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Evaluation of Crude and Fractionated Gut Extract Antigens for Protection Against Camel Tick *Hyalomma dromedarii* (Acari: Ixodidae)

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Abstract: The study aim to evaluate crude and two fractionated gut extract antigens of engorged females of *Hyalomma dromedarii* for protection against tick-feeding. To obtain the two fractionated gut extract antigens (FGE₁ and FGE₂) from Crude Gut Extract (CGE), gel filtration was used. CGE, FGE₁ and FGE₂ were used as immunogens for rabbits with Freund's adjuvant. Six immunized and two control groups were challenged with adult and larvae of *H. dromedarii* (3 immunized and 1 control for each stage). Results showed that the efficacy of immunogens against adult (E%) showed that FGE₁ recorded the highest immune effects on the adults (64.97%) followed by CGE which recorded (30.97%) and the lowest protection recorded with FGE₂ (21.57%), while E% of CGE, FGE₁ and FGE₂ were 85.01, 82.13 and 70.1%, respectively, on larvae. The results of Enzyme Linked Immunosorbent Assay (ELISA) revealed that the level of antibodies for all tested antigens increased gradually until the date of challenge with ticks and then declined. Western blot technique for both adults and larvae showed that the antigen CGE revealed two reactive bands at molecular weights of 50 and 34 kDa before challenge with ticks. These bands still to be reactive after challenge with ticks. Meanwhile, before challenge, the antigen FGE₁ exhibited 3 reactive bands with molecular weights 145.89, 61.09 and 24 kDa and one band at molecular weight of 34 kDa after challenge with ticks. Moreover, the antigen FGE₂ had only one reactive band at molecular weight of 34 kDa before challenge with ticks and two reactive bands at molecular weight of 34 and 24 kDa after challenge with ticks.

Key words: Challenge, ELISA, fractionation, gut, *Hyalomma dromedarii*, western blot

INTRODUCTION

Hyalomma dromedarii is a high desert adapted two-host tick which occasionally uses three hosts. The tick is widely distributed in deserts, semi-desert and steppes, wherever, camels occur (Higgins, 1985). It is a vector of many disease agents such as protozoa (d'Oliveira *et al.*, 1997), bacteria (Montasser, 2005), virus (Gunes, 2006) and rickettsia (Lofis *et al.*, 2006). Current control of ticks is based on the use of acaricides. These chemicals pesticides have serious drawbacks, including the development of acaricide resistance in ticks, toxicity, contamination of food products, human health risks and environmental pollution (Graf *et al.*, 2004). The development of anti-tick vaccines are of the most promising alternative to chemical control and has many advantages as, free of residues, specific, less cross-species action, cheaper, environmental safety and less resistance, lack of human risk and ease of administration (Wikel, 1996; Willadsen, 1997).

The primary step in vaccine development is the identification of suitable antigenic targets (Mulenga *et al.*, 2000). Vaccination with a concealed antigen (e.g., gut-derived antigen) induce specific immunoglobulins that are taken with blood meal as the tick feeds and antibodies interact with the

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concealed antigen on the surface of the gut causing rupture of the gut wall (Nuttal *et al.*, 2006). This action was the principle base that led to discovering the vaccine Bm86 from gut of *Boophilus microplus* in 1986 and then commercially introduced to the market in 1994 (Willadsen, 2001). It was effective against the adult stage of *B. microplus* and the nymphal stage of *B. annulatus*. Also, it was partially protective against *Rhipicephalus* sp. and *Hyalomma* sp. (Nuttal *et al.*, 2006).

Consequently, many trials used gut antigens extracted from other tick species for immunization experiments. These trials were conducted on *H. dromedarii* (Kumar and Kumar, 1995), *H. marginatum marginatum* (Sahibi *et al.*, 1997), *H. anatolicum anatolicum* (Das *et al.*, 2000; Banerjee *et al.*, 2003), *Amblyomma variegatum* (George *et al.*, 1999) and *Rhipicephalus sanguineus* (Szabó and Bechara, 1997; Jittapalapong *et al.*, 2000). The authors used guts extracted from either unfed or partially fed ticks as antigenic materials and there is no trial used gut from engorged females of ixodid ticks. Therefore this study aim to determine the immunological properties of crude gut extracted from engorged females of *H. dromedarii* and its two partially purified peaks. Immunological properties were monitored by three ways; (1) inoculated rabbits with crude and partially purified gut antigens were challenged with ticks and determined the effect of these antigens on biologic parameters of *H. dromedarii*, (2) detection of the level of antibodies against tested antigens by ELISA and (3) detection of immunoreactive bands for each antigen by western blot.

