

Identification and characterization of important cockroach allergens in Egypt.

A Thesis Presented
To the
Department of Entomology
Faculty of Science, Ain Shams University
For the Award of **the Ph.D. Degree**
(In Entomology)

By
Weaam Ali Mahmoud Kamel
(M.Sc.)

Research Assistant, Allergy & Immunology Center,
Al Azhar University.

Supervisors

Prof. Dr. Adel Ibrahim Merdan

Prof. of Entomology, Faculty of Science,
Ain Shams University.

Prof. Dr. El-Desouki E. Fouda

Prof. of internal medicine, Director of
Allergy & Immunology Center,
Al-Azhar University .

Dr. Eman Mohamed Labe

Assistant Prof. of Entomology, Faculty of Science,
Ain Shams University.

Dr. Bouthaina Adel Ibrahim

Assistant Prof. of Entomology, Faculty of Science,
Ain Shams University.

Faculty of Science, Department of Entomology
Ain Shams University

2007

Thesis Examination Committee

Name

Title

Signature

Board of supervision:-

Prof. Dr. Adel Ibrahim Merdan

Prof. of Entomology, Faculty of Science,
Ain Shams University.

Prof. Dr. El-Desouki E. Fouda

Prof. of internal medicine, Director of
Allergy & Immunology Center,
Al-Azhar University .

Dr. Eman Mohamed Labe

Assistant Prof.of Entomology, Faculty of Science,
Ain Shams University.

Dr. Bouthaina Adel Ibrahim

Assistant Prof.of Entomology, Faculty of Science,
Ain Shams University.

