

HEMOCYTES IN SCORPION- *HETEROMETRUS XANTHOPUS*

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ABSTRACT

The present investigation was undertaken to study the hemocytes in scorpion, *Heterometrus xanthopus*. Light and phase contrast microscopic observations have shown seven distinct types of hemocytes: 2% Prohemocytes (PRs), 82% Plasmocytes (PLs), 10% Granulocytes (GRs), 2% Spherulocytes (SPs), 1% Adipohemocytes (ADs), 1% Oenocytoids (OEs), and 2% Coagulocytes (COs). PLs constitutes bulk of the hemocytes in total hemocytes population. The values of THC for male, non pregnant female and pregnant female were 10850 per mm³, 9050 per mm³ and 9760 per mm³ respectively. The males showed higher THC than in non pregnant female. The pregnant females had highest THC value compared to that in males and non pregnant females.

INTRODUCTION

Many physiological functions in arthropoda are carried out in an orderly manner with the help of their body fluids. The number of hemocytes investigated differs according to the subtaxon of Arthropoda. The techniques and procedure for preparations, fixation and staining techniques have impact on the reaction of hemocytes; as a consequence the characteristics on which the nomenclature of hemocytes was based became highly diverse. The identification key used in the present investigation was proposed by Gupta (1985). Many excellent reviews are available on the insect (Jones, 1970, 1977; Gupta, 1985). The study of hemocytes is preferentially focused with insects and other arthropods, but scorpions remained more or less neglected. Scorpion hemocytes were studied by Patil and Shah (2011); Shah and Patil (2011). The hemocytes are very important as far as immunology is considered. Hence the present investigation is focused on the scorpion *Heterometrus xanthopus*.

MATERIALS AND METHODS

The scorpions used in the present investigation were collected from Dadaswadi, Taluka: Atpadi, Dist: Sangli in Maharashtra (India). These are kept in perforated polythene jars containing Hibiscus leaves and fed with small cockroaches. The animals were maintained for months together without any significant mortality.

Hemolymph was collected from the living animal (Padmnabha, 1967). The animal was fixed on the board with the help of rubber bands. The hypodermic syringe was inserted through arhrodial membrane present in the third joint of pedipalp. Depending upon the size of scorpion the volume

of hemolymph collected varied but on an average about 1 to 3 mL could be easily collected.

Technique developed by Patil and Shah, (2011) and Shah and Patil, (2011) was used. Leishman's Stain and Sudan Black B were used. The cytological methods were used for qualitative study while quantitative methods include THC and DHC. Preliminary observations of hemocytes were made by placing a drop of fresh hemolymph on ungreased slide. It was immediately covered with coverglass and ringed with petroleum jelly to exclude air. The preparation was immediately observed under microscope. The coagulocytes were best identified in this preparation. Similarly amoeboid movement could be observed in such preparation.

For THC Neubauer's hemocytometer was used. Fixed dried film was used for DHC. A thin layer of adhesive was applied to degreased slide on which a drop of 2% saline-versene solution was placed. This drop acts as a chemical fixative. A thin smear was prepared for differential staining method. The 100 slides were examined and hemocytes were classified.

RESULTS AND DISCUSSION

Hemocytes types

In the present investigation seven types of hemocytes were observed in *Heterometrus xanthopus* – Prohemocytes (PRs), Plasmocytes (PLs), Granulocytes (GRs), Spherulocytes (SPs), Adipohemocytes (ADs), Oenocytoids (OEs) and Coagulocytes (COs). All seven types of hemocytes are shown in fig. 1-7. The values of THC for male, non pregnant female, pregnant female were 10850 per mm³, 9050 per mm³ and 9760 per mm³ respectively.

Prohemocytes (PRs)

The PRs are generally small and round, but larger in *H. xanthopus*. The size varied from 7-13 μm . A very thin layer of cytoplasm is present with fine basophilic granules. The large compact nucleus is centrally placed. The size of the nucleus varied from 5-10 μm in diameter. DHC showed that PRs constitute 2% of total hemocytes.

Plasmocytes (PLs)

PLs are unique hemocytes in scorpion as polymorphic in nature. It is spindle shaped. During preparation these cells adhere to glass forming cytoplasmic projections, hence accurate measurement was difficult. 2% versene acts as good fixative. It measured 10-35 μm in length and 3-11 μm in width. The cytoplasm is abundant and granular. The granules are Sudan black B positive indicating lipids in it. The oval nucleus was centrally placed and measured about 4-10 μm . The PLs constitute bulk of hemocytes population – 82%.

Granulocytes (GRs)

These are with stainable granules in it. These were oval measured about 10-15 μm in diameter. The varied size indicated different stages of maturation. The nucleus was larger with 5-7 μm in size and eccentric in position. The chromatin was compact and dense. They constitute about 10% of total hemocytes.

Spherulocytes (SPs)

These were characterized by presence of non-refrigent spherules in the cytoplasm.

These were round or oval and size varied between 8-20 μm . The spherules are strongly basophilic. The nucleus was irregular in shape and measured about 3-6 μm . The value of DHC was 2%.

Adipohemocytes (ADs)

ADs were spherical and size ranged between 10-40 μm . The cytoplasm was granular and refrigent in nature. The presence of lipid was deduced from Sudan black B staining. The cytoplasm was vacuolated. Supravital staining indicated presence of large number of mitochondria. The nucleus was large, centrally placed and 4-10 μm in diameter. They constituted only 1% of total hemocytes.

