On The Interaction between Induced Diabetes Mellitus and Schistosomiasis: Mechanism and Protection

Nahla S. El-Shenawy and Maha F. M. Soliman

Zoology Department, Faculty of Science, Suez Canal University, Ismailia, Egypt.

Abstract

In this study mice were percutaneously exposed to 50 *S. mansoni* cercariae. Seven weeks post infection (pi), infected mice were either injected i.p. a single dose of alloxan (200 mg kg^{-1}) or treated with a single dose of alloxan followed, three days later, with daily i.p. treatment of ethanolic extract of *Cleome droserifolia* (0.31 g kg^{-1}) for 18 days. Diabetes mellitus (DM) affected characteristics of *Schistosoma* infection as shown in alteration of oogram pattern, impairing of egg maturation and lowering of faecal egg output. However, extract treatment (EXT) affected the previously mentioned aspects in addition to its remarkable effect on worm load, diminishing of hepatic granulomas and suppressing the formation of involutional granulomas. Results indicated that DM increased the complications of schistosomiasis that was manifested by the augmented increase of serum glucose level, the severe decrease of serum thyroid hormones (tri-iodothyronine; T_3 and tetra-iodo-thyronin; T_4), insulin levels, liver glycogen content and hepatic DNA. In addition, a decrease in hepatic GSH that was accompanied by an increase in serum uric acid were observed in infected-DM mice. Serum total proteins concentrations were obviously decreased and some of protein fractions were also decreased or absent in both infected and infected-DM mice except for gamma globulin that was increased in both groups. EXT succeeded efficiently to alleviate these alterations in infected-diabetic mice, to various extents. The beneficial effects of EXT on thyroid and pancreatic hormones status seem to be contradictory to its beneficial anti-schistosomiasis effects. This contradiction may suggest that EXT exerts its beneficial effects through its direct effects on the parasite, not secondary to its effect on the host. The current results showing differential effects of both diabetes and extract on schistosomiasis, though both are beneficial, serve to corroborate this hypothesis.

Key words: Schistosoma masoni, **diabetes mellitus, parasite load, biochemistry, granuloma, nuclear contents**

Introduction

The physiological relationship between *S. mansoni* and its host is complex moreover, the details of several biochemical processes affecting this relationship are still to be determined (Neves *et al.*, 2002). Host provides a nutrient-rich environment from which the parasite benefits by taking up copious amounts of glucose and other metabolites needed for its development and reproduction. Since the host environment is crucial for the biology of the parasite, therefore, changes in the host metabolism due to other illnesses, or physiological complications could alter the normal course of schistosomiasis (Hulstijn *et al*., 2002). Doubtless, changes in the metabolic pathways of mammalian hosts should affect *schistosomiasis*, as metabolic changes accompanying malnutrition (Rocha, 1982), vitamin deficiency (Turchetti-Maia *et al*., 1983), administration of enzyme inhibitor or hormonal therapy (Chen *et al*., 1991) and insulin-dependent diabetes mellitus (Hulstijn *et al.*, 2001 & 2002).

The importance of several hormones (insulin, steroids and thyroid hormone) on worm development, egg production and the way schistosomes might exploit host signaling molecules to evade the hosts' immune system was emphasized (Mendonca *et al.,* 2000 and Salzet *et al.*,

2000). Moreover, Pearce *et al.* (1991) reported that egg production of *S. mansoni* was associated with a switch from a cellular response (thymic helper 1; Th1) to humoral response (thymic helper 2; Th2) in the host. Besides, the spontaneous development of diabetes mellitus (DM) in non-obese diabetic mice has been shown to be mediated by a Th1 response against beta cell antigens (Cooke *et al.*, 1999).

Cleome species have been used in folk medicine since ancient times. Among the disorders treated are scabies, rheumatism, and inflammation (Hussein *et al.*, 1994). *Cleome droserifolia* has hypoglycemic properties (Yaniv *et al.*, 1987) and possible antimalarial effect (Nicola *et al.*, 1996).

Therefore, this work aimed to determine whether incidence of DM or treatment using *C.droserifolia* extract could influence schistosomiasis and to investigate the relationship between the schistosemiasis and diabetes.

Materials and Methods

Infection, Induction of Diabetes and Treatment

Forty male Swiss albino mice were supplied from experimental research center of Theodor Bilharz Institute, Cairo, Egypt, and weighing 20-22 g. The mice were housed in controlled temperature and fed standard diet *ad labium* and were randomly divided into different experimental groups. Group (I) served as non-infected control (n = 10). Thirty of mice were percutaneously exposed to 50 *S. mansoni* cercariae (Egyptian strain) and were equally divided into 3 groups. Group (II) was non-treated infected mice and group (III) was injected i.p. a single dose of alloxan (200 mg kg^{-1} body weight) (Szkudelski, 2001). Group (IV) was treated with alloxan as previously mentioned in group II, then 3 days later this group was treated daily with ethanolic extract of *C. droserifolia* (Egyptian pharmacopoeia, 1984) at dose rate 0.31 g kg^{-1} for 18 days. This dose was determined according to preliminary studies that proved its effectiveness (unpublished data).

