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Studies on the salivary glands of *Argas persicus*

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Abstract
In the present study morphology and different staining techniques such as Methyl green pyronin (MGP) staining, Giemsa staining and Formalin Azocarmine Alcohol Lactophenol (FAAL) staining were used to visualize the structure of salivary gland of *Argas persicus*. The paired elongate salivary glands of male and female *Argas persicus* comprised of 2 types of alveoli *viz*., type I and type II. The type I alveoli were oval or pear shaped consisting of peripheral and central zones. Type II alveoli had circular or oval outline and consisted of three types of cells surrounding a central lumen and lying on a fine basement membrane In MGP staining, the type I alveoli appeared as pink colour with a centrally located large oval nucleus. In Giemsa staining, the type I alveoli appeared in purple colour with centrally located large oval nucleus. In FAAL staining both type I and II alveoli appeared in orange colour. Type I alveoli showed orange coloured large oval nucleus. Triangular cells were seen in type II alveoli. As compared to MGP and Giemsa stain, the FAAL stain was better to visualize the structure of salivary glands.

Keywords: Salivary glands, Methyl green pyronin, FAAL

Introduction
Argas persicus was first described in 1818 by Lorenz Oken in Meyaneh, Persiaand are now established as having a worldwide distribution, particularly affecting warmer regions because of its close association with *Gallus domesticus*. The fowl tick specially affects domestic fowl, turkey, duck, pigeon, geese, canaries and ostrich hiding in certain wild bird habitats, the cracks and crevices of poultry house, bird nests, roosting sites and also under the bark of many trees. Two types of salivary alveoli are present in adult *Argas persicus*: agranular type I and granular type II alveoli [1]. Two types of alveoli were observed in salivary glands of *Argas persicus* [2, 3]. Some resemblance of type I alveoli to the type II alveoli of ixodid ticks were observed [4-6]. In case of type B alveoli of *Argas arboreus*, three types of cells were observed [7, 8]. *Argas persicus* saliva had anticoagulating properties [9]. Carbohydrate and protein in the i cells of the type II alveoli are the precursors of the anticoagulant contained in the *Argas persicus* salivary secretion [10]. Presence of cytolysins and enzymes in the salivary secretions of argasid ticks appear to be markedly more vigorous and destructive in their actions than those of the ixodid ticks [11].

Feeding habits of 29 argasid ticks were observed by [12]. The fine structure of salivary alveoli has been investigated in argasids [13-17].The methyl green pyronin staining in the whole salivary glands of *Rhipicephalus appendiculatus* and *Hyalomma anatolicum anatolicum* for the presence of *Theileria parva* and *Theileria annulata* were described [18]. A rapid method of methyl green pyronin staining for salivary glands of *Rhipicephalus appendiculatus*ticks infected with *Theileria parva* [19]. Several dyes such as Periodic Acid Schiff [20], Mallory's triple stain [5], Aniline black blue [21] and Giemsa stain [22, 23] have been used in paraffin embedded sections of tick salivary glands. The present study describes the morphology and different staining techniques such as Methyl green pyronin staining, Giemsa staining and FAAL staining were used to visualize the structure of salivary gland of *Argas persicus*. This information is helps to understand the mechanism of saliva production and role of salivary glands in disease transmission from tick to vertebrate host.

Materials and Methods
Ticks were collected from different poultry farms located at various places in Telangana and brought to the laboratory in zip lock bags with small holes for aeration. These ticks were found in the cracks, crevices, below the feed trough, inner side of feed trough, bird cages, litter and on birds. The dissection of *Argas persicus* (ticks) was carried out as per the method described [24] with minor modifications. Salivary glands were removed intact while viewing through a dissecting microscope with fine tipped forceps and rinsed thrice in PBS (pH 7.4) and transferred in to fresh PBS (pH 7.4).

Staining of salivary glands of *Argas persicus*
Methyl Green Pyronin (MGP) Staining
The salivary glands were placed on a clean glass slide with a drop of 1 % solution of bovine serum albumin (BSA) and teased with the help of a needle under the dissection microscope to ensure even spreading of the acini without any overlapping. The glands were then air dried and fixed for 2-5 minutes in Cornoy's fixative. Then glands were rinsed sequentially for 2 minutes in 70 % ethyl alcohol and 2 minutes in distilled water. Then the glands were stained with MGP for 7 minutes. After staining, the glands were rinsed in distilled water and air dried. Final clearing was done in xylene for 1-2 min then mounted in Dibutyl Phthalate Xylene [19].

Giemsa Staining
The salivary glands were placed on a clean glass slide with a drop of 1 % solution of bovine serum albumin (BSA) and teased with the help of a needle under the dissection microscope. Then the glands were air dried and fixed for 30-45 sec in methanol. The smear was stained by adding few drops of Giemsa stain for 40 minutes and washed with tap water and air dried [25].

Formalin Azocarmine Alcohol Lactophenol (FAAL) Staining
Freshly collected salivary glands were washed three times in PBS to remove dirt and the whole salivary gland was stained in FAAL for overnight at 27 ^{0}C and examined by mounting in the same stain [26].

