

**ROLE OF MOSQUITO SALIVARY GLAND  
SECRETIONS ON BLOOD FEEDING BY  
CULICIDAE (O:DIPTERA)**

*A thesis*

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*By*

***KHALED AHMED AL-BADAWI ISMAIL***

**B. Sc. (Entomology)**

**Department of Entomology-Faculty of Science-Ain Shams University**

**SUPERVISORS**

**\*PROF. DR. AHMED HASSAN KASHEF**

**Professor of Entomology-Faculty  
of Science-Ain Shams University**

**\*PROF. DR. BELAL AHMED SOLIMAN**

**Professor of Entomology- Dean of  
Faculty of Education-Suez Canal  
University- Suez**

**\*PROF. DR. AMR AHMED FATHY**

**Professor of Clinical Pathology-Head of Clinical Pathology  
Laboratories-Faculty of Medicine-Menofia University**

Cairo, Egypt

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

ان الله لا يضيع أجر من أحسن عملا

صدق الله العظيم

## **BIOGRAPHY**

**-DATE OF BIRTH** : 8 – 4 – 1966, Cairo.

**-DATE OF GRADUATION:** September 1988.

**-DEGREE AWARDED :** B. Sc. (Entomology-  
Chemistry)

**-GRADE** : Good.

**-OCCUPATION** : Assistant Researcher,  
medical analysis laboratory  
& blood bank, Tibah  
hospital, Abbassia, Cairo.

**-DATE OF APPOINTMENT:** 1990.

**-DATE OF REGISTRATION:** March 1991.

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## Abstract

The role of mosquito salivary gland secretion on human blood feeding process among three mosquito species, namely *Culex* (*Cx.*) *pipiens*, *Aedes* (*Ae.*) *caspius* and *Anopheles* (*An.*) *pharoensis* was investigated. The obtained results showed that the salivary gland extract did not induce blood agglutination. The whole blood clotting time was not affected by the addition of salivary gland extract. Platelet aggregation of human platelet rich plasma was decreased by the addition of salivary gland extract. The platelet aggregation inhibition was more pronounced in *An. pharoensis* than in *Ae. caspius* and *Cx. pipiens* indicating the presence of apyrase enzyme in the three studied species with different activities being higher in case of *An. pharoensis* than in *Ae. caspius* and *Cx. pipiens*. The salivary gland extract of mosquito species did not affect the human blood clotting factors (prothrombin, partial thromboplastin and fibrinogen). Probing and engorgement time of *An. pharoensis* was less than that of *Ae. caspius* and *Cx. pipiens*, confirming the presence of apyrase activity, though in different degrees. The present study elucidates the role of mosquito salivary secretions in facilitating blood feeding, by keeping blood flowing during the blood meal. Parasites may thus profit in order to achieve increased transmission, which eventually lead to enhance the role of mosquitoes as diseases vectors and expose human to infection risk.

**Key words:** mosquito, salivary gland secretion, Apyrase enzyme.

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## I. INTRODUCTION

Mosquitoes search for blood by repeatedly thrusting their mouthparts into the host's deep network of skin vessels (Gordon and Lunsden, 1939; Gordon and Crewe, 1948, Mellink *et al.*, 1982 and Ribeiro *et al.*, 1985). Saliva is ejected during this intradermal probing period. Small hemorrhages accumulate during probing, until the mouthparts eventually coming rest inside a venule, arteriole, or hematoma. Saliva of hematophagous arthropods contains substances that help blood feeding, by counteracting their host's hemostasis that prevent blood loss. Hemostasis is carried out through a redundant process involving platelet aggregation, blood coagulation and vessel constriction (Hugues, 1953, Roskam, 1954, Hutton, 1979 and Ribeiro *et al.*, 1991). Platelets play a central role in hemostasis by clumping within seconds and adhering to the injured blood vessels (Vargaftig *et al.*, 1981). Mosquitoes face their hosts' haemostatic mechanisms when attempting to feed on blood. Accordingly, they antagonize haemostasis by salivary agents that include anti-clotting, anti-platelet and vasodilatory compounds (Hajnicka *et al.*, 2000 and Ribeiro, 2000).

