Journal of Pharmaceutical and Scientific Innovation
www.jpsionline.com
Research Article

CHANGES IN SOME BIOCHEMICAL PARAMETERS IN THE SALIVARY GLANDS OF TWO IXODID TICKS

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Key words: ticks, biochemical parameters, protein bands
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Received on:15/04/12 Revised on: 20/05/12 Accepted on: 12/06/12

ABSTRACT
Extracts of salivary glands of the two types of ticks namely, *Hyalomma anatolicum anatolicum* and a *Rhipocephalus sanguineus sanguineus* , were subjected to the test of some biochemical parameters . The total protein intensity has been estimated in salivary glands extracts in the two types, high percentage was found in *H.anatolicum* compared with *R.sanguineus* forms, (104.12±4.93) and (91.33±3.04) microgram/Cm^3 respectively. The intensity of carbohydrates in salivary gland extracts was high in *H.anatolicum* (0.640±0.009), after comparing with a lower intensity for *R.sanguineuss* (0.360 ±0.004) microgram/Cm^3. The lipid intensity in salivary glands was high in *R.sanguineus* (245.37±8.76) compared with concentrations in *H.anatolicum* (244.80±9.27)microgram/Cm^3.
The total isolation of protein amount in the salivary glands in both types of tick, electrophoresis technique with Poly acrylamid gel with SDS has been implied to trace the most important separated proteins. The results denotes the existence of the four protein bands in *H.anatolicum*, and three protein bands in *R.sanguineus*, and their molecular weights were recognized. The Molecular weights for the four bands were; 76.190, 53.616, 38.168, and 16.614 kDa, respectively, while the molecular weight for the three protein bands were 73.569, 51.760, and 32.779 kDa, respectively.
KEYWORDS: *Hyalomma anatolicum anatolicum,* Ixodid Ticks, Salivary glands

INTRODUCTION

The salivary glands of the hard ticks consist of a pair of acini(cluster) situated at both sides of the haemocoel. The first connected directly to the main salivary canal, the 2nd has small lobes and the 3rd has longitudinal lobes at sides of the canal. The acinus I contains cytoplasmic granules with fibrils, 2 -3 nuclei, water apical cells are present in acini II, and III[1]. They also revealed the presence of complex-protein carbohydrates and agranular cells stain positively with alcian blue as such they suggested the presence of neutral glycoprotein and also contain Tyrosine amino acid and lipoprotein which stain positively with Sudan black-B.
Sauer and Essenberg[2] indicated that the salivary glands are controlled by nervous control and neurotransmitter in which Dopamine is the most important one. Sauer et al.[3] concluded that the main essential materials in saliva in Ixodid tick are cement materials which act as anchor to help the tick to attach to the host skin, and also contain enzymes , anti-coagulant , muscular anti -histamine and prostaglandin. Furthermore, detailed description of the salivary glands cells in males of R. sanguinus in different feeding stages were carried out[4]. Binnigton[5] concluded in his study that saliva lead to destruction of glycogen in the host skin which leads into oozing of blood vessels, as saliva may cause vasodilatation of the blood vessels and the active esterase present in the saliva of H anatolicum lead to permeability of blood vessels via hydrolysis of cholesterol esters.
As concern protein investigation in ticks, Bishop et al.[6] analyzed the cement protein in acinus III , and found that the DNA with 36 kDa protein , and revealed the cement cone in *R. appendiculatus* has 334 amino acids.
Other researchers like[7] estimated the protein in a of number of ticks and they concluded that *A. persicus* possess a protein with 4 bands and with molecular weight 16.076 – 82.76kDa while in female of *H. anatolicum excavatum* consists of 6 bands and the estimated molecular weight was 6.216 – 103.74 kDa.
Guddera et al.[8] when studied the hard tick, *Dermacentor variabilis* they found Lipoglycoheme DvCP with molecular weight of 200 kDa.
Both ultrastructure of the granular cells and the lactene of female *Ixodes ricinus* was studied using SDS technique, exploring the granular secretion of both acinus II and III[9], and they confirm it is a carbohydrate in nature . Amr et al.[10] detected two types of glycoprotein in the larvae of cow ticks, while Uhlir et al.[11] found antigene of glycoprotein in the larvae and adult of *Ixodes ricinus,* and they reached to a conclusion that most of the glycosylated antigens possess N-type glycans.
Studies on lipid estimation in ticks, Hajjar[12] revealed the presence of phospholipids and sterol in biological fluids of *Hyalomma dromedarii* and *H. anatolicum excatum*, also it was found that molting fluid contain phospholipids and cholesterol and fatty acids and sterols. Furthermore,[13] studied the lipid variations in *Amblyomma americanum* during nutrition, and [14] studied the variations in esterase and lipases during embryogenesis of the tick, *Hyalomma dromedarii,* they found 6 isoenzymes of esterases using chromatography . Further investigation on ticks lipids,[15] studied cells of salivary glands of *Amblyomma cajennens* which have mitochondria in the three acini and tried to find a possible relation with lipid activity.
Therefore it appear logic to trace the changes of some biochemical parameters in the salivary glands of two types of hard ticks belonging to family: Ixodidae, infesting Iraqi mammals, namely: *Hyalomma anatolicum anatolicum* and *Rhiphicephalus sanguineus sanguineus* , and also to reveal their protein bands.

