Egypt. Acad. J. biolog. Sci., 2 (1): 33- 39 (2010) **E. Medical Entom. & Parasitology**
Email: egyptianacademic@yahoo.com **ISSN: 2090 - 0783**
Received: 29/6/2010 **www.eajbs.eg.net**

Protein patterns of Admiral- treated and untreated house dust mites: *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (*Acarina: Pyroglyphidae*)[1]

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ABSTRACT

The present work deals with the study of the effect of Admiral "pyriproxyfen" as a juvenile hormone analogue (JHA) on the protein patterns of the two common house dust mites (HDM): *Dermatophagoides pteronyssinus* and *D. farinae*. Analysis revealed lower protein content in either JHA-treated or untreated tritonymphs than in the respective adults. JHA treatment affected protein concentration of both tritonymphs (significantly increased) and adults (significantly decreased). Significantly lower protein contents were detected for *D. farinae* than for *D. pteronyssinus* either in normal or JHA-treated individuals. A total of 49 bands with molecular weights of 10-108 Kilo Daltons (KD) and relative mobilities of 0.99-0.16 were electrophoretically separated from the whole body tissue extracts of normal and treated tritonymphs and adults of the two species. Four common bands (12-60 KD) were suggested as genus-specific. Three *D. pteronyssinus* proteins (21-33 KD) and one *D. farinae* protein (69 KD) were suggested as species- specific. The HDM allergen (14-15 KD) was detected in the two mite species. Several proteins that detected in normal individuals of the two species disappeared in the respective treated individuals that may be due to the effect of treatment with such JHA.

Key Words: House dust mites, *Dermatophagoides pteronyssinus*, *D. farina*, Protein patterns, Juvenile hormone analogues, Pyriproxyfen, *Admiral*.

INTRODUCTION

The house dust mites (HDMs) are of great medical importance due to their incrimination in causing house dust allergy (Sears *et al.,* 1989 and Yong and Jeong, 2009). They are considered as important sources of indoor allergens associated with bronchial asthma (Milian and Diaz, 2004; Abou Gamra, *et al*., 2005 and O'Neil *et al.* 2006) and other allergic conditions like rhinitis and atopic dermatitis (Gamal-Edddin *et al.,* 1982; Beck and Korsgaard, 1989; Morsy *et al*., 1994 and Oshio, *et al*. 2009)

In Egypt, of the different HDM forms, *Dermatophagoides farinae* Hughes "the American HDM" and *D. pteronyssinus* (Trouessart) "the European HDM" of the family *Pyroglyphidae* are the most common species and important as causative of asthmatic bronchitis (Morsy *et al.,*1995 and El-Shazly *et al*., 2006). Such bronchial asthma forms a problem. It was suggested (Gamal-Edddin *et al.,* 1982) that the geographical situation of Egypt and its favorable climatic conditions together with other factors may play a major role in abundance of HDM and consequently HDM allergy occurs more common than to any other allergen in the Egyptian asthmatic patients (Gamal-Edddin *et al.*, 1985).

The juvenile hormone analogue (JHA), Admiral "pyriproxyfen", as an insect growth regulator acts by suppressing embryogenesis (ovicidal activity), inhibiting metamorphosis and adult emergence and suppressing hatching of eggs (sterile activity) of the target insect . It is widely used for controlling several pests of medical and

The first international Conference of Biological Sciences
27-29-Sep. 2010 Cairo – Egypt
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veterinary importance (e.g., Yapabandara and Curtis, 2002 and Mollina *et al.,* 2006).

In Egypt, in spite of the extensive studies on HDMs, very few studies (Mazyad *et al.*, 2006) were carried out to investigate the effect of Admiral, or other JHA on protein patterns of these two important HDMs. For this, the present study was planned to compare the protein patterns of the admiral-treated and untreated *D. pteronyssinus* and *D. farinae* and objected at investigating the efficiency of Admiral which acts as protein de-naturing agent, in reducing the allergenicity of these two important mite species.

MATERIALS AND METHODS

Mites

Mite individuals of *D. pteronyssinus* (*Dp*) and *D. farinae* (*Df*) from laboratory colonies raised on powered macaroni as a feeding medium were used for experimentation. For comparison, tritonymphs and adults of the two mite species were kept on the feeding media treated with the predetermined LC_{50} of Admiral (Kenawy *et al*., 2007).