MATERIALS AND METHODS

This study was carried out in the laboratory of the Department of Parasitology and Animal Diseases, Veterinary Research Division, National Research Center, Egypt during the summer of 2006.

Ticks

Engorged females and nymphs of the camel tick, *Hyalomma dromedarii* were originally collected from ground of camel pens, Burkash village, Giza governorate, Egypt. The identification of females was confirmed in the laboratory according to Hoogstraal (1956) and Estrada-Peña *et al.* (2004). The females were divided into two groups; a group was dissected to obtain the gut that used as a source of antigenic material and another group was incubated under a constant temperature of $27\pm 2^{\circ}\text{C}$, relative humidity of $75\pm 5\%$ and a permanent darkness to obtain eggs and larvae. Engorged nymphs were also incubated at the same conditions until they moult to the next instar (unfed adults). These adults were identified to eliminate other tick species. Larvae and unfed adults were used for challenge infestations.

Preparation of Crude Gut Extract Antigen (CGE)

The CGE antigen was prepared as described by Rosell-Davis and Coons (1989). The living engorged females of *H. dromedarii* were washed with 0.01 M Phosphate Buffer Saline (PBS) (pH 7.2), transported to a Petri-dish filled with a wax and embedded on its surface by their ventral surfaces using melting an amount of wax around their legs. The dorsal surface of tick-integument was removed and the internal organs were immersed with PBS. Guts were isolated under stereomicroscope, washed several times with PBS, placed in cold PBS (4°C) that contained 200 guts and stored at -20°C for further use. Stored guts were thawed and homogenized in a sterile pestle and mortar kept on ice. The material was sonicated by an ultra-sonicator (Vibrionics Pvt. Ltd., Bombay) at $55,000\text{ cycle sec}^{-1}$ with simultaneous cooling on ice. The sonicated tissues were centrifuged at 1400 rpm at 4°C for one hour according to Heller-Haupt *et al.* (1996). Then the resulting supernatant was separated and this was considered the CGE.

Fractionation of Crude Gut Extract Antigen (FGE₁ and FGE₂)

The fractionated gut extract antigens were obtained by gel filtration according to Brown and Askenase (1986). Sephadex G100 (Pharmacia Chemical Co.) were suspended and packed in glass column $60\times 0.5\text{ cm}$ in diameter. The protein solution was allowed to pass into the good packed column

and then 2 mL of buffer was applied several times to wash completely the inner wall of the column. Peaks were collected 1 mL in each tube at a flow rate of 15 mL h⁻¹ until the run was terminated. The protein absorbance at 280 nm was monitored by a Gilford-250 spectro-photometer (Instrument Laboratories, USA). Peak (1) and peak (2) from several runs were pooled separately, concentrated and designated as FGE₁ and FGE₂. The protein content of the crude and fractionated antigens was estimated as protocol of Lowry's method (Lowry *et al.*, 1951).

Experimental design

Rabbits were immunized against *H. dromedarii* ticks according to protocol of artificial immunization Sran *et al.* (1996) (Fig. 1). Thirty-two healthy New Zealand rabbit males (2.5 kg weight for each) used for immunization. Rabbits were divided into eight groups (4 rabbits for each). Groups were inoculated as follows; G1 and G2 with CGE, G3 and G4 with FGE₁ and G5 and G6 with FGE₂ while group G7 and G8 were kept as unimmunized control. Rabbits were subcutaneously inoculated with 300 µg protein two times 15 days apart for groups 1-6. The first injection was emulsified with complete Freund's adjuvant in equal volume and the other one booster dose was emulsified with incomplete Freund's adjuvant.