MATERIALS AND METHODS

To estimate the total protein the method proposed by[16] was used for the salivary glands obtained from the females of the two ixodid ticks. The method depend on the reaction of protein with Follein reagent in basic medium to give a

complex blue color byproduct, the adsorbance was measured using 750 nanometer wave length[17].

For Carbohydrate estimation , the method proposed by Herbert et al.[18] was used which depend on reaction between phenol(5%) with dissolved saccarides and which form complex medium reacted with concentrated sulphuric acid which finally formed colored complex measured by a wave length 488 nanometer.

As concern estimation of lipids the method of Chabrol and Chardonnet[19] were used[20]. This heating of a measured amount of salivary solution with sulphuric acid then the mixture subjected to react with Phosphovaniline reagent to produce a byproduct with red gentian color. Adsorbence were measured in standard solution in 540 nanometer.

For isolation of protein bands, Sodium Dodecyl sulphate-polyacrylamid gel electrophoresis technique (SDS-PAGE) was used as described and used by[21-22] and for the preparation of jell the method reported by[23] was used.

RESULTS AND DISCUSSION

Estimation of Total protein

As seen in table 1 there were significant differences in concentration of protein in the extract of salivary glands in both types of ixodid ticks at a level of ($P<0.o5$). The total protein was high in *H. anatolicum* (4.93+104.12) and low in the *R. sanguneius*(3.04 +91.33) mg/ cc. The difference in protein concentration in the two types of ticks may be due to adaptive behavior of the *R. sanguineus* as this tick is more sensitive to environmental changes as indicated in the life cycle which took less time during parasitizing the rabbits which make them consume more blood which is rich in protein.

As shown in table 1 there were significant differences in carbohydrate in the salivary glands for the two types of ticks at probability($p<0.05$), the results shown that there is an increase in the level of carbohydrate in *H.anatolicum* (0.009-0.640) mg/ cc while in the body tick *R. sanguineus* there is a decrease in carbohydrate level(0.004 +0.360)mg/cc.

As it is clear from above results that the high level of carbohydrate in *H. anatolicum* is indication of carbohydrate consumption as the host of *H. anatolicum* is more active in *R. sanguineus* as it suck large quantity of carbohydrates from the host.

Marie et al.[9] concluded that the granular secretion of certain cells of salivary glands for acinus II, III in ixodid ticks are carbohydrate in nature. The difference in carbohydrate concentration depends on many factors such as efficacy of carbohydrate transport from the host to the tick during attachment, efficacy of mouthparts for suction. Further study is suggested to trace the carbohydrate level in different feeding period and in a variety of host to know precisely the high level of carbohydrate of *H. anatolicum* due to which factor.

As indicated in Table 1 the lipid level in *R. sanguineus* (245.37 +8.76)was significantly differ from that of *H. anatolicum* (244.80+ 9.27)at $p<0.05$ level. This difference may be due to different feeding period similar to the finding of Hajjar[12] such as during dropping of encouraged females and in the period of ecdysis .

As revealed from the results that the level of cholesterol increases in the blood of rabbits during their infection with larvae and adult of *H. anatolicum* and adults of R. *sanguinus* which may be because of the transfer of variety of lipids including cholesterol from the haemolymph of the ticks and from their salivary glands to the blood of the rabbit which may result in the decrease of lipid level in the salivary gland of *R. sanguineus* and may be due to consumption during cement formation which is lipoprotein in nature.

Furthermore studies are essential to detect sites of chemical in the salivary gland especially the histochemical tests in addition to the tests in different variables such as feeding period, encouragement of female, ecdysis, time of attachment and dropping in order to trace the factors directly affect the level of protein , carbohydrate and lipids.

