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THE PROTECTIVE EFFECT OF *SCHISTOSOMA MANSONI* ADULT WORM ANTIGEN ON INFECTED MICE

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ABSTRACT

The present work aims to study the immunogenic effect of *Schistosoma mansoni* adult worm antigen on infected Swiss albino mice. A total number of 150 male mice infected with 100 cercariae of *S. mansoni* was divided into equal (5) groups (A,B,C,D and E). In group A the mice were immunized after 4 weeks of infection (early immunized group) and were perfused after 12 weeks of infection. However, the mice of group C were immunized after 8 weeks of infection(late immunized group) and were perfused after 16 weeks of infection. The two groups B and D were considerd as control groups to A and C respectively. There was an early infected control group E (without immunization) which was perfused after 8 weeks of infection. In addition, a group of 20 mice (non-infected, non-

immunized) was used as a normal group. The dose of immunization(300μg/mouse) was divided into 3 doses (50,100 and 150μg) injected at weekly intervals through the subcutaneous route. The level of protection was measured through parasitological parameters (worm load, state of copulation, viability of ova in tissues, mortality rate among mice and the weight of body-liver-and spleen), as well as the biochemical analysis of liver functions. The obtained results revealed that early immunization was better than late immunization. It displayed a protection level of 35.9% depending on worm load in comparison with 22.5% at late immunization. It also decreased the number of worms in copula and the number of living eggs. It exhibited a higher number of dead ova. It led to less mortality rate and improvement of liver functions.

KEY WORDS: schistosomiasis, protective antigen, worm load, ova count, enzymes.

INTRODUCTION

Schistosomiasis is a chronic and debilitating parasitic disease, it affects approximately 200 million people in the developing world and imposes a substantial public health and economic impact, despite the continuous control efforts (Wang *et al.,* 2004).

Egypt's awareness of schistosomiasis dated back to the time of ancient Egyptians (Mobarak, 1985). The disease heads the list of communicable diseases in Egypt as regards its prevalence, gravity and its repercussions on the nationl economy of the country (Mousa, 1976).

Despite the presence of modern control schemes and the development of a highly effective schistosomicidal drug (Prasiquantel) with minimal side effects, the epidemiological status of schistosomiasis has not been officially lowered. In addition there is evidence of resistance or tolerance in at least two endemic human populations (Egypt and Senegal) (Ismail *et al.,* 1996 and Fallon *et al.,* 1996).

The advance in immunology and molecular biology in the past few years has substantially increased the odds of effective immune prophylaxis. A range of promising candidate vaccine antigens is currently undergoing independent confirmatory testing with scaled up production and human trails will be the logical next step (Bergquist, 1995).

Successful vaccination against schistosomiasis has been demonstrated in several host-parasite models using radiating, attenuated cercariae schistosomula. Significant levels of protection against challenge, both in terms of reduction in the adult worm burden and the pathology obtained from a challenge infection, have been obtained for *Schistosoma mansoni* in mice (Murrell *et al.*, 1979).

It has been over half a century since the human dream of vaccination against schistosomiasis started. The development of effective vaccines against the disease for general use in humans is the thought to be a problem of time scale rather than feasibility (Butterworth, 1992).

The present study is a trail to clarify the antischistosomal effect of adult worm antigen on experimentally infected mice with *S. mansoni* by exploring the following points: worm load, viability of eggs in tissues (tissue bound ova), state of copulation in the adult worms, mortality rate among mice, weight of the (body, liver and spleen), morphological changes in the parasitic worms and biochemical analysis of the liver functions.

MATERIALS AND METHODS

I- EXPERIMENTAL DESIGN AND GROUPING OF ANIMALS

This study included 150 Swiss albino mice divided into equal five groups. All are laboratory bred males, approximately 8 weeks old,

their body weight ranged between 20 to 22 gms. Each animal was exposed to ± 100 cercariae of the Egyptian strain of *Schistosoma mansoni*, using the body immersion method (paddling technique) according to Purnell (1966)

Group A : (Early immunized group)

The animals were immunized after 4 weeks of infection with 3 successive subcutaneous injection (50, 100 & 150 µg) of *Schistosoma mansoni* adult worm antigen mixed with complete Fruend's adjuvant (Sigma) at weekly intervals. Sacrification was done after 12 weeks of infection.

