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Protection Against Schistosoma Mansoni In Mice By using UV-Irradiation In Comparing With Soluble Adult Worm Antigen (SWAP)

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Abstract

Background: The present study aimed to investigate the dynamics of immunoregulatory factors implicated in the murine model of soluble adult worm antigen preparation (SWAP) and UV-irradiated adult worm extract-induced granulomatous hypo-responsivness.

Material and Methods: Native male mice were classified into four groups. The first negative control (non-infected) group, the second positive control (infected) group, the third SWAP-vaccinated group and the fourth UV-irradiated adult worm extract-vaccinated group. The last two groups were injected subcutaneous by 0.2 ml (contained 100 µg protein) of the two used vaccines SWAP or UV-irradiated adult worm extract respectively, four weeks prior to infection. The 2^{nd}, 3^{rd} and 4^{th} mice groups were infected by subcutaneous injection with 100 cercariae of Egyptian strain of S. mansoni. All mice groups were sacrificed 8 weeks post-infection.

Results: Parasitological parameters such as worms count, eggs/gram tissues of liver and intestine were studied. Biochemical parameters such as the activities of liver function tests (AST, ALT, ALP and γGT) were measured. The cellular immune responses were assessed by studying the cytokine measurement. Hepatic histopathological criteria and the morphological changes of adult worms were studied through the scanning electron microscope.

Conclusion: The present results revealed that the UV-irradiated adult worm extract have high efficacy than SWAP as immunizing antigens as shows in our data, which reported that irradiation associated vaccine antigen was shown to achieve a higher protection in mice.

Key words: Schistosomes-Radiation-Liver enzymes.

Introduction

Schistosomes are parasitic platyhelminths (flatworms) of birds and mammals. As a parasitic disease of humans, schistosomiasis ranks second to malaria in global importance. Schistosome larvae (cercariae) must invade and penetrate skin as an initial step to successful infection of the vertebrate host. Proteolytic enzymes secreted from the acetabular glands of cercariae contribute significantly to the invasion process **(McKerrow *et al*., 2008)**.

The best long-term strategy to control schistosomiasis is thought to be the immunization with an anti-schistosomiasis vaccine **(Berquist *et al*., 2002)**. There is no vaccine against schistosomiasis is yet available. Some achievements has been developed in experimental vaccination as demonstration of a strong and specific immunity following immunization with irradiated cercariae or schistosomula **(Santos *et al*., 1999)** or immunization with crude or purified antigenic extracts of various life cycle stages **(Wynn and Hoffmann, 2000)**. UV-irradiated and gamma-irradiated schistosomula of *Schistosoma mansoni* induce high levels of resistance to challenge infection in experimental hosts **(Wales *et al*., 1992)**. It seems that the adult worm antigenic preparation (SWAP) possesses several different molecules responsible for inducing the heterogeneity in cell mediated responsiveness **(Hirsch and Goes, 1996)**.

Since schistosomes do not multiply within the final host, a vaccine that induces even a partial reduction in worm burdens could considerably reduce pathology, limit parasite transmission and be less expensive than repetitive drug treatment **(Berquist *et al*., 2002)**.

The present study is undertaken to use the radiation as a tool to produce vaccine against experimental *Schistosoma mansoni* infection. Ultraviolet irradiated adult worm extract and soluble worm antigen preparation (SWAP) were used as vaccines and study the effects of these vaccines as regarded parasitological, biochemical, immunological, histopathological and scanning electromicroscope aspects on mice challenged by *Schistosoma mansoni* cercariae.

Material and methods

1-Experimental animals:

The present study was done on 80 laboratories bred male, Swiss albino mice, 6-8 weeks old, each weighting 18-20 gram, were obtained from the *Schistosome* Biological supply program unit at Theodor Bilharz Research Institute, Guiza-Egypt (SBSP-TBRI). They were kept under standard laboratory care.

