

## Immune Response in Rabbits Experimentally Infected with *Hyalomma dromedarii* Larvae and Treated with Liquvit Aminopan TRE.I

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**Abstract:** The aim of the present study is to determine the role played by Liquvit aminopan TRE.i preparation in control of *Hyalomma dromedarii* infection in rabbits as an immune potentiator and with high nutritive value. Four groups of ten rabbits each, were used in the experiment; negative control, non-treated infected, vaccinated crude antigen and Liquvit aminopan TRE.i treated groups. Blood and serum samples were taken from each rabbit in each group at different times according to the plan of work to determine the complete blood profile, liver enzymes, alanine transaminase (ALT) & aspartate transaminase (AST), liver histopathological sections and kidney function creatinine. Also, polyacrylamide agar gel electrophoresis SDS-PAGE was done to tick *Hyalomma dromedarii* larval antigen and Western blot (WB) assay. The results showed that the RBC total count, Haemoglobin and total WBC with its differential count were of normal ranges in rabbits of all groups before infection with viable *Hyalomma dromedarii* larvae and in any treatment with tick larva antigen and or Liquvit aminopan TRE.i. The third group immunized with larval antigen showed decrease in the total RBC and Hb while, there was an increase in total WBC. Concerning the fourth group of rabbits administered with Liquvit aminopan showed normal values in the total RBC, Hb, total WBC, liver enzymes; ALT & AST and creatinine. No histopathological changes in liver cells and tissues in all groups of rabbit. The electrotransfer SDS-PAGE of larval protein antigen and Western blot assay are performed in order to study and comparing the induced immune protection by *H. dromedarii* larval antigen and Liquvit aminopan compound.

**Key words:** *Hyalomma dromedarii* • Rabbit • Larval antigen • Immune response • Aminopan therapy

### INTRODUCTION

Tick are economically important pests found in all the world. They are found in the tropics, subtropics, deserts and semideserts [1]. The traditional ticks control is application of acaricides and the chemical tissue residues beside the environmental pollution reflect on animal and human health [2], beside the high annual pesticide cost.

The problem due to the side effect of acaricides, the vaccination trial researches were adopted in order to protect animals and humans. The immunological interactions between host and the Ixodid ticks have been studied and they conclude that the host immune and inflammatory reactions are elicited by tick-derived macromolecules (antigens) injected into the host skin

during tick feeding [3&4]. Electrophoretic separation of homogenates of unfed larvae and nymph of *Rhipicephalus appendiculatus* ticks followed by immunostaining with post-infection sera revealed two bands of 36.5 & 23 KDa in all homogenates [5]. Several differences in the protein profiles of crude extracts from *Hyalomma dromedarii* ticks polypeptides; 86,54 and 40 KDa were the most prominent [6]. A common bands of 205 KDa and other located bands 150 and 75 KDa was observed in eggs, larvae, nymph, salivary glands and midgut antigens of *Rhipicephalus sanguineus* carried out by SDS/PAGE analysis [7]. Some trials of use natural products, amino acids and supportive vitamins and minerals are adopted for control of internal and external parasites. Carnosine, a natural dipeptide found in animal tissues, was reported to act as a beneficial regulator of

many biological processes; anti-oxidant, buffer action protecting against protein modifications [8]. Study the effect of carnosine administration on metabolic parameters in bilharzia-infected hamsters and immune response in rabbits reduces worm burden and egg count in infected hamsters and plays a role in immune defense against *Schistosoma mansoni* infection in rabbit [9 & 10]. Carnosine and Mirazid (herbal fasciolicidal drug) have been used in studying the immune response in *Fasciola gigantica* experimentally infected rabbits. The result of Mirazid presents complete eradication of flukes while Carnosine gives a reduction of 54% in worm burden [11]. Some researchers around the globe are looking for botanical pesticides as an alternative new safe method for tick control. The anti-tick properties of the *Jatropha curcas* seed meal in a percentage of 2.5 was effective against experimental infestation of rabbits with *Hyalomma marginatum marginatum* [12]. The electrophoretic separation by sodium dodecyl sulphate polycrylamid gel electrophoresis (SDS-PAGE) of *H.dromedarii* larval protein resolved polypeptides with molecular weight ranging from 147.78-15 KDa and the anti rabbit sera showed 58.49 and 47.98 KDa by the Western Blot analysis [13]. Identification of *H.dromedarii* adult and larvae antigen protein reveals about 97,66 and 40 KDa and the rabbit antisera reacted with *H.dromedarii* larvae separated protein resolved 95.2, 34.7 and 23.1 KDa polypeptides [14]. *Rhipicephalus annulatus* tick antigens of salivary gland, ovary and larval extracts were resolved by SDS-PAGE electrophoresis and differentiated into 22,18 and 25 KDa polypeptides respectively. Protein bands ranging in molecular weight from 18 to more than 200. KDa. The bands >200,170,117,100,93,70,55,44,37,30 and 27 KDa were common in all extracts. These findings illustrate the recognition of common proteins with molecular weight of 170,117,100,70,37,33 and 30 KDa from different antigens by sera of infested cattle [15].

