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Hematological analysis of Molluscan species *Bellamya bengalensis* and *Lamiellidens marginalis*

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ABSTRACT : Two molluscan species, freshwater snail *Bellamya bengalensis* and a bivalve *Lamellidens marginalis* were used for the hematological analysis. Hemolymph of both the species was collected from the body parts by using nonlethal technique in in-situ condition. Cells observed in the heamolymph were compared morphologically and quantitatively to discuss its role in the mechanism of circulation, transportation and also by means of defense mechanism in the body of both species. Cellular comparisons were made at the species level.

Keywords : Mollusca, Haemocyte, *B. bengalensis*, *L. marginalis*

INTRODUCTION

In spite of the constant progress of studies on molluscan blood cells, there is no uniform and satisfactory classification system. Therefore these cells classified on one hand, the role of blood cells in various functional and development stages, and to the other hand, either by morphological aspects in the body (Adamowicz and Bolaczek, 2003). Hemolymph, the circulatory fluid of molluscs, which is slightly bluish in colour due to presence of respiratory pigment hemocynine. Hemolymph transports nutrients, respiratory gases, enzymes, metabolic wastes and also toxicants throughout the body. Hemolymph with plasma and corpuscles can provide information pertinent to health assessment of animals or population. A large number of colorless stellate amoebocytes or corpuscles also referred as leucocytes are found in plasma, which are collectively called as hemocytes (Gustafson and Stoskop, 2005). Although hematology has been explored considerably and studies have been made on blood of molluscs, there is still much opportunity for investigation of blood differentiation and its mechanism in various body processes of freshwater snails and bivalves by means of their crucial role in body process. In general molluscs play a role in the balance of nature, and also act as biological indicator, in determining the degree of pollution of water and terrestrial environment. By considering the important role of hemocytes in molluscs, we have decided to study hematological analysis of two freshwater species as *Bellamya bengalensis and Lamelliden marginalis.* Comparison of two species quantitatively as well as qualitatively was done at cellular level.

MATERIAL AND METHODS

For the present investigation two molluscan freshwater species *(Bellamya bengalensis and Lamellidens marginalis)* were used. The first Freshwater prosobranch snail *Bellamya bengalensis* belongs to class gastropoda, were collected from 'Rajaram tank', near the campus of Shivaji University Kolhapur, Maharashtra, India. The snails were collected and brought to laboratory in polythene bags. After the cleaning, snails were kept in large plastic trough having 50 lits. The snails were provided with proper ventilation and food, like *Hydrilla*, *Pistia*, etc. After acclimatization for a week, healthy, adult and same sized snails (2.8-3.5 gm. weight, and 23-26 mm shell height) were sorted out and used for experimental studies.

Similarly the second molluscan freshwater bivalve species *Lamellidens marginalis* belongs to class pelecypoda inhabiting along the bank of Krishna river was specifically collected from south bank of Haripur and north of Ankali villages, Dist. Sangli, Maharashtra, India. After collection, the shells of these bivalve molluscs were cleaned to remove the fouling algal mass and mud and were kept for acclimatization for a week. The bivalves were provided with proper ventilation and food in the form of zooplanktons. The healthy, adult and same sized bivalves (9.5cm to 10.5 cm length in shell size) were used for experiment.

NONLETHAL METHOD OF HEMOLYMPH COLLECTION

Collection of hemolymph from the gastropod snail Bellamya bengalensis was carried out in following manner. Prior to bleeding snail were washed with cold water in order to remove the feaces and excess mucus. It was then held in the left hand and then the syringe with a needle size 26 G½ (0.45mm × 13mm) was inserted in foot, through the operculum. The pressure is applied by withdrawing the plunger of the syringe and needle was slowly moved deeper until drops of pale blue hemolymph were aspirated. After the collection of required hemolymph the snail was returned to its enclosure.