Challenge with Adults

Adult ticks were fed on the immunized and control groups 1, 3, 5 and 7. Ticks were placed inside a feed capsule consisting of a plastic tube (2.5 cm of diameter and 3 cm of height) glued on the shaved backs of rabbits (2 capsules for each). Wooden collar used on rabbits to prevent grooming (Szabó and Bechara 1997). Twenty adults (10 for each capsule; 5 females and 5 males) were fed on each rabbit. Ticks were checked daily to observe their feeding. Biological parameters; feeding period, body weight of engorged females, egg mass and egg hatchability were recorded. The percent of rejection and reproductive index were calculated according to Singh and Ghosh (2003). DT (the percentage reduction of engorged females), DO (the percentage reduction of mean weight of eggs), DR (the percentage reduction of mean weight of adult females) and E% (the efficacy of immunogens) were calculated according to the methods of Ghosh *et al.* (2005).

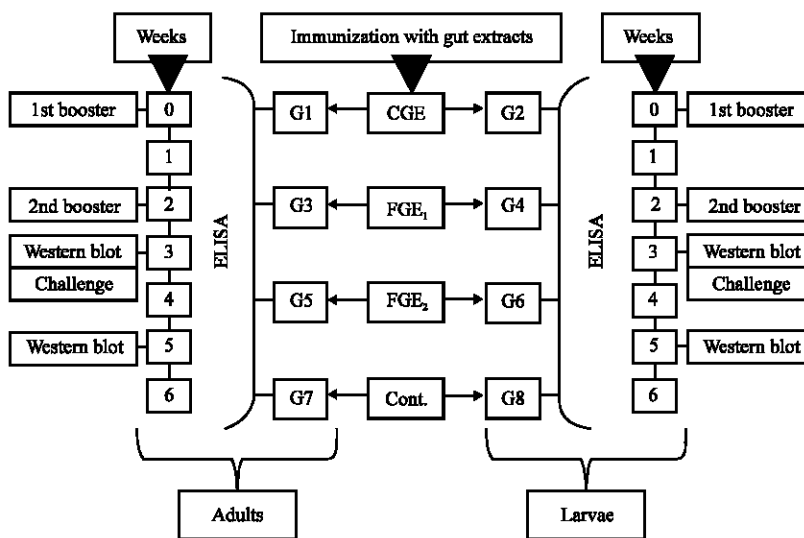


Fig. 1: The experimental immunization design including the time of collecting sera for ELISA and western blot tests

Challenge with Larvae

Larvae were fed on the immunized and control groups 2, 4, 6 and 8. The feeding technique described as above. Two hundred larvae (100 for each capsule) were fed on each rabbit. One-week post feeding, ticks were checked daily. Feeding period, number and weights of dropping nymphs were recorded. The percentage of rejection and DT% were calculated as above. MO% (the percent of reduction in moulting of engorged nymphs) and E% were calculated according the method that described by Ghosh *et al.* (2005).

Enzyme Linked Immunosorbent Assay (ELISA)

Blood samples were weekly collected from all rabbits. Sera were separated and tested by ELISA for the detection of the level of antibodies against antigens CGE, FGE₁ and FGE₂. The test was carried out according to Zimmermann *et al.* (1985) with some modifications. The optimal reaction condition as regards sensitizing antigen concentration, antibody and conjugate dilutions were chosen for use with micro-ELISA after preliminary checker board titration. In the present study, the optimum condition were 20 µg mL⁻¹ coating buffer antigen concentration, 1:100 serum dilution, 1:3000 alkaline phosphatase labeled goat anti-rabbit IgG as conjugate and 1 mg P-nitrophenyl phosphatase dissolved in 1 mL substrate buffer as substrate. All incubation steps were carried out at 37°C in a moist chamber. OD values were measured at 405 nm. The positively threshold value was determined as double fold of mean negative sera.

Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The antigens, CGE, FGE₁ and FGE₂ (10 µg well⁻¹ antigen⁻¹) were electrophoresed using 10% SDS-PAGE under reducing conditions (Laemmli, 1970). Antigens were stained by Commassie stain for 2 h. The gel was washed several times with destaining buffer till the band become clear.