Preparation of the mite samples

Samples of the mite body tissues (one gm. each) of admiral-treated and untreated (normal) tritonymphs and adults were separately homogenized in a cold glass homogenizer containing 1 ml. of coca solution (4 gm. phenol per liter of 0.9 normal saline). Samples were then centrifuged at 5000 r.p.m. for 5 minutes. The supernatants were transferred each to an Eppendorf tube (3 ml. volume) and kept freezing at –20°C until used for protein analysis.

Determination of the total protein concentrations

The total protein concentrations (mg. / gm. tissue) were determined by a dye-binding assay (Bradford, 1976) based on the changing color of a dye reagent according to the different protein concentrations. A commercially available kit (Pointe Scientific INC, Johna, Lincoln park, Michigan 48146 USA) was used. The protein concentration in each mite sample was measured spectrophotometrically against a standard of bovine gamma globulin at 590 nm.

Separation of proteins

Proteins of the different body extracts of the two mite species were separated using polyacrylamide gel electrophoresis (PAGE) following the method described by Abdel-Hamid (1996). Prior to electrophoresis, the total protein of each sample was adjusted to 1mg. protein / ml by dilution with solibilization buffer (pH 6.8). Low (25-40 KD) and high (10-116 KD) molecular weight standards were prepared in the solibilization buffer for determination of the weights of proteins on the gel (Lambin *et al.* 1976). Electrophoresis was done in 1 mm-thick slabs which consisted of a resolving gel containing 8% acrylamide and a stacking gel containing 4% acrylamide. Bromophenol blue was used as a tracking dye. Twenty µl of each sample were added to each well of the gel. Two or 3 wells in each slab were used for the molecular weight standards and the blank. Electrophoresis was run out at 27 ± 2°C at 20 mA / plate for about 4 hours. After electrophoresis, the protein fractions were stained with COBB (coomassie brilliant blue). The relative mobility (Rm) of the protein bands were calculated from [Rm = distance migrated by the protein band / Distance migrated by the tracking gel]. The densitometric scanning of the separated protein patterns were made to estimate the relative molecular weights of the identified bands using Epson GT-9500 scanner.

Statistical analysis

Means and standard deviations (SD) of protein concentrations were computed and compared by the one-way Analysis Of Variance (ANOVA). For more than two sets of data and if means were significantly different, they were further exposed to multiple pairwise comparisons by Bonferroni method. All analysis were carried out using GPIS (Graph Pad In Stat, version 1.0 by H. J. Motulsky) computerized program. Whatever, the significance levels, these were restricted to a maximum of $P< 0.01$.

RESULTS

Total protein concentrations

The concentrations of the total protein (mg./ gm. tissue) were spectrophotometrically determined and compared for admiral-treated (at LC_{50}) and untreated tritonymphs and adults of the two mite species (Tables 1 & 2).

Protein patterns

A total of 49 bands (M.wt. 10-108 KD, Rm 0.99-0.16) were electrophoretically separated from the whole body tissue extracts of normal and admiral-treated tritonymphs and adults of *Dp* and *Df* by using COBB stain (Fig. 1).

Table 1: Total protein concentrations (mg. / gm. tissue) in normal and admiral-treated *Dermatophagoides pteronyssinus* and *D. farinae*.

Stage	Mean±SD [1,2] / Range			
	D. pteronyssinus		*D. farinae*	
	Normal	Treated	Normal	Treated
	Tritonymph			
Female	85.3±4.0[a] 81–89	95.3±5.0[a] 90–100	77.0±4.6[a] 72–81	86.0±5.6[a] 80–91
Male	85.7±5.9[a] 79–90	95.7±7.0[a] 89–103	79.3±6.5[a] 73–86	87.0±5.6[a] 81–92
All	85.5±4.5[a] 79–90	95.5±5.5[a] 89-103	78.2±5.2[a] 72–86	86.5±5.0[a] 80–92
	Adult			
Female	206.7±8.0[b] 199–215	194.3±6.1[b] 183–197	183.3±6.0[b] 177–189	151.3±10.6[b] 140–161
Male	208.7±10.5[b] 198 – 219	194.3±6.1[b] 189– 201	185.0±7.0[b] 180–193	149.3±6.5[b] 143–156
All	207.7±8.4[b] 198–219	192.8±6.3[b] 183–201	184.2±5.9[b] 177–193	150.3±7.9[b] 140-161

