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Special Issue

A potential Marine Species on Coast for Isolation of Platelets for Human - *Tegillarca rhombea*

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Abstract

Marine molluscs play an important role in the ecology of oceans and seas. They are less likely to survive in an acidic environment caused due to the changing climatic conditions in the sea. Climate change is expected to have profound effects on the coastal and oceanic habitats of these organisms. The blood cockle, *Tegillarca rhombea* is one of the important clams from the aquaculture point of view. This protein rich, low fat organism is also unique in its blood composition similar to humans. Present paper analyses the, haemocytes, thrombocyte counts, clotting time and other blood characteristics of the blood cockle. The blood of *T. rhombea* showed nucleated RBCs (diameter 22.19 µm) with a few dark granules dispersed in the cytoplasm. The average count of red blood cells was 0.06 X 10^{12} l^{-1}, white blood cell count 13.04 X 10^{12} l^{-1} and non-nucleated platelets count was 40.67 X 10^{9} l^{-1}. Average diameter of the white blood cell was recorded as 15.19 µm whereas, that of platelets was 2.59 µm. Percentages of lymphocytes, granulocytes and MID cells were 66.38, 18.86 and 14.74, respectively. The average value of hemoglobin was 2.52 g dl^{-1}. Average Hematocrit (HCT) was 30%, MCV 52.5 fl, MCH value 4.2 pg, average MCHC 8.4 g dl^{-1}, PCV 6.48%, PCT or thrombocrit 0.04%, mean platelet volume MPV 9.8 fl and average PDWc was recorded as 32.82% during the study period. Average clotting time was noted as 25-30 min. Current findings, especially about the platelets or thrombocytes are promising in terms of the possibilities of their use in treatment of patients on antiplatelet agents under certain conditions. *T. rhombea* possesses potential as a candidate species for harvesting or isolating the platelets as well as providing hemoglobin to patients suffering from anemia.

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Introduction

Many species of clams occur abundantly along Indian coast, particularly in the estuaries and backwaters, forming sustenance fisheries. The clams are rich in protein, glycogen and minerals which are easily digestible. During the current scenario of changing climatic conditions, the coastal as well as the oceanic habitats of these invertebrates are expected to get affected leading to decline in their stocks. These clams are good candidate species for coastal aquaculture as they fulfill important ecological role and provide a significant source of livelihood to the local artisanal communities.

Tegillarca rhombea belongs to the family Arcidae and it is popularly known as blood clam owing to the red colour of its flesh which is due to the presence of hemoglobin in the blood (Kanchanapangka *et al.* 2002, Gabriel *et al.* 2011). The presence of platelets in the blood of *T. rhombea* is much more interesting in the studies pertaining to the hemostasis or blood clot formation and complete interactions of injured vessels, platelets coagulation factors and fibrinolysis platelets that are involved in blood clotting (Richard *et al.* 2006). Platelets aggregate as haemostatic plug at the site of vascular injury, whereby bleeding is limited or arrested in advance of plasma coagulation. This function of platelet aggregation is literally 'vital'. Platelets aggregate intravascularly as arterial thrombi in response to haemorrhage into fissures or ruptures in atherosclerotic plaques. Pathologically, therefore, platelet aggregation is potentially lethal (Born and Patrono 2006).

During recent years, new antithrombiotic or anti platelet agents have been developed and introduced in clinical medicine to overcome the limitations in existing drugs. These are used during the treatment of blood clot formation in the blood vessels and their possible effects on various organs such as heart and brain resulting in organ failure. The use of anticoagulant and anti platelet agents and intravenous unfractionated heparin sulfate, is now common treatment of several diseases (Populard *et al.* 2006). The preoperative risk of bleeding with anti platelet acute hemorrhage agents depends upon the surgical procedure. Since these anti clot agents do not have a specific antidote, their effect can be reversed only by the transfusion of platelets and other blood products. Research on finding alternative sources of platelets has gained momentum in recent years. At present, platelets are harvested from the donated blood through fractionalization of blood and are stored for further use. The presence of hemoglobin and other related blood products in the blood clams belonging to family Arcidae, hence present a possible candidate species for harvesting the platelets needed for treatment of patients on antiplatelet agents under certain conditions. With this background, the present investigation was carried out to study the blood chemistry and the whole blood elements of the blood clam, *T. rhombea* found commonly along the south west coast of Maharashtra, India.

