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MORPHOLOGICAL AND HISTOCHEMICAL CHARACTERIZATION OF HAEMOCYTES OF FRESH WATER PRAWN, *Macrobrachium dayanum* (CRUSTACEA-DECAPODA)

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Author HSL designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author SS managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Haematological profile of the *M. dayanum* will be helpful in monitoring the health status of this prawn species which is a potential animal for fresh water aquaculture as well as a good model for monitoring the pollution level of the fresh water ecosystems. The haemocytes of the freshwater prawn, *Macrobrachium dayanum* were classified into seven types based on morphology and behaviour like formation of pseudopodia, encapsulation of foreign particles clumping etc., under phase contrast microscope and their reactions with different stains. The seven types of haemocytes included prohaemocytes, plasmatocytes, spindle cells, granular haemocytes, spherule cells, adipohaemocytes and the cystocytes. The structural details and their significance in haemocyte classification along with probable functions like transport of food, phagocytosis and encapsulation of foreign particles, defense against various infections, hardening of exoskeleton, prevention of blood loss by promoting immediate clotting, confinement of invasive organism and detoxification**,** of different haemocyte types have been discussed.

Keywords: *Macrobrachium dayanum*; Crustacea; haemocytes; classification; functions.

1. INTRODUCTION

Haemocytes are the major component of the circulation of Arthropods, which perform various vital functions like transport of food, phagocytosis and encapsulation of foreign particles, defense against various infections, hardening of exoskeleton, prevention of blood loss by promoting immediate

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clotting, confinement of invasive organism and detoxification [1,2,3,4,5,6]. Numerous light and electron microscopic studies have been reported concerning the classification of arthropod haemocytes, especially that of insects [7,8,9,10,3,11, 12,13,14,15,16,17,18] but the classification schemes for crustaceans are not uniform [19].

Classification of haemocytes in Decapod Crustaceans is mainly based on the presence of cytoplasmic granules, on the basis of which they have been classified as Hyaline cells, semi granular cells and granular cells [19,20,21]. In *M. rosenbergii* three types of haemocytes were defined by [17] *viz.* hyaline haemocyte, granular haemocytes and undifferentiated haemocytes whereas hyaline haemocyte, small granule haemocytes and large granule haemocytes were described by [22] in *M. rosenberghi* and *M. acanthurus*.

Because of inconsistency in haemocyte classification of crustaceans [19,23], we have adopted the classification proposed by [24], for insect haemocytes which appears more suitable for other groups of arthropods [25, 26, 14, 15,27,28]. Classification scheme for arthropods and suggestions of [3,29,30,31,32,20,4] were also taken into considerations as described in the observation of haemocytes of *M. lamarrei* [23]. Fresh water prawn, *Macrobrachium dayanum* (Henderson) is a small Palaemonid prawn, early available throughout year, a good laboratory model for environmental monitoring and potential animal for fresh water aquaculture [33,34,35], specially to non coastal areas [36,37]. For successful culture and the knowledge of immune system and health parameter is a primary requisite. Present work has been taken to study the Haemocyte of fresh water prawn, *Macrobrachium dayanum* (Henderson) (Crustacea-Decapoda) [38] which will be helpful in monitoring the health status of prawns without sacrificing them.

2. MATERIALS AND METHODS

The freshwater prawns, *M. dayanum* were collected from river Gomti at Lucknow (U.P.) INDIA, with the help of local fisherman and were brought to laboratory (N-26°5'59" E-80°56'17") in large plastic containers. Studies were performed on both freshly collected as well as laboratory maintained [39] animals of inter moult stage of moulting cycle.

Haemolymph samples were obtained from normal prawns, preheated (48 to 50°C) and chilled (4°C) prawns. Haemolymph was obtained from the dorsal carapace just above the pericardial sinus or from the inter segmental arthrodial membrane between the first and second abdominal segments at dorsal side by puncturing with sterilized needle (Direct method) as well as by inserting a fine capillary or parafinised syringe in between thorax and abdomen from the side holding horizontally (indirect method) [23].