Treatment regimens were started seven weeks post-infection (pi). Three days after administration of alloxan, blood glucose levels were measured in all animals; alloxan-treated mice having blood glucose level less than 120 mg dl^{-1} was excluded from this study.

Parasite load and Kinetics of Faecal Eggs Releasing

Seventy days after cercarial exposure, all animals were sacrificed. Adult worms were recovered from the portal and mesenteric veins by perfusion (Smithers & Terry 1965). For intestinal egg counts (oogram pattern), 1 cm long fragments from the beginning of the distal part of the small intestine were crushed between two glass slides (Machado-Silva *et al.*, 1991). According to Prata (1957), one hundred per oogram were randomly chosen and quailfied under bright field microscopy (100X).

Feacal sample were collected three times per week starting from week 7 pi till the end of experiment from each mouse individually. The faeces were processed according to the Kato-Katz technique (Katz *et al.,* 1972) where three slides per animal were inspected.

Biochemical Alterations

Blood glucose concentrations were measured using Glucometer Elite test (Hulstijn *et al.*, 2002). Serum total proteins were determined using Bio-Rad protein assay reagent (Bio-Rad Laboratories, Hercules, CA. USA). Serum uric acid was determined at 546 nm using enzymatic colorimetric method (uricase-PAP) (Fossati *et al.*, 1980). Serum insulin concentrations were estimated using an enzyme-linked immunosorbent assay (ELISA) kit (Mercodia AB, Uppsala, Sweden) with rat insulin as a standard (Starr *et al.*, 1978). Levels of serum T_3 and T_4 were measured using ELISA kit (Mercodia AB, Uppsala, Sweden) according to Chopra *et al.* (1971) and Liewendahi (1990), respectively. Liver glycogen and reduced glutathione (GSH) levels were determined according to Seifter *et al.* (1951) and Beutler *et al.* (1963) respectively.

Blood Electrophoretic Pattern

Technique of cellulose acetate zone electrophoresis was used for analyzing plasma protein (Rand & Murray, 1999). Electrophoresis of sample in electrolyte buffer (Tris-barbital-sodium barbital, Electra HR buffer Helena, Laboratories, U.K., Cat # 5805) pH 8.8, was performed for 25 minutes and the current was adjusted to 250 volts. Separated protein bands were visualized in characteristic position after being stained (Ponceau's fixative dye solution, Helena Biosciences, Sunderland, Tyne and wear, SR 53XB) for 5 minutes. Then, they were rinsed in 95% glacial acetic acid: ethanol (3:7; V/V). Densitometer scanning converted bands to characteristic peaks of albumin, α_1-globulin, α_2-globulin, β- globulin and γ-globulin.

Kinetics of Granuloma Formation and nucleic acid Analysis

Specimens of Liver were fixed in 10% buffered formalin for histological examination using haematoxylin and eosin. Granuloma size was determined in histological sections by using an optical micrometer. 70 granulomas with central ovum were examined; data are presented as mean cross-sectional diameter $\pm$ standard error (SE). Density of total granuloma formation and number of each stage in an area equal to 1 mm^2 were analyzed by bright field microscopy. Granulomas were classified according to Lenzi *et al.* (1998) being discriminated to exudative and involutional stages. For visual evaluation of hepatocytic nucleic acids content, liver sections were stained using methyl green pyronine (Bancroft & Stevens, 1996).

Statistical analysis

The statistical program SPSS 11.0 for Windows was used for data processing. Data was given as mean $\pm$ SE. One-way analysis of variance followed by Tukey–Kramer multiple comparisons test was used.

Results

Results of worm recovery from each infected group were shown in Table 1. DM had no effect on worm recovery while extract treatment (EXT) seemed to affect the worm burden slightly with a reduction rate 13.5% as compared to infected-DM; this reduction was observed mainly in number of total female worms ($P < 0.01$). Consequently, the sexual bias (male per female worms) was changed in EXT mice.

Table 1: Male and female worms recovered from mice infected with 50 cercariae of *S. mansoni* at week 10 pi under different treatment regimens

Groups	Male worms hepatic	Female worms hepatic	Total Worms hepatic	Male worms Promesent.	Female worms Promesent.	Total Worms Promesent.	Mean total worms
Infected	5.4 ± 0.5	2.8 ± 0.4	8.2 ± 0.9	8.5 ± 0.4	4 ± 0.28	12.5 ± 0.2	20.7 ± 0.9
Infected - DM	5.3 ± 0.45	2.12 ± 0.35	7.4 ± 0.8	8.5 ± 0.9	3.4 ± 0.4	11.9 ±1.1	19.3 ±1.9
EXT	4.5 ± 0.8	3.00 ± 0.5	7.5 ± 1.3	6.5 ± 0.7	2.7 [a] ± 0.3	9.2 ± 0.9	16.7 [a] ± 1.01 9

Results are given as mean $\pm$ SE of eight mice in each group. [a] ($p < 0.05$) infected-DM versus EXT.

