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Biochemical changes of Culex pipiens females infected with Plasmodium cathemerium

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Abstract: *The present work aims to study the biochemical changes of Culex pipiens females infected with Plasmodium cathemerium. For achievement this research, females of Culex pipiens were infected with Plasmodium cathemerium to study the effect of Plasmodium on Culex pipiens females protein profile quantitative and qualitative at 12h, 1d, 4d, 5d and 6d post infection for mid-gut samples, while salivary glands collected after 3d, 4d, 5d, 6d and 7d post infection. The results showed a significant gradual decreases in total protein concentration within the infected samples at all tested times. In case of mid-gut-samples, the total protein concentration of the infected samples decreased on comparing with control samples with exception after 5 d.p.i. the total protein concentration increases, while, in case of salivary glands –samples total protein concentration of the infected samples decreased on comparing with control samples. Protein of Cx. pipiens (control and infected) were separated using Native and SDS-PAGE for mid-gut and salivary glands samples at different times postinfection. The results of native-PAGE demonstrated that there were differences in the overall protein banding pattern in the infected females as compared to control. The results of the SDS-PAGE clarified that the molecular weight of the separated proteins ranged from 494 to 12 KDa for mid-gut samples and from 147 to 8 KDa for salivary glands samples and Plasmodium infection had led to change in the proteins pattern of Culex pipiens mid-guts and salivary glands as compared to control.*

Keywords: *biochemical changes, Culex pipiens, Plasmodium cathemerium.*

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I. Introduction

Mosquitoes are indicated as hematophagous insects in the group of arthropods and they consider the most medically important arthropods for transmitting infectious diseases **(Youdeowei and Service, 1983).** They also act as major vectors for many diseases such as malaria, filariasis, dengue, Japanese encephalitis and other fevers (for example: Chikungunya, West Nile fever and yellow fever). More than half of the world's population lives under the risk of becoming infected by mosquitoes that carry the causative agents of such diseases. In terms of morbidity and mortality caused by vector-borne diseases, mosquitoes are the most dangerous animals confronting mankind. They threaten more than three billion people, in the tropical and subtropical regions and have also substantially influenced the development of mankind, not only socio–economically but also politically. Undoubtedly, insect-transmitted pathogens leading to epidemics and pandemics have been instrumental in the development, decline, and fall of empires, for, *e.g.* Greece and Rome. Malaria was the dominant health problem in the latter days of the Roman Empire. The Roman marshes were notorious for "malaria" (bad air) **(Bruce-Chwatt and de Zulueta, 1980).**

The mid-gut is the first insect tissue that the parasite contacts during its migration, and it is the first cellular barrier that the parasite must overcome to be transmitted to a new vertebrate host. It has been proposed that the creation and release of genetically altered mosquitoes, which are refractory to parasite or virus transmission, may be a promising new method for controlling the transmission of mosquito-borne diseases **(Crampton *et al.*, 1994; Gwadz, 1994 and Collins and Paskewitz, 1995).**

Mid-gut proteins of *An. tessellatus* and *Cx. quinquefasciatus* mosquitoes have been characterised by gel electrophoresis **(Ramasamy *et al.*, 1991)** but little is known of the glyco-protein constituents of the mid-gut.

Using an in vitro culture system, the migration of malaria ookinetes observed on the surface of the mosquito mid-gut and invasion of the mid-gut epithelium. Ookinetes display constrictions during migration to the mid-gut surface and a gliding motion once on the luminal mid-gut surface. Invasion of a mid-gut cell always occurs at its lateral apical surface. Invasion is rapid and is often followed by invasion of a neighboring mid-gut cell by the ookinete. The morphology of the invaded cells changes dramatically after invasion, and invaded cells

die rapidly. Mid-gut cell death is accompanied by activation of a caspase-3-like protease, suggesting cell death is apoptotic. The events occurring during invasion were identical for two different species of *Plasmodium* and two different genera of mosquitoes; they probably represent a universal mechanism of mosquito mid-gut penetration by the malaria parasite **(Zieler and Dvorak, 2000)**.

Identification and characterization of the molecules involved in the interactions between *plasmodium* and gut epithelium of malaria vectors is an important step in designing new vaccines. It has been demonstrated that various proteins on the mid-gut of *Anopheles* vectors are involved in invasion of ookinetes onto mid-gut epithelium, which is crucial for transforming *plasmodium* from ookinetes into oocysts. These processes are necessary to establish parasites inside the *Anopheles* body **(Carter *et al.*, 2007).** Different glycoproteins and proteins of the mosquito mid-gut are known as a receptor for the *plasmodium* ligands, including lamininγ1 **(Vlachou *et al.*, 2001 and Mahairaki *et al.*, 2005a)**, integrin **(Mahairaki *et al.*, 2005b)**, chondroitin sulfate proteoglycans **(Dinglasan *et al.*, 2007)**, carboxypeptidase B (CPB) **(Lavazec *et al.*, 2005)** and calreticulin (CRT) **(Rodriguez *et al.*, 2007).** *P. berghei* ookinetes interact with annexin B11 **(Kotsyfakis *et al.*, 2005)** and *P. berghei* P25 (Pbs25) binds laminin1, present in mid-gut basal lamina of *An. gambiae* **(Vlachou *et al.*, 2001)**, suggesting the existence of receptor–ligand interactions as part of the oocyst development. However, the molecular interactions involved in mid-gut internal surface recognition await description, and these findings in the rodent malaria model require validation in human malaria parasites.