Materials and methods

Blood clams were collected from beds of Kajali and Kalbadevi estuaries of Ratnagiri (Lat. 16° 59' 10" N and Long. 73° 18' 25" E) along the Southwest Coast of Maharashtra, India. The study was conducted from February 2015 to March 2016 except June, July due to heavy rains and non availability of the clams. Weekly random samples ranging between 55 to 68 mm were collected by hand dredge net as well as by hand picking. The shells were opened and the blood was collected with syringe from 10 clams per month. Standard haematological procedures (Brown 1980) and Clotting assay (Suganthi *et al.* 2009) were employed in the assessment of the various blood parameters.

Results

Only a few species of bivalve molluscs are reported to contain hemoglobin in their blood cells. *T. rhombea* is one such clam which shows the presence of hemoglobin. The averages of the blood counts are presented in Table 1. The blood cell counts included red blood cells (RBC), white blood cells (WBC) such as lymphocytes, granulocytes and MID cells which included less frequently occurring and rare cells correlating to monocytes, eosinophils, basophils, blasts and other precursor white cells that fall in a particular size range and platelets (Thrombocytes). The blood parameters also included hemoglobin (g dl^{-1}), Hematocrit (HCT) that measured the percentage of blood that consists of red blood cells, MCV that measured the volume of erythrocytes in cubic micrometers (microns) or femtoliter (fl), MCH measuring the content of hemoglobin in single erythrocytes in pictogram (pg), MCHC that measured the concentration of hemoglobin in erythrocytes, reflecting the degree of saturation of the red blood cell hemoglobin (g dl^{-1}), PCV measuring the packed cell volume (%), PCT measurement of platelet crit or trombocrit, MPV i.e. mean platelet volume (fl) and PDWc i.e. the relative width of the distribution of platelets in volume index of the heterogeneity of platelets. The monthly average values of the diameters of the blood cells are given in Table 2.

Fresh blood smears of this clam were studied under the light microscope (Plate 1). Red blood cells of the *T. rhombea* were found to be nucleated and ovoid to round with average diameter of 22.19 µm. The nucleus was round with a few dark granules dispersed in the cytoplasm. The average count of red blood cells was $0.06x10^{12}$ l^{-1}.

In White blood cells, granulocytes and agranulocytes such as Lymphocytes were observed. Monocytes, eosinophils, basophils, blasts and other precursor white cells were counted as MID cells. Average diameter of the white blood cell was recorded as 15.19 µm. Percentages

Table 1. Average values of the blood counts from the blood clam *T. rhombea.*

Blood components	Average
WBC (x 10^9 l^{-1})	13.04
Lymphocytes	8.49
MID	2.64
Granulocytes	1.90
Lymphocytes %	66.38
MID%	18.86
Granulocytes %	14.74
RBC (x 10^{12}.l^{-1})	0.06
PLT (x 10^9.l^{-1})	40.67
Hb (g dl^{-1})	2.52
HCT %	30
MCV (fl)	52.5
MCH (pg)	4.2
MCHC (g dl^{-1})	8.4
PCV %	6.48
PCT %	0.04
MPV (fl)	9.8
PDWc %	32.82

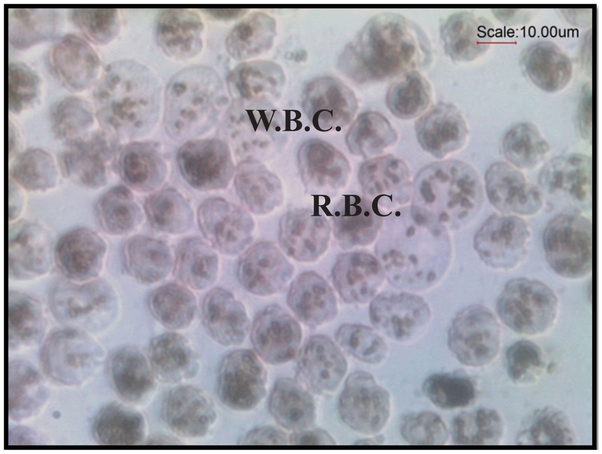

Plate 1. The blood cells in blood clam *T. rhombea*
R.B.C. - Red blood cell; W.B.C. - White blood cells