Intestinal oogram pattern was given in Table 2. Both DM and EXT increased the percentage of total dead eggs. DM mainly affected dead immature eggs while the effect of extract was on dead mature ones (Table 2). The percentage of live mature and immature eggs was rather different between groups, as well as the percentage of live and dead eggs. Low percentages of live mature and immature eggs were found in infected-DM mice as compared to fectedgroup while EXT resulted in low percentage of live immature ones.

Table 2: Intestinal oogram pattern of mice infected with 50 cercariae of *S. mansoni* at week 10 pi under different treatment regimens.

Groups	shell	Dead immature	Indefin-able	Dead mature	Total dead	Early immature	Late mature	Mature	Total live
Infected	2.8 ± 0.6	6.7 ± 1.5	3 ± 0.7	1 ± 0.4	13.5 ± 2.3	18.3 ± 1.9	37.5 ± 4.8	30.7 ± 2.3	86.5 ± 2.3
Infected - DM	2.25 ± 0.3	40.75 ± 2.7 [a]	2.75 ± 0.2	5.50 ± 1.0 [a]	51.25 ± 1.5 [a]	11.50 ± 1.6 [a]	22.0 ± 2.5 [a]	15.25 ± 2.8 [a]	48.75 ± 1.5 [a]
EXT	1.5 ± 4.2	24.25 ± 2.5 [b]	1.5 ± 0.4	15.25 ± 2.4 [b]	42.5 ± 3.5	8.5 ± 1.4	21.5 ± 0.6	27.5 ± 3.4 [b]	57.5 ± 3.5

Results are given as mean ± SE of eight mice in each group. [a] ($p < 0.001$) infected versus infected-DM groups; [b] ($p < 0.001$) infected-DM versus EXT groups.

The kinetics of fecal egg output (Fig. 1) was characterized by an increasing in the number of eggs with the duration of infection. Starting from week 8 pi (one-week post treatment) egg elimination was observed in both infected-DM and EXT groups. This elimination became very high in week 9 and extended to week 10 pi in infected-DM group as compared to infected ($p < 0.01$) and EXT ($p < 0.05$) groups. In EXT group, the elimination of eggs reached to a stable phase with egg-laying lower than infected group.
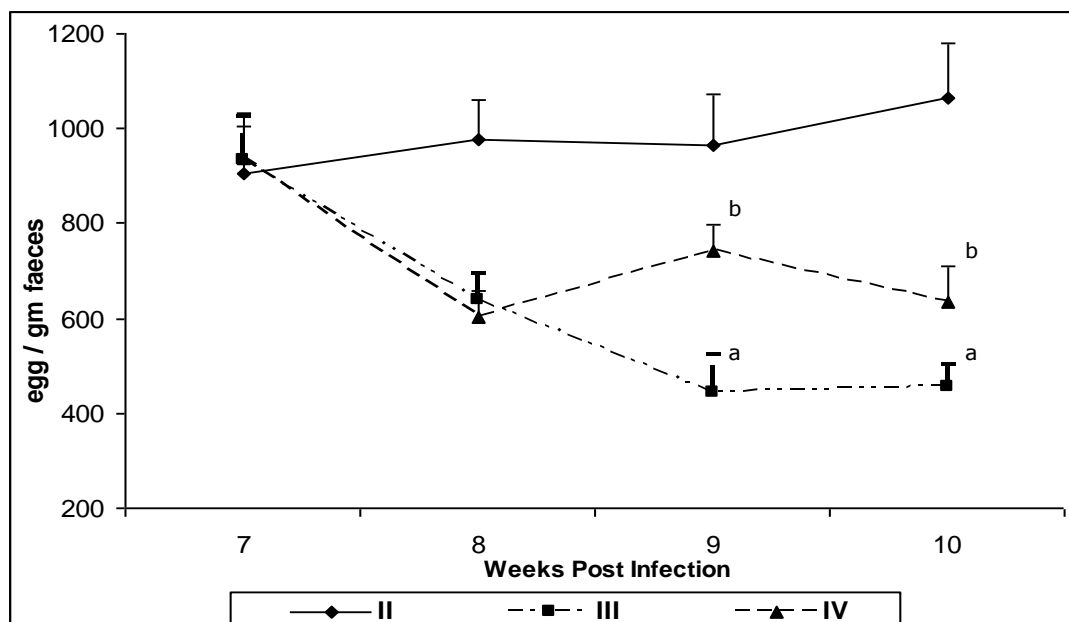

Fig. 1: Kinetics of faecal eggs releasing from mice infected with 50 cercariae of *S. mansoni* following different treatment regimens at week 7, 8, 9 and 10 pi. II: Infected; III: Infected-DM; IV: Infected-DM and EXT. [a] ($p < 0.01$) infected versus infected-DM mice; [b] ($p < 0.05$) EXT versus infected-DM mice (n = 8).