I.2- Fresh adult *Schistosoma mansoni* worms, SWAP and Cercaria for challenge infection:

Fresh adult *Schistosoma mansoni* worms, SWAP-vaccine and cercaria for challenge infection were provided by *Schistosome* Biological supply program unit at Theodor Bilharz Research Institute, Guiza-Egypt (SBSP-TBRI).

I.3- Ultra violet rays (UV-irradiation) source:

A Duo- UV-source, (Abnehmbar removable UV-254 nm. From Desaga Heidelberg, W. Germany) giving dose equivalent to 220 Mw/cm-2/min. was used, at the National Center for Radiation Research (NCRRT), Cairo, Egypt.

II. Methods:

II.1-Vaccine preparation:-

Ultraviolt-irradiated *Schistosoma mansoni* adult worm extract:

Adult *Schistosoma mansoni* worms were suspended in 50 mL Phosphate buffer saline solution (pH 7.2) and exposed to UV-irradiation at dose equivalent to 220 Mw/cm-2/min for 3 min. was used **(Maghraby *et al.*, 1999)**. UV-irradiated worms were homogenized with a glass homogenizer pre-cooled for 5 minutes. The homogenate was centrifuged at 6500 g for one hour at 4ºC **(Salih *et al.*, 1978)**. The supernatant was collected and the antigen solution was used for measuring the protein content using the Bio-Rad protein assay kit.

II.2-Vaccination method:-

Eighty mice used for experimental vaccination and challenge were divided into four equal groups each containing 20 mice.

1- Mice of **group 1** were injected subcutaneous with 0.2 ml of sterile saline solution as negative control group.

2- Mice of **groups 2** were injected subcutaneous with 0.2 ml of sterile saline solution as positive control group.

2- Mice of **group 3** were vaccinated by SWAP via subcutaneous injection of 0.2 ml (contained 100 µg protein).

3- Mice of **group 4** were vaccinated by UV-irradiated adult worm extract via subcutaneous injection of 0.2 ml (contained 100 µg protein).

Mice of 2, 3, and 4 groups were exposed to experimental subcutaneous injection with live *S. mansoni* cercariae (100/mouse) after 4 weeks from vaccination.

All groups were scarified at the end of eight week post-infection. Blood was collected for serum preparation. Evaluation of the effect of vaccines will depend upon:-

II.3- Parasitological parameters:-

II.3.A- Worms count was studied in infected mice by perfusion (hepatic) method **(Kloetzel, 1967)**.

II.3.B- Egg count in liver & intestinal tissue **(Cheever, 1968)**.

II.4- Biochemical parameters: Including liver function tests.

II.4.a- Determination of serum amino transferase activity levels **(Reitman and Frankel, 1957)**.

II.4.b- Determination of serum alkaline phosphatase activity **(kind and king, 1954).**

II.4.c- Determination serum gamma gultamyl transferase activity **(Rosalki, 1975)**.

II.5- Immunological parameters:

II.5.A- Determination of serum Tumor Necrosis factor-alpha (TNF-α) **(MacKichan and DeFranco, 1999)**. The Biosource. International, Inc.

II.5.B- Mouse Interlukin-10 (IL-10) **(Zeng *et al.*, 1998)**. The Biosource. International, Inc.

II.6-Histopathological parameters: Including histopathological changes in the liver tissue.

Liver sample from each animal was removed and fixed in 10% buffered formaline solution. Then processed in pathology lab and embedded in paraffin wax to be sectioned. Liver sections were microscopically studied to evaluate the pathological changes including portal tracts and the schistosomal granulomatous reactions **(Botros *et al*., 1986)**.

II.7- -Scanning Electron microscope (E/M) study:

Morphological changes of recovered adult worms of all groups were studied. Worms were fixed in 4% gluteraldehyde and 0.2 M Na-cocodylate (v/v) and then processed for SEM according to **Fallon *et al.* (1996)**. Processed worms were dried with CO2 and Methyl alcohol using critical point dryer (Samdri-PVT-3B). Specimens were then subjected to gold coating by JEOL-JFC-1100E ion sputtring device and examined using JEOL-JSM-5400 scanning electron microscopy at the National Center for Radiation Research (NCRRT), Cairo, Egypt.