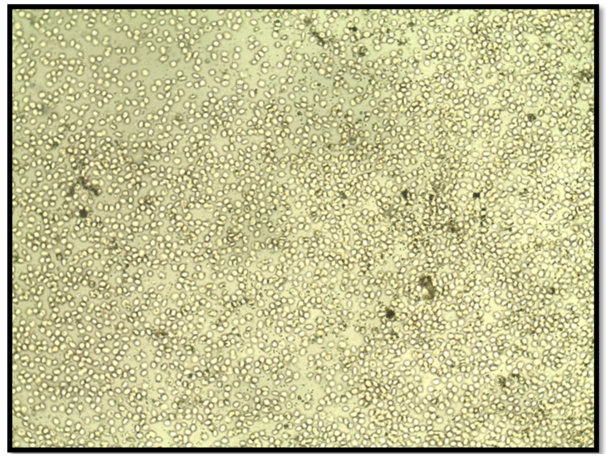

Plate 2. Platelets in blood clam *T. rhombea*

Table 2. Monthly average diameters (in µm) of the blood cells of *T.rhombea*

Months / Blood Cells	Feb. 2015	March 2015	April 2015	May 2015	Sept 2015	August 2015	Dec 2015	Jan. 2016	Feb. 2016	March 2016	Avg.
R.B.C.	22.77	20.29	23.71	21.76	22.92	21.88	22.13	19.80	23.15	23.46	22.19
W.B.C.	16.41	14.77	16.59	16.81	15.34	14.21	15.27	15.76	14.71	12.02	15.19
Platelets	2.38	2.51	1.88	2.21	3.07	2.66	2.71	2.86	2.68	2.86	2.59

of lymphocytes, granulocytes and MID cells were 66.38, 18.86 and 14.74, respectively. The average white blood cell count was 13.04×10^{12} l^{-1}. The average count of non-nucleated platelets was $40.67 \times 10^{9} l^{-1}$ and the average diameter was 13.78 µm. Platelets or thrombocytes were mostly found scattered individually but sometimes adhered to each other forming clusters (Plate 2).

The average value of hemoglobin was 2.52 g dl^{-1}. Average Hematocrit (HCT) was 30 %. MCV indicating the volume of erythrocytes was 52.5 fl. The average MCH value was 4.2 pg, average MCHC showing the concentration of hemoglobin in erythrocytes was 8.4 g dl^{-1}, PCV value was 6.48 %, PCT indicating trombokrit was 0.04 %, mean platelet volume MPV was 9.8 fl and average PDWc was recorded as 32.82 % during the study period. Average clotting time was noted as 25-30 min.

Discussion

Generally bivalves absorb oxygen from water directly into the tissues, without the help of oxygen transport pigments like hemoglobin or hemocyanin. These pigments facilitate oxygen transport into the tissue where the water is very muddy and oxygen may be deficient. Hence bivalves are associated with clean water; rather they are used as the indicator species of clean and unpolluted aquatic habitats. Members of Family Arcidae, commonly known as blood cockles show the presence of the hemoglobin pigment which is otherwise a common characteristic of vertebrates. It is simply dissolved (not in corpuscles) in both the blood and tissues and makes the muscle red (Morton 1960).

The present work not only indicated the presence of hemoglobin but also the blood cells such as R.B.C., W.B.C. and platelets or thrombocytes. Red blood cells contain hemoglobin that is associated with the transport of oxygen and carbon dioxide. White blood cells are responsible for recognition and neutralization of alien components of the immune defense against viruses and bacteria, the removal of dead cells from the body. The platelets of this species are of much importance as they are involved in hemostasis. Platelets were irregular to round in shape and showed the presence of dense granules and dense bodies.

To analyse the overall composition of the blood of *T. rhombea*, the blood parameters were also studied during the present work. Average value of the hematocrit showing the ratio of corpuscles to plasma was 30%, whereas the Trombocrit that represented the total platelet mass was 0.04%. It is believed that the hematocrit reflected the ratio of red blood cells to the volume of blood plasma as well as mostly red blood cells make up the volume of blood cells. In similar work, blood components and clotting time of *Anadara inequivalvis* were studied (Suganthi *et al.* 2009). Microscopic analysis showed Platelet cells with 1.77X10^3 mm^3 of blood. The whole blood component possesses rich chemical elements (Ca, Zn, K, Cl, Cu, P, S and Mg). The platelets are involved in the coagulation cascade. Blood components play important role in metabolism, act as biocatalysts for enzymes, hormones, proteins, bone and blood formation.