Several studies have reported that glycan-proteins and proteins on the microvilli of the mid-gut of *Anopheles* mosquitoes are involved in the invasion of ookinetes to mid-gut epithelial cells and in the transformation of ookinetes into oocysts **(Wilkins and Billingsley, 2010).**

In a previous study **(Brennan *et al.*, 2000),** monoclonal antibodies (mAbs) against female-specific *An. gambiae* salivary gland proteins were tested for their ability to affect interaction between *Plasmodium* sporozoites and *An. gambiae* salivary glands. One such mAb (2A3), recognizing a ~100 kDa protein, resulted in approximately 73% reduction of the average number of sporozoites per infected salivary gland. Additional parasite ligands necessary for sporozoite invasion of the salivary glands have been identified, the thrombospondin-related anonymous protein (TRAP). TRAP is important for attachment to and invasion of salivary glands by parasites **(Ghosh *et al.*, 2009).** Other proteins that have a role in salivary gland invasion include cysteine repeat modular proteins and apical membrane antigen/erythrocyte binding-like (MAEBL) **(Kariu *et al.*, 2002 and Thompson *et al.*, 2007).** Attempts to identify candidate receptors for sporozoite invasion have been partially successful. A previous study reported the isolation of one such target antigen, *Ae. aegypti* salivary gland surface protein 1 (aaSGS1), and its apparent involvement in *P. gallinaceum* invasion and identified previously unknown *An. gambiae* homologues of aaSGS1 **(Korochkina *et al.*, 2006).** In a separate set of experiments, monoclonal antibodies raised against *A. gambiae* salivary glands led to the identification of a 50-kDa protein, termed saglin **(Brennan *et al.*, 2000 and Okulate *et al.*, 2007).** Saglin facilitates sporozoite invasion by interacting with TRAP **(Ghosh *et al.*, 2009).** While saglin may participate in the invasion of salivary glands by *Plasmodium* organisms, growing evidence suggests that several salivary gland molecules are likely to be involved in sporozoite entry into this organ. Further characterization of molecular interactions between sporozoite and salivary glands may clarify the invasion process and lead to novel targets to interrupt the malaria transmission cycle. **Montero-Solis *et al.* (2004)** identified a saliva soluble glycoprotein (Gp65-1) in *An. albimanus*, which is abundant and specifically expressed in the medial and distal lateral lobes of adult female glands. In overlay experiments, this molecule binds to a recombinant *P. vivax* circumsporozoite protein (CSP) repetitive region peptide. Using monoclonal antibodies, Gp65-1 was located on the nucleus of sporozoites resident in the secretory cavity of salivary glands. This location opens the possibility of a regulatory role in sporozoites maturation for Gp65-1.

Mosquito saliva and salivary glands are central to parasite-vector-host interactions with previous studies indicating that it can be exploited to impede malaria transmission in line with the "area-wide vector control" paradigm. Saliva contains pattern recognition proteins (PRPs) and potent antimicrobial proteins (AMPs) that may play a role in the regulation of mid-gut microbiota **(Rosinski- Chupin *et al.*, 2007);** serine proteases and protease inhibitors that may play a role in blood meal digestion in the mid-gut **(Arcà *et al.*, 2005);** and xanthurenic acid that has been shown to stimulate male gametocyte exflagellation in vitro **(Hirai *et al.*, 2001).** In addition, functional genomics and proteomics have availed a vast and verifiable platform of knowledge on the molecular components of mosquito saliva and their putative roles **(Francischetti *et al.*, 2002; Valenzuela *et al.*, 2003; Calvo *et al.*, 2004; Arcà *et al.*, 2005; Calvo *et al.*, 2006; 2007 and 2009).**

Materials And Methods

1- Insects and *Plasmodium* infection:

Mosquito samples were obtained from breeding habitat in Giza Governorate, Egypt. Mosquito larvae reared and colonized in the in sectary of the Department of zoology, Faculty of Science, South Valley University. Adults were identified morphologicaly using taxonomic keys of **Harbach (1985).** The stock colony

of the adult mosquitoes was maintained under laboratory conditions (27 ± 2 °C and 60-70% RH) for supplying clean adults of known ages, according to the method described by **Adham *et al.*, (2003).**

Mosquitoes adult females were allowed to feed fully on positive sparrows whose blood contains ripe gametocytes and negative sparrows (control samples) and held at 28 °C (the optimum temperature for sporogony).

2- Mid-gut and salivary glands sample collection:

Mid-gut samples collected after 12h, 1d, 4d, 5d and 6d post infection, while salivary glands collected after 3d, 4d, 5d, 6d and 7d post infection and used for biochemical studies. According to **Coleman *et al.* (2007),** dissection of mid-gut and salivary glands tissues from mosquitoes requires prior preparation of 1X Phosphate Buffered Saline (1X PBS) solution and anesthetization of mosquitoes by subjecting to a temperature of 4°C, until immobilized.

3- Quantitative Protein Analysis:

The total protein content of the mid-gut and salivary glands was quantified spectrophotometrically in both the control and *Plasmodium* -infected samples at 12h, 1d, 4d, 5d and 6d post infection for mid-gut samples, while salivary glands at 3d, 4d, 5d, 6d and 7d post infection according to the method described by **Bradford (1976)**. This method is based on the observation that Coomassie Brilliant Blue G-250 exists in two different colour forms, red and blue. The red colour is converted to the blue colour upon binding of the dye to protein. This binding causes a shift in maximum absorption of the dye from 465 to 595 nm. The intensity of the colour was measured at 595 nm.

4- Native Polyacrylamide Gel Electrophoresis (PAGE):

Native-PAGE of both control and *Plasmodium* -infected samples at 12h, 1d, 4d, 5d and 6d post infection for mid-gut samples, while salivary glands at 3d, 4d, 5d, 6d and 7d post infection was carried. It was carried out using 15% polyacrylamide gels pH 4, using a discontinous buffer system accordding to **Gabriel, 1971**. The gels were run at 200 V until the tracker dye (Bromphenol blue) was running off the gel (approximately 2.5 h).

