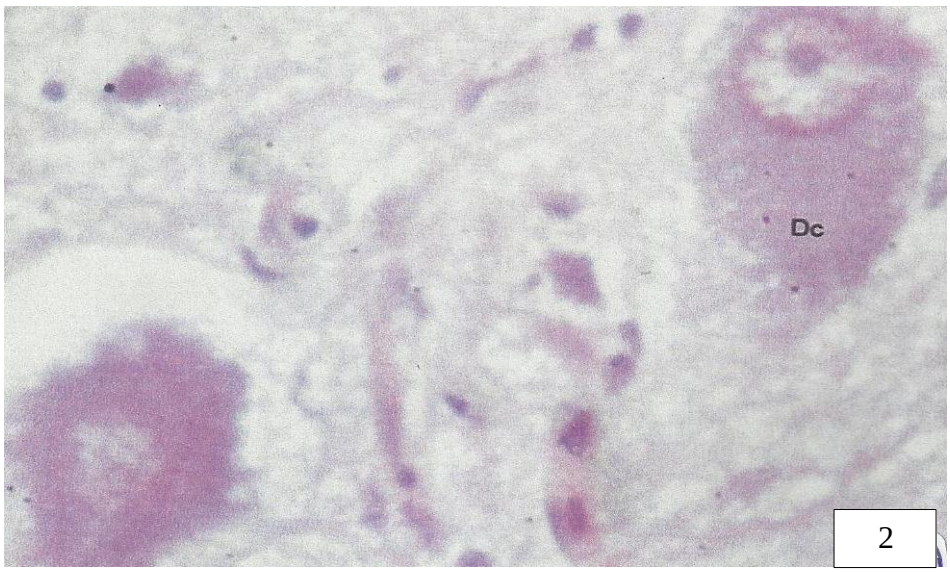

Fig. (2). Part of a longitudinal section of the caudal neurosecretory system of *B. luteus* with Dahlgren cell(Dc) in the stage II of secretion. The cytoplasm with moderate staining. H-E, 400 X.

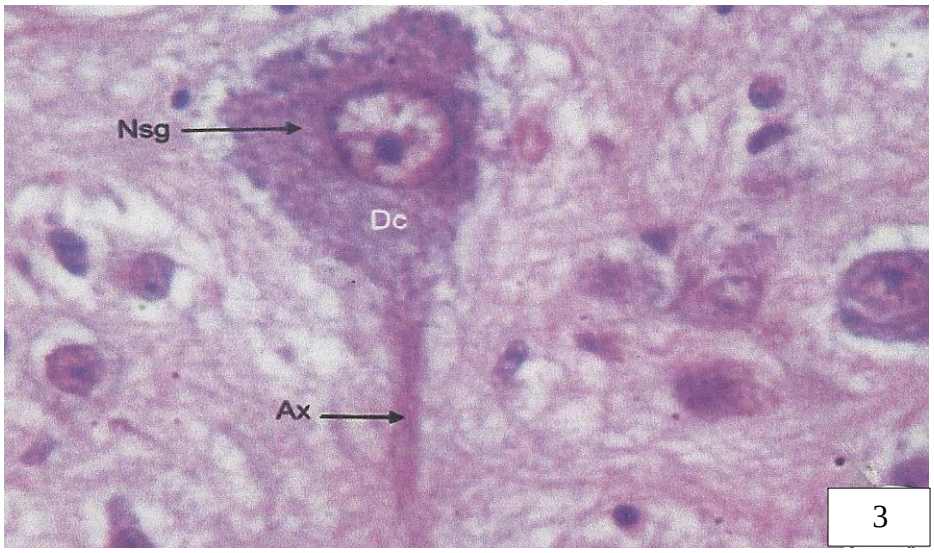

Fig. (3). Part of longitudinal section in the neurosecretory system of *B. luteus* in the stage II of secretion showing Dahlgren cell (Dc) and dense secretory granules (Nsg), secretory material seen in axon (Ax). H-E, 400 X.