Western Blot

The profile of reactive bands were recognized by sera collected from immunized rabbits with CGE, FGE₁ and FGE₂ antigens on the 3rd week PI (before challenge with adults and larvae) and 5th weeks PI (two week PC). The antigens, CGE, FGE₁ and FGE₂ (10 µg for each) were electrophoresed using 10% SDS-PAGE under reducing conditions (Laemmli, 1970). The test was conducted according to Towbin *et al.* (1979). The gut antigens were electrically transferred onto nitrocellulose (NC) membrane. NC sheets were cut into 0.5 cm strips followed by blocking in 5% bovine serum albumin in PBS for 2 h on a rocker platform. Rabbit sera diluted at 1:200 in 5% BSA/PBS-T were reacted with transferred gut antigen on NC strips for 2 h on a rocker platform. Following washing, peroxidase labeled anti-rabbit IgG diluted at 1:1000 (Bio-Rad Co.) in PBS-T was added to NC strips for 1 h on a rocker platform. The chromogen AEC (Sigma) substrate was added to NC strips and allowed to develop for 30 min. The reaction was visualized by the naked eye.

Statistical Analysis

Significant differences in mean values from immunized and control rabbits were determined using student t-test by SPSS computing program (Anonymous, 1999).

RESULTS

Feeding and Reproductive Performance of Adult Females

Table 1 shows the immunological protections of crude and fractionated gut extract antigens (CGE, FGE₁ and FGE₂) separated from engorged females of *H. dromedarii* on feeding and reproductive performance of adult females. Both antigens CGE and FGE₁ achieved significant rejection percentage ($p < 0.01$) comparing with that in control, while FGE₂ had insignificant rejection. Females those fed on

Table 1: Feeding and reproductive performances of adult females of *H. dromedarii* fed on immunized and control rabbits

Parameters	Control group Mean±SE (Range)	Immunized group Mean±SE (Range)	
		Crude Gut Extract (CGE)	Fractionated gut extract FGE ₁ FGE ₂
Rejection (%)	0	20±0.00** (20-20)	50±5.77** (40-60)
Feeding period (day)	9.9±0.26 (8-12)	8.9±0.23* (8-12)	9.2±0.22* (8-10)
Engorgement weight (mg)	865.6±26.6 (544.5-1245.6)	805.1±46.1 (228.7-1122.3)	729.9±69.6 (97.1-1086.0)
Egg mass (mg)	538.3±17.2 (351.5-785.7)	464.5±31.3** (90.8-677.1)	377.1±40.6** (29.4-593.5)
Reproductive index	62.20±0.47 (56.20-66.37)	56.10±1.29** (39.70-66.58)	49.25±1.84** (30.28-59.94)
Egg hatchability (%)	94.99±0.72 (80.40-99.80)	47.93±4.36** (0.00-98.60)	69.73±5.23** (0.00-98.7)

*: Significant (p<0.05) **: High significant (p<0.01)

Table 2: The percent of reduction in biological parameters of adults of *H. dromedarii*

Parameters	CGE	Fractionated gut extract	
		FGE ₁	FGE ₂
DT	20.00	50.00	5.00
DR	6.99	15.68	8.70
DO	13.71	29.95	17.44
E (%)	30.97	64.97	21.57

DT = Reduction (%) of engorged tick numbers, DR = Reduction (%) of mean weight of adult females, DO = Reduction (%) of mean weight of eggs, E (%) = The efficacy of immunogens against ticks

immunized rabbits with CGE and FGE₁ showed significant decreasing (p<0.05) in their feeding period and there was an insignificant difference between the feeding period of FGE₂ and control. By comparing with control, all immunized antigens had insignificant reduction on engorged weight of females. In spite of this finding, egg mass or egg number was significantly reduced for all three tested antigens (p<0.01), consequently, reproductive index of immunized antigens was significantly lower (p<0.01) than in control group. Egg hatchability was significantly reduced for all antigens (p<0.01). Generally, CGE and FGE₁ were the highest effective antigens. However, CGE antigen was more effective on feeding period and egg hatchability, while FGE₁ was more effective on rejection (%) and reduction in egg mass (%). These results were confirmed by the values those were calculated in Table 2 as DT, DR, DO and E. The values of DT were equal the values of rejection because there was not any rejection in control group. The highest values of DR and DO were recorded in FGE₁ followed by FGE₂ and then CGE. The values of E (%) showed that FGE₁ recorded the highest immunological effective followed by CGE and then FGE₂.