II.8- Statistical analysis:

Results are expressed as the mean ± SE. Data were statistically analyzed for variance and the least significant difference (LSD) using one way analysis of variance (ANOVA) according to **(Snedecor and Cochron, 1989)**. An IBM computer with a software system SPSS version 13 was for these calculations.

Results

1- The results of parasitological parameters, males, females, couples and total worms counting. In addition to Eggs /grams tissue in liver and intestine counting.

The results indicated that, in the SWAP-vaccinated and UV-irradiated adult worm extract-immunized groups there was a very high significant reduction ($p< 0.001$) of the males, females, couples and total worms number and the Eggs /grams tissue in liver and intestine compared to positive control, but with variable degree where (a slightly reduction of males, females, couples and total worms number and the Eggs /grams tissue in liver in intestine was observed in UV-irradiated adult worm extract-immunized compared to SWAP-vaccinated group (Table 1).

2- The results of biochemical parameters of serum liver enzyme

Estimation of serum AST, ALT, ALP and γ-GT activity:

The results explain that, at eight weeks post-infection, there was a very high significant increase ($P<0.001$) of AST, ALT, ALP and γ-GT activity in infected positive control group versus the uninfected negative control group. Whereas, there was also a very high significant increase ($P<0.001$) of AST, significant increase ($P<0.05$) of ALT, high significant increased ($P<0.01$) of ALP and γ-GT in the SWAP-vaccinated group and significant increase ($P<0.05$) of AST, ALP and γ-GT and no significant increased of ALT, in the UV-irradiated adult worm extract vaccinated groups as compared to uninfected negative control group. There was significance decrease ($P<0.05$) of AST, and no significance decreased of ALT, ALP and γ-GT in UV-irradiated adult worm extract vaccinated groups compared to SWAP vaccinated group (Table 2).

3- The results of measurement of splenic cytokine production:

The results explain that, at eight weeks post-infection, there was a very high significant increase ($P<0.001$) of TNF-α and IL-10 concentration in infected positive control group versus the uninfected negative control group. Likewise, there was also a very high significant increase ($P<0.001$) of TNF-α and IL-10 in the SWAP and UV-irradiated adult worm extract vaccinated groups as compared to uninfected negative control group, and very high significant decrease ($P<0.001$) of TNF-α content and very high significant increase ($P<0.001$) of IL-10 content as compared to infected positive control group. Indeed, There was significance decrease ($p<0.05$) of TNF-α level in UV-irradiated adult worm extract vaccinated groups compared to SWAP-vaccinated group. On the other hand, there was significance ($p<0.01$) increased of IL-10 level in UV-irradiated adult worm extract vaccinated group compared to SWAP-vaccinated group (Table 3).

4- The results of histopathological changes in the liver.

The results explain that, at eight weeks post-infection, liver sections of infected control group showed scattered lobular granuoloma around liver ovum, multiple smaller lobular granulomas, large fibrocellular granuoloma in the hepatic lobules around living& degenerated ova,

amulgamated (adherent) fibrocellular gramulomes, and Multiple small scattered lobular fibrocellular granulomes formed around living degenerated ova as represented in figures (1, 2, and 3). While small scattered lobular granulomas, single cellular granulomas, small single cellular granuloma in the hepatic lobule formed around degenerated ovas, Cellular fibrocellular granulomas formed around degenerated & calcified ova and lobular granulmes formed around calcified ovum of mouse vaccinated with SWAP (Fig. 4 and 5) and Cellular lobular granuloma formed around degenerative ovum, enlarged portal tract by dilated blood vessels and intensive inflammatory reaction, Lobular cellular & fibrocellular granulomes in UV-irradiated adult worm extract vaccinated group (Fig. 6, 7 and 8).

