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

Hameed A.[1]; Muhammad F.[1*]; Muhammad A.A.[1, 2]; Shafi M.[3]; Sultana R.[4]

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1-Center of Excellence in Marine Biology, University of Karachi
2-Coastal Development and Fisheries Department, Government of Balouchistan
3-Lasbella University of Agriculture, Water and Marine Sciences
4-PCSIR, Laboratory Complex Karachi
*Corresponding author's Email: balouch_23@yahoo.com

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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

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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.

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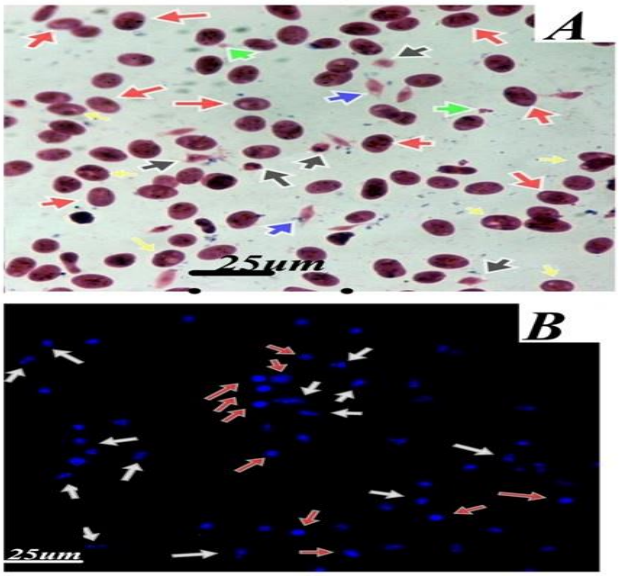

Figure 2: (A)Red arrows show the red blood cells, black arrows are the different types of white blood cells, yellow arrows are vacuolated cells, green are platelets and blue arrows indicate the euglanoied shape cells. (B) DAPI stained cells, the red arrows show the nucleus of red blood cells and white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

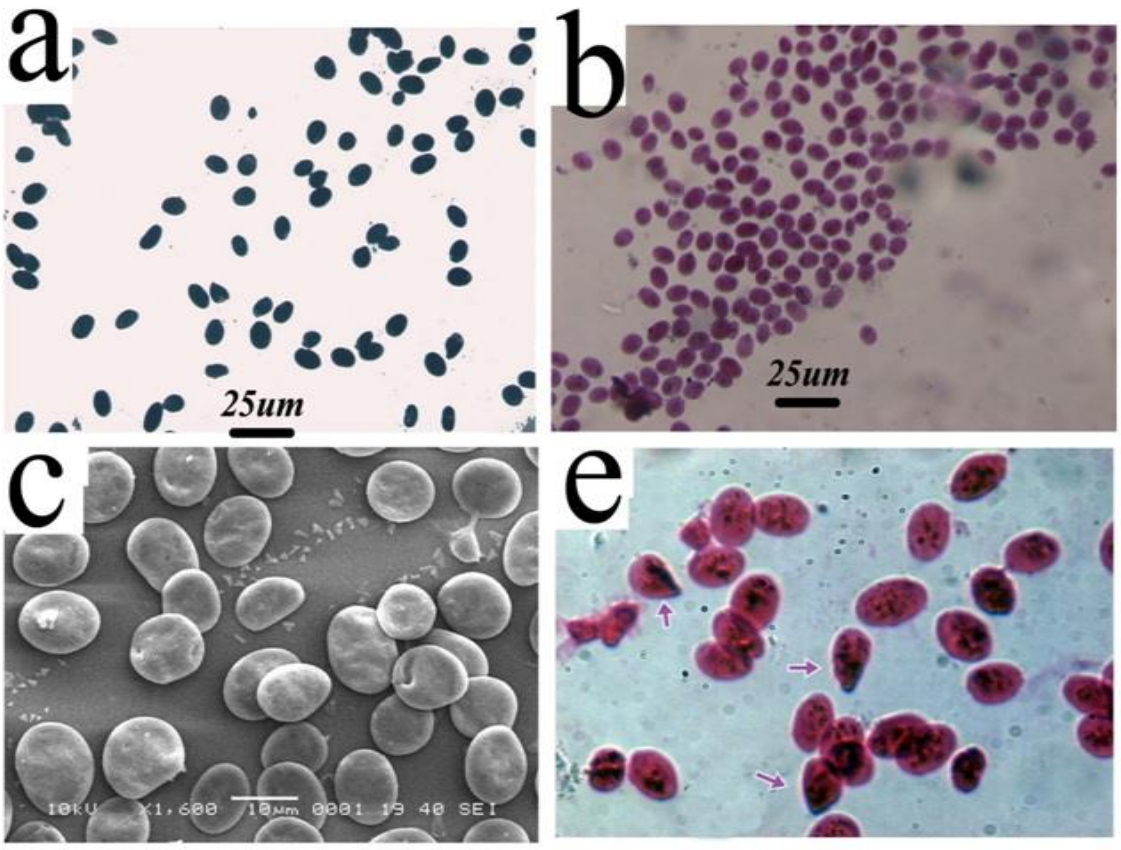

Figure 3: (a) All types of *Anadara antiquata* blood cells are Sudan Black B positive (b) PAS positive (c) Scan electron microscopic images showing the majority of erythrocytes (e) the marginal band of red blood cells.

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