Serum glucose levels of all groups were summarized in Fig. 2. Both infection and its association with DM increased glucose level. EXT significantly reduced the fasting blood glucose levels as compared to infected-DM group ($P <$ 0.001). Significant decreases in liver glycogen content was accompanied with high glucose level in infected and infected-DM groups while extract-treatment signify-cantly enhanced liver glycogen content (Fig. 2).
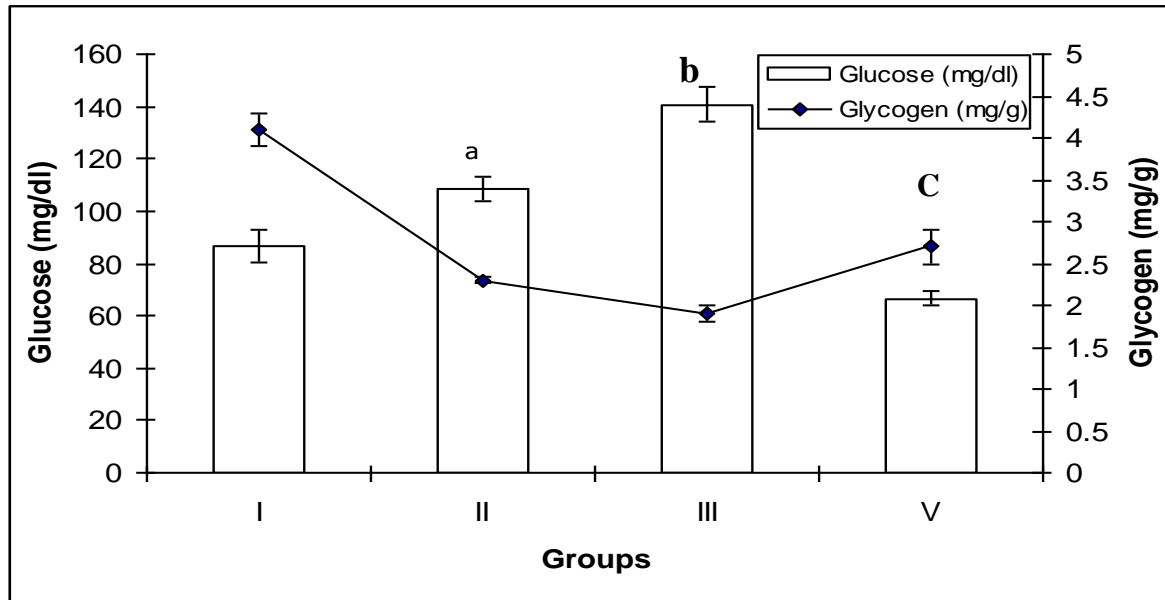

Fig. 2: Serum glucose and glycogen contents of mice infected with 50 cercariae of *S. mansoni* at week 10 pi under different treatment regimens. I: control negative; (II- IV) as described in Fig. 1. [a]($p < 0.05$) negative control versus infected; [b]($p < 0.02$) infected versus infected-DM mice; [c] ($p < 0.001$) infected-DM versus EXT, (n =8).

Levels of serum T_3, T_4, T_3/T_4 ratio, and insulin were decreased in both infected and infected-DM mice as compared to control and infected groups, respectively. Increases in all hormones levels were observed following EXT (Table 3). There was no significant difference in serum T_4 level between infected and infected-DM mice (Table 3). Results revealed a highly significant decrease in fasting serum insulin level in infected mice (50%) as compared with the normal group, while infected-DM mice had 13.6% depression of insulin as compared with infected mice (Table 3). EXT enhanced the insulin level of infected-DM mice by 27%. Results revealed that the level of serum uric acid of infected-DM mice has been reduced with EXT (Table 3). Hepatic GSH levels of infected and infected-DM mice were significantly decli-ned as compared to control and infected mice, respectively while the levels were increased after EXT (Table 3).

Table 3: Biochemical parameters of mice infected with 50 cercariae of *S. mansoni* at week 10 pi under different treatment regimens.