5- The results of Scanning Electron-Microscope Study on Schistosoma morphological changes:

The results explain that, at eight weeks post-infection, the adult worm from the infected control group have no change in own morphology, while adult worms from both SWAP and UV-irradiated adult worm extract vaccinated groups showed some damage in own morphology. Scanning EM in the present study represents abnormalities in the adult male worms like abnormal suckers, flattens of gynaecophoric canal, strictures, detached of spines and some area of erosion on the posterior surface of the worm as represented in figures (9, 10, 11, 12, 13 and 14).

Table (1): Effect of SWAP & UV-irradiated adult worm extract on Parasitological parameters in experimental mice.

		Mean ± SE % of reduction from positive control					
Mice group		**Male**	**Female**	**Couple**	**Total**	**Eggs in liver**	**Egg in intestine**
Positive control	N=20	9.05 ±0.45	8.65 ±0.71	7.2 ±0.54	24.4 ±1.86	3566.55 ±325.83	4095.05 ±382.59
Immunized with SWAP	N=20	a*** 1.55 ±0.146 82.7%	a*** 1.7 ±0.09 80.2%	a*** 1.15 ±0.07 84%	a*** 4.4 ±0.33 82%	a*** 1073.85 ±108.58 70%	a*** 990.7 ±77.81 76%
Immunized with extracts from UV-irradiated worms	N=20	a*** 1.0 ±0.07 88.8%	a*** 1.25 ±0.07 86%	a*** 1.15 ±0.09 84%	a*** 3.2 ±0.31 87%	a*** 453.45 ±39.02 88%	a*** 419.55 ±36.86 89.8%

- Each value represents the mean of 20 mice ± SE.

(a): Significant different from positive control group at $p < 0.001$***.

Table (2): Effect of SWAP & UV-irradiated adult worm extract on biochemical parameters in experimental mice.

		Mean ± SE % of change from negative control			
Mice group		**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**γGT (U/L)**
Negative control	N=20	34.31 ±2.072	34.08 ±2.77	7.37 ±0.57	4.38 ±0.21
Positive control	N=20	a*** 70.41 ±5.13 105%	a*** 65.3 ±6.13 91%	a*** 13.73 ±0.83 86%	a*** 8.56 ±0.67 95%
Immunized with SWAP	N=20	a*** b*** 51.377 ±4.12 50%	a* b*** 45.38 ±3.11 33%	a** b*** 10.5 ±0.71 42%	a** b*** 6.43 ±0.52 46%
Immunized with extracts from UV-irradiated worms	N=20	a* b*** c* 41.69 ±3.11 21%	b*** 40.9 ±3.91 20%	a* b*** 9.32 ±0.81 26%	a* b*** 5.58 ±0.39 27%

- Each value represents the mean of 20 mice ± SE.
(a): Significant different from negative control group at p<0.05 *, p< 0.01 ** and p< 0.001***.
(b): Significant different from the positive control group at p< 0.001***.
(c): Significant different from the SWAP group at p<0.05 *.

Table (3): Effect of SWAP & UV-irradiated adult worm extract on Immuonological parameters in experimental mice.

		Mean ± SE % of changes from negative control group	
Mice group		**TNF-α (pg/mL)**	**IL-10 (pg/mL)**
Negative control	N=20	17.73 ±0.7	84.45 ±6.4
Positive control	N=20	a*** 63.07 ±5.4 255%	a*** 439.17 ±37.9 420%
Immunized with SWAP	N=20	a*** b*** 35.23 ±2.9 98%	a*** b*** 536.24 ±48.44 534%
Immunized with extracts from UV-irradiated worms	N=20	a*** b*** c* 30.63 ±2.78 72%	a*** b*** c** 600.05 ±51.67 610%

- Each value represents the mean of 20 mice ± SE.
(a): Significant different from negative control group at p< 0.001***.
(b): Significant different from the positive control group at p< 0.001***.
(c): Significant different from the SWAP group at p<0.05 * and p< 0.01 **.