Various preparation like hanging drop [24], thick and thin film [40], moist chamber preparations [41], Vaseline sealed thin and thick preparation [42] and fresh undiluted or diluted preparation [43] were made to observe morphology and behaviour under phase contrast microscope (Olympus). Observations were made on fresh and fixed as well as stained preparations. Haematoxylene and Eosin, Wright's Eosin Methylene blue, Giemsa's and Malory's triple staining techniques [44] and [23,45,46] were used for light microscopic studies.

Histochemical tests were carried out on air dried and wet films with or without prior fixation. Histochemical test for identification of lipids, carbohydrates, proteins and enzymes like Acid and Alkaline phosphatase were routinely performed as described in [44,47] and [45,46]. Cells were observed and photographed on Olympus Trinocular Microscope (Olympus) and the line diagrams were made with the help of Camera Lucida (ELEITZ WETZLAR). Measurements of the size of cells and nucleus were made with the help of ocular and stage micrometer. At least 30 cells of each cell type were measured that all data was analyzed using MINITAB software on a PC.

3. RESULTS

The haemocytes of the freshwater prawn *Macrobrachium dayanum* (Henderson) (Fig. 1) when observed under the light microscope were found mainly of two types, agranulocytes and the granular haemocytes; however using various light microscopic, histological and histochemical preparations examined with light microscope and fresh haemolymph examined under phase contrast microscope the haemocytes of freshwater prawn *M. dayanum*, appeared to be further distinguishable into different types. The classification of haemocytes and the terminology adopted according to [24] and [3,29] described by [23] for the classification of haemocytes of *M. lamarrei*.

After studying the haemocyte morphology and behaviour under Phase contrast microscope, their staining reactions with different stains, utilizing the relative size calculated using the *Camera lucida* and micrometer under light microscope along with histochemical observations (Tables 1&2, Plate 2) following 7 types of cells were distinguished in the haemolymph of *Macrobrachium dayanum*.

Fig. 1. Photograph of the freshwater prawn, *Macrobrachium dayanum* (average size 5.64±0.42 cm)

3.1 Prohaemocytes (PH) (Plate 1, Fig. 1)

Prohaemocytes of *M. dayanum* were regular in shape and smooth in appearance. The cell size was 14.55±0.82µmX 11.89±0.48µ having a nucleus of the size 11.43±0.64 µ X 9.96±0.55. These haemocytes bear discoid round or oval nucleus and with small nucleo-cytoplasmic ratio. No granules or any other types of cytoplasmic inclusions were found to be present in the smaller Prohaemocytes but a few vacuoles and one or two granules appeared in the larger Prohaemocytes (Plate 2, Figs. 1, 2, 3). Presence of this type of inclusion probably indicated development of these cells and differentiation into the other cell types. In wet film preparation the Prohaemocytes appeared spherical or oval structure floating around freely. Prohaemocytes can be regarded therefore as the stem cells. Another interesting feature of these cells is that unlike other cells described below, these cells did not quickly disintegrate in wet haemolymph preparations and remain entangled between the clotted structures for longer periods.

3.2 Plasmatocytes (PL) (Plate 1, Fig. 2)

Plasmatocytes were one of the two more common type of haemocytes observed in the blood of the freshwater prawn and the size of Plasmatocytes was 29.82±1.57µ into 14.69±0.55µ with a nucleus of the size 17.10±0.93µX 14.64±0.47µ. These were pleomorphic in nature with around to elongated nucleus and where able to change their shape *in vitro*, such as round, pear shape, fusiform and podocyte type (Plate 2, Figs. 4-9). The nucleocytoplasmic ratio was quite high. The cytoplasm was either homogenous or with fever very fine granules which were dispersed around the discoid nucleus or present scattered in the cell. In round cells these fine granules were concentrated around the nucleus but in elongated cells concentration of these granules was towards one pole i.e. with the elongation (Plate 2, Fig. 9). Small or large vacuoles were also seen especially at the elongated end. After few minutes of haemolymph withdrawal these cells send out one or more cytoplasmic extensions. In wet preparations most of the Plasmatocytes seemed like podocyte, within 2 to 5 minutes of haemolymph withdrawal.