Oenocytoids (OEs)

These were extremely fragile in nature, hence was difficult to locate and observe under light microscope. The morphological details presented here emerged from phase contrast

microscopy. 2% versene used to preserve and study the morphological details. The size varied from 10-45 μm . The nucleus was small with diameter 4-10 μm . The DHC value was 1%.

Coagulocytes (COs)

These were also fragile like OEs and were observed under phase contrast microscope. The size varied between 12-32 μm . The cytoplasm was hyaline and granular. The nucleus was round with compact chromatin and size ranged between 3-7 μm . The DHC value was 2%.

Selection of proper technique becomes important since several methods are available for the study of arthropod hemocytes (Shapiro, 1979; Gupta, 1985). During the course of this investigation it was noticed that any one technique was

not suitable for all the hemocytes. Therefore either the combinations of techniques or a suitable modification of a single technique became necessary.

In the present study wet fixed preparations were stained by Pappenhem's panchrome which was found to be most effective as it brought about the basophilia more clearly. 2% versene preserve hemocytes and also responsible for prevention of hemolymph coagulation.

THC studies in the present investigation were included with an objective to find out whether any relation between hemocytes number, sex and pregnancy has exist in *H. xanthopus*. The males showed higher THC than in non pregnant female. The pregnant females were having highest THC value than that in males and non pregnant females.

The aquatic chelicerata have only one type of hemocytes – Granulocyte (Gupta, 1985). The onychophora showed five types- PRs, PLs, GRs, SPs, OEs. Seven types of hemocytes of *H. xanthopus* were recorded on the basis of distinctive morphological and cytological features revealed by light and phase contrast microscopy. Seven types of hemocytes are reported in *Mesobuthus tamules tamules* (Patil and Shah, 2011) and same number of types in *Mesobuthus tamales concanensis* (Shah and Patil, 2011). The PRs have been reported in all insect orders (Gupta, 1985a), same was also reported in *H. xanthopus*. PRs having high nuclear to cytoplasmic ratio, are considered as stem cells (Srivastava and Richards, 1965) give rise to some other hemocytes (Jones, 1959). But it is not observed in *H. xanthopus*.

Some authors have difficulty in differentiating PRs from PLs.

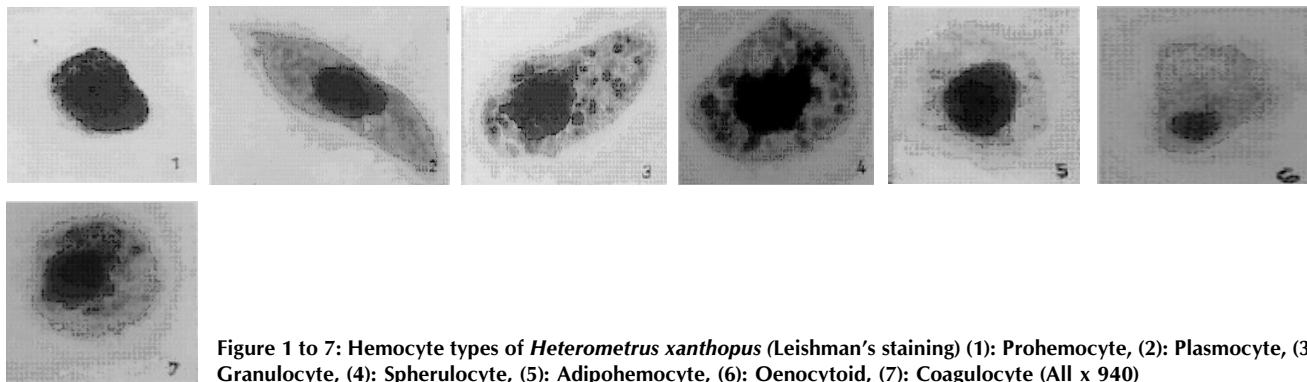


Figure 1 to 7: Hemocyte types of *Heterometrus xanthopus* (Leishman's staining) (1): Prohemocyte, (2): Plasmocyte, (3): Granulocyte, (4): Spherulocyte, (5): Adipohemocyte, (6): Oenocytoid, (7): Coagulocyte (All x 940)

PRs has been considered as young PLs In *H.xanthopus*, the PRs and PLs having all the typical features of their own and separate identities. Being pleomorphic PLs are known to present themselves in different forms. The large number of PLs in the scorpion might be because of their phagocytic activities.

The GRs are considered as plesiomorphic hemocytes and only the hemocytes type that has been reported from all arthropod groups and Onychophora (Gupta, 1985a).

Hollande (1909) coined the term SPs, the cells containing spherules in it. The present investigation confirms the conspicuous spherules filling in the cytoplasm. The ADs and OEs were described in many insects (Gupta, 1985b). Andrade *et al.* (2003) did not report ADs from *A. gemmatalis*. The consideration of Ads as a separate category has often been questioned and it has been regarded as a functional stage of the GRs (Raina and Bell, 1974). In the present investigation we have reported it as separate hemocytes. As indicated above the COs has been observed, both with light and phase contrast microscopes.

Seven types of hemocytes have been observed in the scorpion *H xanthopus*. Thus the scorpion hemolymph appears to be different from other arthropod groups. The scorpion, belonging to the oldest arthropod group of terrestrial Chelicerata can be considered to have retained this primitive feature.

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