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Morphological and structural characterization of blood cells of *Anadara antiquata*

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Introduction

The blood cockle belongs to the genus *Anadara* of family Arcidae. In Pakistan *A. antiquata* can easily be found in Phitti Creek and Sonmiani locations (Jahangir *et al.*, 2014). It has also been reported in Dar es Salaam, Tanzania (Toral-Barza and Gomez, 1985; Mzighani, 2005). This is a valued species around the globe and rich in glycogen, mineral and protein.

A. antiquata carries haemoglobin (Kanchanapangka *et al.*, 2002; Gabriel *et al.*, 2011) hence it is commonly termed as blood cockle. Its economic importance is rising. Therefore, it attracts investigators to study its dimensional aspect of biology (Jones, 1970; Silas *et al.*, 1982). Furthermore, knowledge on its biology, physiology, and health status provide basic information for effective management (Gabriel *et al.*, 2011).

Haematological studies often provides effective and sensitive index to see environmental, physiological, pathological and biochemical changes in organism (lwama *et al.*, 1976; Akinrotimi *et al.*, 2007). Unusual changes in blood profile can interpret metabolic and health status of animals (Babatunde *et al.*, 1992).

Earlier investigations had shown that blood cockles are capable of surviving at least one month at 20 ^{0}C, in case of oxygen depletion (Thillart *et al.*, 1992) because haemoglobin enables the organism to bind oxygen (Brooks *et al.*, 1991; Vooys *et al.*, 1991; Zwaan *et al.*, 1991). Zwaan and Cortesi (1993) investigated that oxygen storage allows survival of about 12 hours compared to the bivalves which lack haemoglobin.

There is limited knowledge about the morphology of the blood cells of Arcidae species (Holden *et al.,* 1994). Griesbach (1891) and Cuenot (1891) focused on the white blood cells of *A. tetragona, A. noae* and *Solen legume*. The morphology of red blood cells and white blood cells of *Arca inflate* were investigated by Sato (1931) and Ohuye (1937). The red blood cells of *A. transversa* was studied by Dawson (1933), Cohen and Nemhauser (1980) and Nemhausern *et al*., (1983) worked on the erythrocyte of *Anadara* spp by using electron microscopy. Besides them, Gabriel *et al.,* (2011) worked on haematological characteristics of the blood cockle *A. senilis* from Niger delta and mentioned three types of cells. They stated that haematological characters increase with the increase of shell. Mohite and Meshram, (2015) studied the haematological characterises of *Tegillarca rhombea*, and they discussed the red blood cell (cell have round nucleus), white blood cell (kidney bean-shaped nucleus) and non-nucleated tiny particles called platelets. The white blood cells were further classified into two categories granulocyte and agranulocytes.

The present investigation provides information about the morphology and cytochemical analysis of blood cells of *A. antiquata*.

Materials and methods

Study area

We collected live samples of *A. antiquata* at different intervals from the intertidal sandy-muddy flats at Sonmiani Bay from January 2015 to December 2015. The samples were acclimatized and reared in the laboratory for further experiments.

Blood sampling

The blood samples were taken from the posterior and anterior adductor muscle, using a sterile syringe with a 25-guage needle according to the procedure of Lowe and Pipe (1994) (Fig. 1).

Figure 1: Blood cell sampling from *Anadara antiquate.*

Light microscopy

Blood smears were placed on slides and air-dried at room temperature, fixed and washed carefully. The slides were stained with Giemsa, periodic acid schiff (PAS) and with Sudan Black B. Observations were made under a light microscope.

Fluorescence microscopy

Standard procedures were employed to stain the cells with DAPI stain (4, 6-diamidino-2phenylindole (Kapuscinski, 1995) to see the nucleus morphology using fluorescent microscopy.

Scan electron microscopy (SEM) observation

The cells were fixed in 2.5% glutaraldehyde rinsing two-times each for 13 minutes with phosphate buffer solution. Samples were dehydrated through an ethanol series, critical point drying was omitted and air drying procedure was followed and then the samples were coated with gold (Au), and examined using SEM.

Results and discussion

The results revealed three types of blood cells: red blood cell, white blood cells and platelets which are in accordance with early authors (Holden *et al.,* 1994; Kanchanapangka *et al.*, 2002; Mohite and Meshram, 2015). Red blood cells were oval, round, tear drop and elongated in shape (Fig. 2a). A more or less similar description was given by Kanchanapangka *et al.* (2002). The tear drop type of cells are considered the marginal band of microtubules, physically associated with a pair of centrioles where cells looks like a tear drop and are vacuolated (Figs. 2a,3c) as described by Nemhausern *et al. (*1983) and Holden *et al*. (1994). RBC cells were in abundance; as were described in *Scapharca inaequivalvis* (Holden *et al.*, 1994). The red blood cells of the arcid clam specialized for transportation of respiratory pigments have various cellular organelles and nuclei (Mangum and Mauro, 1985).