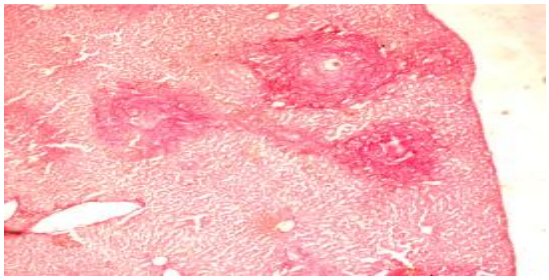

Fig. (1): Section in liver shows scattered lobular granuoloma of infected mouse (control group) (Sirius red X50).

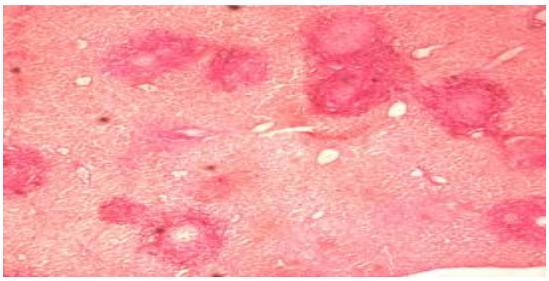

Fig (2): Multiple lobular granulomas in the liver of infected mouse (control group) (Sirius red X 50).

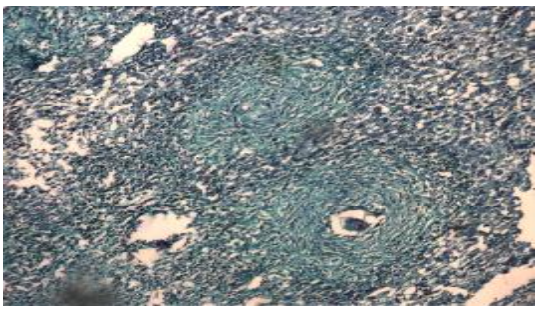

Fig (3): Shows Large fibrocellular granuoloma in the hepatic lobules around living& degenerated ova (Control group) (Masson trichrome X100).

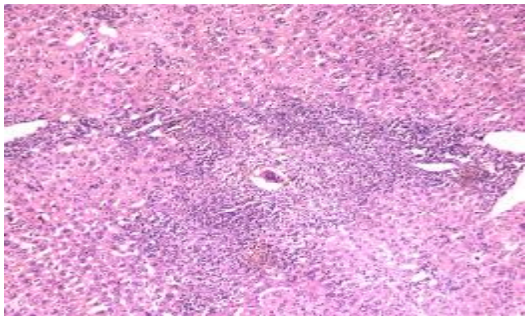

Fig (4): High power view of single cellular granulomas of mouse vaccinated with SWAP around ovum (H. & E.X100).
(Masson trichrome x100).

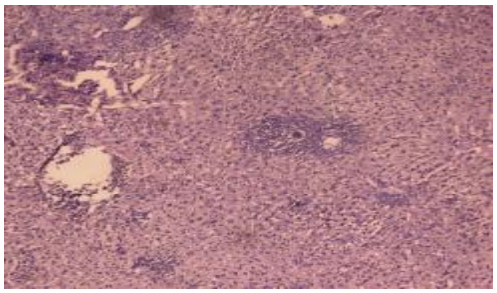

Fig (5): Shows small single cellular granuloma in the hepatic lobule formed around degenerated ova in mouse vaccinated with SWAP (H. & E. X50).

Fig (6): Cellular lobular granuloma formed around degenerative ovum (UV-irradiated adult worm extract vaccinated group) (Masson trichrome X 50).

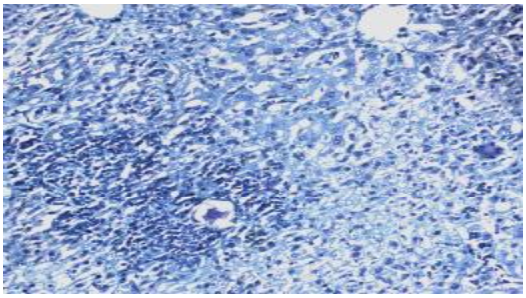

Fig (7): Shows lobular cellular granuloma of mouse vaccinated by UV-irradiated adult worm extract formed around degenerative ovum (Masson trichrome X200).