Feeding and Developmental Performance of Larvae

Table 3 shows the immunological protections of CGE, FGE₁ and FGE₂ on feeding and developmental performance of larvae. All antigens showed highly significant rejection (%) at p<0.01. The differences between feeding period of the tested groups and control group were insignificant. Moulting percentage of engorged nymphs resulting from releasing larvae was significantly lower for FGE₁ at p<0.01 and CGE at p<0.05 while for FGE₂ was equal that in control group.

According to rejection (%) and number of engorged nymphs resulting from releasing larvae, CGE was the highest effective antigen followed by FGE₁ and then FGE₂. On the other hand, FGE₁ was higher effective on moulting than CGE. Data in Table 4 indicated that CGE was the highest immunogenic antigen followed by FGE₁ and then FGE₂ according to the calculated values of DT, MO and E.

Table 3: Feeding and developmental performances of larvae of *H. dromedarii* fed on immunized and control rabbits

Parameters	Control group Mean±SE (Range)	Immunized group Mean±SE (Range)		
		CGE	Fractionated gut extract	
			FGE ₁	FGE ₂
Rejection (%)	24.00±1.15 (22.00-26.00)	88.25±1.88** (85-91.50)	85.35±1.82** (82.20-88.50)	77.25±1.30** (75.00-79.50)
Feeding period (day)	14.5±0.29 (14-15)	14.5±0.29 (14-15)	13.3±0.42 (12-14)	12.8±0.25 (12-14)
No of larvae fed to engorged nymphs/rabbit	152±2.31 (148-156)	23.5±3.75** (17-30)	29.5±** (23-36)	45.5±2.60** (41-50)
Moultling (%)	100±0.00 (100-100)	96.65±1.27* (93.30-100)	92.10±1.93** (87.00-97.20)	100±0.00 (100-100)

*: Significant (p<0.05) **: High significant (p<0.01)

Table 4: The percent of reduction in biological parameters of larvae of *H. dromedarii*

Parameters	Larva		
	CGE	Fractionated gut extract	
		FGE ₁	FGE ₂
DT	84.5	80.6	70.1
MO	3.3	7.9	0
E (%)	85.0	82.1	70.1

DT = Reduction (%) of engorged tick numbers, MO = Reduction (%) of moulting of engorged nymphs resulted from releasing larvae, E (%) = The efficacy of immunogens against ticks

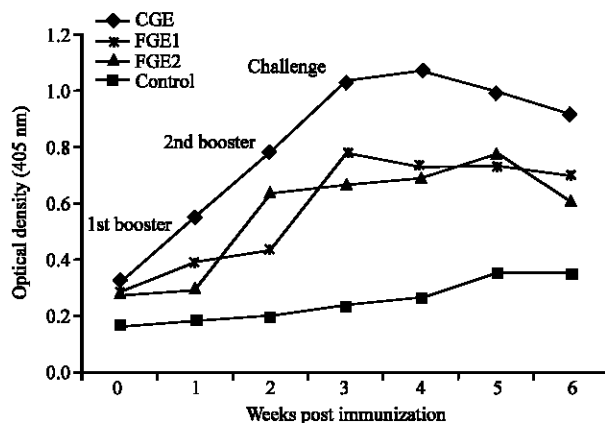


Fig. 2: Profile of antibody response of immunized rabbits with CGE, FGE₁ and FGE₂ and unimmunized rabbits tested by ELISA before and after challenge with *H. dromedarii* adults

Detection of Antibody in Sera of Immunized Rabbit's Challenge with Adults and Larvae

Figure 2 and 3 show the levels of antibodies against CGE, FGE₁ and FGE₂ through two boosters and challenge with adults and larvae. The level of antibodies against all tested antigens increased gradually after the 1st booster until the date of challenge with adults and larvae (3rd week). The level of anti-CGE reached the maximum on the 4th week Post Immunization (PI) or one week Post Challenge (PC) with adults or larvae and then declined gradually. The level of anti-FGE₁ declined gradually after challenge with adults, while in case of challenge with larvae, it increased gradually until the last week PI. The level of anti-FGE₂ increased until the 5th week PI and declined for challenge with both adults and larvae.