1-Mean value of 3 replicates x 1 gm. tissue each, SD: Standard Deviation.
2- Vertically, means with the different letters are significantly different (Bonferroni method, $P<0.01$); Conclusion: (Tritonymph ♂ = Tritonymph ♀ = All tritonymphs) < (Adult ♂ = Adult ♀ = All adults).

Table 2: Analysis of variance for comparison of the protein concentrations in normal and admiral-treated stages of *Dermatophagoides pteronyssinus* and *D. farina*

Stage	Normal x Treated		*pteronyssinus* x *farinae*	
	pteronyssinus	*farinae*	Normal	Treated
	ANOVA: *F* (*d.f.*)[(1)]			
	Tritonymph			
♂	7.20 (1,4)[ns]	4.67 (1,4)[ns]	5.58 (1,4)[ns]	4.64 (1,4)[ns]
♀	3.56 (1,4)[ns]	2.40 (1,4)[ns]	1.57 (1,4)[ns]	2.81 (1,4)[ns]
All	12.95 (1,10)**	8.00 (1,10)*	6.83 (1,10)*	8.84 (1,10)*
	Adult			
♂	5.94 (1,4)[ns]	20.66 (1,4)**	16.23 (1,4)*	28.80(1,4)**
♀	4.17 (1,4)[ns]	41.78 (1,4)**	10.55 (1,4)*	76.26 (1,4)**
All	11.95 (1,10)**	70.06 (1,10)**	31.25 (1,10)**	105.80 (1,10)**

1 ns: not significant; *: $P< 0.05$; **: $P< 0.01$

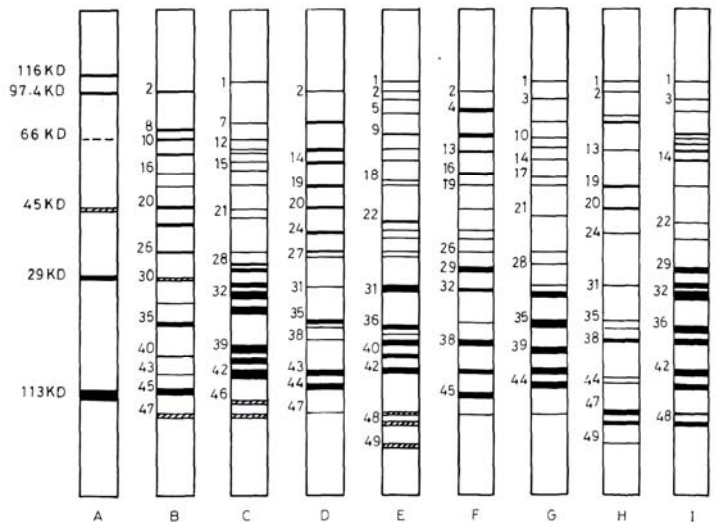

Fig. 1**:** Diagrammatic illustration of electrophoretic protein fractions of the normal (N) and admiral-treated (T) tritonymphs (Trny) and adults (Ad) of *D. pteronyssinus* (*Dp*) and *D. farina (Df)*: (A) Marker proteins, (B) N *Df* Trny, (C) N *Df* Ad, (D) N *Dp* Trny, (E) N *Dp* Ad, (F) T *Df* Trny, (G) T *Df* Ad, (H) T *Dp* Trny, (I) T *Dp* Ad.

DISCUSSION

It was observed that development from tritonymphs (normal or admiral-treated) to adults of the two species was accompanied by a significant increase (ANOVA, $P<0.01$) in the concentration of body proteins. Multiple comparisons however, revealed that within the tritonymph or adult groups, no difference (Bonferroni $P>0.05$) in protein concentrations of males and females of the same stage. This may indicate no sex-dependence of protein concentration in either tritonymphs or adults of the two examined species. From this, it can be concluded that the mean protein concentrations of the *Dp* or *Df* stages (treated or normal) can be arranged as follow: (tritonymph ♂ = tritonymph ♀ = all tritonymphs) < (adult ♂ = adult ♀ = all adults). According to Tovey *et al.* (1981), the whole body and cuticles of adult mites contain the major proteins (which act as allergens) than those of the other stages and their eggs contain a very little amount of proteins.