Fig (8): Higher power view of Lobular cellular & fibrocellular granulomes of mouse vaccinated with UV-irradiated adult worm extract group (Masson trichome X100).

Fig (9): Shows Scanning EM of abnormal male adult worm *Schistosoma mansoni* with abnormal gynaecophoric canal.

Fig (10): Shows Scanning EM of abnormal male adult worm *Schistosoma mansoni* with abnormal suckers.

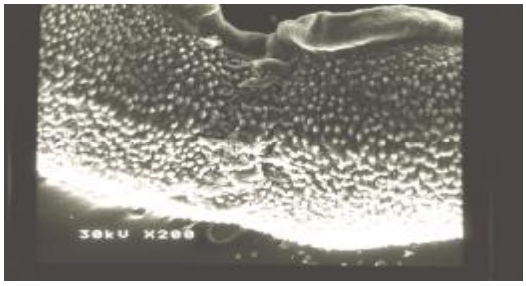

Fig (11): Shows Scanning EM of posterior surface of abnormal male adult worm *Schistosoma mansoni* with stricture.

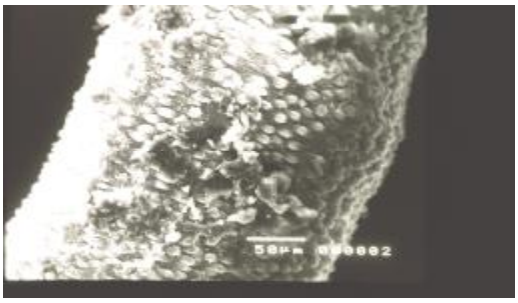

Fig (12): Scanning EM of posterior surface of abnormal male adult worm *Schistosoma mansoni* shows erosion.

Fig (13): Scanning EM of posterior surface of abnormal male adult worm *Schistosoma mansoni* show detached spines.

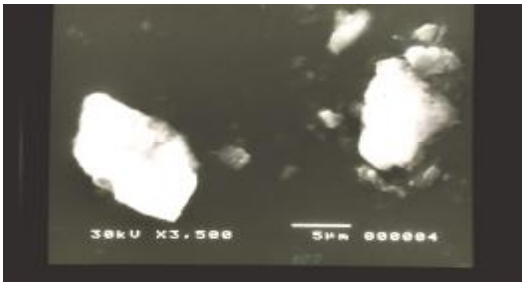

Fig (14): Scanning EM show abnormal separated spines of male adult worm *Schistosoma mansoni.*

Discussion:

Many world health agencies agree that the development of an antischistosomiasis vaccine should be sought. Several studies are in progress in this field, testing different antigens of the parasite and different vaccination strategies **(Bergquist *et al*., 2002)**.

In the present study the UV-irradiated adult worm extract group has very highly percentage reduction in worm burden, and a very highly percentage reduction of ova count was observed in the liver and the intestine tissues compared to positive control (non-immunized infected) group. On the other hand, the SWAP-vaccinated group has less percentage reduction in worm burden, also a less percentage reduction of ova count was observed in the liver and the intestine tissues compared to UV-irradiated adult worm extract group, these reflect the high efficacy of UV-irradiated adult worm extract as protective antigen than the SWAP-vaccine where the activity of liver function enzymes, liver histopathology and serum cytokine concentration ensure the host reaction to parasite eggs that are

laid in the portal venous system and subsequently trapped in the liver and intestine. This agree with **Cheever *et al.* (1997)** who reported that in murine schistosomiasis, the pathology resulting from infection with the helminth parasite *Schistosoma mansoni* is predominantly caused by the host reaction to parasite eggs that are laid in the portal venous system and subsequently trapped in the liver and intestine. The associated egg-induced fibrosis could lead to portal hypertension, which causes much of the morbidity and mortality associated with this disease.