5- SDS Polyacrylamide Gel Electrophoresis (PAGE):

Sodium dodecylsulfate (SDS-PAGE) of control and *Plasmodium* -infected samples at 12h, 1d, 4d, 5d and 6d post infection for mid-gut samples, while salivary glands at 3d, 4d, 5d, 6d and 7d post infection was carried out. SDS-PAGE of denatured proteins was carried out using 15% polyacrylamide gels pH 8.8, in a discontinous buffer (**Maizel and Jr, 1971**). The acrylamide/ bisacrylamide ratio was 50: 1. The gels contained no SDS before electrophoresis. The protein samples were pretreated with 1% SDS and 1% β-mercaptoethanol for 5-10 min. at 100 °C. The gels were run at 150 V until the tracker dye (bromphenol blue) was leaving the gel (approximately 2 h.). All gels were fixed in 20% 5 sulfosalicylic acid, stained with Coomassie Brilliant Blue R250 and destained in 7% acetic acid.

Results

1-Quantitative protein analysis:

Quantitative protein analysis of the mid-gut and salivary glands homogenate of control and *Plasmodium* - infected samples of *Cx. Pipiens* was estimated at 12h, 1d, 4d, 5d and 6d post infection for mid-gut samples and 1d, 4d, 5d 6d and 7d post infection for salivary glands samples (**Table, 1 and 2 and Figs. 1 and 2**). Generally, significant gradual decreases in total soluble protein concentration were observed within the infected samples at all tested times.

Firstly, total protein concentration of mid-gut-samples decreased within the control samples to time 5 d.p.i. then it increases at 6 d.p.i., also total protein concentration of mid-gut samples decreased within the infected samples to time 4 d.p.i. then it increases at 5 d.p.i. On comparing of control with the infected samples we observed decrease in the total protein concentration with exception in the case of 5 d.p.i. the total protein concentration increases (**Table 1, Fig. 1**).

Secondly, total protein concentration of salivary glands -samples decreased within the control samples but at time 5 d.p.i. and 6 d.p.i. it gives the same value, also total protein concentration of salivary glands samples decreased within the infected samples to time 4 d.p.i. then it increases at 5 d.p.i. and then gives the same value at the other times. On comparing of control with the infected samples we observed decrease in the total protein concentration (**Table 2, Fig. 2**).

Table (1): Quantitative protein analysis of the *Plasmodium*-infected mid-guts of C*x. pipiens* at different times post infection.

Times post infection		**12h**	**1d**	**4d**	**5d**	**6d**
Protein concentration (µg/µl) Mean ± S.E.	**Control**	**3.8±0.19**	**3.7±0.09**	**2.7±0.07**	**2.5±0.07**	**2.7±0.12**
	***Plasmodium*-infected**	**3.4±0.12**	**3.3±0.12**	**2.3±0.09**	**2.6±0.15**	**2.5±0.09**

Table (2): Quantitative protein analysis of the *Plasmodium*-infected salivary glands of C*x. pipiens* at different times post infection.

Times post infection		**1d**	**4d**	**5d**	**6d**	**7d**
Protein concentration (µg/µl) Mean ± S.E.	**Control**	**4.7±0.07**	**3.0±0.09**	**2.9±0.12**	**2.9±0.15**	**2.7±0.06**
	***Plasmodium*-infected**	**4.0±0.12**	**2.1±0.07**	**2.5±0.09**	**2.5±0.15**	**2.5±0.12**

2- Native and SDS PAGE:

Mid-guts and salivary glands proteins of control and *Plasmodium* –infected females were separated using native and SDS-PAGE at 12h, 1d, 4d, 5d and 6d post infection for mid-gut samples and 1d, 4d, 5d 6d and 7d post infection for salivary glands samples. The results of native-PAGE demonstrated differences in the overall protein banding pattern in the infected females as compared to control. The results of the SDS-PAGE clarified that the molecular weight of the separated proteins ranged from 494 to 12 KDa and *Plasmodium* infection had led to the induction of different proteins as compared to control.

Results represented in Fig. (3) shows the native PAGE of *Plasmodium* –infected mid-gut samples which electrophoresed after 12h, 1d, 4d, 5d and 6d post infection as they separated into different number of bands (Table 3), While Fig. (4) shows native PAGE of *Plasmodium* –infected salivary glands samples electrophoresing after 1d, 4d, 5d 6d and 7d post infection as they separated into different number of bands (Table 4).

Results registered in Fig. (5) shows the SDS-PAGE of *Plasmodium* –infected mid-gut samples which electrophoresed after 12h, 1d, 4d, 5d and 6d post infection as they separated into different number of bands with molecular weight ranged from 494 to 12 KDa (Table 3), While Fig. (6) shows SDS-PAGE of *Plasmodium* –infected salivary glands samples electrophoresing after 1d, 4d, 5d 6d and 7d post infection as they separated into different number of bands with molecular weight ranged from 147 to 8 KDa (Table 4).

Table (3): Total number of bands separated by native PAGE of the *Plasmodium* –infected mid-gut samples of *Cx. pipiens* at different times post infection.

Times post infection		**12h**	**1d**	**4d**	**5d**	**6d**
Total number of bands	**Control**	**8**	**8**	**3**	**3**	**4**
	***Plasmodium*-infected**	**12**	**10**	**3**	**4**	**5**

Table (4): Total number of bands separated by native PAGE of the *Plasmodium* –infected salivary glands samples of *Cx. pipiens* at different times post infection.

Times post infection		1d	4d	5d	6d	7d
Total number of bands	Control	8	10	7	10	7
	Plasmodium-infected	9	7	9	4	7

Table (5): Total number of bands separated by SDS PAGE of the *Plasmodium* –infected mid-gut samples of *Cx. pipiens* at different times post infection.

Times post infection		12h	1d	4d	5d	6d
Total number of bands	Control	8	7	10	9	9
	Plasmodium-infected	6	5	10	11	7

Table (6): Total number of bands separated by SDS PAGE of the *Plasmodium* –infected salivary glands samples of *Cx. pipiens* at different times post infection.