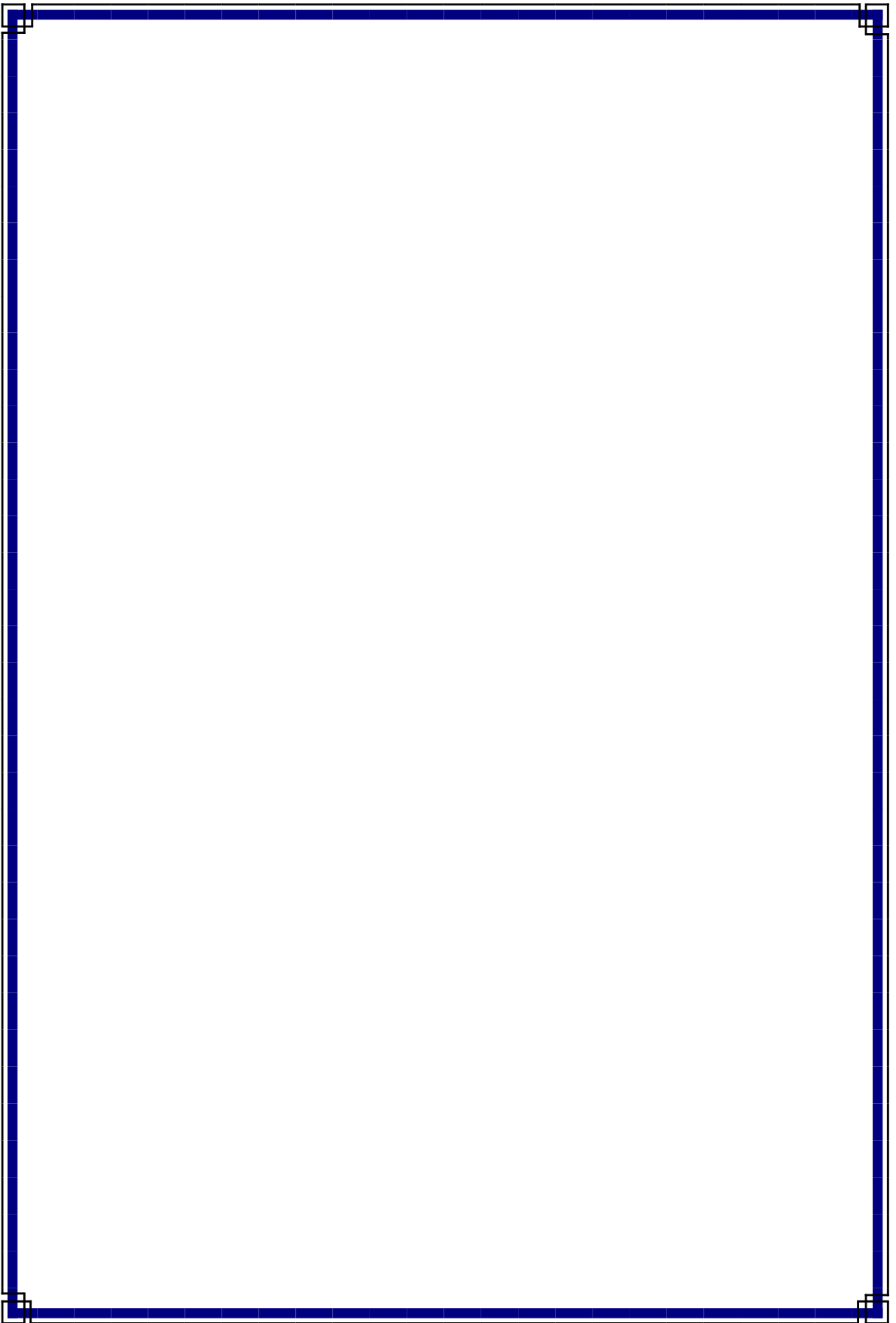
Biography

Name : Weaam Ali Mahmoud Kamel

Degree awarded : B.Sc. (Entomology-Chemistry)1981.
M.Sc. Entomology , 2001
Faculty of Science,
Ain Sham University,
Egypt.

Occupation : Research assistant, Allergy
& Immunology Center,
Al Azhar University .

Date of registration : October, 2002 .
For Ph.D. Degree



Abstract

The present study is concerned with evaluation the role of cockroach in allergy .This study revealed the identification, characterization and purification of the crude cockroach allergen extracts. The most important of the common native cockroaches are the American and German cockroach (*Periplaneta americana* & *Blatella germanica*), were used to prepare the crude allergen extracts collected from different household in low-cost public housing where all had visible cockroach infestation and higher cockroach index trapped, occupied by people with asthma and have detectable serum total IgE.Ab.(more than 150 IU/ ml). The collected cockroach whole body of both sexes were killed by freezing and then lyophilized to get dry powdered samples for the preparation of the crude cockroach allergen extracts. Total protein measurement of the local prepared crude cockroach allergen and the commercial crude cockroach (American and German cockroaches), was the same ranging between (2.8 - 3.0 mg/dl.) ,then the two allergens were diluted with phosphate buffer saline which were given the best result with analysis of Sodium DodecylSulfate - PolyAcrylamide Gel Electrophoresis (SDS.PAGE), compared with the total protein of the commercial one.The amino acids analysis of the local crude and the commercial were done, the results revealed that two crude cockroach allergen extracts were very closely related in presence of glycine amino acid, the most notable differences were found in other amino acids due to the commercial CR. mixed with phenol and glycerol. High Performance Liquid Chromatography (HPLC) analysis for both the local CR and the commercial one revealed many distinct peaks which are corresponding to each others, in which there were many peaks closely the same in retention time. The golden male hamster have been used as a model of human allergy because similarities exist in the response to a variety of contractile and relaxant substance. Animal models have been developed to investigate the histopathogenic role of cockroach allergens in inducing allergy.Male hamsters sensitized with the crude cockroach allergen extracts have great effect on changed skin features with separation of the sheet root of hair follicles. Degranulation of mast cells, increased eosinophil counts and increased production of Total IgE. Ab. Exposure and sensitization to cockroach appears to be linked to socioeconomic condition and low income families, most of whom reported cockroach infestation in their homes. So the cockroach has been recognized as an etiologic factor and demonstrated a health problem to allergic subjects living in cockroach-infested environments.