Group B :

It was considered as a control group to (A) and also sacrificed after 12 weeks of infection.

Group C : (Late immunized group)

Animals were immunized after 8 weeks as group (A) but sacrificed after 16 weeks of infection.

Group D :

It was considered as a control group to (C) and sacrificed after 16 weeks of infection.

Group E : (Early infected control group)

It included only infected mice and was sacrificed after 8 weeks of infection.

Another group of 20 mice with no infection no immunization was used as a normal control group.

At the end of each group, all animals were sacrificed and the following parameters were evaluated.

1-Mortality rate. 2-Weight of the body, liver and spleen.

3-Worm load. 4-Tissue bound ova.

5-Biochemical analysis of some liver functions (alanine aminotransferase, ALT ; aspartate aminotransferase, AST ; alkaline phosphatase , ALP and bilirubin).

II- PREPARATION OF *S. MANSONI* ADULT WORM ANTIGEN

Worms were extracted from the portal system and liver through perfusion technique using citrated saline according to Smithers and Terry (1965) from previously infected hamsters obtained from Theodor Bilharz Research Institute.

They were homogenized for 5 minutes at room temperature. The resulting suspention was centrifuged for 60 minutes at 4°C and the supernatant was re-centrifuged again for another 30 minutes using a cooling centrifuge. The supernatant fluid was considered as the crude antigen and its protein content was determined according to Bradford technique (Bradford, 1976).

It was adjusted to a concentration of 200μg protein/ml using pyrogen free physiological saline, sterilized through membrane filtration and lastly stored in aliquots at –70°C till use.

Assessment of the effect of antigen

1. Worm load

The number of worms recovered from each immunized group of mice was compared with a non-immunized control group, and protection was expressed as a percentage according to the following formula.

Percent immunity =$\frac{\text{mean number of worms from the control group – mean number of worms from immunized group}}{}$ x100

mean number of worms from the control group (Smither *et al.* , 1989).

2. Sex ratio

The perfused worms were collected in a Petri- dish containing physiological saline (0.85%), where number of males, females and worms in copula was counted and tabulated.

3. Mortality rate

It was represented by dividing the number of dead animals at the end of the experiment by the total number of mice.

4. Weight of the body, liver and spleen

At the end of each group all animals were weighted. Their liver and spleen were extracted and weighted also. All results were recorded and tabulated.

5. Tissue bound ova

According to Pellegrino *et al.* 1962, changes in the number and character of eggs (oogram) provided a simple, sensitive and reliable tool for assessment of immunizing effect against *S.mansoni.*

III- BIOCHEMICAL ANALYSIS OF LIVER FUNCTIONS

Blood was collected through the left ventricle of the heart in clean dry centrifuge tubes and sera were separated and stored at – 20°C till use. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to Reitman and Frankel (1957). The alkaline phosphatase (ALP) was measured according to Kind and King (1954) and the total bilirubin concentration was detected according to Jendrassik (1938).

STATISTICAL ANALYSIS

Statistical analysis was done using an IBM compatible computer system having SPSS, (8.0) for windows.

RESULTS

- **Mortality rate**

As appears in fig.(1), the mortality rate reached its lower level 13.4% in group A (the early immunized group) comparing to 20% in group C (late immunized group). Both control groups (B, D) had a mortality rate of 30% and 40% respectively. Group E had a mortality rat of 15%.

- **The weight**

No significant difference was detected for mean animal body weight or mean weight of the liver and spleen in both immunized groups when compared to non-immunized ones (control) (fig. 2).

- **Worm load**

The data obtained are presented in fig.(3).

Total worm

All groups displayed positive significant correlation($P<0.05$) except group A & B as well as C & E which gave non significant correlation ($P>0.05$).

Males

Only correlation between group C & E was non significant ($P>0.05$) while other groups had positive significant correlation ($P<0.05$).

Females

Correlation between group B & C was only non significant ($P>0.05$) while other groups had positive significant correlation ($P<0.05$).

Copula

All groups had significant correlation ($P<0.05$).