It was reported (Leysen *et al.,* 1974 and Mitchell *et al.,* 1985) that admiral as an insect growth regulator has similar effects on mite's development as natural JH. In general, JH is present in the mites for completing progression from one stage to another. However, admiral applied to the tritonymphs of the two species resulted in disturbance of their hormonal balance, and this, in turn, affected the protein synthesis in the tritonymphs (significantly increased, $P<0.01$ for *Dp* and $P<0.05$ for *Df*) and in the developed adults (significantly decreased, $P<0.01$ for both *Dp* and *Df*) compared to the normal individuals. From such observation, the rate of asthma attack may be reduced due to decrease of the adult proteins and so, reduce the allergenicity of the dust mites after treatment with such JHA. Similarily, Mazyad *et al*. (2006) observed that the total protein concentrations and protein patterns of *D. farinae* were different in normal and admiral-treated mites and concluded that this may result in reducing the allergenic of treated mites

Significantly lower protein contents ($P<0.05$/$P<0.01$) were detected for *Df* than for *Dp* either in normal individuals or admiral-treated ones (except for tritonymphs (♂ or ♀) of normal or treated groups, $P>0.05$). This may indicate more alergenicity of *Dp* than *Df*. Similarly, Guerin *et al.* (1992) determined the relative importance of *Dp* and *Df* as a cause of allergic sensitivity and asthma in Mauritius and concluded that the *Dp* is the principal one.

In the present study, a total of 49 bands (10-108 KD) were separated from the whole body tissue extracts of normal and admiral-treated tritonymphs and adults of *Dp* and *Df*. Approximately a similar molecular weight distribution (10-110 KD) was reported by Munhbayarlah *et al.* (1998). Hong *et al.* (1991) separated over 30 protein bands of the whole body extract of *Df* with M.wt between 93 and 12 KD. Musken *et al.* (2003) observed that extracts of *Dp*, *Df* and 10 other mite species showed a complex protein pattern in the M.wt. range of 95 to 10 kD.

The protein components of the two species were somewhat different however, with a small component showed identical molecular weights. Four protein bands (M.wt. 12-60 KD and Rm 0.92-32) were commonly detected in the normal stages of both mite species. This may suggest that these proteins are characteristic for the genus *Dermatophagoides*. On the other hand, three protein bands (M.wt. 21-33 KD and Rm 0.75-0.56) and one band (M.wt. 69 KD and Rm 0.29) were detected only in all normal individuals of *Dp* and *Df*, respectively. Such results may suggest that these bands may be specific for the respective species. Such suggestion, however require further confirmation though comparison with the other HDM genera and species.

Several protein bands were detected in normal individuals of the two species which disappeared in the respective admiral-treated individuals. For purpose of comparison to detect any effect of the JHA on protein patterns, the protein bands of tritonymphs and adults were accumulated. For *Dp*, eight bands (M.wt. 19-102 KD and Rm 0.79-0.18) were detected in normal individuals and disappeared in the treated ones which showed other eight bands (M.wt. 11-108 KD and Rm 0.94-0.16). For *Df*, fourteen bands (M.wt. 13-81 KD and Rm

0.89-0.27) were detected only in normal individuals compared to twelve bands (M.wt. 15- 93 KD and Rm 0.85-0.20)

detected only in the treated individuals. Such bands that newly appeared or completely disappeared in admiral-treated individuals of the two mite species may be due to the influence of such treatment with the JHA which is generally known to cause disturbance of the hormone balance, and may in turn, affects the protein synthesis in the tritonymphs and adults. However, with unavailability of previous observations, such changes in protein pattern associated with admiral treatment could not be proved, the case which requires further investigation.