In vertebrates, aggregation largely determinates haemostasis (Mustard and Packkam, 1977 and Vargaftig *et al.*, 1981). Platelet aggregation is itself a redundant process and can be initiated by several agonists produced at the lesion site such as: adenosine diphosphate (ADP) (released by injured cells), collagen (exposed to platelets on the subendothelial structures of lacerated vessels) or thrombin. Because of the importance of ADP in platelet aggregation, many blood sucking arthropods contain potent salivary apyrase which degrade adenosine triphosphate (ATP) and ADP to adenosine monophosphate (AMP) and orthophosphate. These enzymes have been shown to have anti-platelet activity (Ribeiro, 1987 and 1989b).

Accordingly, the objective of the present study is to elucidate the role of mosquito salivary gland secretions on blood feeding process in comparative way between three species namely *Culex (Cx.) pipiens*, *Aedes (Ae.) caspius* and *Anopheles (An.) pharoensis*,

considering their importance as main vectors of filariasis (Gad *et al.*, 1994), Rift Valley fever (Turell *et al.*, 1996 and Gad *et al.*, 1999), and malaria (El Said *et al.*, 1986), in Egypt, respectively.

To achieve this goal the following aspects will be investigated:

1. The effect of mosquito saliva from different female species on:
  - 1.1 Blood agglutination.
  - 1.2 Blood clotting.
  - 1.3 Platelet aggregation.
2. Detection and measuring apyrase activity of mosquito saliva from different species and at various time intervals.
3. Probing and engorgement time of feeding mosquito females.

## II. LITERATURE REVIEW

### 1. Human Haemostatic Mechanism

**Hugues (1953)** reported that in the circulating blood, the platelets are normally carried along separately from each other and there is no evidence that they adhere to normal vascular endothelium. When the wall of a blood vessel is injured, platelets adhere to it immediately and to each other to form aggregates on the damaged intima.

**Roskam (1954)** reported that when the vessel wall is broken, aggregates of platelets tend to seal the opening and so help to arrest bleeding. Such aggregates have been called “haemostatic plugs”.

**Hutton (1979)** stated that in man and many other animals, haemostasis is achieved by a highly integrated process involving the blood vessels themselves, together with the blood platelets and a number of plasma proteins which participate in the coagulation and fibrinolytic pathways.

**Hajnicka *et al.* (2000)** detected that the saliva of mosquitoes contains potent immunomodulatory activities that counter their hosts haemostatic, inflammatory and immune responses to facilitate blood-feeding.

### 2. Effect of Mosquito Saliva from Different Species on Human Blood Agglutination

**Cornwall and Patton (1914)** demonstrated the presence of agglutinins in different mosquito species. They stated that the salivary gland secretion of both of *Ae. rossi* and *Ae. jameasi* induce a strong and immediate agglutination effect on the human red cells.

**Yorke and Macfie (1924)** detected the action of the salivary secretion of mosquitoes and *Glossina (G.) tachinoides* on human blood. They reported the degree of agglutination of the erythrocytes occurred on mixing an emulsion of salivary gland with citrated human blood. They found that in case of *An. maculipennis*, complete and immediate agglutination of the erythrocytes was observed, the agglutination of red cells due to salivary secretion of *An. maculipennis* takes place about equally well at 37°C as at laboratory temperature of about 15°C, while in the case of *Cx. pipiens*, *Theobaldia annulata*, *Stegomyia fasciata* and *G. tachinoides*, no trace of agglutination of the erythrocytes was seen.

**Nayar and Knight (1997a)** demonstrated hemagglutinins in the salivary gland extract from strains of the mosquito, *An. quadrimaculatus*, susceptible and refractory to the filarial parasite, *Brugia malayi*, having higher titers against human A+, B- and O+, and sheep erythrocytes than against rabbit and jird erythrocytes.

**Nayar and Knight (1997b)** determined hemagglutinins in six species of mosquitoes. They found that high titers of hemagglutinins were found in the salivary gland extract and in the body fluid of *Ae. taeniorhynchus* and *An. quadrimaculatus* but low levels of hemagglutinins were present in the body fluid of *Ae. aegypti*. Hemagglutinins activity was not found in the other three species of mosquitoes, *Cx. quinquefasciatus*, *Cx. nigripalpus*, and *Ae. albopictus*.

### **3. Effect of Mosquito Saliva from Different Species on Human Whole Blood Coagulation**

**Yorke and Macfie (1924)** incubated salivary gland extract of mosquitoes and *G. tachinoides* with human blood 1: 2 volumes in a water bath at 37°C, the control tubes containing one volume of physiological saline and two volumes of blood. In case of control tubes, blood was found clotted after five minutes. The salivary secretion of