## MATERIAL AND METHODS

**Animals:** Forty male Mixed Balady rabbits, each weighing approximately (1.5-2 Kg), were obtained from private animal house. They were routinely examined for intestinal helminthes and ectoparasites. The rabbits received balanced ration and water and kept in separate cages with separate boxes. The rabbits were left for one week for its acclimatization and observing its health condition.

**Ticks:** *Hyalomma dromedarii* (Acari: Ixodidae): ticks used in the present study were collected from camels in quarantine of the main abattoir before slaughtering.

Adult female and male ticks were partial fed on the skin of rabbits and reared in laboratory at 80-85% relative humidity and 28°C in cotton-plug vials in desiccator over a saturated solution sodium chloride. Larvae were obtained after 14 days and used in the experiment; viable tick larvae for experimental infection of rabbits [16].

**Liquivit aminopan TRE.i** : Pharmaceutical liquid antioxidant compound produced by INDUSTRIA ITALIANA INTEGRATORI TRE.i S.p.A. MODENA-ITALY COMPANY and is formulated by the following elements: Amino acids; L-Lysine Hcl, DL Methionine, L-Threonine and L-Tryptophane./ supportive anti oxidant vitamins; A, D<sub>3</sub>, E, B<sub>2</sub>, B<sub>12</sub>, Niacin, Biotin, Folic acid, Pantothenic acid and choline. The liquid compound is added to drinking water in a dose of 1cm/liter 15 days for rabbits in group 4.

**Experimental Design:** Forty rabbits were divided into four groups; 10 each as the following:

**Group 1:** Non-infected-non treated control.

**Group 2:** Non-treated- *Hyalomma dromedarii* infective larvae.

**Group 3:** Crude antigen larvae injected. Each rabbit is injected intramuscularly in a dose of 327.02 µg protein antigen 3 times 2 weeks intervals.

**Group 4:** Liquivit aminopan TRE.i treated compound. Rabbits in each group were necropsied in time according to its plan.

***Hyalomma Dromedarii* Crude Larvae Antigen:** was prepared according to Hudson *et al.*, 1994 [17] and the protein content was determined according to Lowry *et al.*, 1951 [18] and it was 1423.4 µg/ml.

**Samples:** Whole blood and serum were taken from rabbits in different groups at the beginning of the experiment and before any treatment in each group for determination of blood pictures, liver enzymes and creatinine. Also liver samples from experimental rabbits were taken at necropsy and embedded in paraffin wax and then sectioned and stained with H&E for histopathological studies. The rabbit blood and serum samples were taken from group 2 before and 2 weeks after infection with viable *Hyalomma dromedarii* larvae and in group 3 before and 2 weeks after protection with larvae antigen challenged infection with viable *Hyalomma dromedarii*

larvae and in group 4 before administration of Liquivit aminopan TRE.i antioxidant compound which continued for 15 days and then challenged infection with viable *Hyalomma dromedarii* larvae.

#### Immunoassay:

- Sodium dodecyl sulphate Polyacrylamid gel electrophoresis (SDS-PAGE) separation of soluble *Hyalomma dromedarii* larvae antigen according to Laemmli,1970 [19] in order to determine the separated polypeptides of antigen reflecting the character of protein.
- The different anti-sera in experimental rabbit in different groups were passed against larval antigen and recognized by electroimmune transfer blotting (EITB) (Western blot) according to Uhler *et al.* 1994 [20]. The molecular weight of the obtained polypeptide bands in SDS-PAGE and western- blot were calculated using image software; [www.bio-rad.com/pred/en/US/LSR/PDP/Image-Lab-Software](http://www.bio-rad.com/pred/en/US/LSR/PDP/Image-Lab-Software).