3.3 Spindle Cells (SC) (Plate-1, Fig. 3)

These were the haemocytes rarely observed in the circulating haemolymph during normal condition but their findings increased rapidly during stress conditions especially after injury or Monday these cells were characterized with their specialist print and save to body having such a good place to Nucleus and pointed ends the size of the cells was 41.68±1.47µX 10.99±0.41µ with the nucleus of the size 20±0.98µ X 9.48±0.36µ but sometimes the length was found up to 58.9µ (Plate 2, Figs. 19, 20). These haemocytes when allowed to settle on the slide, expanded into flattened angular forms with blonde cytoplasmic extension that taper into fine points at peripheral angles. These haemocytes have been clustered in large numbers around some cellular debris with the longitudinal axis of each cell pointing to the centre of the cell aggregation.

3.4 Granular Haemocytes (GH) (Plate -1, Figs. 4,5)

Majority of the haemocytes were the granular haemocytes containing varying numbers of phase-light and phase-dark granules. These cells were also pleomorphic mostly round or oval, spherical, discoid, crescentric or even irregular in outline. The granulocytes may also be further classified as small granule haemocytes (SGH) containing fine but large number of granules and large granule haemocytes (LGH) containing fewer but large sized granules. The size of small granule haemocytes was 26. 0.5±1.59µ X 21.37±1.31 µ with nucleus of the size 20.79±1.24µX 16.80±0.88µ whereas that of large

Table 1. Summary of staining reactions in haemocytesof freshwater prawn, *macrobrachium dayanum*

S. No.	Stains	Haemocyte type																				
		Pro		Plasmato				Granular				Spherule cell			Adipohaemocytes			Cystocytes			Spindle cell		
		Nu.	Cyt.	Nu.	Cyt.	Gra.	Va.	Nu.	Cyt.	Gra.	Nu.	Cyt.	Sph.	Nu.	Cyt.	Glo.	Nu.	Cyt.	Va.	Nu.	Cyt.	Va.
1	**Leishman's**	Blue	Deep Blue	Light Violet	Deep sky Blue	Fine dark Red	-	Magenta	Light Blue	Orange refractile	Magenta	Light Violet or Pink	Deep Orange with Red periphery	Deep Violet	Orange to Red	Bright refractile Orange	Light Blue & Nucleolus Deep Blue with Dark Blue Chromatin	-	Light Bluish Pink	Light Blue with scattered chromatin	Faint Blue	-
2	**Giemsa's**	Deep Blue	Reddish Blue	Blue to Violet with Deep Blue chromatin patches	Sky Blue with light Blue patches	Few small dots	-	Sky Blue	Pink	Deep Violet or Orange refractile	Sky Blue	Pink	Bright Orange Red	Blue	Light Pink	Bright Orange to Magenta	±	Pink	With Blue periphery	Blue or Violet	Pinkish Blue	-
3	**Wright's Eosine-Methylene Blue**	Violet	Pinkish Blue or light Violet	Deep Violet	Light Blue	-	-	Violet	Blue	Orange	Light Violet	Faint Pink	Reddish orange	Deep Violet	Deep Violet	With Orange red periphery	±	±	±	-	-	-
4	**Haematoxylene & Eosine**	Deep Blue	Deep Reddish blue	Blue	Pink with Bluish patches	-	-	Light Blue	Bluish Pink	Reddish Blue	Light Blue	Dark Red	Orange	Blue	Light Sky Blue	Muddy Black or Orange	Light Blue with dark Blue Chromatin Patches	Light Sky Blue	-	Deep Blue	Pink	-

Nu.=Nucleus; Cyt.=Cytoplasm; Gra.=Granules
Va.=Vacuole; Sph.=Spherule ; Glo.=Globule

Table 2. Summary of staining reactions in haemocytesof freshwater prawn, *macrobrachium dayanum*