الخلاصة

استهدفت الدراسة الحالية تقييم دور الصرصور كمسبب للحساسية، وأوضحت الدراسة تعريف ودراسة الخصائص و تنقية الأنتيجين المسبب للحساسية المستخلص من الصرصور. وقد تم استخدام نوعان من الصراصير هما الصرصور الألماني و الأمريكي لتحضير المستخلص الخام المسبب للحساسية ، وقد تم تجميعهم من عدة منازل مختلفة يتواجد بها صراصير ويقطنها أفراد ذو مستوي معيشي متدني وقد لوحظ عند تحليل الدم لمعظم هؤلاء الأفراد وخاصة الاطفال أن مستوي الأمينجلوبولين الكلي إيه (IgE) أعلى من نسبة المعدل العادي وهو أكثر من (150 وحدة عالمية / مل). يتم تجميد ثم تجفيف أجزاء الجسم كاملا ذكر أو انثى للصراصير المجمعة للحصول على بودرة جافة لتحضير المستخلص المسبب للحساسية . تم قياس البروتين لهذا المستخلص المحضر و المستخلص المستورد من الخارج لكل من الصرصور الأمريكي والألماني، فكانت القراءات لكلاهما تتراوح بين (2.8- 3.0 مليجرام/دسم) ، ثم تخفيف المستخلصين بمحلول ملحي حيث أعطت أفضل النتائج عند التحليل بالهجرة الكهربية ، مقارنة بقياس البروتين الموجود في المستخلص المستورد. تم تحليل الأحماض الأمينية لكل من المستخلص المحضر بالدراسة والمستورد وأعطت النتائج وجود حمض الجليسين الأميني في كلاهما ، كما ظهرت بعض الأختلافات في أنواع اخرى للأحماض الأمينية. تم عمل التحليل الكروتوجرافي لكلا من المستخلصين ، أوضحت النتائج عدة نقاط متطابقة لكلاهما. تم استخدام حيوان التجارب ذكور فئران الهامستر الذهبي كمثال شبيهة للإنسان بتأثرة بمسببات الحساسية وأيضا لتأثرة الإيجابي للمواد المقلصة والمهدئة ، وذلك لمعرفة مدى تأثرة بمسببات الحساسية ضد الصراصير. لذا تم حقن ذكور الفئران بالمستخلص المسبب للحساسية ضد الصراصير وقد اعطت النتائج ظهور اختلافات واضحة في تركيب الجلد الداخلي مع حدوث إنشقاق في أماكن منبت الشعر الداخلي وتمزق للخلايا المستتية وزيادة عدد خلايا الازينوفيل مع ارتفاع في نسبة قياس الامينوجلوبين إي (IgE) في الدم للفئران أعلى من المستوى الطبيعي 0 وخلصت النتائج في هذه الدراسة أن التعرض والاستجابة لمسببات الحساسية ضد الصراصير له علاقة بالمستوى المعيشي المتدني والدخل البسيط للعائلات . حيث أنه من الصعب عند السيطرة على تجمعات الصراصير تقليل مستوى الحساسية ، لذا فإن الصرصور يعتبر من العوامل البيئية المسببة للحساسية و المشاكل الصحية التي تصيب الأفراد الذين يعيشون في بيئة يتواجد بها الصراصير.

Contents

	Page
Abstract -----	
List of Figures -----	
List of Tables -----	
List of abbreviations -----	
I-Introduction -----	1
II- Literature Review -----	4
- Antigens -----	4
- Allergens -----	5
Cockroach and allergy-----	6
- Socioeconomic status and race as risk factors for cockroach allergen exposures -----	14
- Preparation of cockroaches allergen extracts-----	20
- Sensitization with cockroaches allergen extracts -----	24
-Sodium DodecylSulfate Poly Acrylamide Gel Electrophoresis (SDS-PAGE)-----	29
-Amino acids-----	31
- High Performance Liquid Chromatography (HPLC)-----	32
-Atopy -----	33
-Atopic dermatitis -----	34
- Immunologic mechanisms of tissue damage-----	35
- Animals models-----	36
-Eosinophil-----	38
- Mast cells-----	40
-Total Immunoglobuline E.(IgE. Ab.)-----	41
III- Materials and Methods -----	46
Test insect-----	46
1- Sampling techniques-----	48
2- Extraction of cockroaches allergens -----	48
3 –Characterization of cockroach allergen extracts -----	50
A- Determination of antigenic property of cockroach allergen extracts with human serum antibodies -----	50

B-Protein concentration -----	52
C- Detection of protein by (SDS-PAGE)-----	54
D-Amino acids analysis-----	58
E- High Performance Liquid Chromatography (HPLC)-----	58
F-Clinical significance and immune response to	
-cockroach allergens (golden male hamster)-----	61
-Experimental design-----	65
IV- Results-----	80
-Identification and characterization of cockroach allergens-----	
A-Antigen- antibody reaction -----	83
B-Protein concentration -----	83
C-Detection of protein by (SDS-PAGE)-----	86
D-Amino acids analysis-----	94
E-High Performance Liquid Chromatography (HPLC)-----	98
F-Clinical significance and immune response to	
cockroach allergens-----	104
-Histological studies on section of male hamster's skin-----	104
The behavior of experimental animals-----	106
- Mast cells. -----	116
Determination of Eosinophil Count -----	120
-Immunological studies-----	122
Determination of total serum IgE-Ab-----	122
-Determination of antigenic property of cockroach allergen	
extracts with hamsters serum antibodies -----	126
V- Discussion and Conclusion-----	129
VI-English Summary-----	139
VII- References-----	145
Arabic Summary.	
Arabic Abstract .	