Results
Morphology of Salivary Glands of *Argas persicus*
The paired elongate salivary glands of male and female *Argas persicus* comprised of 2 types of alveoli *viz*., type I and type II (Fig. 1). The type I alveoli were oval or pear shaped (Fig. 2) consisting of peripheral and central zones. Each type I alveoli consisted of several cells with indiscernible boundaries, resting on a delicate basement membrane (Fig. 2). These cells formed a peripheral zone with striations almost perpendicular to alveolar boundary where numerous fine granules occurred (Fig. 2). Type II alveoli had circular or oval outline and consisted of three types of cells surrounding a central lumen and lying on a fine basement membrane (Fig. 3). These cells appeared triangular in outline with the triangle base at the alveolar periphery (Fig. 3).

Staining of salivary glands of *Argas persicus*
Methyl Green Pyronin (MGP) Staining
In MGP staining, the type I alveoli appeared in pink colour with a centrally placed large oval nucleus (Fig. 4).

Giemsa Staining
In Giemsa staining, the type I alveoli appeared in purple colour with centrally placed large oval nucleus (Fig. 5). Each type I alveolar duct connected to lobular duct which in turn was connected to main salivary duct (Fig. 5).

Formalin Azocarmine Alcohol Lactophenol (FAAL) Staining
In this staining both type I and II alveoli were appeared in orange colour. Type I alveoli of *Argas persicus* was showing the orange coloured large oval nucleus, peripheral zone and central zone (Fig. 6). Triangular cells were present in type II alveoli (Fig. 7).

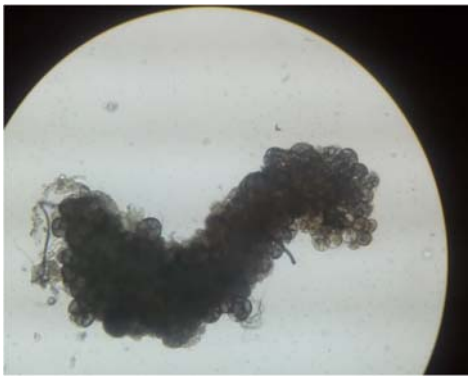

Fig 1: Photomicrograph showing salivary glands of *Argas persicus* (40X).

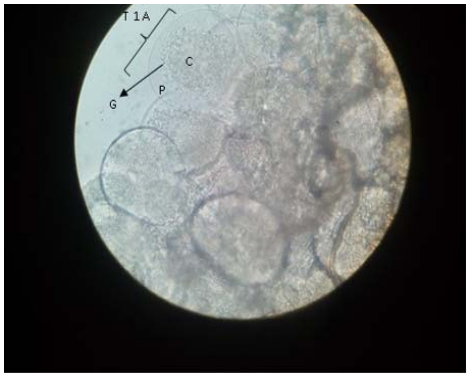

Fig 2: Photomicrograph of Type I alveoli (T 1 A) of *Argas persicus* showing central zone (C), peripheral zone (P) and fine granules (G) in peripheral zone (40X).

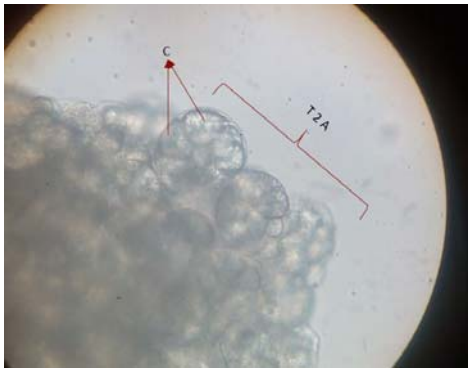

Fig 3: Photomicrograph of Type II alveoli (T 2 A) of *Argas persicus* showing triangular cells (C) (40X).

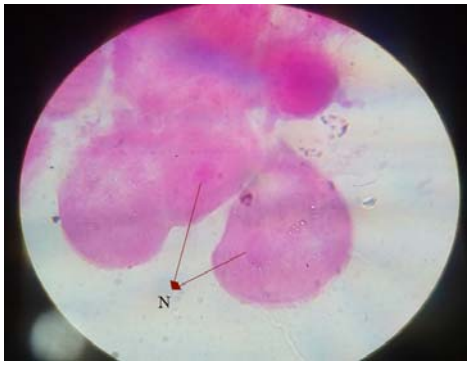

Fig 4: Photomicrograph of methyl green pyronin staining of Type I alveoli of *Argas persicus* showing pink coloured large oval nucleus (40X).