In case of bivalve molluscs, *Lamellidens marginalis* hemolymph was collected by gently prying the shell to open approximately 5 to7 mm. with a thin knife. The shell was held open with tissue forceps. The foot was visible between slightly gaping of shell valves, as a highly muscular white surface, then it was gently penetrated with a 26 G½ (0.45 mm × 13mm) sized needle. Required quantity of

heamolymph was easily collected using gentle intermittent suction.

DIFFERENTIAL CELL COUNT

A drop of collected hemolymph was taken on a clean and dry slide at one end, with the help of a second slide (spreader slide) held at 45° drawn a thin uniform film of the blood. It was air- dried perfectly. An area of uniform film marked with a wax pencil and stained with Leishman's stain for one minute. Then distilled water was added to the stain (in ratio 1 : 1). Stain was allowed to stand for 7-10 minute up to appearance of greenish metallic scum of mixture. Thereafter stain was drain off and film washed for 10-15 seconds with distilled water. The colour of film was pink. The slide was observed under microscope for differential cell count, by using high power or oil immersion objective.

CALCULATION

Percent of different Types of leucocytes

$$= \frac{\text{No. of a type W.B.C.} \times 100}{\text{Total No. of W.B.C.}}$$

RESULT AND DISSCUSSION

A. Qualitative Analysis

In case of freshwater snail, *Bellamya bengalensis* and bivalve *Lamellidens marginalis* classification criteria based on morphological characters and behavioral pattern of the haemocyte. Main two types of hemocytes were identified in stained spontaneous monolayer namely, Agranulocytes and Granulocytes.

(1) Agranulocytes. The agranulocytes are less abundant and had a limited ability to spread on glass slides, thus showing a round shape. Agranulocytes were smaller than granulocytes and had large nuclei and reduced basophilic cytoplasm.

Their nucleus-cytoplasm (n/c) ratio was high. The basophilic cytoplasm generally, did not contain granules. These are also called as hylinocytes which comprise 5 % of the total hemocytes population and represents young blastic cells.

(2) Granulocytes. Granulocytes are larger than agranulocytes, also called as spread cells, constitutes about 95% hemocytes population. These cells are polymorphic in nature with polymorphic nucleus and numerous granules in the cytoplasm, forming long pseudopodia with axial streaks. The cells are adhering to substratum and has capacity to change their shapes and size, the cells represented a population of mature cells. Some cells stained with Leishmans stain, showed eccentrically situated oval or kidney shaped nucleus and high affinity towards acidic stain. These cells were acidophilic granulocytes. Where as nucleus of some granulocytes shows affinity towards basic stains and were stained blue colour referred as basophilic granulocytes.