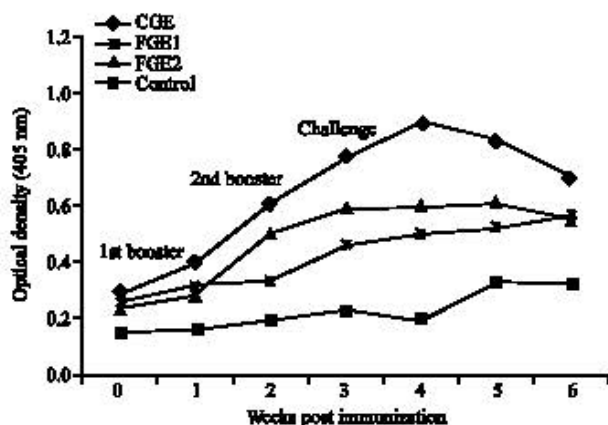


Fig. 3: Profile of antibody response of immunized rabbits with CGE, FGE₁ and FGE₂ and unimmunized rabbits tested by ELISA before and after challenge with *H. dromedarii* larvae

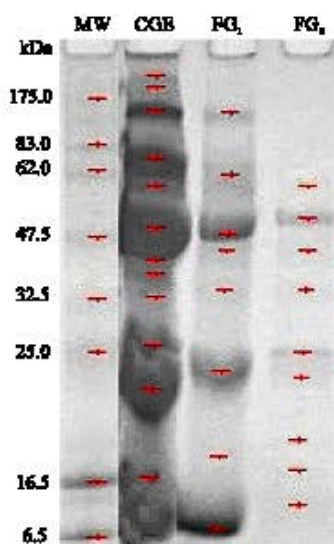


Fig. 4: SDS-PAGE analysis showing the polypeptide pattern of molecular weight marker (MW), Crude Gut Extract (CGE), partially purified gut extract of peak 1 (FGE₁) and partially purified gut extract of peak 2 (FGE₂)

In general, the level of anti-CGE was significantly higher ($p < 0.01$) than other antigens. The level of antibodies against FGE₁ was close to FGE₂ for both adults and larvae. Furthermore, anti-FGE₁ was slightly higher than anti-FGE₂ especially during the feeding period of adults which completed during the first two weeks PC, while anti-FGE₂ was higher than anti-FGE₁ in case of challenge with larvae but the antibodies for these two antigens reached to the same level on the last week PI.

Electrophoretic Profile: Figure 4 shows that the electrophoretic separation from antigens of CGE, FGE₁ and FGE₂ resolved 12, 8 and 9 protein bands, respectively. The respective molecular weights were 221.8, 195.96, 151.71, 71.8, 58.89, 50.00, 41.67, 38.47, 34.00, 25.74, 22.58 and 16.80 kDa for

CGE; 145.89, 61.09, 48.15, 44.03, 34.58, 24.00, 18.12 and 7.87 kDa for FGE₁ and 58.89, 51.51, 43.89, 34.00, 24.00, 23.34, 19.26, 17.26 and 12.40 kDa for FGE₂.

Western Blot Analysis

Figure 5 and 6 show the profile of reactive bands of CGE, FGE₁ and FGE₂ antigens recognized by sera collected from immunized rabbits with these antigens. All sera strongly reacted with antigens before and after challenge with ticks. Sera collected from immunized rabbits exhibited the same number of bands and molecular weights for each adults and larvae. The antigen CGE revealed two reactive bands at molecular weights of 50.00 and 34 kDa before challenge with ticks. These bands still to be reactive after challenge with ticks. Meanwhile, before challenge, the antigen FGE₁ exhibited 3 reactive bands with molecular weights 145.89, 61.09, 24 kDa and one band at molecular weight of 34 kDa after challenge with ticks. Moreover, the antigen FGE₂ had only one reactive band at molecular weight of 34 kDa before challenge with ticks and two reactive bands at molecular weight of 34 and 24 kDa after challenge with ticks.