Parameters	Negative control	Infected	Infected-DM	EXT
Serum				
T_3 (ng dl^{-1})	270.8 ± 7.4 (254.8 – 288.6)	228.7 ± 11.1 [a] (200.6 – 248.4)	183.5 ± 2.9 [b] (176.9 – 190.4)	269.8 ± 3.1 [c] (261.2 – 274.6)
T_4 (nmol dl^{-1}l)	43.4 ± 0.7 (41.8 – 45.0)	37.4 ± 0.3 [a] (36.6 – 37.8)	35.8 ± 0.3 (35.3 – 36.7)	40.1 ± 0.7 [c] (38.6 – 41.5)
T_3/T_4 ratio	20.1 ± 0.4) (18.7 – 20.6)	20.6 ± 0.1 (18.3 – 22.4	15.8 ± 0.2 [b] (15.5 – 16.2)	21.7 ± 0.5 [c] (20.3 – 22.8)
Insulin (µU ml^{-1})	27.7 ± 0.6 (25.8 – 28.8)	13.9 ± 0.4 [a] (12.8 -14.8)	11.1 ± 0.3 (10.4 – 12.0)	15.3 ± 0.6 [c] (14.5 – 17.3)
Uric acid (mg dl^{-1})	3.1 ± 0.1 (3.1 - 3.4)	4.9 ± 0.1 [a] (4.6 – 5.0)	4.5 ± 0.1 (4.3 – 4.9)	4.1 ± 0.1 (3.9– 4.5)
Liver				
GSH (mg g^{-1})	4.7 ± 0.1 (4.4 – 4.9)	3.1 ± 0.1 [a] (2.9 – 3.5)	2.7 ± 0.2 (2.5 – 3.6)	3.9 ± 0.4 [c] (3.0 – 4.6)

Results are given as mean ± SE of eight mice in each group. [a]($p < 0.001$) infected mice versus negative control; [b]($p < 0.01$) infected-DM versus infected mice; [c] ($p < 0.001$) infected-DM versus EXT. The minimum and maximum values are shown in parentheses

As seen in table 4, serum total proteins were increased after EXT. Serum albumin and alpha globulin concentrations were significantly decreased (53.7 % and 9.4%, respectively) in infected group (P < 0.001) as compared to control. Beta globulin was absent and gamma globulin increased in infected mice as compared to control, while, these fractions of protein remained nearly in the same level in infected-DM mice. EXT induced highly significant increase of all these parameters except for gamma globulin concentration that was highly decreased (Fig. 3). A/G ratio was higher in the case of infected and infected-DM mice than that of control and EXT groups, respectively (Table 4).

Table 4: Serum total protein and its fractions of mice infected with 50 cercariae of *S. mansoni* at week 10 pi under different treatment regimens

Parameters	Negative control	Infected	Infected-DM	EXT
Total protein	8.9 ± 0.2 (8.3 – 9.4)	5.1 ± 0.4 [a] (4.0 – 6.0)	3.1 ± 0.1 [b] (2.9 – 3.3)	5.7 ± 0.1 [c] (5.2 – 5.8)
Albumin (%)	76.2	53.7 [a]	51.8	63.5 [c]
α (%)	17.7	9.4 [a]	7.7 [b]	11.4
β (%)	29.6	--	--	14.4 [c]
γ (%)	19.0	24.4 [a]	25.6	18.4 [c]
A/G ratio	1.11	1.74	1.43	1.20

Results are given as mean ± SE of eight mice in each group. [a]($p < 0.001$) negative control versus infected; [b]($p < 0.02$) infected versus Infected-DM mice; [c] ($p < 0.01$) infected-DM versus EXT. The minimum and maximum values are shown in parentheses.
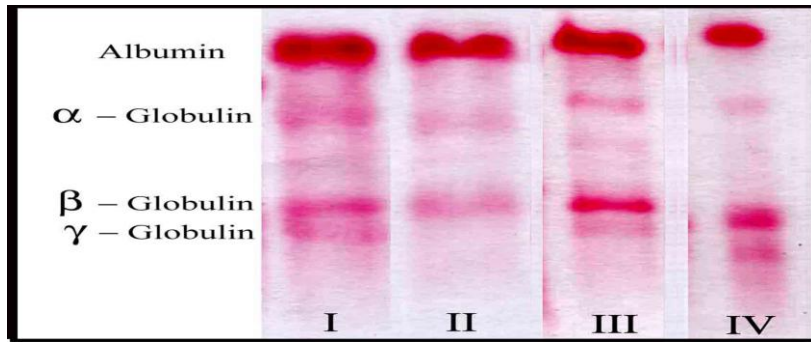
Fig. 3: Serum protein electrophoritic pattern of mice infected with 50 cercariae of *S. mansoni* at week 10 pi under different treatment regimens in stained histological sections. Groups I-IV as ascribed in Fig. 2.