Times post infection		1d	4d	5d	6d	7d
Total number of bands	Control	10	14	14	13	13
	Plasmodium-infected	9	16	13	11	12

Discussion

The main objective of the current work is to study the changes in protein profile of *Plasmodium* -infected *Culex pipiens* females. Quantitative protein analysis of the mid-guts and salivary glands of control and *Plasmodium* -infected *Culex pipiens* females was estimated at different times post infection. Quantitative protein analysis of the mid-gut and salivary glsands samples of control and *Plasmodium* -infected samples of *Cx. pipiens* was estimated at different times post infection. On comparing of control with the infected samples we observe decrease in the total protein concentration. Agreeable results were reported by **Abuldahab *et al.* (2011)** who noticed a significant decrease in the level of total soluble protein in the larvae of *M. domestica* treated with bacteria at different time intervals as compared to control, **El-Sobky *et al.* (2006)** stated that the protein content of second instar larvae of *Cx. pipiens* decreased after bacterial treatment and **Abo El-Mahasen (2007)** demonstrated that a marked decrease in the total protein content in the whole body homogenate of *Cx. pipiens* larvae treated with *B. thuringiensis*. In contrast to the present results, **Brogden *et al.* (2003)** reported that after immunization, the level of antimicrobial activity in the haemolymph increases significantly.

Polyacrylamide gel electrophoresis (PAGE) has been extensively used as an excellent tool for the separation of proteins from all living organisms (**Zacharius *et al.*, 1969**). The vast majority of recent studies on insect proteins have used electrophoretic techniques. Polyacrylamide gel, with the advantages of high sensitivity and resolving power, is generally the most efficient medium (**Wyatt and Pan, 1978**).

Results obtained from the native-PAGE of mid-gut and salivary glsands samples of control and *Plasmodium* -infected samples of *Cx. pipiens* at different time intervals demonstrated that there were changes in the bulk protein content of the infected females as compared to control. Similarly, SDS-PAGE results showed characteristic protein bands appeared in *Plasmodium* - infected samples after 4 days post infection with relatively low and high molecular weights in response to invasion of ookinete to the epithelium mid-gut. Thus, the *Plasmodium* infection was capable of changing the profiles of whole body proteins qualitatively. The appearance of different bands in treated females may be attributed to the induction of new proteins in the immune reactions. These results agree with (**Rasmuson and Boman, 1979**) who stated that the synthesis of new immune proteins may be a result of simultaneous induction of infected-bacteria.

Reference

[1]. **Abo El-Mahasen, M. M. (2007):** Biochemical effects of some biological agents on *Culex pipiens* larvae (Diptera: Culicidae). The Egyptian Journal of Experimental Biology (Zoology), **3**: 27-34.

[2]. **Abuldahab, F.; Abozinadah, N. and Al-Haiqi, N. (2011):** Impact of *Bacillus thuringiensis* β – exotoxin to some biochemical aspects of *Musca domestica* (Diptera: Muscidae). African Journal of Bacteriology Research, **3**(6): 92-100.

[3]. **Adham, F. K.; Gabre, R. M.; Ayaad, T. H. and Galal, F. H. (2003):** The effects of laboratory *Hepatozoon gracilis* infection on the fecundity, mortality and longevity of *Culex* (*Culex*) *pipiens Linnaeus* (Diptera:Culicidae) in Egypt. J. Egy. Soc. Parasitol., 33(2) : 353 – 360.

[4]. **Arcà, B.; Lombardo, F.; Valenzuela, J. G.; Francischetti, I. M.; Marinotti, O.; Coluzzi, M. and Ribeiro, J. M. (2005**): An updated catalogue of salivary gland transcripts in the adult female mosquito, *Anopheles gambiae*. The Journal of Experimental Biology, **208**: 3971-3986.

[5]. **Bradford, M. M. (1976):** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, **72:** 248-254.

[6]. **Brennan, J. D.; Kent, M.; Dhar, R.; Fujioka, H. and Kumar, N. (2000):** *Anopheles gambiae* salivary gland proteins as putative targets for blocking transmission of malaria parasites. Proceedings of the National Academy of Sciences, **97**(25): 13859-13864.

[7]. **Brogden, K. A.; Ackermann, M.; McCray, P. B. and Tack, B. F. (2003):** Antimicrobial peptides in animals and their role in host defences. International Journal of Antimicrobial Agents, **22**: 465-478.

[8]. **Bruce-Chwatt, L. J. and Zulueta de, J. (1980):** The rise and fall of malaria in Europe. A historico-epidemiological study. University Press, Oxford, pp. 240.

[9]. **Calvo, E.; Andersen, J.; Francischetti, I. M.; deL Capurro, M.; deBianchi, A. G.; James, A. A.; Ribeiro, J. M. and Marinotti, O. (2004):** The transcriptome of adult female *Anopheles darlingi* salivary glands. Insect Molecular Biology, **13**: 73-88.

[10]. **Calvo, E.; Dao, A.; Pham, V. M. and Ribeiro, J. M. (2007):** An insight into the sialome of *Anopheles funestus* reveals an emerging pattern in anopheline salivary protein families. Insect Biochemistry and Molecular Biology**, 37**: 164-175.

[11]. **Calvo, E.; Pham, V. M.; Lombardo, F.; Arcà, B. and Ribeiro, J. M. (2006):** The sialotranscriptome of adult male *Anopheles gambiae* mosquitoes. Insect Biochemistry and Molecular Biology, **36**: 570-575.

[12]. **Calvo, E.; Pham, V. M.; Marinotti, O.; Andersen, J. F. and Ribeiro, J. M. (2009):** The salivary gland transcriptome of the neotropical malaria vector *Anopheles darlingi* reveals accelerated evolution of genes relevant to hematophagy. BMC Genomics, **10**(1): 57.

[13]. **Carter, V.; Nacer, A. M.; Underhill, A.; Sinden, R. E. and Hurd, H. (2007):** Minimum requirements for ookinete to oocyst transformation in *Plasmodium*. International Journal for Parasitology, **37** (11): 1221–1232.

[14]. **Coleman, J.; Juhn, J. and James, A. A. (2007):** dissection of midgut and salivary glands from *Ae. aegypti* mosquitoes. Journal of Visualized Experiments, **5**: e228.

[15]. **Collins, F. H. and Paskewitz, S. M. (1995):** Malaria: current and future prospects for control. Annual Review of Entomology, **40**: 195–219.