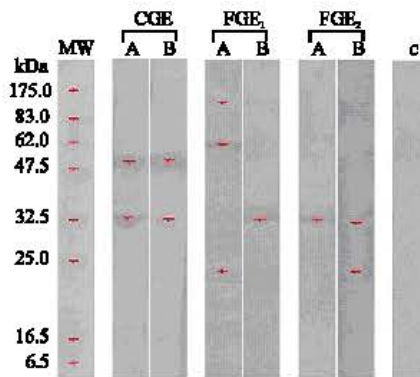


Fig. 5: Western blot, reactive bands recognized by the sera from rabbits immunized with Crude Gut Extract (CGE), partially purified gut extract of Peak 1 (FGE₁) and partially purified gut extract of Peak 2 (FGE₂) before (A) and after (B) challenge with adults and © negative control

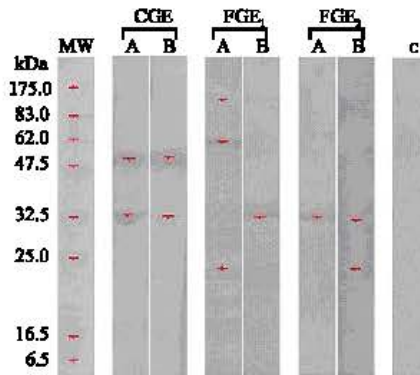


Fig. 6: Western blot, reactive bands recognized by the sera from rabbits immunized with Crude Gut Extract (CGE), partially purified gut extract of Peak 1 (FGE₁) and partially purified gut extract of Peak 2 (FGE₂) before (A) and after (B) challenge with larvae and © negative control

DISCUSSION

This study aim to check whether the CGE and its two partially purified peaks (FGE₁ and FGE₂) of engorged females of *H. dromedarii* contain protective antigens. To determine this purpose the tested antigens were inoculated in experimental rabbits with complete Freund's adjuvant at zero time and incomplete Freund's adjuvant after two weeks and then challenge with both adults and larvae of *H. dromedarii* on the third week. Sera were collected weekly to evaluation the levels of antibodies against these antigens by ELISA test. Also, these sera were used in detecting the reactive bands for each antigens before and after challenge by using western blot technique.

Present results showed that all tested antigens exhibited rejection ranged 77-88% against larvae while only CGE and FGE₁ recorded significant rejection (20-50%) against adults. Rejection percentages in larvae were higher than those in adults because we believe that the rejection % in larvae included the larvae those fed and dead before reaching to engorged nymphs. The results obtained by Manzano-Román *et al.* (2006) confirm this opinion. They recorded that the inoculation of the gut membrane extract of *Ornithodoros erraticus* with Freund's adjuvant into pigs and mice induced a protective response able to kill 80% of the immature stages. They explained that the mode of action of the gut membrane extract is the result damage to the gut wall. This damage would be insufficient to kill adults and it able to kill a high rate in the immature form up to 83.4%.

The results recorded by Kumar and Kumar (1995) and Sahibi *et al.* (1997) agreed with the results of tick feeding. They recorded 24% rejection against adults of *H. dromedarii* and *H. marginatum marginatum* when rabbits and calves were inoculated with crude gut extract of partial fed females. This agreement may attribute to the consideration of *H. dromedarii* and *H. marginatum marginatum* are large ticks and their responses to antigens were equal. In contrary, Banerjee *et al.* (2003) and Szabó and Bechara (1997) recorded higher rejection than the present study when they used gut antigens. The first authors recorded 34% rejection against adults of *H. anatolicum anatolicum* and the second authors recorded 87.8% against adults of *Rhipicephalus sanguineus*. The disagreement may attribute to *H. dromedarii* is larger than these two tick species and it was more tolerant. The fractionated FGE₁ revealed higher rejection than CGE agreed with Singh and Ghosh (2003) those found that purified glycoproteins (29 and 34 kDa) which isolated from larvae of *H. anatolicum anatolicum* recorded 75% rejection against adults.