Fig.(1):Mortality rate among different groups

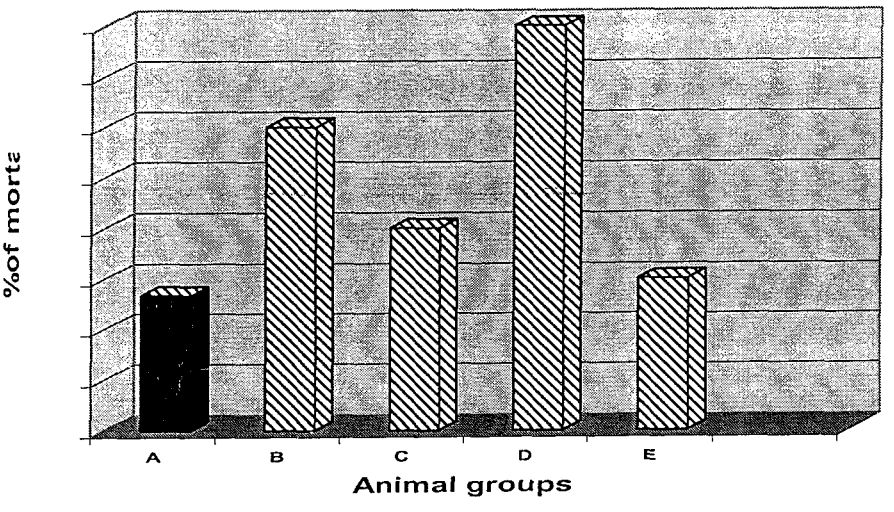


Fig. (2):Mean body weight of animals and weight of the liver and spleen in different groups.

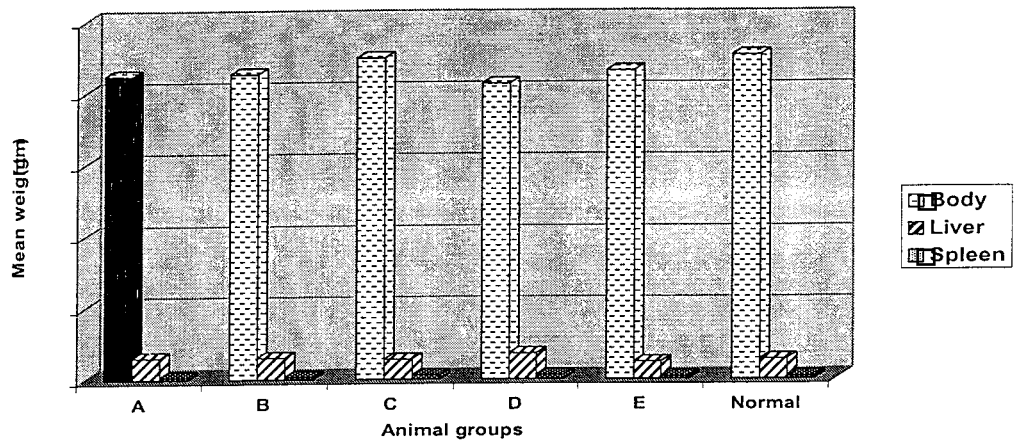
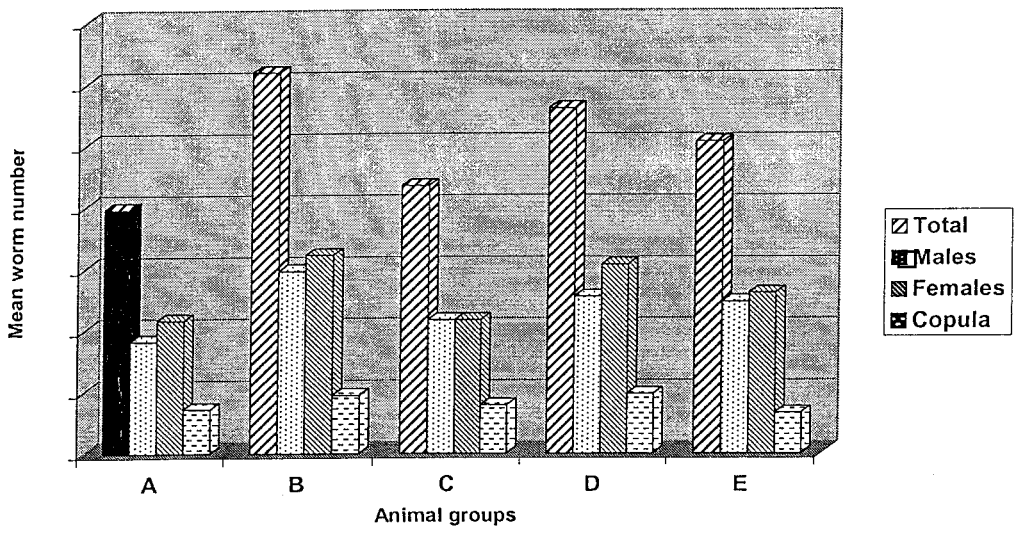