[16]. **Crampton, J. M.; Warren, A.; Lycett, G. J.; Hughes, M. A.; Comley, I. P. and Eggleston, P. (1994):** Genetic manipulation of insect vectors as a strategy for the control of vector-borne disease. Annals of Tropical Medicine and Parasitology, **88**: 3–12.

[17]. **Dinglasan, R. R.; Alaganan, A.; Ghosh, A. K.; Saito, A.; van Kuppevelt, T. H. and Jacobs-Lorena, M. (2007):** *Plasmodium falciparum* ookinetes require mosquito midgut chondroitin sulfate proteoglycans for cell invasion. Proceedings of the National Academy of Sciences, **104**(40): 15882-15887.

[18]. **El-Sobky, M. M.; Ismail, H. I. and Assar, A. A. (2006):** Histochemical effects of some biological agents on *Culex pipiens* larvae. Journal of the Egyptian Society of Parasitology, **27**(2): 461-479.

[19]. **Francischetti, I. M.; Valenzuela, J. G.; Pham, V. M.; Garfield, M. K. and Ribeiro, J. M. (2002):** Toward a catalog for the transcripts and proteins (sialome) from the salivary gland of the malaria vector *Anopheles gambiae*. The Journal of Experimental Biology**, 205**: 2429-2451.

[20]. **Gabriel, O. (1971):** Analytical disc gel electrophoresis. Methods in Enzymology, **22**: 565-578.

[21]. **Ghosh, A. K.; Devenport, M. and Jethwaney, D. (2009):** Malaria parasite invasion of the mosquito salivary gland requires interaction between the *Plasmodium* TRAP and the *Anopheles* saglin proteins. PLoS Pathogens, **5**(1): 1000265- 1000277.

[22]. **Gwadz, R. W.** (**1994):** Genetic approaches to malaria control: how long the road?. The American Journal of Tropical Medicine and Hygiene, **50**: 116–125.

[23]. **Harbach, R. E. (1985)**: Pictorial keys to the genera of mosquitoes, sub-genera of *Culex* and the species of *Culex* (*Culex*) occurring in south-western Asia and Egypt, with anote on the sub-generic placement of *Culex deserticola* (Diptera : Culicidae). J. Mosq. Sys., 17(2): 83-107.

[24]. **Hirai, M.; Wang, J.; Yoshida, S.; Ishii, A. and Matsuoka, H. (2001):** Characterization and identification of exflagellation-inducing factor in the salivary gland of *Anopheles stephensi* (Diptera: Culicidae). Biochemical and Biophysical Research Communications, **287**: 859-864.

[25]. **Kariu, T.; Yuda, M.; Yano, K. and Chinzei, Y. (2002):** MAEBL is essential for malarial sporozoite infection of the mosquito salivary gland. Journal of Experimental Medicine, **195**:1317–23.

[26]. **Korochkina, S.; Barreau, C.; Pradel, G.; Jeffery, E.; Li, J.; Natarajan, R.; Shabanowitz, J.; Hunt, D.; Frevert, U. and Vernick, K. D. (2006):** A mosquito specific protein family includes candidate receptors for malaria sporozoite invasion of salivary glands. Cellular Microbiology, **8**(1): 163-175.

[27]. **Kotsyfakis, M.; Ehret-Sabatier, L.; Siden-Kiamos, I.; Mendoza, J.; Sinden, R. E. and Louis, C. (2005):** *Plasmodium berghei* ookinetes bind to *Anopheles gambiae* and *Drosophila melanogaster* annexins. Molecular Microbiology, **57**:171–179.

[28]. **Lavazec, C.; Bonnet, S.; Thiery, I.; Boisson, B. and Bourgouin, C. (2005):** cpbAg1 encodes an active carboxypeptidase B expressed in the midgut of *Anopheles gambiae*. Insect Molecular Biology, **14** (2): 163–174.

[29]. **Mahairaki, V.; Lycett, G.; Sinden-Kiamos, I.; Sinden, R. E. and Louis, C. (2005b):** Close association of invading *Plasmodium berghei* and beta integrin in the *Anopheles gambiae* midgut. Archives of Insect Biochemistry and Physiology, **60** (1): 13–19.

[30]. **Mahairaki, V.; Voyatzi, T.; Siden-Kiamos, I. and Louis, C. (2005a):** The *Anopheles gambiae* gamma1 laminin directly binds the *Plasmodium berghei* circumsporozoite- and TRAP-related protein (CTRP). Molecular and Biochemical Parasitology, **140** (1): 119–121.

[31]. **Maizel, J. V. and Jr, P. B. (1971):** Methods in virology. Academic Press, New York and London, pp. 179-246.

[32]. **Montero-Solis, C.; Gonzalez-Ceron, L.; Rodriguez, M. H.; Cirerol, B. E.; Zamudio, F.; Posan, L. D.; James, A. A. and Hernandez-Hernandez, F. C. (2004):** Identification and characterization of gp65, a salivary-gland-specific molecules expressed in the malaria vector *Anopheles albimanus*. Insect Molecular Biology, **13**: 155–164.

[33]. **Okulate, M. A.; Kalume, D. E.; Reddy, R.; Kristiansen, T.; Bhattacharyya, M.; Chaerkady, R.; Pandey, A. and Kumar, N. (2007):** Identification and molecular characterization of a novel protein Saglin as a target of monoclonal antibodies affecting salivary gland infectivity of *Plasmodium* sporozoites. Insect Molecular Biology, **16**(6):711–722.

[34]. **Ramasamy, R.; Nadesalingam, P. and Ramasamy, M. S. (1991):** Antigenic similarity between the mosquito vectors of malaria and filariasis. Journal of Medical Entomology, **28**(6): 760-762.

[35]. **Rasmusom, T. and Boman, H. G. (1979):** Insect immunity: purification and some properties of immune protein P4 and haemolymph of *Hyalophora cecropia* pupae. Insect Biochemistry, 9: 259-264.