CGE and FGE₁ reduced significantly ($p < 0.05$) the feeding period of adults only. Connat (1991) attributed this reducing in time of feeding to the influence of anti saliva response nor to the response against gut components that have been regurgitated during blood meal. Moreover, Sahibi *et al.* (1997) found the same result that mentioned in previous author when immunized calves were challenged with the adults of *H. marginatum marginatum*.

This study revealed that all tested antigens had insignificant reduction on engorgement weight of females and showed significant reduction on egg mass, reproductive index and egg hatchability. This finding clarified that the antigens had not significant effective against ingestion of blood meal and its effect appeared after feeding completely on the reproductive performance. The previous studies used gut extract antigens for protection against tick species were Rodriguez *et al.* (1995) and Jittapalapong *et al.* (2004) against *B. microplus*, Manzano-Román *et al.* (2006) against *O. erraticus*, Kumar and Kumar (1995) against *H. dromedarii*, Sahibi *et al.* (1997) against *H. marginatum marginatum* and Jittapalapong *et al.* (2000) against *R. sanguineus*. All of these researches confirmed our results in this point. Additionally, the last two authors reported that the gut extract had immunological effects on fecundity more than its feeding period and amount of blood meal as present results.

The results stated that the reduction of engorgement number of adults (DT%) was equal to the values of rejection because the control group did not recorded any rejection, while DT% of larvae ranged 70-85.5. Reduction of engorgement weight of females (DR%) and Egg mass (DO%) ranged 6.99-15.68% and 13.71-29.95%, respectively. Engorged nymphs recorded slightly moulting (MO%) only at FGE₁ (7.9%) and CGE (3.31%). Generally, the efficacy of immunogen of adults (E%) was the highest at FGE₁ (64.97%) followed by CGE (30.97%) and then FGE₂ (21.57%), while E% for larvae was the highest at CGE (85.01%) followed by FGE₁ (82.13%) and then FGE₂ (70.1%). These results agreed with the reports of other works like De Vos *et al.* (2001) who used the commercial gut-derived vaccine Bm86 against *H. dromedarii*, Kumar and Kumar (1995) who used crude gut extract from partial fed females of *H. dromedarii*, Singh and Ghosh (2003) who immunized with 29 and 34 kDa glycoproteins isolated from larvae of *H. anaticum anaticum* and Ghosh *et al.* (2005) who used the same previous purified antigens against *H. anaticum anaticum* and *B. microplus*.

The level of antibodies declined after 4-6 weeks PI (1-3 weeks PC) may attributed to the immunoglobulin-binding proteins (IGBPs) which released with saliva during tick feeding. IGBPs taken up by ticks in their blood meal passes through the tick midgut into the haemocoel retaining biological activity (Ben-Yakir *et al.*, 1986). In agreement with present results Inokuma *et al.* (1999) found that 24 kDa was a common reactive band for larvae, nymph, male, female whole body and salivary gland extracts of males and females of *R. sanguineus*. Moreover, Ghosh *et al.* (2005) found the glycoproteins of 29 and 34 kDa had protective effects against *H. anaticum anaticum* and *B. microplus*.

CONCLUSION

All crude and fractionated gut antigens extracted from engorged females of *H. dromedarii* showed protection against feeding and reproductive performance of ticks. FGE₁ recorded the highest effects on rejection, feeding period, egg mass, engorgement weight of females, reproductive index followed by CGE and then FGE₂, while CGE was the highest effective on egg hatchability followed by FGE₁ and then FGE₂. The level of antibodies increased gradually until the date of challenge and then declined gradually. CGE and FGE₁ had higher reactive bands than FGE₂ before challenge with ticks. They recognized 2 at molecular weights of 50 and 34 kDa for CGE and 3 reactive bands at molecular weights of 145.89, 61.09 and 24 for FGE₁ comparing with one band at molecular weight of 34 for FGE₂. It is recommended that gut of engorged females of *H. dromedarii* strongly can be used in the development vaccine process against this tick and further investigations are required to more purification of immunogenic gut antigens.

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