S. No.	Histochemical tests	Haemocyte type																					
		Pro		Plasmato				Granular				Spherule cell			Adipohaemocytes			Cystocytes			Spindle cell		
		Nu.	Cyt.	Nu.	Cyt.	Gra.	Va.	Nu.	Cyt.	Gra.	Va.	Nu.	Cyt.	Sph.	Nu.	Cyt.	Glo.	Nu.	Cyt.	Vac.	Nu.	Cyt.	Gra.	Va.
1	**Protein**																						
	Ninhydrin Schiff's	++	++	++	+	-	++	-	-	+++	-	+	-	++	-	-	+	+	-	-	++	…	…	…
	Aquous Bromophenol Blue	+++	+++	++	+	-	-	++	-	++	-	+	-	++	+	-	+	++	-	-	++	++	+	…
	Hg-Bromophenol Blue	+++	+++	++	++	-	++	++	+	+++	-	+	-	++	-	+	+	++	-	-	+	++	+	-
2	**Carbohydrates**																						
	PAS	+	+	+	-	++	-	-	-	+++	-	-	-	+	…	…	…	+	-	-	-	-	++	-
	Best's Carmine	+	+	+	+	+	-	+	++	+++	-	-	+	++	…	…	…	-	+	-	-	±	±	±
3	**Lipoides**																						
	Sudan Black-B (in 70%)	-	-	-	-	-	-	+	+	-	-	-	-	++	-	+	+++	-	-	-	-	…	…	…
	Acetone Sudan Black-B	-	-	-	-	-	-	-	+	-	-	-	-	++	-	+	+++	-	-	-	+	…	…	…
	Sudan-IV	-	+	-	+	-	+	-	++	+	-	-	+	++	-	+	+++	-	-	-	-	…	…	…
4	**Enzymes**																						
	Acid Phosphatase	-	-	-	++	-	-	++	-	+++	++	-	+	++	-	-	-	-	+	-	++	…	…	…
	Alkaline Phosphatase	-	-	-	-	-	-	±	±	±	±	-	-	-	-	-	-	±	±	±	±	…	…	…

Nu.=Nucleus; Cyt.=Cytoplasm; Gra.=Granules
Va.=Vacuole; Sph.=Spherule ; Glo.=Globule