[36]. **Rodriguez, M. D. C.; Martínez-Barnetche, J.; Alvarado-Delgado, A.; Batista, C.; Argotte-Ramos, R. S.; Hernández-Martínez, S. and Rodríguez, M. H. (2007):** The surface protein Pvs25 of *Plasmodium vivax* ookinetes interacts with calreticulin on the midgut apical surface of the malaria vector *Anopheles albimanus*. Molecular and Biochemical Parasitology, **153**(2): 167-177.

[37]. **Rosinski-Chupin, I.; Briolay, J.; Brouilly, P.; Perrot, S.; Gomez, S. M.; Chertemps, T.; Roth, C. W.; Keime, C.; Gandrillon, O.; Couble, P. and Brey, P. T. (2007):** SAGE analysis of mosquito salivary gland transcriptomes during *Plasmodium* invasion. Cellular Microbiology, **9**(3): 708–724.

[38]. **Thompson, J.; Fernandez-Reyes, D.; Sharling, L.; Moore, S. G.; Eling, W. M.; Kyes, S. A. and Waters, A. P. (2007):** *Plasmodium* cysteine repeat modular proteins 1–4: complex proteins with roles throughout the malaria parasite life cycle. Cellular Microbiology, **9**(6): 1466-1480.

[39]. **Valenzuela, J. G.; Francischetti, I. M.; Pham, V. M.; Garfield, M. K. and Ribeiro, J. M. (2003):** Exploring the salivary gland transcriptome and proteome of the A*nopheles stephensi* mosquito. Insect Biochemistry and Molecular Biology, **33**: 717-732.

[40]. **Vlachou, D.; Lycett, G.; Sidén-Kiamos, I.; Blass, C.; Sinden, R. E. and Louis, C. (2001):** *Anopheles gambiae* laminin interacts with the P25 surface protein of *Plasmodium berghei* ookinetes. Molecular and Biochemical Parasitology, **112** (2): 229–237.

[41]. **Vlachou, D.; Lycett, G.; Sidén-Kiamos, I.; Blass, C.; Sinden, R. E. and Louis, C. (2001):** *Anopheles gambiae* laminin interacts with the P25 surface protein of *Plasmodium berghei* ookinetes. Molecular and Biochemical Parasitology, **112** (2): 229–237.

[42]. **Wilkins, S. and Billingsley, P. F. (2010):** Mosquito cell line glycoproteins: an unsuitable model system for the *Plasmodium* ookinete-mosquito midgut interaction?. Parasites and Vectors, **3** (1): 22.

[43]. **Wyatt, G. R. and Pan, M. L. (1978):** Insect plasma proteins. Annual Review of Biochemistry, **47**: 779-817.

[44]. **Youdeowei, A. and Service, M. W. (1983):** Management of vectors. Pest and Vectors Management in the Tropics. Longman, **420**: 265-280.

[45]. **Zacharius, R. M.; Zell, T. E.; Morrison, J. H. and Woodlock, J. J. (1969):** Glycoprotein staining following electrophoresis on acrylamide gels. Analytical Biochemistry, **30**: 148-152.

[46]. **Zieler, H. and Dvorak, J. A. (2000):** Invasion *in vitro* of mosquito midgut cells by the malaria parasite proceeds by a conserved mechanism and results in death of the invaded midgut cells. Proceedings of the National Academy of Sciences, **97**: 11516–11521.

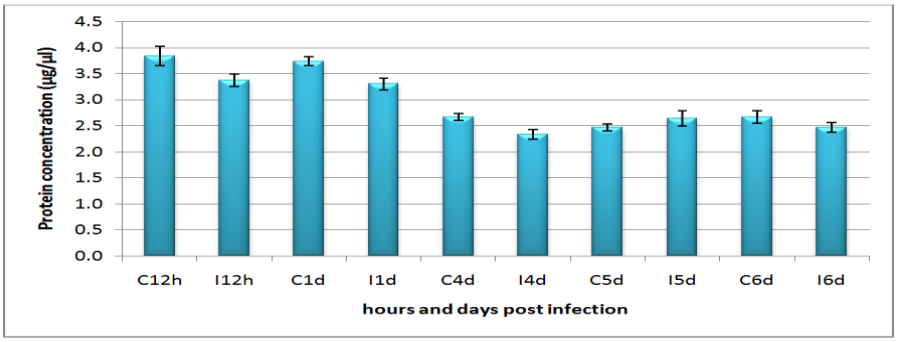

Fig. (1): Quantitative protein analysis of the *Plasmodium*-infected mid-guts of C*x. pipiens* at different times post infection.

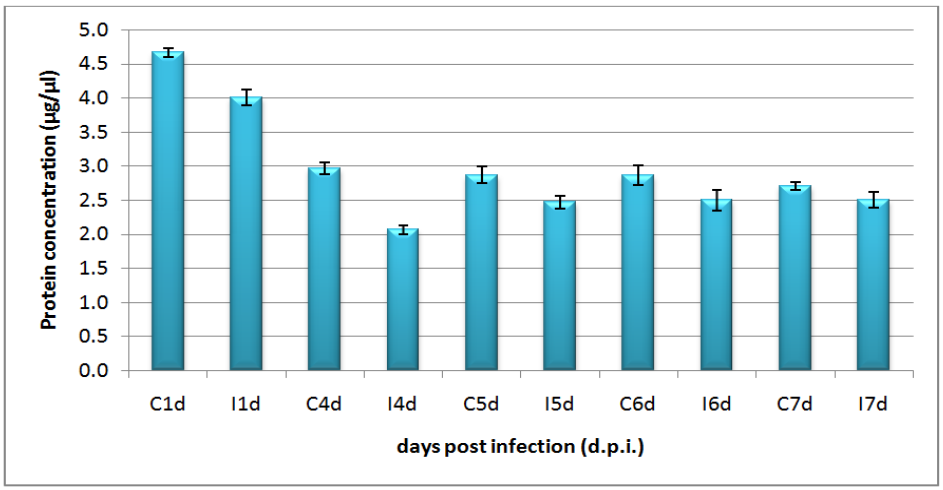

Fig. (2): Quantitative protein analysis of the *Plasmodium*-infected salivary glands of C*x. pipiens* at different times post infection.