The white blood corpuscles which were lesser in count than red blood cells showed kidney bean-shaped nuclei (Fig. 2a), which is in agreement with results of *T. rhombea* (Mohite and Meshram, 2015). Gabriel *et al.* (2011) and Suganthi *et al.* (2009) mentioned two types of white blood cells; granulocyte and agranulocyte. While Cuenot, (1891) and Griesbach, (1891) stated that these cells have a role in phagocytosis, granulocytes are more active. We observed both types of white blood cells (Fig. 2a).The non-nucleated platelets were also viewed in *A. antiquata*, (Fig. 2a) which are tiny particles. These cells are the main source of haemostasis (Suganthi *et al.*, 2009).

The blood cells of *A. antiquata* are Sudan black B and PAS positive which means these cells have lipid and glycogen contents in their cytoplasm (Figs. 3, A and B). It is like non haemoglobin carrying invertebrate blood cells (Muhammad *et al.*, 2013).

The DAPI results suggested round nucleus in RBC and small, kidney bean and irregular shaped nucleus are considered white blood cells (Fig. 2b).These results are in agreement with Pengsakul *et al.* (2013). SEM results showed similar morphology of RBC to that of light microscopy.

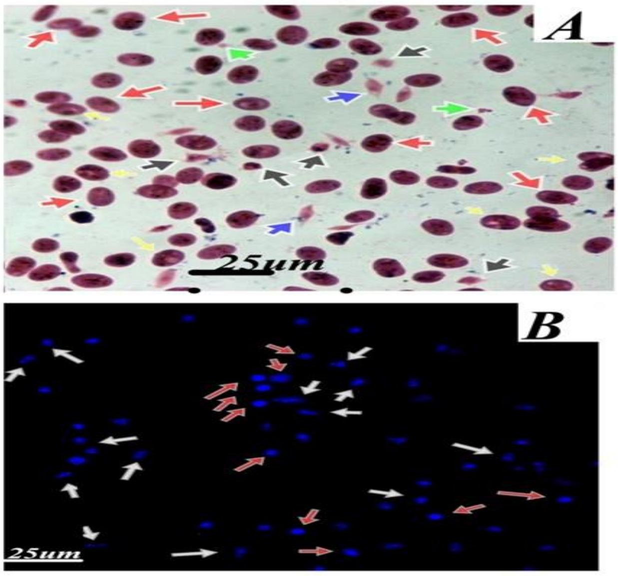

Figure 2: (A)Red arrows show the red blood cells, black arrows are the different types of white blood cells, yellow arrows are vacuolated cells, green are platelets and blue arrows indicate the euglanoied shape cells. (B) DAPI stained cells, the red arrows show the nucleus of red blood cells and white arrows are white blood cells.