Fig.(3): Mean number of worm load in different groups.



- **Morphological changes**

No morphological changes have been detected in both immunized groups.

- **Tissue bound ova**

The obtained data are presented in table (1) and fig. (4).

Dead ova

Groups A & D and C & D as well as D & E exhibited non significant correlation ($P>0.05$) whereas other groups had significant correlation ($P<0.05$).

Immature eggs

All groups had positive significant correlation ($P<0.05$).

Mature ova

All groups had positive significant correlation ($P<0.05$) except groups A & B and B & C and B & D which gave non significant correlation ($P>0.05$).

- **Biochemical analysis of liver functions**

The data obtained are presented in table (2).

Aspartate aminotransferase (AST)

Statistical results revealed significant correlation among all groups ($P<0.05$).

Alanine aminotransferase (ALT)

There was a positive significant correlation between all groups ($P<0.05$) except between group A & B which gave non significant correlation ($P>0.05$).

Table (1) : Comparison between tissue bound ova among different groups (300 ova each)

Group	Dead eggs		Immature stage I		Immature stageII		Immature stageIII		Immature stageIV		Total Immature		Mature Egg	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A	106.4	8.2	13.7	5 6	26.3	5.6	27.4	3.9	30.7	5.7	16.1	7.2	90.2	22.5
B	82.4	5.3	11.1	3.5	24.2	5.2	27.0	4.2	13.2	5.7	14.3	6.7	124.1	7.6
C	88.5	3.1	10.9	2.8	20.7	9.9	29.0	3.6	38.8	10.3	16.4	8.7	112.2	5.8
D	52.7	5.7	25.5	10.0	26.7	5.8	37.3	12.0	34.3	6.0	26.9	8.7	120.3	8.5
E	63.6	5.7	10.0	4.0	23.4	10.4	30.9	4.1	40.3	10.6	13.0	8.1	131.7	7.4