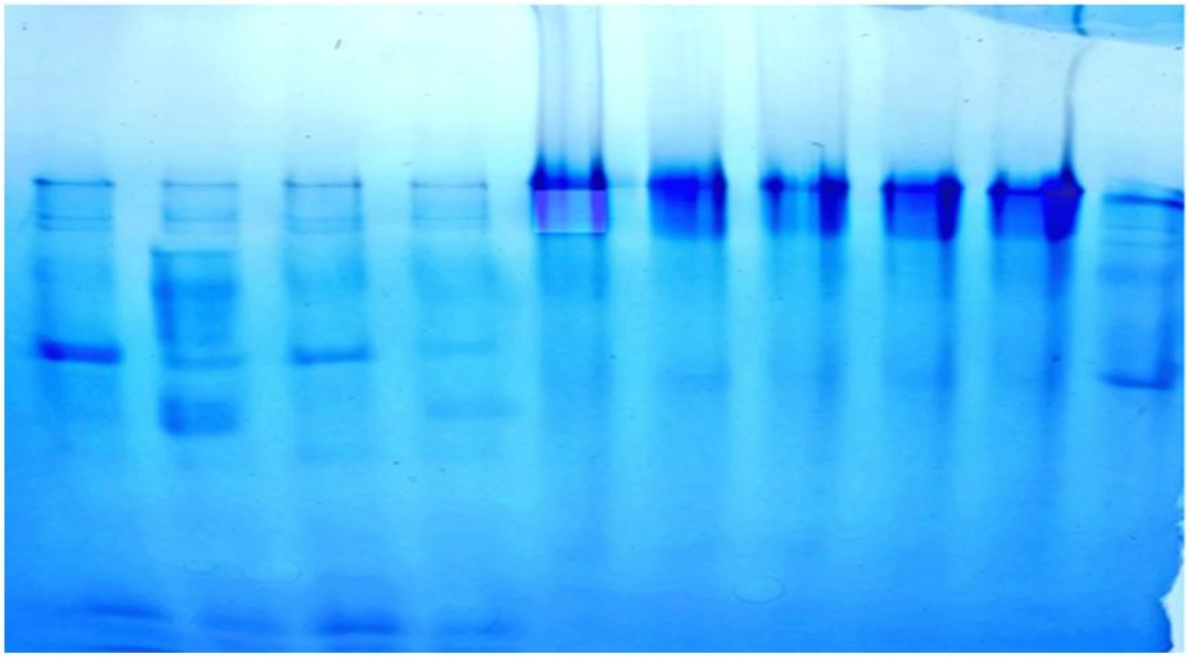

Fig. (3): Electrophoretic native gel (15%) of mid-gut of *Cx. pipiens*. Lanes 1, 3, 5, 7 and 9 normal mosquitoes without any infection at 12h, 1d, 4d, 5d and 6d post blood feeding, lanes 2, 4, 6,8 and 10 *Plasmodium* -infected mosquitoes at 12h, 1d, 4d, 5d and 6d post infection.

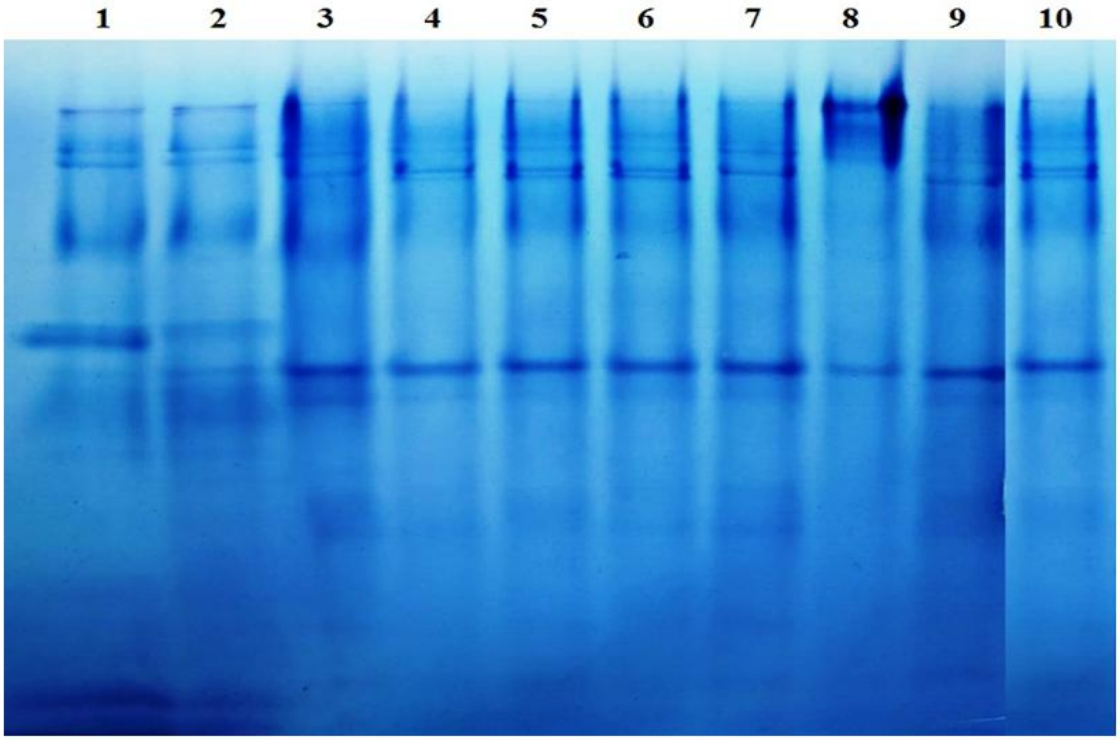

Fig. (4): Electrophoretic native gel (15%) of salivary glands of *Cx. pipiens*. Lanes 1, 3, 5, 7 and 9 normal mosquitoes without any infection at 1d, 4d, 5d, 6d and 7d post blood feeding, lanes 2, 4, 6,8 and 10 *Plasmodium* -infected mosquitoes at 1d, 4d, 5d, 6d and 7d post infection.