### RESULTS

From the data displayed in Table 1, the erythrogram of rabbits in control group 1; non- infected ones and in the other 3 groups before deal with the experiment are within normal ranges of RBC, Hb, PCV, MCV, MCH and MCHC. Haemoglobin is significantly decreased in infected group 2 rabbits ( $10.1 \pm 0.1$ ) and on the other side Hb is significantly increased in Aminopan after treatment and after challenged rabbits in group 4 ( $14 \pm 0.7$ ). The PCV is significantly decreased ( $31.8 \pm 0.77$ ) in rabbits group 2 after infection while is normal ranges in rabbit of other groups. The MCV is significantly increased ( $77.5 \pm 0.07$ ) after infected group 2 rabbits and on the other side is significantly decreased ( $52.7 \pm 0.33$ ) after challenged Aminopan treated rabbits group 4. The MCHC is within normal ranges but is significantly increased in after challenged Aminopan treated rabbits. The platelet count was in normal range ( $10550 \pm 10$ - $1350 \pm 30$ ) in all groups of rabbit. The leuckogram of rabbit showed significant increase in total WBC in rabbits group 2 after infection and in challenged rabbits group 3 after antigen injection ( $9.7 \pm 0.03$  and  $9.5 \pm 0.11$  respectively). The differential leuckocytic count are within normal range in all groups of rabbit.

The liver enzymes (ALT & AST) and creatinine are of normal ranges in all rabbits during experiment.

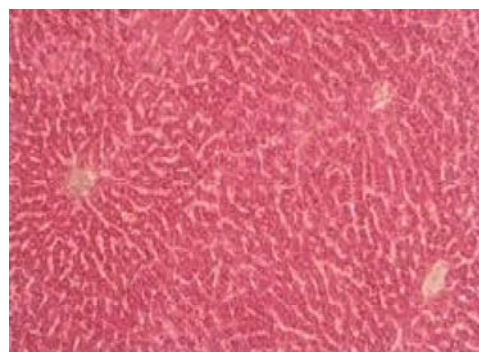


Fig 1: Normal cellular and tissues of infected and treated rabbits (low power).

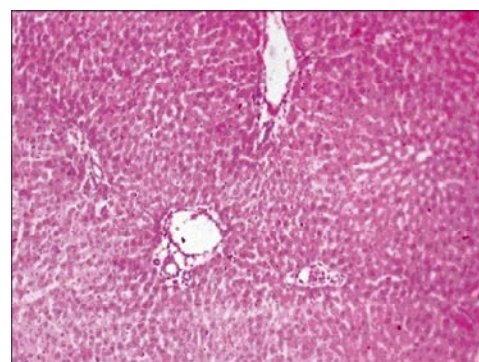


Fig 2: Normal cellular and tissues of infected and treated rabbits (high power).

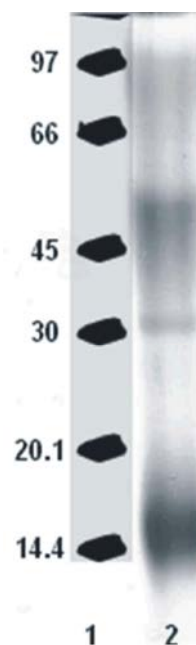


Fig 3: Electrophoretic profile (SDS-PAGE): Lane1, molecular weight standards in kDa. Lane2, *H. dromedarii* larval antigen.

Table 1: Blood pictures, Liver enzymes and Creatinine values in the involved experimental Rabbits