The various stages of granuloma evolution were present in both infected and treated groups with clear predominance of exudative type in EXT mice (Fig. 4 & 5). EXT significantly reduced the size of hepatic granulomas compared to infected-DM mice (P< 0.01) while weak reduction was observed in infected-DM mice (P< 0.05) as compared to infected ones (Fig. 5 & 6). In contrary, number of granuloma / 1 mm^2 was not affected by different treatment regimens except marginally in infected-DM compared to infected mice (P= 0.05) (Fig. 6). Both Infection and induction of DM showed marked reduction in the nucleic content of hepatocytes while no reduction was observed after EXT (Fig. 7).
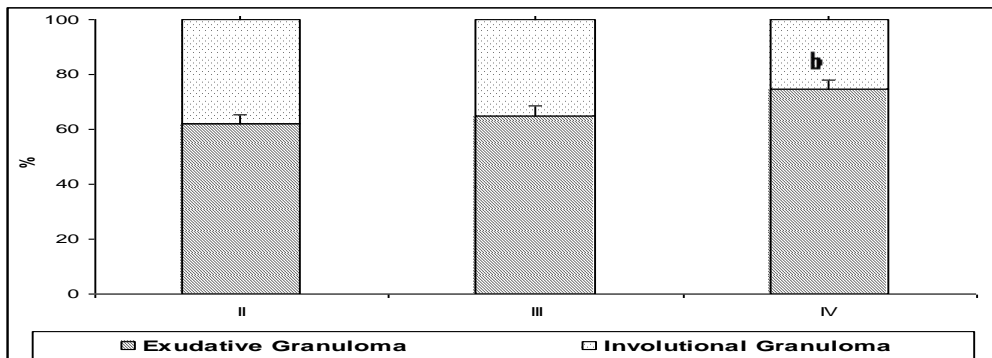

Fig. 4: Percentage of different stages of hepatic granuloma formation / 1mm^2 of mice infected with 50 cercariae of *S. mansoni* at week 10 pi under different treatment regimens in stained histological sections. Groups II-IV as described in Fig. 1. [b] (p < 0.01) EXT versus infected-DM mice. (n= 8).
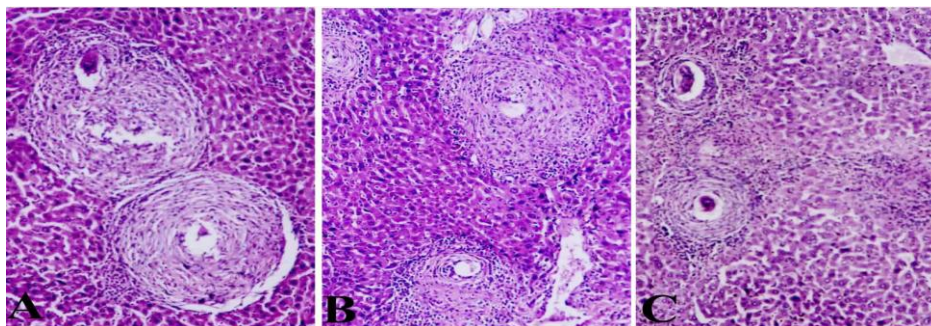

Fig. 4: Hepatic granuloma formation in mice infected with 50 cercariae of *S. mansoni* at week 10 pi under different treatment regimens in stained histological sections. A: infected mice; B: infected-DM and C: EXT mice (X 200).
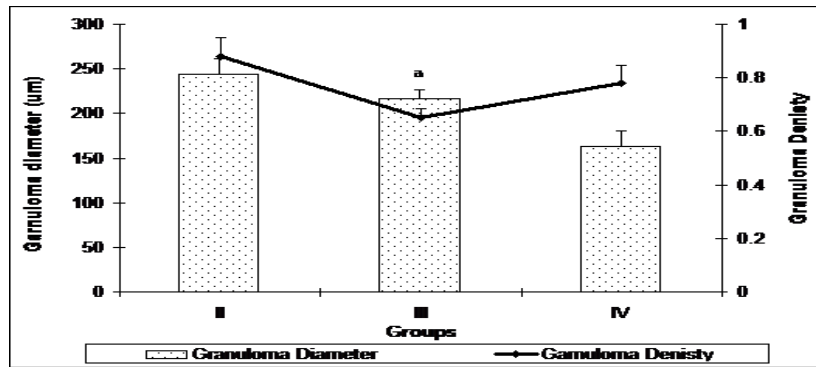
Fig. 6: Hepatic granuloma diameters and density/1mm^2 of mice infected with 50 cercariae of *S. mansoni* at week 10 pi under different treatment regimens in stained histological sections. Groups I-IV as described in Fig. 1. [a] (p < 0.05) infected versus infected-DM mice; [b] (p < 0.01) EXT versus infected-DM mice. (n= 8).
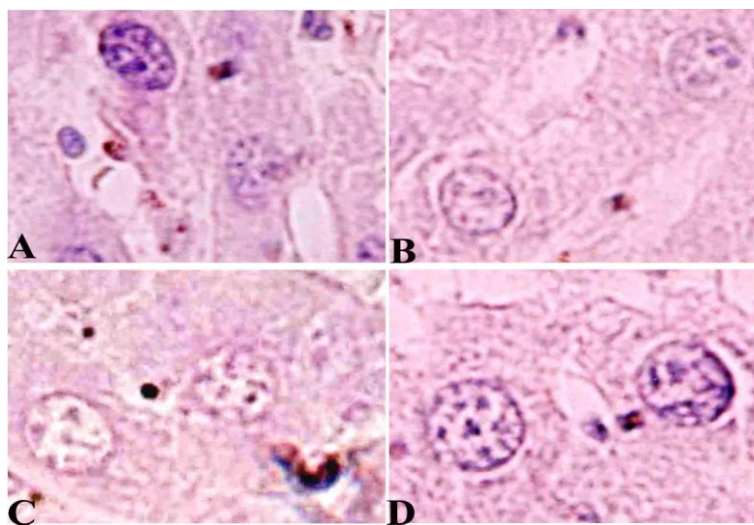