List of Figures

Figure 1	: Dynex instrument used in ELISA reader	53
Figure 2	: Rearing cage of the golden male hamsters and the balance for weighing the tested animals	64
Figure 3	: The steps followed in staining of the paraffin section taken from male hamster's skin with Hematoxylin and Eosin stain	68
Figure 4	: Mast cell, it's small cells, may be oval, rounded or irregular in shape. It's usually present in groups around blood vessels	71
Figure 5	: The method of spreading of blood film to stain it with Leishman's stain for counting of Eosinophils.....	75
Figure 6	: Microscopic field from blood film showing the Eosinophil content.....	75
Figure 7	: Total number of the trapped cockroaches / month of the year which were collected from different households.....	82
Figure 8	: Showing a comparison between antigenic property of cockroach allergen extracts with normal human serum antibodies compared with patient serum total IgE-Ab.....	85
Figure 9	: A photograph of SDS-PAGE of separated components of crude cockroach extract	87

Figure 10	:	Molecular weight analysis of Lane 1 for gel marker (high & low, M.Ws.) as standard	88
Figure 11	:	Molecular weight analysis of Lane 2 for commercial crude cockroach allergen extracts contained 0.5 mg/ml of protein.....	89
Figure 12	:	Molecular weight analysis of Lane 3 for the local prepared crude cockroach allergen extracts contained 0.4 mg/ml of protein.....	90
Figure 13	:	Molecular weight analysis of Lane 4 for the local prepared crude cockroach allergen extracts contained 0.5 mg/ml of protein....	91
Figure 14	:	Molecular weight analysis of Lane A for the local prepared crude cockroach allergen extracts contained 0.5 mg/ml of protein....	92
Figure 15	:	Molecular weight analysis of Lane G for the local prepared crude cockroach allergen extracts contained 0.5 mg/ml of protein....	93
Figure 16	:	Amino acids analysis of the commercial crude cockroach allergen extracts.....	95
Figure 17	:	Amino acids analysis of the local prepared crude cockroach allergen extracts.....	97
Figure 18	:	Purification of the commercial crude cockroach allergen extracts by HPLC gel filtration and fractions were analyzed for protein content.....	99
Figure 19	:	Purification of the local prepared crude cockroach allergen extracts by HPLC gel filtration and fractions were analyzed for protein content.....	100

Figure 20	: Retention Time is compared between the local prepared crude cockroach extracts and the commercial crude cockroach extracts with the percentage of Peak Area	103
Figure 21	: A photomicrograph of a T. section of normal control male hamster's skin group one.....	107
Figure 22	: A photomicrograph of a T.S. of normal control male hamster's skin group one showing the epidermis of the skin, the basal columnar cells with oval nuclei	108
Figure 23	: A photomicrograph (higher magnification of the previous) of control male hamster's skin group one showing a part of hair and erector pili muscle	109
Figure 24	: A photomicrograph of a T.S. of male hamster's skin group two which were injected weekly for three months with commercial crude extracts of cockroach	110
Figure 25	: A photomicrograph of a T.S. of male hamster's skin Group two which were injected weekly for three months with commercial crude extracts of cockroach.....	110
Figure 26	: A photomicrograph of a T.S. of male hamster's skin group two which were injected weekly for three months with commercial crude extracts of cockroach allergens showing abnormality of a hair follicle with a separation of a major part of the hair (h) and the coiled part.....	112
Figure 27	: A photomicrograph of a T.S. of male hamster's skin group three which were injected weekly for three months with the local prepared crude extracts of cockroach allergens	113
Figure 28	: A photomicrograph of a T.S. of male hamster's skin group three which were injected weekly for three months with the local prepared crude extracts	114

