Effect of ablation of cerebral ganglia and injection of there extract on the Ascorbic Acid content of fresh water bivalve *Lamellidens marginalis* during winter season

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Abstract

Considering the metabolic shifts in freshwater bivalve shell fishes, due to neuro-endocrine stress we report here the seasonal variation in ascorbic acid content from mantle, hepatopancreas, gonad, foot and gill of adult freshwater bivalve *Lamellidens marginalis*, collected from Kaigaon during winter (December-January).Adult bivalves of approximately same size were distributed in four group 1st treated as control 2nd as cerebral ganglia ablated 3rd as dist water injected and 4th as cerebral ganglionic homogenate injected. The ascorbic acid content was determined on 5th and 10th day from mantle, hepatopancreas, gonad, foot and gill different group. On 5 th day, the ascorbic acid content was significantly decreased from mantle in both cerebral ganglia ablated and ganglionic extract injected group respectively. While the content from gonad showed significant increase in ganglionic extract injected group On 10th day the ascorbic acid Content was significantly increased from hepatopancrcasand in cerebral ganglia ablated and ganglionic extract injected group respectively The content from gonad was also significantly increased in cerebral ganglia ablated and i n ganglionic extract injected group The result are discussed in the light of metabolic shift in the bivalves due to neuro-endocrine manipulation.

Introduction : The cerebral and visceral ganglia in these animal have been shown to play an important role in the rate of water filtration and regulation of biochemical metabolites.(Hautteville P & Lubet 1974 Mane U.H.1986,Shinde N.G.& Gaikwad D.M.2014) The Evidence for the occurrence of a wide variety of neurotransmitters in different tissues of Lamellibranchs including the nerve ganglia has been discussed (Leak and Walker, 1980). From a functional point of view, Lubet (1956) was the first to suggest that a relationship might exist between the neurosecretory cells in the cerebropleural and visceral ganglia and reproduction in two marine mussels, *Mytilus edulis* and *Chlamysvaria*. Studies on changes in the biochemical constituents in relation to the reproductive cycle in bivalve molluscs have been carried out extensively and have reviewed by many workers (Bayne, 1976; Sastry, 1979). Perusal of literature shows that the ascorbic acid content in different tissues of freshwater bivalve mollusc has received little attention. effects on the content of ascorbic acid i.e. rate of protein metabolism in bivalve molluscs (Bayne,1976.) Most of the vital activities in bivalves are regulated by neuro-endocrine center. The ascorbic acid content of the animal reflects their general metabolic rate. Protein (ascorbic acid) Ingested through food are hydrolysed in the digestive system to their constitutive amino acid by proteiolytic enzymes. These amino acids are then accumulated for carbon and nitrogen catabolism. It is evident that the energy content of excreta comprises a significant component of total energy loss. The effect of bilateral cerebralactomy and its extracts injection on lipid content in freshwater bivalve species Lamellidens corrianus (Lea).(N.G.Shinde and D.M. Gaikwad 2014).Very few literatures are available on the

effect of cerebralectomy on biochemical constituent and reproductive cycle (Lubet, 1959, 1965; Nagabhushanam and Mane, 1975) To extend the knowledge in this field, an attempt has been made in the present study to demonstrate the effect of bilateral cerebralactomy and its extracts injection on ascorbic acid content in freshwater bivalve species *Lamellidens marginalis*. In present study the biochemical analysis like ascorbic acid was estimated from different body parts of *Lamellidens marginalis*. This species abundantly distributed along the banks of Godavari river of Jayakwadi backwaters at Kaigaon Aurangabad, occurs throughout the year abundantly and thus used for the present study.