Parameters	Different Rabbit groups								
	G. 2			G. 3			G. 4		
	Gr. 1 Control	Before Inf.	After Inf.	Before Ag Inj.	fter Ag Inj.	Challenge after Ag Inj.	Before Aminopan treatment	After Aminopan treatment	Challenge after Aminopan treatment
Complete blood picture									
RBC(106/ $\mu$ l)	6.71 $\pm 0.06$	6.5 $\pm 0.09$	4.1 * 0.05	6.1 $\pm 0.07$	5.8 $\pm 0.07$	5.77 $\pm 0.07$	5.9 $\pm 0.05$	7.31 $\pm 0.02$	7.94 $\pm 0.01$
Hb(g/dl)	13.77 $\pm 0.3$	13 $\pm 0.1$	10.1* $\pm 0.1$	12.5 $\pm 0.3$	11.8 $\pm 0.3$	10.4* $\pm 0.5$	11.5 $\pm 0.1$	14* $\pm 0.7$	14.5* $\pm 0.11$
PCV %	45.2 $\pm 0.27$	38.7 $\pm 0.11$	31.8* $\pm 0.77$	41.9 $\pm 0.71$	40.1 $\pm 0.5$	39.1 $\pm 0.55$	42.5 $\pm 0.11$	47.7 $\pm 0.51$	41.7 $\pm 0.27$
MCV (fl)	52.7 $\pm 0.40$	59.6 $\pm 0.30$	77.5* $\pm 0.07$	68.6 $\pm 0.01$	69.1 $\pm 0.31$	67.5 $\pm 0.16$	72.3 $\pm 0.15$	65.2 $\pm 0.32$	52.7* $\pm 0.33$
MCH (pg)	20.6 $\pm 0.16$	20 $\pm 0.01$	24.6 $\pm 0.09$	20.7 $\pm 0.11$	20.3 $\pm 0.71$	18.2 $\pm 0.09$	19.4 $\pm 0.13$	19.1 $\pm 0.17$	18.3 $\pm 0.44$
MCHC (g/dl)	30.3 $\pm 0.7$	33.5 $\pm 0.11$	31.4 $\pm 0.71$	29.8 $\pm 0.11$	29.4 $\pm 0.55$	26.5 $\pm 0.17$	27.5 $\pm 0.13$	28.8 $\pm 0.21$	33.7* $\pm 0.11$
Platelet count	11070 $\pm 50$	11500 $\pm 10$	13050 $\pm 30$	10550 $\pm 10$	11710 $\pm 10$	11770 $\pm 50$	11400 $\pm 50$	11550 $\pm 10$	12000 $\pm 10$
WBC(103/ $\mu$ l)	6.3 $\pm 0.44$	6 $\pm 0.51$	9.7* $\pm 0.03$	7 $\pm 0.02$	9 $\pm 0.45$	9.5* $\pm 0.11$	6.5 $\pm 0.5$	6.7 $\pm 0.5$	6.1 $\pm 0.7$
Diff.WBC	56.3	52.9	61.5	53.1	56.5	56.5	53.5	51.5	53.5
Neutrophils %	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
Lymphocytes %	38.4 $\pm 0.7$	42.1 $\pm 0.1$	33 $\pm 0.1$	41.5 $\pm 0.1$	35.7 $\pm 0.3$	35.7 $\pm 0.5$	40.5 $\pm 0.1$	43.5 $\pm 0.3$	46.5 $\pm 0.5$
Monocytes %	3.3 $\pm$	3	3.5 $\pm$	3.4 $\pm$	5.8 $\pm$	5.8 $\pm$	4	3	3
Eosinophils %	2	2	2	2	2	2	2	2	2
Basaphils %	0	0	0	0	0	0	0	0	0
Liver enzymes	9.1	10.5	10.9	9.5	9.2	8.2	9.5	10.4	9.1
ALT(unit/l)	$\pm 0.2$	$\pm 0.9$	$\pm 0.5$	$\pm 0.5$	$\pm 0.5$	$\pm 0.8$	$\pm 0.4$	$\pm 0.3$	$\pm 0.5$
AST(unit/l)	20.7 $\pm 0.6$	24.7 $\pm 0.3$	25.5 $\pm 0.5$	23.7 $\pm 0.1$	23.5 $\pm 0.4$	25.5 $\pm 0.5$	24.3 $\pm 0.7$	25.1 $\pm 0.3$	25.1 $\pm 0.3$
Kidney function	0.81	0.75	0.55	0.77	0.7	0.84	0.81	0.77	0.7
Creatinine (ml mol/l)	$\pm 0.05$	$\pm 0.03$	$\pm 0.04$	$\pm 0.07$	$\pm 0.05$	$\pm 0.07$	$\pm 0.01$	$\pm 0.33$	$\pm 0.50$

\* means with number superscripts are significantly different at  $P < 0.05$ .

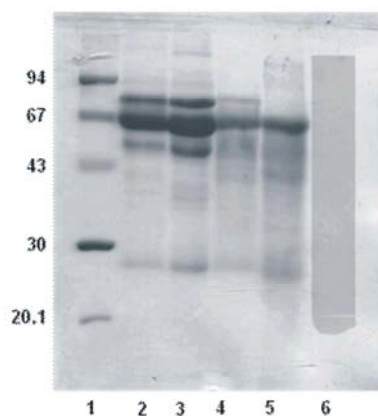


Fig 4: Immunoreactive bands (WB) identified by rabbit groups antisera. Lane 1, molecular weight standards in kDa. Lane 2, infected rabbits by *H.dromedarii* larvae. Lane 3, larval protein protected antigen. Lane 4, larval protein protected antigen challenged. Lane 5, rabbits received aminopan challenged. Lane 6, rabbits negative sera.