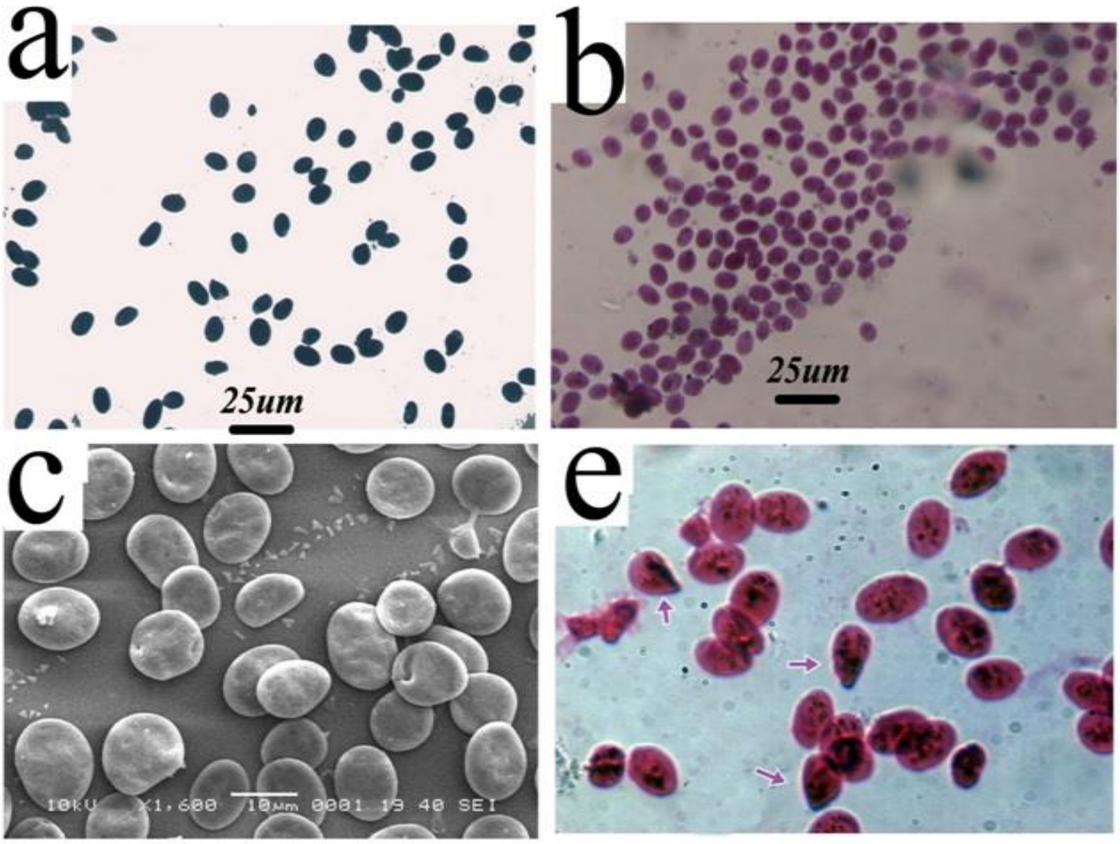

Figure 3: (a) All types of *Anadara antiquata* blood cells are Sudan Black B positive (b) PAS positive (c) Scan electron microscopic images showing the majority of erythrocytes (e) the marginal band of red blood cells.

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References

Akinrotimi, O.A., Gabriel, U.U., Anyanwu, P.E. and Anyanwu, A.O., 2007. Influence of sex, acclimation methods and period on haematology of *Sarotherodon melanotheron* (cichlidae), *Research Journal of Biological Sciences,* 2(**3**), 348-352.

Babatunde, G.M., Fajimi, A.O. and Oyejide, A.O., 1992. Rubber seed oil versus palm oil in broiler chicken diet. Effect on performance nutrient, digestibility, haematology and carcass characteristics. *Animal Feed Science Technology*, 35, 133-146.

Brooks, S.P.J., Zwaan, A., de Thillart, G., van den., Cattani, O., Cortesi, P. and Storey, K.B., 1991. Differential survival of *Venus gallina* and *Scapharca inaequivalvis* during anoxic stress: covalent modification of phosphofructokinase and glycogen phosphorylase during anoxia. *Journal of Comparative Physiology*, 161, 207-2

Cohen, W.D. and Nemhauser, I., 1980. Association of centrioles with the marginal band of a molluscan erythrocyte. *Journal of Cell Biology*, 86, 286-29.

Cuenot, L., 1891. Etudes sur le sang et les glandes lymphatiques dans la serie animale. *Archivesde Zoologie Experimentale et Generale,* 2, 9-19

Dawson, A.B., 1933. Supravital studies on the colored corpuscles of several marine invertebrates. *Biological Bulletin*, 64(**2**), 233-242.

Gabriel, U.U., Akinrotimi, O.A. and Orlu, E.E., 2011. Haematological characteristics of the bloody cockle *Anadara senilis* (L.) from Andoni Flats, Niger delta, Nigeria. *Science World Journal,* 6(**1**), 1-4.

Griesbach, H., 1891. Beitrage zur histologie des blutes. *Archiv fur Mikroskopische Anatomie und Entwicklungsmechanik*, 39, 22-29.

Holden, J.A., Pipe, R.K., Antonio, Q. and Graziella, C., 1994. Blood cells of the arcid clam, *Scapharca inaequivalvis*. *Journal of the Marine Biological Association of the United Kingdom*, 74, 287¬299

Jahangir, S., Siddiqui, G. and Ayub, Z., 2014. Temporal variation in the reproductive pattern of blood cockle *Anadara antiquata* from Pakistan (northern Arabian Sea). *Turkish Journal of Zoology*, 38, 263-272

Jones, S., 1970. The molluscan fishery resources of India. *Proc Symposium on Mollusca MBAI*, 12, 906-918.

Kanchanapangka, S., Sarikaputi, M., Rattanaphani, R. and Poonsuk, K., 2002. Cockle (*Anadara granosa*) red blood cell: structure, histochemical and physical properties. *Thailand Journal of Veterinary Practitioner*, 14(**3**), 2545-2546.

Kapuscinski, J., 1995. DAPI: a DNA-specific fluorescent probe. *Biotechtic & Histochemistry*, 70, 220–233.