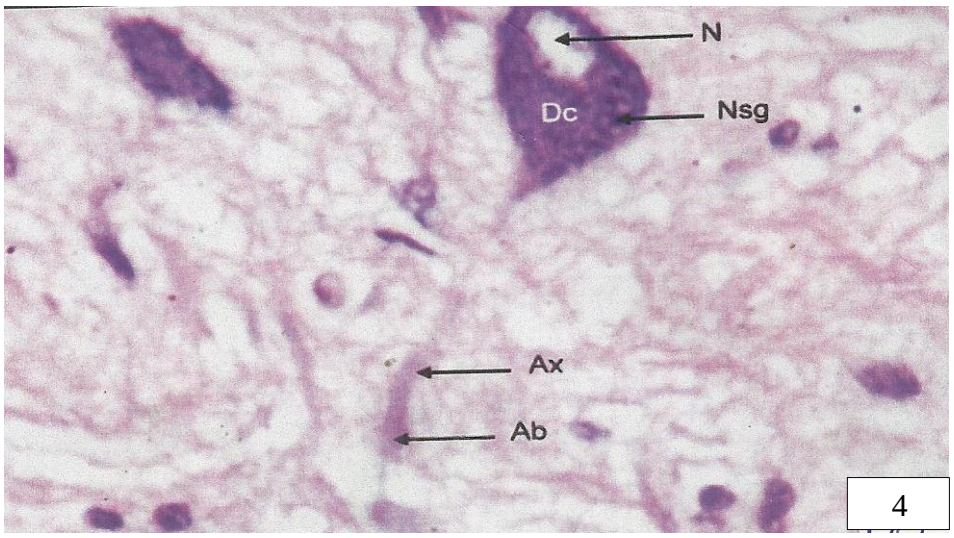

Fig. (4). Part of longitudinal section of the caudal neurosecretory of *C. trutta* showing Dahlgren cell with neurosecretory granules (Nsg), and some accumulated in the axonal bulb (Ab) of axon (Ax). H-E, 400 X.

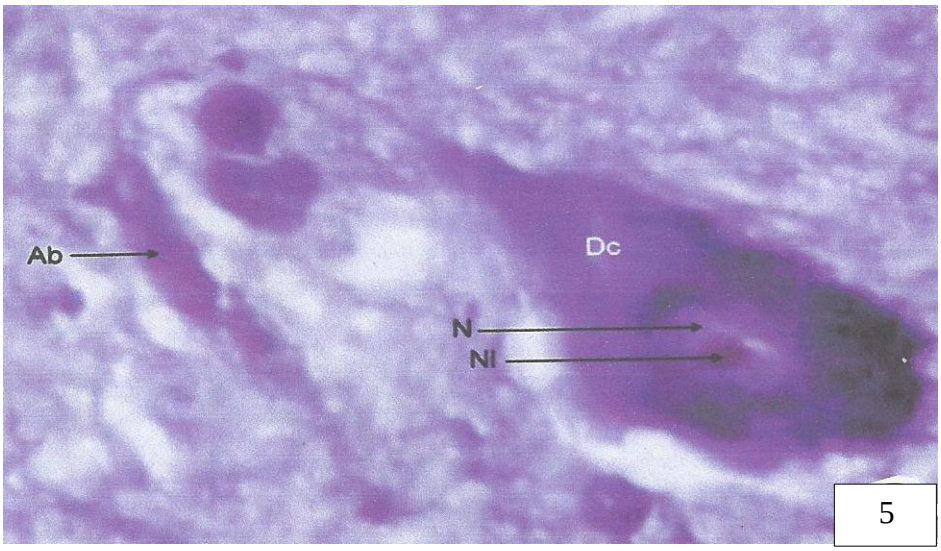

Fig. (5). Dahlgren cell (Dc) of *C trutta* with clear nucleus (N) and nucleolus (NI), secretory granules accumulated in the axonal bulb (Ab), these granules were intensively stained with Aldehyde fuchsin (AF), 400 X.

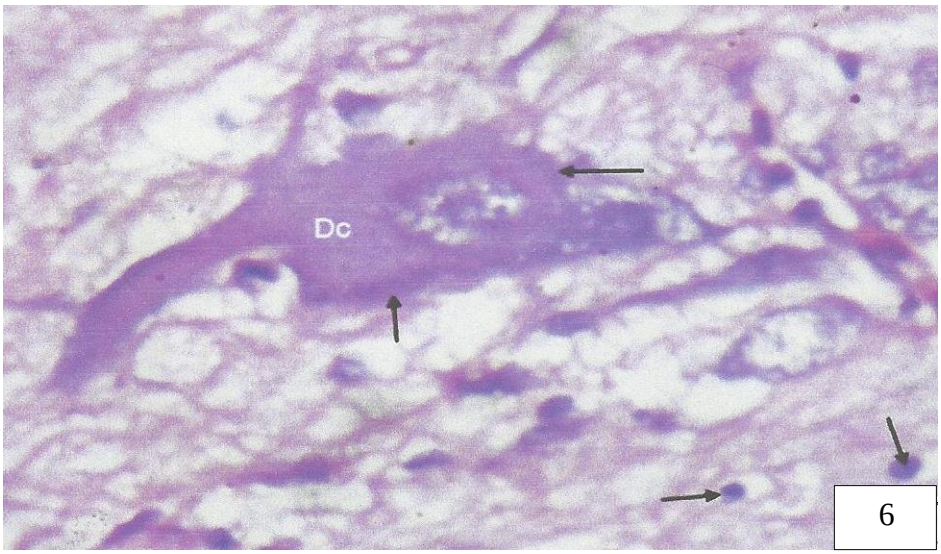

Fig. (6). Part of the caudal neurosecretory system of *B. luteus* with clear Dahlgren cells(Dc) filled with secretory granules in the stage III of secretion, the cytoplasm is homogenously stained with H-E. Secretion was seen around cell body (←), 400 X.