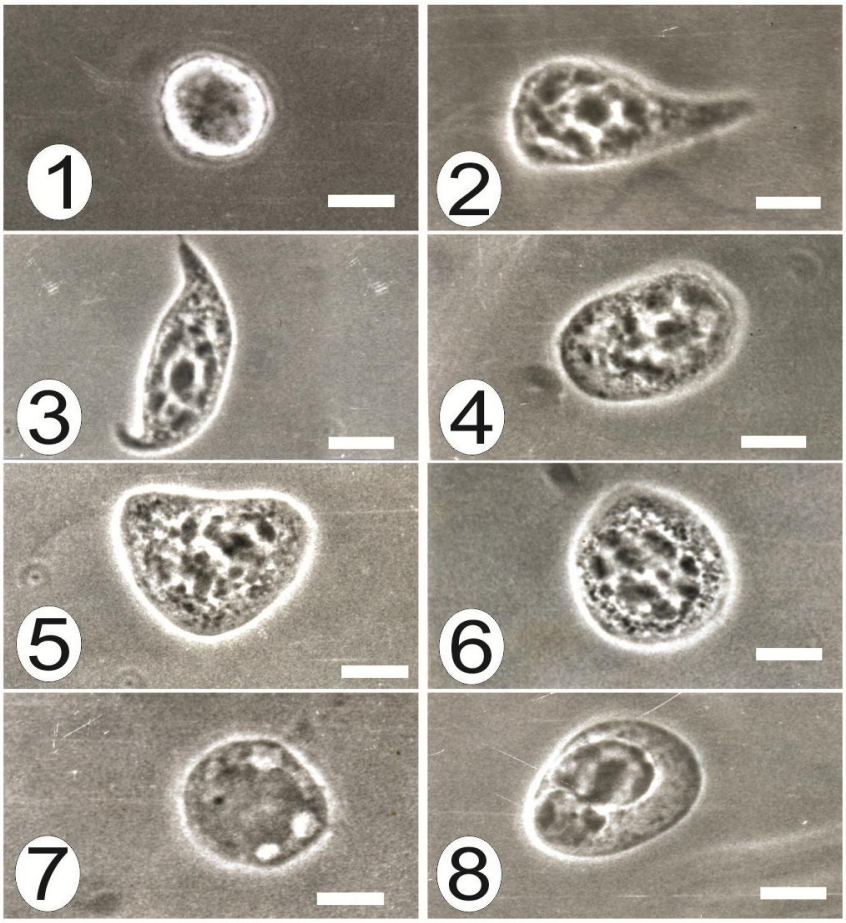

Plate 1. Explanation of figures: Photomicrographs of the haemocytes of *M. dayanum* (Scale bar 0.01mm) Fig. 1. Prohaemocyte, Fig. 2. Plasmatocyte, Fig. 3. Spindle cell, Figs. 4 and 5. Granular haemocytes, Fig. 6. Spherule cell, Fig. 7. Adipohaemocyte and Fig. 8. Cystocyte

granule haemocytes was 23.91±0.889 µX 19.54±0.94 µ with a nucleus of the size 12.59±0.89µ X 8.59±0.43µ (Plate 2, Figs. 10, 11, 12).

In fresh haemolymph preparations the granular haemocytes appeared as pale yellow shining bodies. These were always packed with coarse granules almost invariably obscuring the nucleus. In between these granules a few smaller vacuoles were also present. In wet preparations, granular haemocytes form fine filamentous cytoplasmic extensions and not broad cytoplasmic extension after some times of haemolymph withdrawal, as do the Plasmatocytes. The blabbing of these haemocytes just after few seconds of withdrawal was also observed, followed by the movement of the granules towards the cell periphery. After this these cells, expanded granules darkened up gradually and the nucleus became prominent. The adherence of these cells in wet moist preparations was also apparent.

3.5 Spherule cells (SPH) (Plate 1, Fig. 6)

These were round or sometimes oval cells slightly larger than granulocytes with a size 31.52±0.23µX 23.49±0.55µ with the nucleus sizing 19.56±0.21 µX 17.22±0.31µ. Spherule cells can be easily identified in wet preparation under phase microscope on account of the presence of round prominent signing spherules in them, much larger than the granules of the granular haemocytes and usually spherical in shape (Plate 2, Figs. 13, 14). The spherules were invariably packed in the haemocytes and thereby completely obscuring the nucleus. In wet preparations after some times of withdrawal these cells spread out on the glass surface, their nucleus become more distinct and spherules arranged themselves around the nucleus. The nucleus usually centrally placed. Occasionally spherules were seen liberated from the spherule cells due to their rupturing.