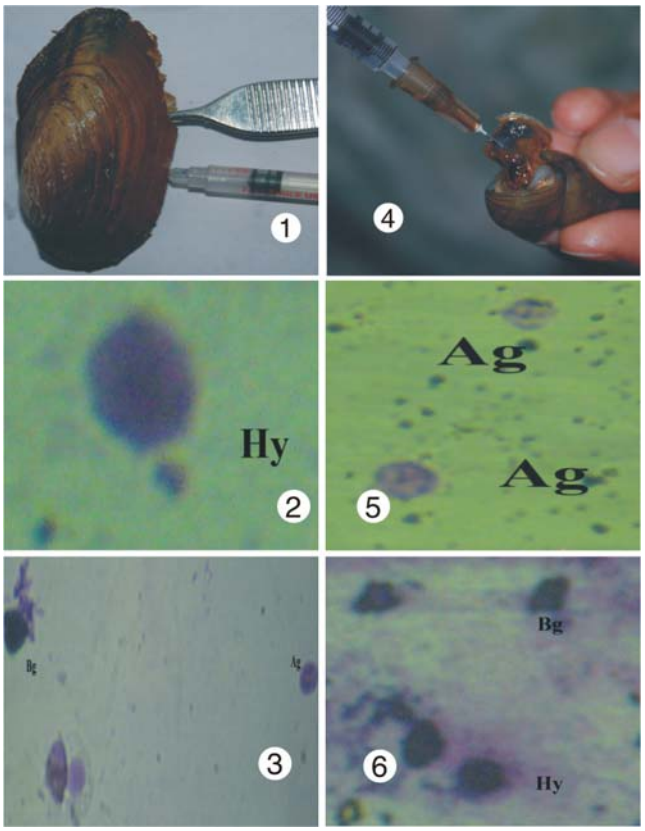

Fig. 1, 2, 3. Heamolymph collection from *L. marginalis,* blood cells, 4, 5, 6. Heamolymph collection from *B. bengalensis*, blood cells.

QUANTITATIVE ANALYSIS

In the quantitative analysis, it was observed that, in case of hemolymph of freshwater snail B. bengalensis, hyalinocytes found about 2 % of total hemocyte population. Where as remaining 98 % of population was of granulocytes. The number of acidophilic granulocytes found less than basophilic granulocytes, which was about 80 % of total granulocytes.

In case of freshwater bivalve *L. marginalis*, hyalinocytes population was 4 %, in comparison with this, observed Granulocytes were about 96 % of total hematocytes population. The number of acidophilic granulocytes found less than basophilic granulocytes, which was about 65 % of total granulocytes in the hemolymph. We found that, some granulocytes showed cytoplasmic granules stained orange in colour. Large multinucleate granulocytes were also found in a single monolayer of hemocytes. In addition to this, cell with yellow-brown granules were seen on several slides. Also cells with orange inclusions are probably phagosomes containing degredated products.

According to Adema *et. al.*, (1992) the numbers of circulating hemocytes varies between and within species and were depends on environmental factors. It was also found that number of hemocytes in the species depends on several factors as, age, temperature, infections (Noda and Loker,

1989), parasite invasion (Drozdowski and Zbikowska, 1994), injuries (Sminia, 1981), water content in the tissues (Zbikowska, 1998), and general condition of the organism (Barracco *et. al.*, 1993). Older individuals may have a twice higher number of blood cells compared to young animals.

The study reveals that, the use of nonlethal technique for hemolymph collection, from foot is effective and allows the repeated bleeding in molluscs. At a time, as the welfare of invertebrates is increasingly attracting attention, it is very important that, procedures such as bleeding noninvasive as possible. According to Idakieva and Chakarska, (2009) hemolymph of molluscs can be collected by making diagonal slits on foot. Berg, (1995) and Naimo *et. al*., (1998), developed some relatively noninvasive technique for hemolymph collection from mantle and foot respectively. Similarly Gustafson and Stoskpf (2005) recorded collection of hemolymph from anterior adductor muscle of molluscs Ellipto complanata and found no adverse effect of repeated hemolymph sampling on growth and servivality of animal.

By considering functional aspects hemocytes can be distinguished as stem cells, phagocytes, trophic cells, haemostatically active cells which are responsible for blood hemostasis, and (Glinski and jarosz, 1997). On the basis of morphology, two basic cell types are recognized among molluscs hemocytes (Carballal, *et. al.*, 1997). Agranulocytes (hyalinocytes) and granulocytes. (Adamowicz and Bolaczec, 2003). Some time morphology based classification is not enough to classify molluscan hemocytes, therefore several authors have used other criteria such as the enzymes content (Moore and Lowe 1977, Bayne *et. al.*, 1979, Pipe 1990 a) antigenic characterization (Yoshino and Granath 1983, 1985, Dikkeboom, *et. al*., 1985, 1988, Morvan 1991, Noel et al. 1994) or lectin related properties (Pipe 1990 b). Hematological analysis in our study found that number of agranulocytes was more in *L. marginalis* than *B. bengalensis*.Where as number of granulocytes was more in *B. bengalensis* than L. marginalis which indicates that, hemoral defense mechanism was stronger in freshwater snail *B. bengalensis* as compared to bivalve *L. marginalis*.

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