MATERIALS AND METHODS : The freshwater bivalve molluscs, *Lamellidens marginalis* (Lamark) were collected during winter season, from Godavari River at Kaigaon, 50 Km. away from Aurangabad city. The animal of 80-90 (mm in shell length) were selected and stocked in well aerated reservoir water for 24 h. in the laboratory condition. After 24 h. the animals were grouped in four sets, each containing 30 animals. The first served as control with intact cerebral ganglia (Intact control), and others three as experiments, i.e., ablation of both cerebral ganglia, sham operated animals and injection of cerebral ganglionic extracts to ablated animals. small cut of 0.5 mm was done at the location of cerebral ganglia and the ganglion was removed with a fine sterilized forceps. For injection of cerebral ganglionic extracts, cerebral ganglionic-extract was prepared in ice cold distilled water (10 ganglia in 1.00 ml cold distilled water) was centrifuged and the supernatant (0.2 mi/animal i.e. equivalent to 2 ganglia/animal) was injected into the foot (muscular region) of cerebral gangliactomized bivalves. In sham operated control animals were injected by 0.2 ml cold distilled water. The result for control and sham operated groups were similar and hence a comparison was made between gangliactomized and control group and extract injected and control group only. Soon after the operation and injection of ganglionic extracts, 30 animals of cerebralectomy, 30 animal of extract injected and 30 animals of control (non-operated) were transferred to separate aquaria. Animal from control, ablation of cerebral ganglia & injection of their extract group were sacrificed for estimation of ascorbic acid For biochemical divergence particularly protein analysis, four animals from each groups,were dissected and removed tissue like Mantle, Hepatopanceas, Gonad and Foot. The ascorbic acid estimation was done according to method proposed by Roe *et. al.* (1967) from the 100mg wet tissues of the individual bivalve. The results are expressed as mg content per 100mg (mg/100mg) wet tissue. Triplicate values were subjected for statistical confirmation using Student "t" test (Dowdswell, 1957) percentage differences were also calculated in monsoon season. The physico-chemical characteristics of water used in experiments i.e. Temperature and pH of the water were recorded daily during the water renewal, and total carbonate and dissolved oxygen were also determined using methods described by APHA et.al., (1985)

RESULTS AND DISCUSSION

The result of the present study were shown in figure with respect to physico-chemical parameters of water used in experiment , i.e. temperature, pH and dissolved oxygen content in winter Season. As follows temperature was 20.00^{o}C-24.00 ^{o}C, pH was recorded 7.9 – 8.5, and dissolved oxygen content was recorded during winter season 6.42-7.00 mg/lit. Effect of bilateral cerebralectomy and ganglionic extract injection were studied to determine the ascorbic acid content in different tissues of freshwater bivalve mussels *Lamellidens marginalis* .On 5 th day, the ascorbic acid content was significantly decreased (0.3515 ± 0.0014, 16 15 %, P < 0.001) and (0.3892 ± 0.0128, 7.15%,P < 0.05) from mantle in both cerebral ganglia ablated and ganglionic extract injected group respectively. While the content from gonad showed significant increase (0.4621 ± 0.0074, 12.52 %. P < 0.05) in ganglionic extract injected group. w hereas the content from foot was significantly decreased (0.3635 ± 0.0128. 7.62 %, P < 0.05) in cerebral ganglia ablated group on 5th day. On 10th day the ascorbic acid Content was significantly increased

from hepatopancrcas,(0.4235 ± 0.0074, 12.52 %. P < 0.05) and (0.4964 ± 0.0196. 31.88 %. P < 0.01) in cerebral ganglia ablated and ganglionic extract injected group respectively The content from gonad was also significantly increased (0.4278 ± 0.0128. 11.13 %, P < 0.05) in cerebral ganglia ablated and (0.4707±0.0196,22.26 %. P < 0.01) i n ganglionic extract injected group

Table:-1 Physico – chemical characteristics of water during Winter seasons

Sr. No.	Seasons	Months	Temperature (ºC)	pH.	Hardness (ppm)	Dissolved oxygen content (mg/lit.)
1	Winter	December	20 – 24.5 ºC	7.9 – 8.5	105 – 110	6.42-7.00
		January	21.5 – 23.5 ºC	7.9– 8.7	115-128	6.68-8.00

Table –2 Effect of ablation of cerebral ganglia and injection of their extracts on ascorbic acid content of *Lamellidens marginalis*on 5th day during winter season. (Bracket values represents percentage difference). • = P<0.001, •• = P<0.01, ••• = P<0.05 (CG=Cerebral Ganglia)

