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Research Article

COMPARATIVE ANALYSIS OF HAEMOCYANIN PROTEIN CONCENTRATION OF THE FUNNEL WEB SPIDER *ARGIOPE ANASUJ*A AND ORB WEB SPIDER *HIPPASA AGELENOI*DES FROM KARNATAKA

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ABSTRACT
Haemocyanin is a major component in invertebrates like insects and spiders for oxygen transport. A comparative study of hemocyanin protein concentration of two species of spiders' *viz., Hippasa agelenoides* and *Argiope anasuja* was estimated by Lowry's method and molecular weight estimated by SDS-PAGE analysis. The Lowry method revealed that *he*mocyanin concentration was more in the *H. agelinoides* (59µg) compared to *A.anasuja* (44 µg). SDS-PAGE revealed 66 kDa thick band for *H. agelinoides* and light band for *A.anasuja.* This preliminary estimation could throw light on the future applications in metabolism, phylogenetics and other evolutionary studies concerning arachnofaunal body fluids.

Key Words: *Hippasa agelenoides, Argiope anasuja, Hemolymph, Hemocyanin, SDS-Page*

INTRODUCTION
Invertebrates comprise the majority of the world's fauna, and are popular as research animals. Hemolymph is a transparent fluid of the invertebrate circulatory system, transports nutrients, hormones, oxygen, cells etc., and contains copper. Fresh spider hemolymph appears transparent bluish due to the presence of copper ion in the respiratory pigment hemocyanin. Hemocyanins are large allosteric respiratory proteins that occur freely dissolved in the hemolymph. Oxygen binding of hemocyanins is mediated by a pair of copper atoms that are coordinated by six histidine residues. Each arthropod hemocyanin subunit 72-75kDa folds into three domains characterized by different folding motifs (Jaenicke *et al.,* 1999; Decker *et al.,* 2007; Paoil *et al.,* 2007; Rehm *et al.,* 2012).
The body fluids, venom of spiders contain both neurotoxic, cytotoxic components which are linked with glucose, nucleic and free acids, inorganic ions such as Ca2+, Mg2+, Na+, K+, Cl-, and neurotransmitters (Jalal *et al.,* 2010). Besides being oxygen carriers, some hemocyanins function as a phenoloxidase (tyrosinase/catecholoxidase) which requires activation. Chelicerates such as spiders and scorpions lack a specific phenoloxidase, and in these animals activated hemocyanin might catalyse melanin synthesis *in vivo* (Decker *et al.,* 2007). Spider's hemocyanin can binds to ecdysone at low affinity (Jaenicke *et al.,* 1999). Various studies had been done on the hemocyanin of the spiders. However there are very few studies on the south Indian spiders. The present study is a preliminary and intended to compare the hemocyanin protein in the hemolymph of two different species of south India (*H.agelinoides* a funnel web spider and *A. anasuja*, an orb web spider) by Lowry method and SDS-PAGE analysis.

MATERIALS AND METHODS
Spider collection and handling: Spiders were collected from different regions of Karnataka in India. Collection was done early in the morning from January 2012 to June 2012 and identified up to species. A sample size, n=10/ each species were utilized for the present analysis.
Hemolymph Collection and processing: Hemolymph collection from adult spiders and processing was followed as described by Yigit and Benli, (2008) and Jalal *et al.,* (2010) with some modifications to suit experimental setup. The live spiders were anesthetized with alcohol; the forth waking legs were separated

from the coxa. The outwards flowing liquid was collected with micropipettes, which varied from 5-25µl. It is a white, viscous and odorless liquid .The tubes were labeled and centrifuged at 12,000 × g for 10min.The resultant cell free hemolymph was refrigerated at approximately 3°C and analysed within 72h (Barron, 1999).It was diluted with distilled water (1:4) for further analysis.

Protein Estimation

Hemolymph proteins of selected spider species were separated by SDS following Yigit *et al*., (2004). Clear supernatant was collected and protein concentration was measured by Lowry method, (1951), using bovine serum albumin as standard at 660nm. Electrophoretic separation of proteins was performed using 12% acrylamide gel with 1 mm thickness following dissociating and discontinuous buffer system. Protein bands were visualized by Commessei blue staining protocol.