The present data shows, a very high significant increase in all parameter of liver functions enzymes activity in infected positive control group versus the uninfected negative control group and amelioration and in all parameters of liver functions enzymes activity of SWAP and UV-irradiated adult worm extract immunized groups was observed, as compared to infected positive control group. This is due to the vaccination which relieves the syndrome of schistosomiasis. This results agreed with **(El-Hawary *et al.*, 1972** and **Salah *et al.*, 1976)**, who mentioned that, the schistosomal infection of the liver results in cirrhosis characterized by fibrosis and absence of regeneration. Subsequently, serum transaminases and alkaline phosphatase are frequently elevated in bilharzial hepatic fibrosis. **Ebeid *et al.* (2000)** and **Ezzat *et al.* (2001)**, mention also that, Alanin aminotransferase (ALT) is a useful indicator of hepatocellular damage, and its assay is considered as helpful screening for liver damage. Early elevation of serum level of ALT may be considered as a sensitive clue for functional changes in hepatosplenic bilharziasis rather than other conventional liver function tests **(El-Haieg *et al.*, 1978).**

Kardorff *et al.* (1997), reported that, Gamma glutamyl transferase (γ-GT) is a sensitive indicator of liver disease. Also **Ashton *et al.* (2001)**, found that the elevated level of serum γ-GT in the infected group of schistosoma was considered as a marker for hepatocellular injury with severs periportal fibrosis. Such elevation may reflect early liver tissue damage from egg secreted proteases, due to the great number of egg deposition occurred after challenge infection. The previous observation was agreed with the elevation values observed in the present study.

In the present study, data shows that very high elevation of type-2 response (IL-10) in positive control group as compared to negative control group. On the manner, SWAP and UV- irradiated adult worm extract immunized groups recorded high and very high significant increase when compared with positive control this reflects the important role of vaccine to induce type-2 response (IL-10). These results was agreed with **Pearce *et al.* (1998)**, who found that, because parasite eggs induce a strong type-2 response, it was hypothesized initially that type-2 rather than type-1 cytokines play an integral role in granuloma formation. Also its agreed with **Hoffmann *et al.* (2000)**, who mentioned that, in murine *Schistosomiasis mansoni*, IL-10 reduces hepatocyte damage induced by the parasite's eggs and is essential for maintaining a nonlethal chronic infection.

The present data shows that high increase level of TNF-α in positive control, SWAP and UV-irradiated adult worm extract immunized groups, the elevated levels of circulating TNF-α appear to correlate with schistosome maturation and oviposition, and the circumoval granulomatous response of the murine host. These results agree with **Amiri *et al.* (1992)** who reported that, TNF-α has a pleiotropic effect on the immune response against schistosomiasis: it restores the ability of T cell deficient mice to mount a granuloma around schistosome eggs. Therefore, the primary role of TNF-α in schistosomiasis is a protective effect. **Workineh and Asrat (2007)**, reported that, the increased level of the inflammatory TNF-α after egg excretion may be an indication of its effect in complications of schistosomiasis in the liver, because TNF-α can induce cell-mediated immune responses that could enhance inflammatory reactions due to the activation effects on macrophages, eosinophils and lymphocytes. The previous observation , was agreed with the elevation values observed in the present study.

In the present data the UV-irradiated adult worm extract-immunized group has decrease of TNF-α compared to SWAP-immunized group. While UV-irradiated adult worm extract-immunized group have high increase of type-2 response (IL-10) compared to SWAP-immunized group, this reflects the balance response between TNF-α and IL-10 in schistosomiasis infected mice, and agreed with **Zouain *et al.* (2002)** who reported that, the association of TNF-α and IL-10 has also been found to modulate the *in vitro* granuloma reaction in humans or in experimental schistosomiasis.

In the present study, histopathological examination of liver tissues showed improvement of liver tissues in both UV-irradiated adult worm

extract and SWAP vaccinated groups, as compared to positive control group, this reflect important role of vaccines for amelioration of liver tissues of *S. mansoni*-infected mice, and agreed with **Andrade and Warren (1964)** who reported that, one of the features of schistosomiasis immunobiology is the gradual and spontaneous reduction in the size of granulomatous inflammation around the continuously incoming eggs.