The histological picture of rabbit liver in different rabbit groups showed normal cellular and tissue structures and do not differ from normal non-infected non-treated rabbits (fig1&2). The liver lobules appear hexagonal in shape with thin separated septae. The parenchymal cells or hepatocytes appear poylygonalwith oval nuckleus and acidic cytoplasm and joined forming normal anastomosing plate radiating from a central vein in the center of lobule. At the vertices of the lobule are regularly distributed portal triads, containing a bile duct, hepatic artery, portal vein and lymphatics.

The immune protection of rabbits against *Hyalomma dromedarii* larvae infection in the experiment presents in (fig.3 & 4). The electrophoresis SDS-PAG assay of *Hyalomma dromedarii* larval antigen separates into 11 bands with the molecular weights: 97, 85.12, 73.15, 66, 57.20, 45, 38.66, 30, 20.10, 18 and 14.4 KDa (Fig 3). The immunoblot reactions were detected by WB assay. The recognized immunoreactive bands in infected rabbits are six in number; 81.61, 67, 57.30, 47.10, 41.50 and 27.15 KDa. In protective antigen rabbits, the separated bands are six in number; 81.61, 67, 57.30, 41.5, 40 and 27.15 KDa. In protective antigen challenged, the separated bands are six in number; 81.61, 67, 57.30, 47.10, 41.50 and 27.15 Kda. In rabbits received amino pan challenged, the separated bands are three in number; 67, 57.30 and 40 KDa (Fig 4).

## DISCUSSION

The erythrogram ; RBC, Hb, PCV, MCV, MCH and MCHC values was improved in rabbits received Liquivit aminopan and in particular, Hb is significantly increased ( $P<0.05$ ). After challenge in the same rabbits group with viable *Hyalomma dromedarii* larvae, Hb & MCHC is significantly increased but the MCV is significantly decreased ( $P<0.05$ ). Concerning the rabbits group protected with *Hyalomma dromedarii* larvae antigen, no significant differences in the erythrogram values except Hb value in challenged rabbits was significantly decreased. The erythrogram ; RBC, Hb, PCV values was significantly decreased but the MCV is significantly increased without significant difference in on MCHC indicate macrocytic normochromic anemia.

It is obvious from above mentioned results that the rabbits protected with tick larval antigen had no marked changes in the erythrogram,either after injection of antigen or after challenge. The erythrogram is markedly improved after Liquivit aminopan supplementation in water and also the challenge of rabbits with viable

*Hyalomma dromedarii* larvae did not affect the blood profile of rabbits. The leuckogram of rabbits in different group are within normal and no significant differences, except the WBC is significantly increased in experimentally infected rabbits and in challenged rabbits protected tick antigen.

The liver enzymes; ALT&AS, creatinine kidney function and liver histology are in normal values in different groups of rabbit.

The immunoblot reactions were detected by western blot assay. The recognized immunoreactive separated bands of infected and antigen protected challenged rabbit groups showed sharing in all molecular weights; 81.61, 67, 57.30, 47.10, 41.50 and 27.15 KDa.The shared separated bands in antigen protected rabbits group are the same in mentioned 2 groups except, 41.50 is replaced by 40 KDa. Concerning the aminopan challenged rabbits group, the separated bands are reduced to only 3; 67, 57.30 and 40 KDa sharing with 2 molecular weights 67 and 57.30 in infected, antigen protected and antigen protected challenged rabbits meanwhile, the molecular weight 40 KDa is sharing with those of antigen protected rabbits. These mean that, crude *Hyalomma dromedarii* larval antigen is benefit in inducing immune defence against *Hyalomma dromedarii* infection and give chance for vaccine candidate but, the more; 6 separated bands needs further studies. The results of supplementation of aminopan compound to rabbits group defend the harmful effects of ticks on animal host; no anemia and the liver enzymes and kidney function creatinine values are normal. In addition, the compound play direct and indirect roles as an immunopotentiator against tick infection as appeared in WB assay; the separated bands are reduced to 3 in number; 67, 57.30 and 40 KDa, all are sharing with other molecular bands in other different conditions.

## CONCLUSION

The study proves that Liquivit aminopan TRE.i patent compound succeeds in protection of animals against *Hyalomma dromedarii* infection due to its high nutritive constituents of amino acids and vitamine and also the immune stimulator.

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