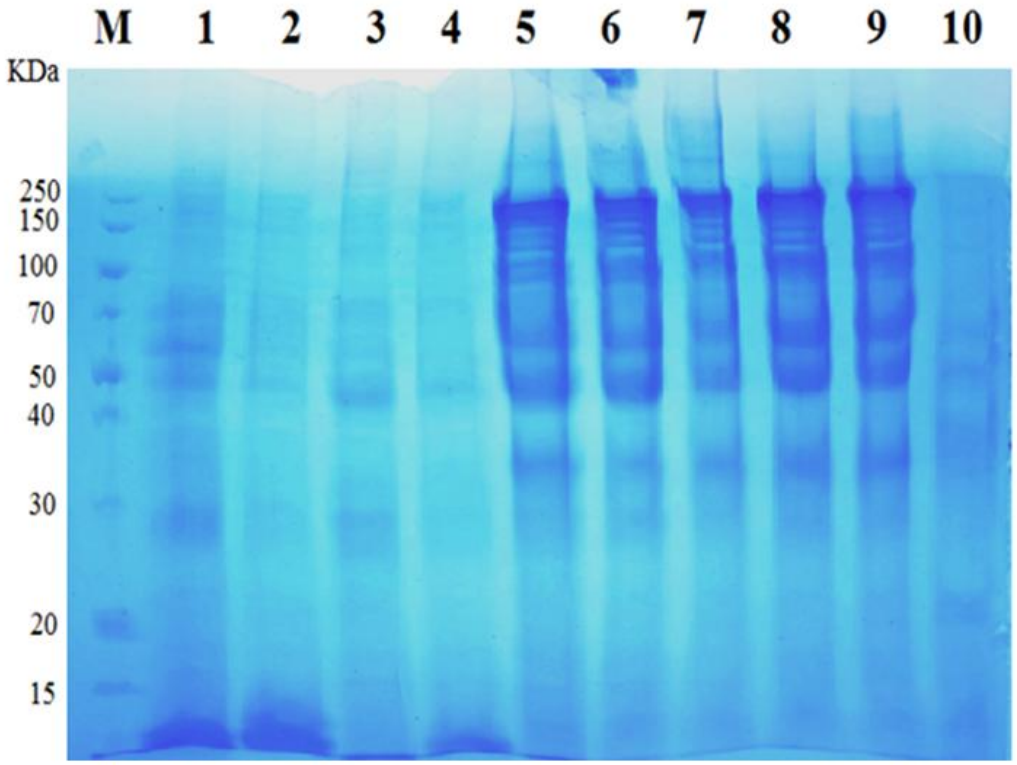

Fig. (5): Electrophoretic SDS gel (15%) of mid-gut of *Cx. pipiens*. Lane M: protein marker, lanes 1, 3, 5, 7 and 9 normal mosquitoes without any infection at 12h, 1d, 4d, 5d and 6d post blood feeding, lanes 2, 4, 6,8 and 10 *Plasmodium* -infected mosquitoes at 12h, 1d, 4d, 5d and 6d post infection.

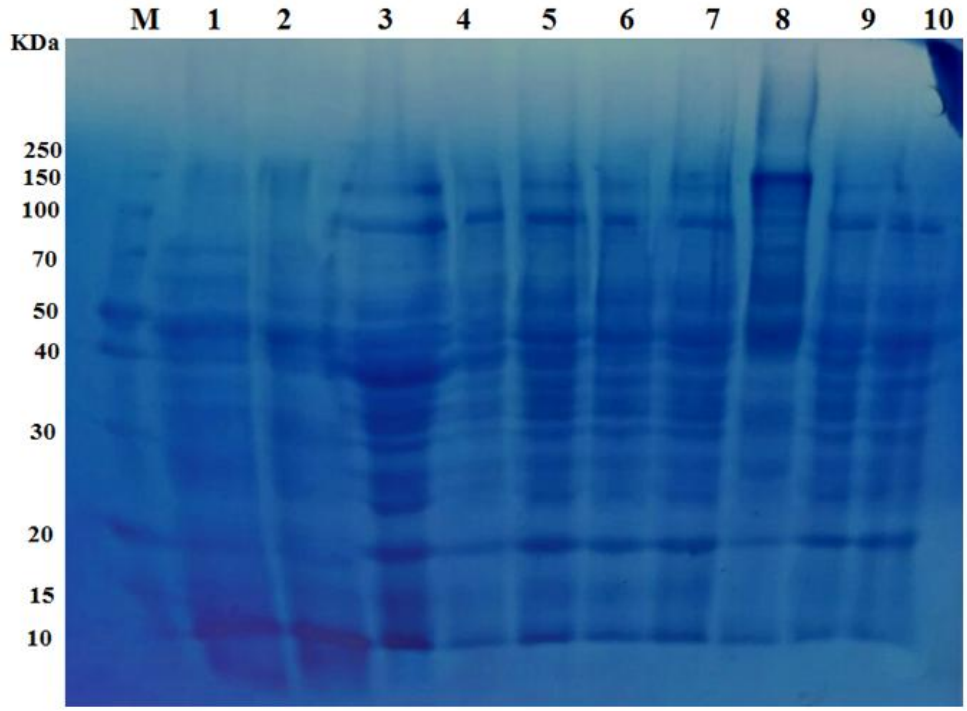

Fig. (6): Electrophoretic SDS gel (15%) of salivary glands of *Cx. pipiens*. Lane M: protein marker, lanes 1, 3, 5, 7 and 9 normal mosquitoes without any infection at 1d, 4d, 5d, 6d and 7d post blood feeding, lanes 2, 4, 6,8 and 10 *Plasmodium* -infected mosquitoes at 1d, 4d, 5d, 6d and 7d post infection.

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