Scanning electron microscopy in the present study represents abnormalities in the adult male worms likes abnormal suckers, flattens of gynaecophoric canal , strictures, detached of spines and some area of erosion on the posterior surface of worm of UV-irradiated adult worm extract-immunized group more than SWAP-vaccinated group. This agrees with **Xiao *et al.* (2002)** who reported that, adult male worms showed severe deformation in anterior and ventral suckers and a wide abnormal gynacoventral groove, in addition, intensive corrugation and lesions in the tegumental surface have been observed in the male worms accompanied with increased loss of spines and tegumental disruption.

The present study suggested that the UV-irradiated adult worm extract have high efficacy than SWAP as immunizing antigens.

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الوقاية ضد الإصابة ببلهارسيا المستقيم في الفئران باستخدام الأنتيجينات المحضرة من الديدان البالغة المعرضة للأشعة فوق البنفسجية مقارنته بالأنتيجينات الذائبة

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هدّفتْ الدراسةُ الحاليةُ لتَحرّي ديناميكية عوامل التنظيم المناعى فى الفئران التى تم تحصينها من خلال الحقن تحت الجلد بكل من الانتيجينات الذائبة المحضرة للديدان البالغة و المستخلص الناتج من الديدان البالغة المعرضة للاشعة الفوق بنفسجية للوقاية ضد الاصابة ببلها رسيا المستقيم فى الفئران.

ثم صُنّفتْ ذكور الفئران إلى أربعة مجموعات؛ المجموعة الأولى الضابطه (غير مصابة), المجموعة الثانية الإيجابية (مُصَابة), المجموعة الثالثة المحصنة ب 0.2 ملليتر (المحتوى على 100 ميكروجرام بروتين) من الانتيجينات الذائبة المحضرة للديدان البالغة , و المجموعة الرابعة المحصنة ب 0.2 ملليتر (المحتوى على 100 ميكروجرام بروتين) من المستخلص الناتج من الديدان البالغة المعرضة للاشعة الفوق بنفسجية. مع الاخذ فى الاعتبار انه قد تم تحصين الفئران فى كل من المجموعتين الثالثة و الرابعة قبل اربعة اسابيع من الاصابة حيث انه تم اصابة كل من المجموعة الثانية, الثالثة و الرابعة ب 100 من العائل المصيب لبلهارسيا المستقيم لكل فأر من المجموعات المصابة, بعد مرور ثمانى اسابيع من الاصابة تم ذبح الفئران وذلك لتتبع التغيرات الناتجة فى التجربة.

تم تتبع كل من التغيرات الطفيلية مثل عدد الديدان, عدد البيض لكل جرام من الانسجة الخاصة بالكبد والامعاء والتغيرات البيوكيميائية مثل تقييم النشاط الوظيفى لانزيمات الكبد (AST, ALT, ALP and γGT) , ودراسة الاستجابة المناعية الخلوية من خلال قياس التغيرات الناتجة على نوعين من السيتوكين وهما عامل تنخر الورم-الفا والانترليوكين-10 والتغيرات المرضية لانسجة الكبد واخيرا التغيرات الناشئة فى الشكل الخارجى للديدان من خلال استخدام الميكروسكوب الالكترونى الماسح.

دلت النتائج ان التحصين بالمستخلص الناتج من الديدان البالغة المعرضة للاشعة الفوق بنفسجية قد اعطى وقاية افضل فى الفئران المصابة ببلهارسيا المستقيم مقارنة باستخدام الانتيجينات الذائبة المحضرة للديدان البالغة هذا يؤيد ان استخدام الانتيجينات المشعة يعطى نتائج افضل من الانتيجينات غير المشعة مما يستلزم التوصيه باستخدام الاشعه فوق البنفسجية لتحضير اللقاحات للوقاية من مرض البلهارسيا.