Table –3 Effect of ablation of cerebral ganglia and injection of their extracts on ascorbic acid content of *Lamellidens marginalis* on 10h day during winter season. (Bracket values represents percentage difference)
• = P<0.001, •• = P<0.01, ••• = P<0.05 (CG=Cerebral Ganglia)

Kulkarni *et.al.* (1988) studied the effect of temperature and pH on ascorbic acid content of *Indonaia caeruleus.* The impact of pollutants on ascorbic acid content in various tissues of freshwater bivalve, *Corbicula striateffa* was studied by Zambre (1991). Jadhav *et.al* . (1996) studied the effect of carbaryl on ascorbic acid content in the various tissues of the freshwater bivalve *Corbicula striatella.* Perusal of literature shows that the ascorbic

acid content in different tissues of freshwater bivalve mollusc has received little attention .Hence considering the multiple role of ascorbic acid (Chinoy and Seetalakshmi,1977; Kachole et al.,1977; Ali et al.,1983; More and Mudkhede, 2009; Pardeshi and Gapat, 2012, Vedpathak et al.,2007 and Kamble et al.,2011)

In the reproductive organs of gastropod, *Laevicaulis alte*, Chinoy and Seethalakshmi (1977) found

	Tissues	Control normal	CG Ablated	Dist. Water injected	CG Extract Injectd
1	Mantle	0.3978 ± 0.0074	0.3935 ± 0.0148 (1.07)	0.4192±0.0196	0.4407 ± 0.0222 (10.77)
2	Hepato-pancreas	0.3764 ± 0.0128	0.4235 ± 0.0074 (12.52) •••	0.4107± 0.0267	0.4964± 0.0196 (31.88) ••
3	Gonad	0.3849 ± 0.0074	0.4278 ± 0.0128 (11.13) •••	0.4029 ± 0.0207	0.4707 ± 0.0196 (22.26) ••
4	Foot	0.3635 ± 0.0222	0.3807 ± 0.0148 (4.71)	0.3849 ± 0.0074	0.3549 ± 0.0074 (2.35)
5	Gill	0.3678 ± 0.0074	0.3849 ± 0.0196 (4.6)	0.3764± 0.0128	0.3931 ± 0.0066 (6.87) •••

increase in ascorbic acid content due to active conversation of ascorbinogen to free ascorbic acid

accompanished by increased the rate of utilization. These authors further stated that monodehydroascorbic acid and ascorbic acid play an important role in steroidogenesis. Monodehydroascrobic acid by virtue of being a free radical with an unpaired electron has stronger

reducing properties than ascorbic acid and is known to participate in several biosynthetic reactions as a source of electron energy (Chinoy, 1970; 1972) and thus for the general maintenance of the necessary cellular reduction potential. Similar important role of monodehydroascrobic acid and ascorbic acid in bivalves cannot be ruled out since different tissues are different in responses according to endogenous changes. They also suggest the increase demand of energy being provided by the utilization of ascorbic acid since summer season provides drastic environmental conditions to the animal at the habitat.

In the bivalve, *Indonaia caeruleus* ascorbic acid content was significantly increase on variation in temperature and pH range (Koundinya & Ramamurthy, 1980). The present study, based on the percentage differences, the removal of cerebral ganglia and injection of cerebral ganglionic extract affected all the organic constituents from different body parts of *Lamellidens marginalis* during winter season.

	Tissues	Control normal	CG Ablated	Dist. Water injectd	CG Extract Injectd
1	Mantle	0.4192 ± 0.0074 ( ) •••	0.3515 ±0.0014 (16.15) •	0.4535 ± 0.0222 () •••	0.3892± 0.0128 (7.15) •••
2	Hepato-pancreas	0.3978 ± 0.0074 ( ) •••	0.3764 ± 0.0128 (5.38)	0.4021 ± 0.0128 () •••	0.4321 ± 0.0196 (8.61)
3	Gonad	0.4107 ± 0.0196 ( ) •••	0.4021± 0.0128 (2.08)	0.4364 ± 0.0267 () •••	0.4621 ± 0.0074 (12.52) •••
4	Foot	0.3935 ± 0.0074 ( ) •••	0.3635± 0.0128 (7.62) •••	0.4364 ± 0.0267() •••	0.4192 ± 0.0148 (6.53)
5	Gill	0.3935 ± 0.0074 ( ) •••	0.3892 ± 0.0128 (1.08)	0.4064 ± 0.0074 () •••	0.3678 ± 0.0148 (6.53) •••