RESULTS

There was significant variation in protein concentration between the spider species studied. The protein concentration in *H.agelinoides* was 59µg and *A.anasuja* the protein concentration was found to be 44 µg.

Table 1: Protein content in two different species of spiders studied

Sl. No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	*H. agelinoides*	*A. anasuja*
Conc. µg/tube	0	10	20	40	80	120	160	59	44
O.D @ 660nm	0.000	0.113	0.179	0.243	0.541	0.648	0.833	0.359	0.267

SDS PAGE: A variation was found during the present analysis wherein, *H.agelinoides* showed a thick band of molecular weight 70KD while *A. anasuja* showed a band of 66KD in weight.

DISCUSSION

In this study the difference between the concentration of hemocyanin protein of hemolymph of two different species of Karnataka (*H.agelinoides* and *A. anasuja)* have been done. The spider hemolymph contained a mixture of components having toxicological and biological potentials (Jalal *et al*., 2010). The hemocyanin protein of hemolymph of *H.agelinoides was* more than that of *A. anasuja (H.agelinoides* 59µg/tube and *A. anasuja* 44µg/tube). The band showed in the SDS-PAGE of *H.agelinoides* have showed a thick band than Argiope. It reveals that the *H. agelinoides* have a high concentration of hemocyanin than the Argiopa . It indicating that the hemocyanin concentration depends on the habitat of spiders as the *H.agelinoides* are mostly running spiders than that of *A. anasuja*. In conclusion, the functional diversity of hemolymph and concentration of hemocyanin seems to be the most significant variable influencing spider abundance, habitat and diversity. The abundance of a species depends on seasons (weather, temperature, humidity, rain fall, etc.). Therefore hemolymph quantity and quality significantly varied with the due course of time. This preliminary estimation could throw light on the future applications in metabolism, phylogenetics and other evolutionary studies concerning arachnofaunal body fluids.

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REFERENCES

Jaenicke, Roman and Decker (1999). Spider Hemocyanin Binds Ecdysone and OH-Ecdysone. *The Journal of Biological Chemistry* **274**(48) 34267–34271.

Jalal F, Shakila M, Shoukat P, Shahanaz AR and Anjum S (2010a). Partial Characterization of Hemolymph of Different Spider Species of Citrus Orchards. *International Journal of Agriculture Biol*ogy **12**(3**)** 447–450.

Jalal F, Shakila M, Shahanaz AR, Anjum S, Tanveer M and Ibrahim R (2010b). Comparative profile of free ions in venom and hemolymph of citrus spiders from Pakistan. *Pakistan Journal of Zoolo*gy **42**(6) 687-691.

Decker Heinz, Nadja H, Elmar J,_Bernhard L,Ulrich M and Ju¨rgen M (2007). Minireview: Recent progress in hemocyanin research. *Integrative and Comparative Biology* **47**(4) 631–644.

Paoli F, Giomi N, Hellmann E, Jaenicke H, Decker P, Di Muro M, Beltramini (2007). The molecular heterogeneity of hemocyanin: Structural and functional properties of the 4×6-meric protein of *Upogebia pusilla* (Crustacea) *Gene* **398**(1-2) **177–182**.

Ballweber P, Ju rgen M and Thorsten B (2002). Complete Hemocyanin Subunit Sequences of the Hunting Spider *Cupiennius salei*. *The Journal of Biological Chemistry* **277(**17) 14451-14457.

Zakariah M, Mitchell, Clare, Guichard and Rimme (2007). Hemolymph biochemistry reference ranges for wild caught goliath birdeater spiders (*Theraphosa blondi*) and chilean rose spiders (*Grammostola rosea*). *Journal of Zoo and Wild Life medicine* **38**(2) 245-251.

Yigit and Benli, (**2008)**. The antibacterial activity of hemolymph of spider, *Agelena labyrinthica* (Araneae: Agelenidae*). Journal Forestry Faculty* **8**(2*)120–124.*