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
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 LC50 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 \[Rm = \text{distance migrated by the protein band} / \text{Distance migrated by the tracking gel}\]. 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 GOPSIS (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 LC50) 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.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Normal</th>
<th>Treated</th>
<th>Normal</th>
<th>Treated</th>
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<tbody>
<tr>
<td>Tritonymph</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Female</td>
<td>85.3±4.0a</td>
<td>95.3±5.0a</td>
<td>77.0±4.6a</td>
<td>86.0±5.6a</td>
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<tr>
<td>Male</td>
<td>85.7±5.9a</td>
<td>95.7±7.0a</td>
<td>79.3±6.5a</td>
<td>87.0±5.6a</td>
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<tr>
<td>All</td>
<td>85.5±4.5a</td>
<td>95.5±5.5a</td>
<td>78.2±5.2a</td>
<td>86.5±5.0a</td>
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<tr>
<td>Adult</td>
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<td></td>
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<tr>
<td>Female</td>
<td>206.7±8.0b</td>
<td>194.3±6.1b</td>
<td>183.3±6.0b</td>
<td>151.3±10.6b</td>
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<tr>
<td>Male</td>
<td>208.7±10.5b</td>
<td>194.3±6.1b</td>
<td>185.0±7.0b</td>
<td>149.3±6.5b</td>
</tr>
<tr>
<td>All</td>
<td>207.7±8.4b</td>
<td>192.8±6.3b</td>
<td>184.2±5.9b</td>
<td>150.3±7.9b</td>
</tr>
</tbody>
</table>

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

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

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. Similarly, 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 allergenicity 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.

REFERENCES


Protein patterns of Admiral- treated and untreated house dust mites


ARABIC SUMMARY

الطرز البروتيني لحلم الغبار المنزلي: Dermatophagoides farinae و Dermatophagoides pteronyssinus (آكارينا: بيروجلود)، المعامل وغير المعامل بالأدميرال فاري (がありました) – والذي يعمل كمعيّر لطبيعة البروتين – على طرز البروتين لحلم الغبار الأروبيي Dermatophagoides pteronyssinus وحلم الغبار الأروبيي Dermatophagoides farinae. بالتركيز الأمريكي، تم تقارير تركز البروتين الكلي لكل من الحوريات الثالثة والطور بالارتفاع المعاملتين بالأدميرال (بالتركيز 0.6 – 100% اللحوم لكل نوع عالٍ من الحلم. أظهرت التحليل انسحاب محتوى البروتين الكلي في الحوريات الثالثة (المعاملة غير المعاملة) كلا النوعين على الطور الباقم. أدت المعاملة بالأدميرال إلى زيادة تركيز البروتين في الحوريات الثالثة وقصاصاته في الطور الباقم. بقارطة النوع، اكتشف أن تركيز البروتين الكلي في د. فاري (المعامل وغير المعامل) أقل من د. پيرونيسيس. بدراسة مثالي البروتين، البديل أكا. كهربانيا فيصل 49 شريط بروتيني (تراوح أوزانها الجزيئية بين 100 و 1000 كيلو دالتون) وعشرات النسبية بين 16.99.0% من مستخلصات نسج طور الحوريات الثالثة المعاملة وغير المعاملة بالأدميرال لكل نوعين. تم اشراك كلا النوعين من الحلم في وجود 4 شرائح بروتينية (1000 كيلو دالتون) مما يقترح أنه من الممكن أن تكون هذه الشواطئ مميزة للحسين Dermatophagoides farinae، كما تبين أيضا وجود 3 شرائح بروتينية (199 كيلو دالتون) معيدة للحلم د. بيرونسيس بينما شريط بروتين واحد (39 كيلو دالتون) كان معبرا للحلم د. فاري. تبين وجود عدة شراط بروتينية في الأفراد المعرضين لحمل في كلا النوعين من الحلم. ولكلها احتفظت في مثبتاتها من الأفراد المعاملة بالادميرال والعكس بالعكس. هذه الشراط التي ظهرت أو احتفظت بالدائم في الأفراد المعاملة بالادميرال في كلا النوعين من الحلم ربما بسبب تأثير المعاملة بالمشابك الكيمياء لهرمون الشباب.