Conclusions :

On 5 th day, the ascorbic acid content was significantly decreased from mantle in both cerebral ganglia ablated and ganglionic extract injected group respectively. While the content from gonad showed significant increase in ganglionic extract injected group. w hereas the content from foot was significantly decreasedin cerebral ganglia ablated group on 5th day. On 10th day the ascorbic acid Content was significantly increased from hepatopancrcasand in cerebral ganglia ablated and ganglionic extract injected group respectively The content from gonad was also significantly increased in cerebral ganglia ablated and i n ganglionic extract injected group Similar results have been shown by Shinde N.G 2008 , in freshwater bivalves' molluscs in *Lamellidens corrianus f*rom Godavari River near Aurangabad, which support the present investigation. Thus, it can be concluded that cerebral ganglia may plays an important role mostly inhibitory one, regulates the fluctuation in ascorbic acid content form different body parts due to neuro-endocrine manipulation The effect was pronounced in cerebral ganglia ablated and ganglionic extract injected group animals. the ascorbic acid may be tentatively suggested that further research should be carried out for the physiological roles of neuro-hormones in the metabolic economy in case of the freshwater bivalve.

REFERENCES

Ali, S. M., Iliyas, R. and Bhusari, N. B. 1983. Effect of lethal and sublethal concentrations of dimethon on ascorbic acid content of freshwater fish, Channa gachua Marath. Univ. J. Sci. Silver Jubilee Issu. 22: 69-74.

APHA, AWAA and WPCF (1985): Standard Methods: For the examination of water and waste water. 16th Edition, APHA Publication, Washington, USA, pp. 1-1267.

Bayne, B.L. (1976): Marine mussels: their eéology and physiology. Cambridge University Press, Cambridge London, New York, and Melborne, pp. 1-495.

Chinoy, N.J. (1970): Source of electron energy for animal metabolism. II. Role of ascorbic acid in the metabolism of insect tissues. The nucleus, 13: 55-58.

Chinoy, N.J. (1972): Ascorbic acid levels in avian tissues and its metabolic significance. Acta. Zool. (stockh) 53 (1): 121-126.

Chinoy, N.J. and L. Seethalakshmi (1977): Biochemical evidence for the occurance of a possible steroidal mechanism in the reproductive organs of slugs, (Laevicaulis alte). In: Advances in invertebrates Reproduction, Vol. 1 Eds K.G. Adiydi and R.G. Adiyodi, Paralam, Knoth. Kerala, India, pp. 356-366.

Dowdeswell, W.H. (1957): Practical Animal Ecology. Methum and Co.Ltd., London. | Folin Phelon reagent. J. Chem. 193:265-275.

Golterman, H. L.1978. Method of clinical analysis of freshwater, IBP,
Blackwell Scientific publications, Oxford and Edinburgh, pp.127-133.

Hautteville P & Lubet 1974.Analyse experimental, en culture organotypique ,de l'action des ganglions cerebro-pleuraux et visceraux sur le manteou de la noule male *Mytilus edulis* (L)(Mollusque pelecypode).C.R. Seances Soc.Fil.278:2469-2472

Jadhav S., Sontakke YB. and Lomte V.S. (1996) : Effect of carbaryl on ascorbic acid content in the selected tissues, *Corbicula striatella.1. Ecotxicol Environ Monit.,* 6 (2) : 109-112.

Kachole, M. S., Pawar, S. S. and Mahajan, A. G. 1977. The toxicity of eldrin and the effect of pretreatment of Phenobarbital and hexobarbital in the mortality of freshwater fishes.Bull. Environ. Contam. Toxicol. 16: 768-770.