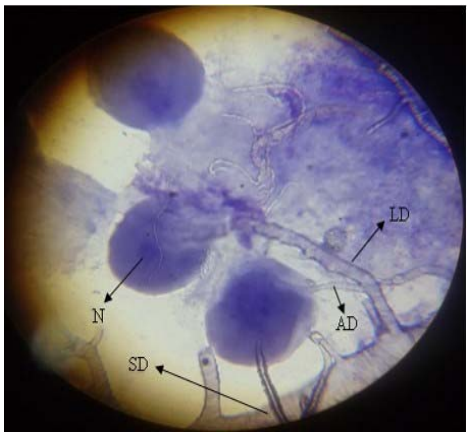

Fig 5: Photomicrograph of Giemsa staining of Type I alveoli of *Argas persicus* showing purple colour large oval nucleus (N), main salivary duct (SD), lobular duct (LD) and alveolar duct (AD) (40X).

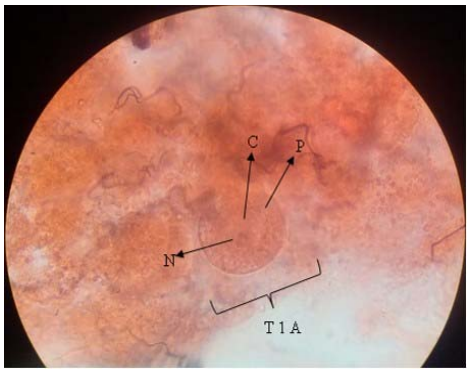

Fig 6: Photomicrograph of Formalin Azocarmine Alcohol Lactophenol (FAAL) staining of Type I alveoli (T 1 A) of *Argas persicus* showing orange colour large oval nucleus (N), peripheral zone (P) and central zone (C) (40X).

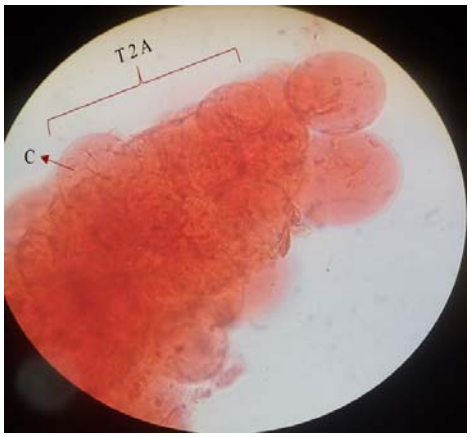

Fig 7: Photomicrograph of FAAL staining of Type II alveoli (T 2 A) of *Argas persicus* showing triangular shape cells (C) (40X).

Discussion

Morphologically there is a difference in the salivary glands of *Argas persicus* and ixodid ticks. The size and number of salivary alveoli of *Argas persicus* was smaller than the ixodid ticks. The types of salivary gland alveoli and their connections to the main salivary duct in *Argas persicus* conform to the general pattern of argasid ticks. The paired elongate salivary glands of male and female *Argas persicus* comprised two types of alveoli *viz*., type I and type II [27-33] but in the ixodid ticks there are three types of salivary alveoli [4-6, 34]. In the present study, the type I alveoli were oval or pear shaped consisting of peripheral zone and central zone. The alveoli consisted of several cells with indiscernible boundaries, resting on a delicate basement membrane. These cells formed a peripheral zone with striations almost perpendicular to alveolar boundary where numerous fine granules occur. Type II alveoli consisted of three types of granular cells (a, b, c). Similar findings of type II alveoli were observed by [1, 7, 35, 36]. The differential histological stains and histochemical techniques of three cell types in type B granule secreting alveoliof *Argas persicus* and also showed metachromatic substances are present in type c cell granules in *Argas persicus* which may have anticoagulant properties which promote blood flow [35].

In the present study, the salivary glands of *Argas persicus* were identified by different staining techniques. Very limited work has been done on this staining techniques for salivary glands of ticks. The methyl green pyronin was comparably better with feulgen's staining for examination of *Theilerial* parasites in salivary glands of *Hyalomma* ticks [18]. The staining technique for salivary glands of *Rhipicephalus appendiculatus* ticks infected with *Theileria parva* and showed that parasite masses in salivary gland acini stained blue or purple, acinar cell cytoplasm stained pink and acinar cell nuclei stained blue [19]. In the present study, the type I alveoli showed only one large oval pink coloured nucleus in methyl green pyronin staining. The structural changes of salivary glands of *Hyalomma anatolicum anatolicum* during attachment and feeding. They reported the greyish to light blue coloured numerous loosely packed large sized granules in b type cells of type II aciniwith Giemsa stain,small to medium sized granules of c_1 type cells were stained different shades of blue with Giemsa's stain andthe granules of c_2 cells stained a dense metachromatic with Giemsa's stain [23]. In the present study, we could visualize the type I alveolar duct connected to lobular duct which in turn was connected to main salivary duct in Giemsa staining. The central zone of type I alveoli showed one large purple coloured oval nucleus. In FAAL staining, the type I alveoli showed a central zone with a large oval nucleus and a peripheral zone. Type II alveoli having a circular or oval outline consisted of more than five triangular cells with triangle base at the alveolar periphery. There is no available information on FAAL staining for salivary glands of ticks, so that we cannot compare the FAAL findings with others. This FAAL staining was better to visualize the structure of salivary alveoli of *Argas persicus* as compared to other staining techniques such as methyl green pyronin and Giemsa stain.

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