Fig. 7: Methyl green pyronine stained liver sections of mice infected with 50 cercariae of *S. mansoni* at week 10 pi under different treatment regimens showing the nucleic contents of hepatocytes. A: negative control; B: Infected; C: infected-DM and D: EXT (X 1000).

Discussion

Relatively few studies have investigated the effect of DM on schistos-omiasis. However, there are many aspects concerning the interaction between the two diseases that still need to be declared and hence was the aim of this work.

Apparently, in the present study both DM and EXT affected the oogram pattern with high percentage of dead eggs seen in both infected-DM and EXT groups. In infected-DM mice, high percentage of immature dead eggs and low live mature ones compared to infected mice suggested impairment of egg development and maturity that could be related to host metabolic changes caused by DM. This in turn might be confirmed by lower faecal egg excretion and equal worm load in infected-DM mice as compared to infected ones. In this regard, Shafrir (1997) mentioned that, a single non-diabetogenic dose of streptozotocin (STZ) could affect specifically the reproductive organs of *S. mansoni* worm with no affect on worm survival. Concerning the changes in oogram pattern, the possible role for insulin in this sense seems doubtful since Clemens and Basch (1989) concluded that glucose uptake by the worm is insulin independent, because no insulin receptors were detected and high concentrations of insulin failed to affect glucose consumptions of worms in vitro. However, non-enzymatic glycosylati-on of proteins of the worm due to

hyperglycaemia of the host cannot be discarded as a causative mechanism behind the impairment of the reproductive capacity of the female worm or the ability of the egg to mature (Hulstijn *et al.* 2001). On the other hand, EXT decreased survival rates of worms, increased the percentage of dead mature eggs and lowered fecal eggs output as compared to infected group suggesting a different mechanism of action compared to alloxan. However, this is the first report of this natural extract action on schistosemiasis that needs further investigation.

Current results showed that biochemical parameters indicating liver functions were disturbed with *Schistosoma* infection and DM induction. The increased serum glucose of infected-DM as compared to infected mice (Fig. 2) might be derived from glycogenolysis and / or gluconeogenesis of diabetic mice (Beck-Nielson *et al*., 1994). These mechanisms were extensively reported to be the causative reasons eventually leading to hyperglycemia in different diabetic states (Guyton & Hall, 2000). Additionally one or more of these mechanisms could be operating in infected mice eventually leading to the observed hyperglycemia (Fig 2). However, EXT significantly suppressed the rise of peripheral blood glucose concentrations as compared to infected-DM. Decreasing the blood glucose in diabetic rat due to the EXT has been previously mentioned (Nicola *et al*., 1996) which gives an additional support to our findings. The obtained depletion of liver glycogen stores of infected or infected-DM mice (Fig. 2) was concomitant with the results of Abdel-Moneim *et al*. (1997) on DM mice and that could be due to the loss of glycogen synthestase-activiting system (Annamala & Augusti, 1980) and/or increased activity of glycogen phospho-rylase (Abdel-Moniem *et al*., 2001). Results revealed enhancement of insulin levels after administration of the extract, which could be mechanism behind the hypoglycemic effects of *C. droserifolia*. The role of EXT in enhancing insulin levels could be secondary to property as an antioxidant (Nicola *et al*., 1996). Therefore, EXT could have a protective effect on the pancreating cells against oxidative stress-induced cellular damaged which certainly affects the synthetic capacity of these cells.