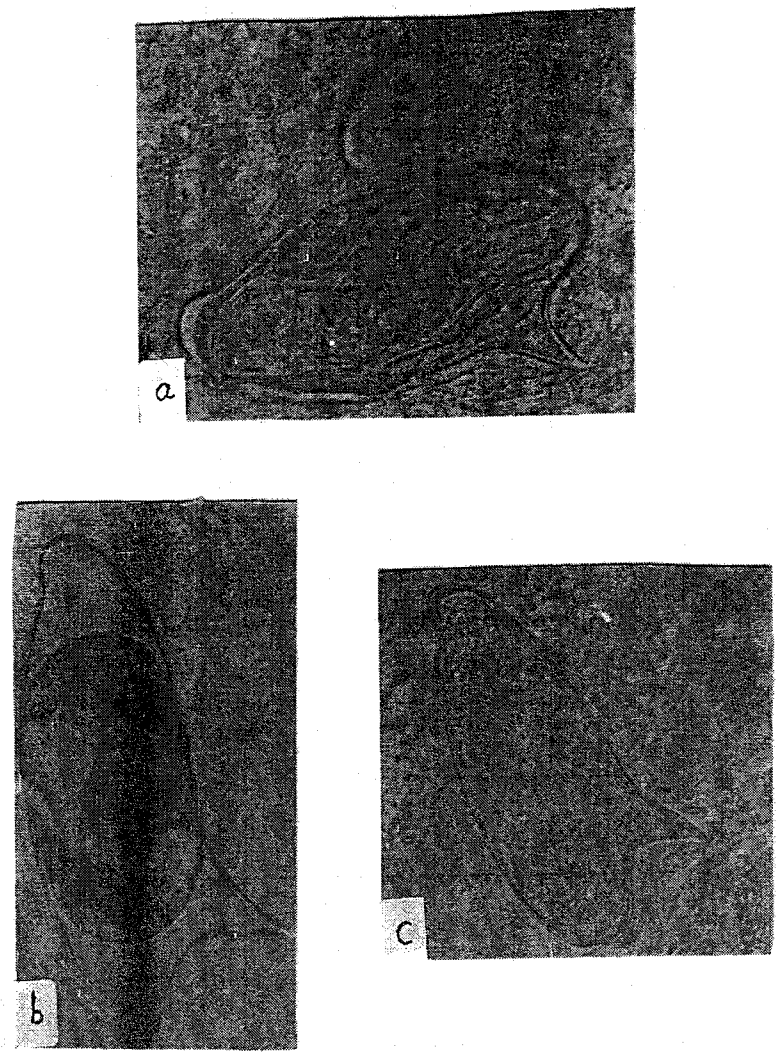


Fig.(4): *Schistosoma mansoni* eggs in different stages of development.

Table(2): Comparison between biochemical results among different groups.

Groups	AST*		ALT**		BR***		ALP****	
	mean	SD	mean	SD	mean	SD	mean	SD
(A)	74.2	9.6	79.6	1.9	1.2	0.1	47.9	3.3
(B)	84.1	8.7	80.7	3.8	1.5	0.1	55.3	2.9
(C)	75.5	6.3	81.2	2.6	1.2	0.1	52.3	2.7
(D)	99.8	4.4	99.2	2.3	1.6	0.2	62.6	10.7
(E)	71.0	4.2	76.2	4.6	1.0	0.1	49.0	1.9
Normal	64.3	4.0	66.9	2.0	1.0	0.1	43.2	2.6

*Aspartate aminotransferase. **Alanine aminotransferase.

***Bilirubin. ****Alkaline phosphatase.

- **Bilirubin**

Groups A & C and B & C as well as E & normal group displayed non significant correlation ($P>0.05$) while other groups gave positive significant correlation ($P<0.05$).

Alkaline phosphatase

All groups had positive significant correlation ($p<0.05$) except between group B and normal group which gave non significant correlation ($P>0.05$).

DISCUSSION

Schistosomiasis ranks as a first problem affecting the health and economy of many populations all over the world. In Egypt, it represents a major national problem regarding its high prevalence, high intensity and frequency of infection (WHO, 1993 and El-Khoby *et al.*, 1998).

The present work revealed a significant reduction of about 35.9% in the number of recovered worms at early immunized group (A) in comparison with about 22.5% reduction in recovered worms at late immunized group (C). This means that immunization during the development of the worm is better than that after maturation of the parasite. The present results are in agreement with that of Shoemaker *et al.* (1990). They used 28000 daltons antigen and the protection level was about 30% in mice.

Gamal-Eldin and Aboul-Atta (1979), recorded abnormal copulatory and locomotory behaviour in the form of erratic and agitated worms which indicated loss of mechanism of orientation among the worms. No similar results have been recorded in the present work. Moreover no morphological changes have been detected in the worms at both immunized groups.

It was evident that early immunization had the maximum number of dead ova followed by late immunization. Comparing the results of tissue bound ova with those obtained from worms in copula,it was notised that rate of dead eggs seemed to run opposite to the number of worms in copula while the reverse was true with viable eggs. Thus, the

fewer was the number of worms in copula, the higher was the number of dead eggs and the fewer were the viable eggs. So it can be concluded that, the decrease or increase in viable eggs under the effect of antigen were closely related to the number of worms in copula. It seemed that the protective power of the antigen used may play a role in the affecting of female gonads or in destroying the eggs.

Sher *et al.* (1977) displayed that the cause of death of eggs was the possibility of damaging female gonads due to antibody response to the antigen, leading to the production of abnormally dead eggs.