Platelets are actually not true cells but merely circulating cytoplasmic fragments without nucleus (Ross and Reith 1990). But they contain many structures that are critical to stop bleeding. Once a platelet is activated it attracts other nearby platelets, which in turn become activated. Platelet adherence and aggregation are driven by the prostaglandin thromboxane A2, which is synthesised within the platelet from arachidonic acid. It is this synthetic pathway that is blocked by aspirin (Schror 1997). ADP plays a key role in haemostasis and thrombosis.

Coagulation potentially results in hemostasis, the cessation of blood loss from a damaged vessel, followed by repair. The mechanism of coagulation involves activation, adhesion, and aggregation of platelets along with deposition and maturation of fibrin. Disorders of coagulation are disease states which can result in bleeding or obstructive clotting (Lillicarp *et al.* 2009). Platelets immediately form a plug at the site of injury; this is called primary hemostasis. Secondary hemostasis occurs simultaneously. Additional coagulation factors or clotting factors beyond Factor VII respond in a complex cascade to form fibrin strands, which strengthen the platelet plug (Furie 2005). While bleeding through a cut or injured blood vessel might be caused by accidents, thrombosis is caused due to unnatural formation of a blood clot in a blood vessel. The blood vessel can be a vein or an artery. The symptoms that occur with a clot depend on where the clot occurs, the size of the clot, and whether the clot breaks off and travels to another part of the body (a process called embolization).

Anticoagulants and antiplatelet drugs eliminate the risk of blood clots. They help prevent or break up dangerous blood clots that form in the blood vessels or heart. Antiplatelets interfere with the binding of platelets, or the process that actually starts the formation of blood clots. Anticoagulants interfere with the proteins in the blood that are involved with the coagulation process. Antiplatelet drugs are intended to prevent and/or reverse platelet aggregation in arterial thrombosis, most prominently in myocardial infarction and ischaemic stroke. In practice, a favourable balance between the beneficial and harmful effects of antiplatelet therapy is achieved by treating patients whose thrombotic risk clearly outweighs their risk of bleeding complications (Patrono *et al.* 2004).

Antiplatelet drugs are most effective for arterial clots that are composed largely of platelets. All antiplatelet drugs can cause bleeding, hypersensitivity and allergy. They may also cause bronchospasm and worsen pre-existing asthma, gastrointestinal (GI) disturbance and bleeding. Antidotes for antiplatelet drugs are needed if severe bleeding occurs in a patient on antiplatelet treatment and transfusion of platelets is often used in an attempt to restore platelet function and haemostasis.

Dual antiplatelet therapy, consisting of aspirin and a P2Y12 antagonist, is critical for the treatment of ACS, the most common cause of cardiovascular mortality and morbidity worldwide (Turpee 2006). However, like anticoagulants, antiplatelet therapies are known to increase the risk of bleeding complications (Wallentin *et al.* 2009), which is why specific antidotes are desired also for antiplatelet agents. Platelet transfusion was found to restore platelet function in patients under aspirin (Wallentin *et al.* 2009). Platelet transfusions are used to stop bleeding in people with low platelet counts or prevent bleeding when they are about to have an operation or a procedure. They are also used to prevent spontaneous bleeding in those with extremely low platelet counts.

In this context the present study sheds new light on the possibilities of harvesting platelets or thrombocytes from the blood clam, which have a diameter similar to human platelets. In similar studies, a general trend in the relationship between blood parameters and body size of the blood clam, *T. rhombea* showed that the values of its haematological parameters increased with increase in the size of the clam (Mohite and Meshram 2015). The haemoglobin count ranged between 2.03 - 2.10 g dl^{-1} to 6.65 - 8.87 g dl^{-1} in the size groups ranging from 20 to 61 mm and above. Similarly the platelet count also ranged from 27 X 10^8 to 60 X 10^8 μl^{-1} in the same size groups. The current study has confirmed the presence of blood platelet necessary for the action of coagulation and homeostasis.

Conclusions

Changing climatic conditions would be affecting the marine invertebrates due to the changes in their habitats leading to physiological changes. The declining stocks of the species like *T. rhombea* could be revived through aquaculture practices along the coastal areas of Maharashtra. This clam being unique in its blood composition similar to humans, offers not only nutritive value but also has medicinal importance. Present study showed that *T. rhombea* can be a potential bivalve species from which platelets similar to that of human blood could be harvested. These could become a part of the antidote therapy desired for antiplatelet agents. The culture of these bivalves in the estuarine areas needs to be studied further in order to enhance their production and use in the pharmaceutical industry.

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