Figure 29	: A photomicrograph of a T.S. of male hamster's skin group three which were injected weekly for three months with the local prepared crude extracts.....	115
Figure 30	: A photomicrograph of a T.S. of normal control male hamster's skin group one showing group of mast cells	117
Figure 31	: A photomicrograph[higher magnification] of a T.S. of male hamster's skin group two showing distorted mast cells with deeply stained nuclei	118
Figure 32	: A photomicrograph[higher magnification] of a T.S. of male hamster's skin group three showing group of abnormal shaped triangular mast cells (arrow).....	119
Figure 33	: Showing a comparison between eosinophiles count in different male hamsters group.-----	121
Figure 34	: Standard curve of Total IgE-Ab. by ELISA method.....	123
Figure 35	: Mean value of total IgE Ab. reading by ELISA method of injected hamsters serum with the crude cockroach allergen extracts.....	124
Figure 36	: Showing a comparison between antigenic property of cockroach allergen extracts with hamsters serum antibodies compared with hamsters serum total IgE-Ab.-----	128

List of Tables

Table 1	: Total number of captured cockroaches from different households.....	81
Table 2	: Antigenic property of cockroach allergen extracts with human serum Ab. compared with human serum total IgE-Ab.....	84
Table 3	: There is a high significant difference between human serum antibodies compared with human serum total IgE-Ab.-----	85
Table 4	: The calculated molecular weight(MWs) analysis of Lane 1 for gel marker.....	88
Table 5	: The calculated molecular weight(MWs) analysis of Lane 2	89
Table 6	: The calculated molecular weight (MWs) analysis of Lane 3	90
Table 7	: The calculated molecular weight (MWs) analysis of Lane 4	91
Table 8	: The calculated molecular weight (MWs) analysis of Lane A	92
Table 9	: The calculated molecular weight (MWs) analysis of Lane G	93
Table 10	: Amino acids analysis of the local prepared crude cockroach allergen extracts	96
Table 11	: Amino acids analysis of the commercial crude cockroach allergen extracts	96

Table 12	:	Amino acids types related to different retention times and concentrations of the local prepared crude cockroach allergen extracts.	97
Table 13	:	HPLC gel fractionated of the commercial crude cockroach allergen extracts.	99
Table 14	:	Purification of the local prepared crude cockroach allergen extracts by HPLC gel filtration.....	101
Table 15	:	Purification of the commercial crude cockroach allergen extracts by HPLC gel filtration.....	101
Table 16	:	Purification of the local prepared crude cockroach allergen extracts and * the commercial HPLC gel filtration and fractions were analyzed for the protein content.....	102
Table 17	:	There is a high significant difference of eosinophiles count between group of male hamsters group two & three compared with group one by using t. test at $P=0.001$.-----	121
Table 18	:	The statistical analysis of total serum IgE-Ab. showing the significant T. calculated during the experimental time	125
Table 19	:	Antigenic property of cockroach allergen extracts with hamsters serum Ab. compared with hamsters serum total IgE-Ab.....	127
Table 20	:	There is a high significant difference between human serum antibodies compared with human serum total IgE-Ab.-----	128

List of Abbreviations

In the present study the following abbreviations were used :

Ab	: Antibody.
Ag	: Antigen.
AST-CR	: Allergy skin test for cockroach.
AWBE	: American cockroach whole body extract.
BAL	: Bronchoalveolar Lavage .
BALF	: Bronchoalveolar Lavage Fluid .
Bp	: Base pairs.
BSA	: Bovine Serum Albumin.
CRa-M	: Mixture of equal of CRa-A & CRa-G .
CIE	: Crossed immunoelectrophoresis .
CV	: Coefficient of variation .
Der p I	: Antigen P ₁ of <i>Dermatophagoides pteronyssinus</i>
Der f	: Antigen F ₁ of <i>Dermatophagoides farinae</i> .
Der m	: Antigen M ₁ of <i>Dermatophagoides microceras</i> .
EDTA	: Ethylenediamine tetraacetic acid.
ELISA	: Enzyme-linked immunosorbent assay .
FAST-CR	: Fluorescence allergosorbent test for cockroach .
FEV	: Forced expiratory volume .
GST	: Glutathione transferase.
HPLC	: High performance liquid chromatography.
IgE-Ab	: Immunoglobuline E. Antibody .
PAGE	: Polyacrylamide gel electrophoresis .
PBS	: Phosphate-buffered saline.
KDa	: Kilo dalton = 10 ³ daltons .
RAST	: Radioallergosorbent test .
RIA	: Radioimmunoassay .
SDS	: Sodium dodecyl sulphate .
TBs	: Tris-buffer saline .
TEMED	: N,N,N',N'-tetramethylene diamine .