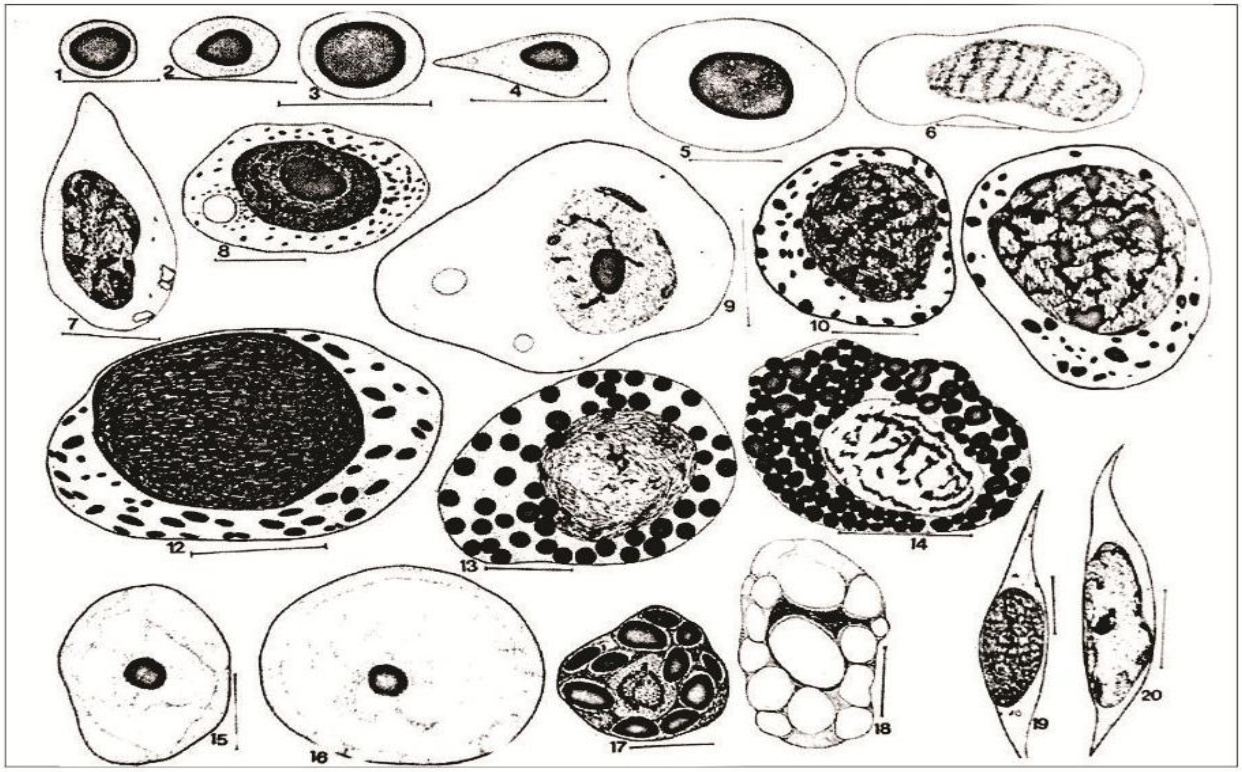

Plate 2. Explanation of figures: Diagramatic representation of the haemocytes of *M. dayanum* (Scale bar 0.01mm) Figs. 1, 2 and 3. Prohaemocytes, Figs. 4 to 9. Plasmatocytes, Figs. 10 to 12 - Granular haemocytes, Figs. 13 to 14. Spherule cells, Fig. 15 to 16- cystocytes, Figs. 17 to 18. Adipohaemocytes, Figs. 19 to 20. Spindle cells

3.6 Adipohaemocytes (ADH) (Plate -1, Fig. 7)

Adipohaemocytes are the rarest haemocyte types found not to be very common in the circulating haemolymph. Adipohaemocytes were characterized by the presence of large round or irregular shaped fat globules in them. The size of these cells was 29.66±1.47µX 20.79±1.24µ with nucleus of the size 12.39±1.29µX 12.23±1.18µ(Plate 2, Figs. 17,18). Nucleus of these cells found usually centrally placed with irregular periphery due to the presence of fat globules in it. These were the cells with least changes in wet preparations and disappear only after the complete coagulation of haemolymph.

3.7 Cystocytes (CYS) (Plate -1, Fig. 8)

Cystocytes were the least common type of haemocytes rarely present in the undiluted wet haemolymph preparations. The size of the cells was 24.39±1.26µX 21.33±0.97µ with a nucleus of the size 16.17±0.76µX 12.37±0.46µ (Plate 2, Figs. 15, 16). Cystocytes were characterized by their cartwheel shaped nucleus. This cartwheel shaped structure was due to the specific arrangement of the chromatin material and the nucleolus. The nucleolus usually centrally placed and a large amount of chromatin was on the nuclear membrane attached with the nucleolus by chromatin filaments, which were broadly separated with each other.

4. DISCUSSION

Prawns, shrimps and lobsters are among the most important constituents of the fisheries, playing important economic role and are on the top of foreign exchange earning items among fisheries products. Due to economic importance their use in aquaculture is rapidly expanding in several countries including India. Extensive culture methods, adopted for prawn culture and increasing load of pollutants particularly heavy metals to our water bodies is causing physiological stress to the aquatic animals and consequently predisposing them to infections and toxic manifestations. To reduce depleting effects of pathogens and toxicants it is essential to have proper knowledge of defense mechanisms and immune system of these crustaceans.