During the present work, mortality rate among mice reached its lower level in the early immunized group comparing to the late immunized one.

No significant improvement of the animal weight or weight of liver and spleen have been noticed in both immunized groups when compared to non-immunized ones.

Concerning the liver functions, the present study revealed an increase in bilirubin concentration and ALT, AST and ALP enzyme activities in the sera of infected non-immunized mice. This is in agreement with Hamed and Hetta (2005), who attributed that increase to the release of the enzymes from the damaged livers into the circulation as a result of increased cell membrane permeability. However, the present results exhibited some improvement in all liver functions (AST, ALT, ALP and bilirubin) at early immunized group comparing to late immunized and non-immunized groups.

In conclusion, immunization of *S. mansoni* infected mice with adult worm antigen resulted in partial protection in mice through

the reduction of the worm load and the decrease in the number of worms in copula which led to reduction of the viable eggs in tissues.

This partial protection can play a role in the strategy of control of schistososiasis beside other methods of control like chemotherapy, snail eradication and health education.

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تأثير التطعيم الوقائي بمستخلص الديدان الناضجة لبلهارسيا المستقيم علي الفئران المصابة

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يعتبر مرض البلهارسيا من الأمراض واسعة الانتشار في المناطق الحارة ومن بينها مصر.
والبحث الحالي يُعد محاولة لدراسة التأثير المناعي بالتطعيم بمستخلص ديدان البلهارسيا علي الفئران السويسرية البيضاء المصابة ببلهارسيا المستقيم. وقد تم استخدام مائة وخمسون فأراً مقسمة إلي خمس مجموعات متساوية علي النحو التالي:

مجموعة (أ) مكونة من ٣٠ فأراً تم تطعيمهم بعد أربعة أسابيع من العدوى ويتم تشريحهم بعد اثني عشر أسبوعاً من الإصابة.

مجموعة (ب) مكونة من ٣٠ فأراً وتعتبر مجموعة ضابطة للمجموعة (أ).

مجموعة (ج) مكونة من ٣٠ فأراً تم تطعيمهم بعد ثمانية أسابيع من الإصابة ويتم التشريح بعد مرور ستة عشر أسبوعا من العدوى.

مجموعة (د) مكونة من ٣٠ فأراً وتعتبر مجموعة ضابطة للمجموعة (ج).

المجموعة (هـ) وتتكون من ٣٠ فأراً وتمثل مجموعة ضابطة لكل المجموعات حيث يتم التشريح بعد ثمانية أسابيع من الإصابة.

وبالإضافة للمجموعات السابقة فقد تم استخدام مجموعة غير مصابة وبدون تطعيم مكونة من ٢٠ فأراً.

هذا وقد كانت الجرعة الكلية للطعم المستخدم ٣٠٠ ميكروجرام لكل فأرقسِمت إلي ثلاث جرعات (٥٠ ، ١٠٠ ، ١٥٠ ميكروجرام) تعطي أسبوعيا عن طريق الحقن تحت الجلد.

ولقد تمت دراسة تأثير الطعم الوقائي علي الفئران المصابة من خلال:

العدد الكلي للديدان خاصة التي توجد في حالة ازدواج.

حيوية البويضات الموجودة في الأنسجة.

معدل الوفيات بين الفئران.

وزن الجسم ووزن الكبد والطحال.

دراسة معدلات وظائف الكبد.

هذا ولقد أظهرت الدراسة أن التطعيم المبكر (بعد أربعة أسابيع من العدوي) قد أعطي نتائج جيدة مقارنة بالتطعيم المتأخر (بعد ثمانية أسابيع من العدوي) حيث أنه:

أعطي معدل حماية قدره (٣٥,٩%) من خلال عدد الديدان البالغة مقارنة بالتطعيم المتأخر الذي أعطى حماية قدرها (٢٢,٥%) فقط ، مع النقص في عدد الديدان الموجودة في حالة ازدواج.

أدي إلي وجود كميات أكبر من البويضات الميتة في جدار الأمعاء.

أدي إلي نقص في معدل الوفيات بلغ (١٣,٤%).

أظهر تحسنا واضحا في وظائف الكبد.