References

1-Al-Daham K.N. (1977). Fishes of Iraq and Arabian Gulf. Vol.1.Gulf center studies-university of Basrah (in Arabic).

2- Arms, K. and Camp, P. (1982). Biology. 2nd ed. Saunders College Publishing, 128-129.

3-Ashworth, A. J., Banks, J. R., Brierley, M. J. and McCrohan, C. R. (2005). Electrical activity of caudal neurosecretory neurons in sea water and fresh water – adapted *Platichthys flesus in vivo*. J. Exp. Biol. 208: 267-275.

4-Bern, H. (1985). The elusive urophysis: Twenty-five years in pursuit of caudal neurohormones. Amer. Zool., 23 (3): 763-769.

5-Chen, H. and Mu, R. (2008). Seasonal morphological and biochemical changes of Dahlgren cells implies a potential of caudal neurosecretory system (CNSS) in the reproduction cycle of teleostean fish. Fish Phys. Bioc., 34 (1): 24-37.

6-Coad B.W. (2010). Freshwater Fishes of Iraq. Pensoft. Sofia-Moscow 6.

7-Culling, C. F. A., Allison, R. T. and Barr, W. T. (1985). Cellular pathology technique. Butterworth and Co. (Publishers) Ltd. London.

8-Ewen, A. B. (1962). An improved aldehyde-fuchsin staining technique for neurosecretory products in insects. Trans. Amer. Micros. Soc., 18: 44-96.

9-Gorgees, N. S. and Rahemo, Z. I. F. (1983). Histomorphology of the caudal neurosecretory system and urophysis of the freshwater teleost, *Chondrostoma regius* (Heckel). Zool. Jb. Anat., 109: 397-406.

10-Gurr, E (1962). Staining Animal Tissue; Practical and Theoretical Loenard Hill, London.

11-Fridberge, G. and Bern, H. A. (2008). The Urophysis and the caudal neurosecretory system of fishes. Biol. Rev., 43 : 175-199. Online.

12-Ichikawa, T., Lederis, K. and Kobayashi, H. (1984). Primary structures of multiple forms of urotensin II in the urophysis of carp *Cyprinus carpio*. Gen. Comp. Endocrinol., 55: 133-141.

13-Ichikawa, T., McMaster, D. and Lederis, K. (1982). Isolation and amino acid sequence of urotensin I, a vasoactive and ACTH – releasing neuropeptide, from the carp *Cyprinus carpio* urophysis. Peptides., 3 (5): 859-867.

14-Luna, L. G., (1968). Manual of Histological staining Methods of the armed forces institute of pathology, 3rd ed., McGraw – Hill Book company, New York.

15-McCrohan, C. R. Lu, W., Brierley, M. J., Dow, L. and Balment, R. J. (2007). Fish caudal neurosecretory system: a model for the study on neuroendocrine secretion. Gen. Comp. Endocrinol, 153 (1-3): 243-250.

16-Owada, K., Kawata, M., Akaji, K. Takag, A., Moriga, M. and Kobayashi, H. (1985). Urotensin II – immunoreactive neurons in the caudal neurosecretary system of fresh water and seawater fish. Cell and Tiss. Res., 239 (2): 349-354.

17-Parmentier, C., Taxl, J. Balment, R., Nicolas, G. and Calas, A. (2006). Caudal neurosecretory system of zebra fish: ultrastural organization and immunocytochemical detection of urotensins. Cell Tiss. Res. 235 (1): 111-124.

18-Rahemo, Z. I. F, and Ami, S. N. (1992). On the morphology of the urophysis spinalis and the caudal neurosecretory system of the freshwater fish. *Liza abu* (Heckel). Basrah J. Agric. Sci., 5 (1): 89-100.

19-Winter, M. J. Ashworth, A., Bond, H., Brierley, M. J. and McCrohan, C. R. (2000). The caudal neurosecretory system: Control and function of a novel neuroendocrine system in fish. Bioc. Cell Biol., 78 (3): 193-203.

20-Yamada, C., Yamada, S., Ichikawa, T. (1986). Immunohistochemical localization of urotensin I and other neuropeptides in caudal neurosecretory system of three species of teleosts and two species of elasmobranches. Cell Tiss. Res., 244 (3): 687-690.