The active HDM allergens as defined by several authors are low molecular weight proteins or glycoproteins. Hong *et al.* (1991) and Musken *et al.* (2003) indicated that the common and major allergenic component of the whole body extract of *Df* and *Dp* and majority of other studied species was the protein of M.wt. 14-15KD, which was detected in most of the patients' sera sensitive to house dust mites. In dogs sensitized with HDMs, Yamashita *et al.* (2002) identified antigenic proteins of HDMs (15-170 kD) among them, the 15 kD protein that might be identical to group 2 antigens (Der f 2, Der p2) was prominently observed. In the present study two bands with M.wt of 14 and 15 KD (no 44 & 45; Rm 0.85 and 0.87, respectively) were more or less detected in normal and admiral-treated individuals of the two mite species. Since admiral acts as a protein de-naturing agent, it may changed the chemical nature of these two bands in treated individuals that may lead to reduced allergenicity of such important HDM species. Whether this is the case or not, it necessitates further studies. On the other hand, high molecular weight mite allergens with high frequency of IgE reactivity in sera of patients allergic to the 2 mite species were identified and characterized. Tsai *et al.* (1998) identified a 98 KD *Df* allergen and Lee *et al.* (2004) characterized a 103 KD *Dp* allergen. Such bands, however were absent among the 49 bands detected in the present study.

From the present observations, it can be concluded that the decrease of the adult proteins after treatment with such JHA may result in reducing the allergenicity of the treated dust mites.

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ARABIC SUMMARY

الطرز البروتينيه لحلم الغبارالمنزلى : *درماتوفاجويدس بتيرونسينسز* و *درماتوفاجويدس فارينى* (اكارينا: بيروجليفبدى) المعامل وغير المعامل بالأدميرال

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تم دراسة تأثير المشابه الكيماوى الصناعى لهرمون الشباب أدميرال (بيريبروكسفين) – والذى يعمل كمغيّر لطبيعة البروتين – على طرزالبروتين لحلم الغبارالاوروبى *درماتوفاجويدس بتيرونسينس* وحلم الغبار الامريكى *درماتوفاجويدس فارينى* وهما الأكثر شيوعاً فى منازل المرضى الذين يعانون من حساسية الصدر فى القاهرة. تم تقدير تركيز البروتين الكلى لكلٍ من الحوريات الثالثة و الطور اليافع المعاملين بالأدميرال (بالتركيز اللازم لتثبيط ٥٠%) والغير معاملين لكلا النوعين من الحلم. اظهر التحليل انخفاض محتوى البروتين الكلى فى الحوريات الثالثة (المعاملة او غير المعاملة) لكلا النوعين عنه فى الطور اليافع. ادت المعامله بالأدميرال الى زيادة تركيز البروتين فى الحوريات الثالثة ونقصانه فى الطور اليافع. بمقارنة النوعين اتضح أن تركيز البروتين الكلى فى د. فارينى (المعامل وغير المعامل) أقل منه فى د. *بتيرونسينس*. بدراسة نماذج البروتين البسيط امكن كهربائياً فصل ٤٩ شريط بروتينى (تتراوح أوزانها الجزيئية بين ١٠-١٠٨ كيلو دالتون وحركاتها النسبية بين ٠.١٦ و ٠.٩٩) من مستخلصات أنسجة جسم الطور اليافع والحوريات الثالثة المعاملة وغير المعاملة بالأدميرال لكلا النوعين. تبين اشتراك كلا النوعين من الحلم فى وجود ٤ شرائط بروتينة (١٢-٦٠ كيلو دالتون) مما يقترح انه من الممكن ان تكون هذه الشرائط مميزة للجنس *درماتوفاجويدس*، *كما* تبين ايضا وجود ٣ شرائط بروتينة (٢١-٣٣ كيلو دالتون) مميزة للحلم د. *بتيرونسينس* بينما شريط بروتينى واحد (٦٩ كيلو دالتون) كان مميزا للحلم د. فارينى. تبين وجود عدة شرائط بروتينة فى الافراد الغير معاملة فى كلا النوعين من الحلم ولكنها اختفت فى مثيلاتها من الافراد المعاملة بالادميرال والعكس بالعكس. هذه الشرائط التى ظهرت أو اختفت بالكامل فى الافراد المعاملة بالادميرال فى كلا النوعين من الحلم ربما بسبب تأثير المعاملة بالمشابه الكيماوى لهرمون الشباب.