Isolation of total protein in salivary gland of the two types of ticks using electrophoresis compared to standard, their molecular weight 45000 – 67000 D after drawing a standard curve to estimate the molecular weight using electrophoresis with gel SDS-PAGE as seen in Fig. 1 and Table 2.

Four protein bands were detected in salivary gland extract of *H. anatolicum* and 3 in *R. sanguineus* (Fig.1)with different weight of those bands , their size and quantity in the two types of ticks. The 4rth band in *R. anatolicum* of molecular weight 16.614 D which is far from other bands which indicate gene distance and consequently give indication of the differences of the two species which are morphologically are belonging to two separate genera.

As regards the molecular weight of the bands 1st, 2nd, 3rd of *R. sangenius* (73.569/ 51.760/ 32.7798) kDa are close to other three bands of *H. anatolicum* (76.190/ 53.616/ 38.168) kDa. Therefore, the slight close between bands of *H. anatolicum* indicates the presence of more intense protein. These results are close to the results obtained by Parmar A[24] when they found 6 protein bands in *H. anatolicum* (60/ 66/ 148/ 264/ 300/ 300) KD, four of these bands are shared with those of cow tick, *Boophilus microplus*. They also proved that the protein common between the two types estimated by 66 kDa during revealing the direct method of hypersensitivity for the swelling which emerge underneath the skin. While results of Kawther MK[25] proved that the common protein of Hyalomma are 4 bands and for Rhipicephalus are 2 bands. However, El Kady[7] proved the presence of 5 protein bands for *H. anatolicum* and 4 for Ripicephalus . Recently, Norouzi F[26] proved the presence of 4 bands in *H. anatolicum* with a molecular weight (84/ 66/ 66/ 55 kDa respectively). In addition, they proved that the common protein has a 55 kDa molecular weight during detecting the antigene of intestinal extract of *H. anatolicum.*

It is obvious from the above results that estimating biochemical parameters and electrophoresis can be used to distinguish between the tick species within the genus and between different genera. However, it appeared from above that *H. anatolicum* is more active tick if compared with *R. sanguineus* in the biochemical parameters tested.

Table1: the estimation of protein, carbohydrate, and lipid in two types of ixodid ticks.

H. anatolicum	4.93+104.120	0.009+ 0.6410 a	9.27+ 244.80 b
R. sanguineus	3.04+91.33 b	0.004+0.360 b	8.76+245.37 a

The different letters vertically indicates significant differences at $P<0.05$

Table 2: The isolated bands of protein and their molecular weight using electrophoresis using jell SDS-P

Tick species	Protein band	Relative distance	Log molecular weight	Molecular weight/Dalton
H. anatolicum	1	3.1	4.8819	76190.35
	2	4.6	4.7293	53616.69
	3	6.05	4.5817	38168.05
	4	9.6	4.2205	16614.98
R.sanguineus	1	3.25	4.8667	73560.87
	2	4.75	4.714	51760.68
	3	6.70	4.5156	32779.32

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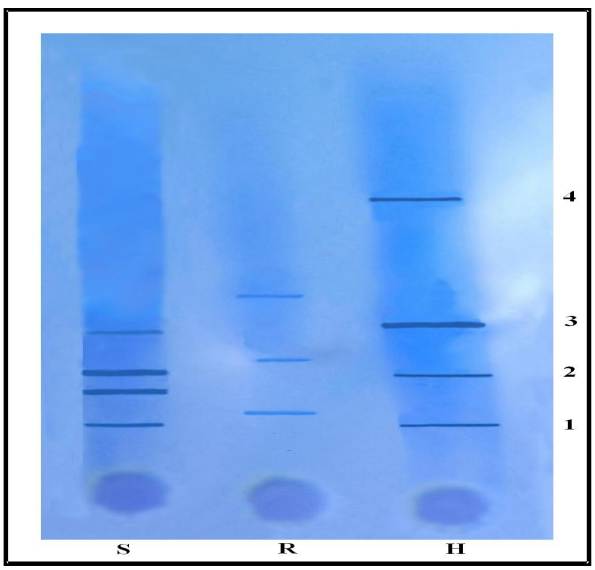

Fig 1: A photograph illustrate the isolated protein bands using electrophoresis with APGE jell with SDS
S: standard protein; H: *H. anatolicum* ; *R. rhipocephalus* ; 1,2,3,4: protein bands