The immune system of crustaceans is primarily related to their blood or Haemolymph and to its circulating cells called Haemocytes. The haemocytes are mainly responsible for cellular immune reactions such as phagocytosis of invading microorganisms, their immobilization in nodular aggregates, and encapsulation of large foreign bodies and healing wounds which is accompanied by an immediate clotting of the haemolymph. To assess the physiological conditions and health status of these animals adequate knowledge of different haemolymph parameters like haemocyte types, total haemocyte counts (THCs), differential haemocyte counts (DHCs) etc., is highly needed for setting proper standards.

In present study with the help of light microscopy, phase contrast microscopy and cytological and cytochemical techniques, 7 types of circulating haemocytes have been found in the haemolymph of *Macrobrachium dayanum*. The characterized haemocytes were Prohaemocytes (PH); Plasmatocytes (PL); Spindle cells (SC); Granular Haemocytes (GH); Spherule cells (SP); Adipohaemocytes (ADP) and Cystocytes (CYS).

Literatures scan reveals the earlier studies on the crustacean haemocytes [48,49,10,50,51,52,53] and [54,55] represent an ambiguous picture of haemocyte types indifferent crustaceans, therefore the classification and nomenclature adopted in the present study is as that proposed by [56] for insect haemocytes which appears suitable also for other groups of arthropods [57,25,26,14,15,27,28]. Suggestions of [3,29] were also taken into consideration as described for crustaceans.

The present work established the occurrence of all the classical haemocyte types in *Macrobrachium dayanum*in contrast to the reporting of earlier workers in the genus *Macrobrachium,* using morphological and cytochemical features with the help of light and electron microscopy [22,58,17,59] except reporting of Ravindranath, for isopod *Ligia exotica*, mole crab *Emerita asiatica* [54,55,56] and Freshwater crab, *Potamon fluviatilis* [60], where almost all classical types of haemocytes were found.

Srivastava ans Narayan, reported only a single cell type and circulating amoebocyte in *Macrobrachium species* [58], whereas Tsing et al., using morphological and cytochemical techniques including ultrastructural details reported three haemocyte types in *M. rosenberghii* [17] along with two other decapod crustaceans. The haemocytes were grouped as the haemocytes with small granules, haemocytes with the large granules and haemocytes with a low level of differentiation. Vazquez, supported the findings of Tsing, but their description was in another way [17,59]. They classified the three haemocyte types of *M. rosenberghii* as hyaline haemocytes, granular haemocytes and undifferentiated haemocytes. The description of Gorgioni & Margherita, about the haemocytes of *M. rosenbergii* and *M. acanthurus* [22] further differs with earlier descriptions as they classified the three-haemocyte types as hyaline

haemocytes, small granule haemocytes and large granule haemocytes.

The Prohaemocytes were the smallest and simplest cell types comparable to the proleucocytes, leucoblasts, proleucocytoids, nucleocytes, and smooth contour chromophobic cells, lymphoid cells, Prohaemocytes and type-Z cells of some insects [10,24,61] and small hyaline leucocytes [62], Prohaemocytes [63,25,26,14,15,64,65,66], Prohyalocytes [67,68] and haemocytes with low level of differentiation [17] of crustaceans.

The prohaemocytes were regarded as immature forms with a small nucleo-cytoplasmic ratio and relatively small number of cytoplasmic inclusions. The simple structural organization of Prohaemocytes prompted [69] to term them as immature cells while [70] and [71] called them as stem cells.

The plasmatocytes of *M. dayanum* were similar to phagocytes of [72], phagocytic cells [73], half granular haemocytes [8], Small granulocytes [74, 17], intermediate cells [75], hyalocytes [67,68] hyaline haemocytes [76, 3,59,77] a granular haemocytes [29], amoebocytes [16], phagocytic cells [73], small granule haemocytes [14] and plasmatocytes [63,26,14,15,66].