Lowe, D.M. and Pipe, R.K., 1994. Contaminant induced lysosomal membrane damage in marine mussel digestive cell: an in vitro study. *Aquatic Toxicology*, 30, 357-365.

Lwama, G.K., Greer, G.L. and Larkin, D.A., 1976. Changes in some hematological characteristics of Coho salmon in response to acute exposure to dehydroabietics acid (DHAA) at different exercise levels. *Journal of Fisheries Research*, 33, 285-289.

Mangum, C.P. and Mauro, N.A., 1985. Metabolism of invertebrate red blood cells: a vacuum in our knowledge. In Circulation, respiration, and metabolism - current comparative approaches (ed. R. Gilles); pp. 280-289.

Muhammad, F., Zhang, F.Z., Shao, Y.M., Shi, L.X. and Shafi, M., 2013. Genesis of hematopoietic tissue and its relation with Haemocytes of *Litopenaeus vannamei* (Boon1931). (Crustacean; Decapod) *Pakistan Veterinary Journal*, 33(**1**), 91-95

Mohite, S. and Meshram, A.M., 2015. On haematological characteristics of blood clam *Tegillarca rhombea* (Born, 1978). *Journal of Aquaculture & Marine Biology*, 3(**2**), 00065.

Mzighani, S., 2005. Fecundity and population structure of cockle *Anadara antiquata* L.1758 (Bivalvia: Arcidae) from a sandy/ muddy beach near Dar es Salaam, Tanzania. *Western Indian Ocean Journal of Marine Science*, 4(**1**), 77–84.

Nemhausern, I., Joseph-Silverstein, J. and Cohen, W.D., 1983. Centrioles as microtubule-organizing bands of molluscan erythrocytes centres for marginal bands of molluscan erythrocytes. The *Journal of Cell Biology*, 96, 979-989

Ohuye, T., 1937. On the coelomic corpuscles in the body fluid of some invertebrates. VII. On the formed elements in the body fluid of some marine invertebrates which possess the red blood corpuscle. *Science Reports of the Tohoku University. Series 4. Biology*, 12, 203-239.

Pengsakul, T., Cheng, Z., Suleiman. A. Y., Tawatsin, A and Thavara, U. 2013. Morphological observations on haemo-lymphocytes in *Oncomelania hupensis* (Gastropoda: Pomatiopsidae). *Pakistan Journal of Zoology*. 45 (5) 1321-1327

Sato, T., 1931. Untersuchungen am blut der gemeinen japanischen archemuschel (Area inflate Rve.) *Zeitschriftfur Vergleichende Physiologie*, 14, 763-783

Silas, E.G., Alagarswami, K., Narasmham, K.A., Appukuttan, K.K. and Muthiah, P., 1982. Bivalve culture in Asia and the Pacific - India. Proceedings of a workshop held in Singapore, pp. 34-43.

Suganthi, K., Bragadeeswaran, S., Prabhu, K., Rani, S.S., Vijayalakshmi, S,T. and Balasubramanian, T. 2009. In vitro assessment of haemocyte and thrombocyte count from the blood clam of *Anadara inequivalvis. Middle-East Journal of Scientific Research,* 4(**3**), 163-167.

Thillart, G., van den, Lieshout, G., van, Storey, K., Cortesi, P. and Zwaan, A.de., 1992. Influence of long-term hypoxia on the energy metabolism of the haemoglobin-containing bivalve *Scapharca inaequivalvis*: critical O_2 levels for metabolic depression. *Journal of Comparative Physiology*, 162B, 297-304.

Toral-Barza, L. and Gomez, E.D., 1985. Reproductive cycle of the cockle *Anadara antiquata*. In Calatagan, Batangas, Philippines. *Journal of Costal Research*, 3, 241–245.

Vooys, C.G.N., de, Zwaan, A., de, Roos, Carpene, E. and Cattani, O., 1991. Anaerobic metabolism of erythrocytes of the arcid clam *Scapharca inaequivalvis* (Bruguiere): effects of cadmium. *Comparative Biochemistry and Physiology*, 98B, 169-175.

Zwaan, A. de., Cortesi, P., Thillart, G. van den, Roos, J. and Storey, K.B., 1991. Differential sensitivities to hypoxia by two anoxia-tolerant marine molluscs: a biochemical analysis. *Marine Biology*, 111, 343-35

Zwaan, A. de. and Cortesi, P., 1993. Energy metabolism of invertebrate erythrocytes: anaerobic metabolism of blood clams. In Surviving hypoxia: mechanisms of control and adaptation fed. P.W. Hochachka *et al*. pp. 41-52. London: CRC Press.