Kamble, V. S., Gavhane U. V. and Rao K. R. 2011. Impact of organochlorine and organophosphorus on Ascorbic acid content in Lamellibranch freshwater molluscs, Lamellidens corrianus (Lea) during monsoon. 1(2): 146-149.

Koundinya PR, Ramamurthy R (1980) Effect of sub-lethal concentration of sumithion and sevin on certain haematological values of srotherondon Mossambicis (Peters). Current Science 49 : 645-646.

Kulkarn D.A., Rao K.R., Vedpathak A.N. and Mane D.H.(1988) : Effect of temperature and pH on ascorbic acid content of the freshwater mussel, *Indonaia caeruleus* (Prashad1918). *J. Adv. Zool.*, 9 (1) : 20-24.

Leak, LD. and Wailcer, R.J. (1980): Invertebrate neuropharmacology Thomson Litho Ltd., East Kilbride, Scotland, Blackie, Glasgow and London, Edward Arnold, pp. 1-357.

Lubet, P. (1956): Effects of l'ablation des centres nerveux surl'emission des game'es schez Mytilus edulis (L) et. Chiamys varia (L.) (Mollusques : Lamellibrancheses). Ann. Des. Sci. Nat., 175-183.

Lubet, P. (1965): Incedences de l'ablation bilaterale des ganglions céIebroides sur Ia gamétogenése et la development du tissue conjuntif chez Ia moule *Mytiius galloprovincialis Lam*. (Moll. Lamellibranches). C.R. Soc. Biol., 159: 397-399.

Lubet, P. (1966): Essai d' analyse expérimentale des perturbations producites par les ablations des ganglions nerveux chez *Mytilus ediilis* D. et Mytilus gallop rovincialis Lmk. (Mollusques Lamellibranches). Ann. Endocrinol., 27: 353-365.

Mane U.H.1986 Neurosecretory phenomena of Indian green mussel,*perana viridis*(L)Proc.8th Internat Malacolol Cong.,Unitas Malacol., Budapest (1983),pp151-156

More Aruna and Mudkhede, L. M. 2009. Changes in ascorbic acid levels of freshwater crab, Baritelphusa guerini, after exposure to sulphates of three metallic salts.Viz.Copper sulphate and cadmium sulphate. Journal of Aquatic Biology. 21: 210-214.

Nagabhusanam, R. and Mane, U. H. 1975. Reproduction in mussel,Mytilus viridis at Ratnagiri. Bull. Dept. Mar. Sci. Univ. Cochin. India. 7: 377

Pardeshi Anilkumar and Gapat Minakshi, 2012. Ascorbate effect of protein during nickel in toxication of freshwater bivalve *Lamellidens corrianus*. Bioscience Discovery. 3(2): 270-274.

Roe, J.R. 1967. In: Methods of Biochemical analysis, Vol.5, Ed.Glick,
Inter science, New York, pp.44.

Sastry, A.N. (1979): Pelecypoda (excluding Ostreidea). In: "Reproduction of marine invertebrates", (Eds.Geese A.C. and pearse, J.S.).Academic Press, NewYork, Vol.5.pp. 113-292.

Shinde, N. G. (2008): Induction of breeding by neuro-endocrine manipulation in some commercially important bivalve mollouscs from Jayakwadi backwater, Ph. D. Thesis, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. pp.1-306.

Shinde,N.G., GaikwadD.M.(2014) Effect of Neuro-Endocrine Manipulation on Lipid Content In Freshwater Bivalve: Lamellidens Corrianus During Summer Season. Indian Journal Of Applied Research Volume : 4 | Issue : 10 | October 2014 | ISSN - 2249-555x pp606-608

Vedpathak, A. N., Jadhav, M. R. and Misal, P. J. 2011. Role of cerebral ganglia in regulation of oxygen consumption of freshwater bivalve molluscs, Indonaia caerulueus (Prasad 1918) From Godavari River during summer. The Bioscan. 6(4): 609-611

Zambre S.P. (1991) : Reproductive physiology of the freshwater bivalve, *Corbicula striatella*. Ph.D. Thesis, Marathwada University, Aurangabad. MS.