The positive reactions for PAS, Best's carmine, Ninhydrin Schiff's, Hg-bromophenol blue, Aqueous bromophenol blue, Sudan Black-B and tests of acid phosphatase show the presence of carbohydrates, proteins and lipoids along with enzymes in the plasmatocytes of these prawns. These results are comparable with the reactions of similar cells in other crustaceans [22,14,15,17]. The high nucleocytoplasmic ratio, eccentric nucleus and presence of one large or a few small blunt pseudopodia were the morphological features of plasmatocytes characterized in present study are very similar to the descriptions of earlier workers.

The granular haemocytes were identified by the presence of granules of different sizes filling the cytoplasm almost completely. The centrally placed and sometimes obscured nucleus is another important feature of granulocytes. The granulocytes of *M. dayanum* correspond to the granular amoebocytes [72], granuleor Cystocytes [65], large granulocytes [29,74,17] eosinophilic granulocytes [68] haemocytes containing elongated and spherical granules and granular haemocytes or granulocytes [8,76,75,63,67,3,26,14,15,16,59,66]. The description of granulocytes as round ovoid cells with a relatively small nucleus and the cytoplasm filled with numerous large refractilegranules that sometimes obscure the nucleus is very similar to the earlier descriptions. Sternshein and also Gorgioni and Margherita described these as large granule haemocytes [22,16].

The presence of large spherical globules or spherules helped in the identification of spherule cells. The spherule cells of *M. dayanum* responded to the spheres and vacuolated cells of [62], spherule cells of [63, 78, 79, 56, 15, 27, 28] and chromophobic granulocytes of [67,68]. Adipohaemocytes were characterized by presence of large globules with faint nucleus and black staining with Sudan Black- B showing the presence of lipoid material inside the globules. The lipoid material in globules stained blackish red with Sudan-IV. These descriptions of adipohaemocytes are very much similar to the descriptions of [26,14,15,80] and [73] who described these as cells containing lipidic inclusions.

The cystocytes of the prawns morphologically appeared identical with the cystocytes of [42,79,81,56,26,14,15, 65] While Stang-Voss, described them as clotting cells [72] and Sternshein & Burton, as coagulocytes [16]. The description of these cells as containing large cytoplasmic vacuoles, a larger nucleus containing a large nucleolus in centre and chromosomal material on Periphery giving it a cartwheel shaped structure was very much supported as that described by [26,14,15].

The spindle cells of prawns contain an elongated nucleus and pointed pseudopodia at both ends. Most of the workers have described these cells along with Plasmatocytes, as fusiform plasmatocytes [7,82,80] and spindle shaped plasmatocytes [14] but morphologically they were quite distinct from Plasmatocytes. Nucleocytoplasmic ratio was almost found equal in spindle cell and pseudopodia were pointed at ends in contrast to blunt pseudopodia and high nucleocytoplasmic ratio of Plasmatocytes. Since these cells were also found in undiluted fresh haemolymph preparations in *Macrobrachium dayanum*, it appeared quite reasonable to categories them into a separate category as described in Noctuids by [61] and [83] as vermiform cells. Vazquez et al., reported most of haemocytes as spindle cells in a freshwater prawn, *Macrobrachium rosenbergii* [59] and called them as hyaline haemocytes and were of view that freshwater crustacean haemocytes do not fit in scheme of haemocyte classification described for marine crustaceans. These cells also take part in encapsulation of foreign materials along with other cells like granulocytes as evident by the observations of [84] and author himself for the cysts of helminth parasite, *Phyllodistomum lucknowensis* parasitising *Macrobrachium lamarrei*. Present study will be helpful in monitoring the health status of freshwater

prawn, *M. dayanum*, a potential animal for freshwater aquaculture.

5. CONCLUSION

This paper provides fundamental information necessary for understanding the immune system of freshwater prawns which is primarily based on the haemocytes. Therefore the characterization and classification of different haemocytes becomes the primary requirement which is taken in account in this paper. The findings will be helpful in better maintenance of the freshwater prawns, the potential animals for freshwater aquaculture.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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