Serum T_3 and T_4 levels were appropriate indicators of thyroid function (Guyton & Hall, 2000). T_3 level decreased approximately by 15% and 19% in infected mice and infected-DM as compared to control and infected mice respectively. These decreases reflect that significant reduction in T_3 /T_4 ratio in the infected-DM group. Several systemic non-thyroid diseases induce subnormal T_3 levels indicating impaired microsomal capacity to convert T_4 to T_3 and the mechanism behind that is the development of oxidative states (Itoh *et al*., 1989). Therefore, it is reasonnable to conclude that infection-induced liver damage could be the mechanism behind the observed decrease in T_3 of infected-DM mice. Schistosomiasis infection (Hassan, *et al*., 1991) and diabetes (Sambandam *et al.,* 2000) suppressed thyroid hormones (T_3 and T_4) and insulin serum levels that regulate the basal metabolic rate. It is clear that the induction of diabetes augmented the effect of infection on reducing thyroid hormones levels. Disturbance in thyroid hormones and/or insulin function should result in general failure of energy metabolism of the host that may affect in way or another energy metabolism of the parasite. This could be a hypothesis that linking T_3 and T_4 depletion accompanying schistosomiasis to impaired reproductive capacity of the worm. However, EXT restored the serum T_3 back to its normal levels (Table 3) which seems to be secondary to its effect as an antioxidant. The effect of EXT in increasing thyroid hormone levels may reflect its effects on enhancing insulin level, since insulin has been reported to be able to stimulate the hepatic T_3 /T_4 conversion and to improve the synthetic capacity of the thyroid cells (Jennings, 1984). The findings of the present study showed that the T_3/T_4 ratio was unchanged in infected mice, which was due to decreased level of both T_3 and T_4. Moreover, T_3/T_4 ratio increased significantly after EXT as compared with infected-DM.

High rate of oxidative processes, formation of hepatic malondialdehydein

due to the peroxidative damage to the liver microsomal membrane lipid and impairment of the antioxidant defense (Gharib *et al.*, 1999; El-Sokkary *et al.*, 2002; El-Shenawy & Soliman, unpublished data) characterize schistosomiasis. Meanwhile, the complications of DM may be attributed to hyperglycemia that may directly or indirectly contribute to excess formation of free radicals (Feillet-Coudray *et al.*, 1999) and impairment of antioxidant systems (Ďuračková, 1999). Among the antioxidant defense mechanisms are GSH and uric acid that remove reactive oxygen species once formed (Bonnefont-Rousselot *et al.*, 2000). In the present study, hepatic GSH decreased significantly by 34% and 13% in infected and infected-DM mice as compared to control and infected groups respectively, which indicates that schistosomiasis concurrently with induction of diabetes liberated more free radicals. In contrast, EXT raised the hepatic GSH by 31% as compared to the infected-DM (Table 3). These observations are in accordance with the findings of Szkudelski *et al.* (1998) who reported that alloxan depleted hepatic GSH content in rat due to the higher levels of free radical generation that convert more reduced GSH to its oxidized form. Possible improvement in insulin action secondary to its enhanced levels and manifested as the hypoglycemic effect of EXT might be attributed to its ability to improve the physical state of plasma membrane and its related activities as glucose transport which is basically controlled by insulin (Guyton and Hall, 2000). Increased uric acid content in infected and infected-DM groups suggests that it acted together with GSH to offer significant protection against oxygen free radical-induced liver injury. Indeed, Sharma & Buetner (1993) have confirmed the role of uric acid as antioxidant in diabetic rats.

More decrease of serum total protein (hypoproteinemia) of infected-DM mice than infected group (Table 4) might be ascribed to the exaggerated liver damage caused by DM to infected mice. In fact decreased amino acids uptake or hepatic protein synthesis was reported to be depressed due to liver disease (Garber,

1980). EXT significantly increased serum total proteins and albumin concentration as compared to infected-DM that might be a result of increasing serum insulin level (Table 3). In this regard, Flaim *el al*. (1985) noticed that decrease of serum total proteins and albumin in diabetic animals was restored to control rates by insulin treatment which accelerates amino acids transport through cells and stimulates the protein manufacturing machinery of the cell (Guyton & Hall, 2000). The present study showed a decrease in alpha and beta globulin needed for the transport of T_3 and T_4 as described by William (2001). Therefore, serum depletion of T_3 and T_4 might be due to lacking of thyroid binding proteins needed for their transport. In addition, the congenital absence of one or more of the plasma protein fractions indicates a defect in blood immunity (William, 2001) which may explain the exacerbation of schistosomiasis due to diabetes on impaired protein synthesis. On the other hand, the observed increased in gamma globulin fraction in case of infected and infected-DM mice may represent responsive mechanism enhancing the immunity of the host.

Granuloma diameter was not highly affected by DM as previously mentioned by Mahmoud (1979) who noticed marked reductions in the areas of inflammation around *S. mansoni* eggs in chemically-induced as well as mutation diabetes in the infected mice. In contrary, EXT diminished granuloma sizes extensively and decreased number of involutive granulomas as compared to the infected-DM mice that might suggest a possible antifibrotic role of *C. droserifolia*. Since granuloma formation has been shown to be affected though cell-mediated immune response (Wakelin, 1998), therefore, the EXT could operate through its interference with immune response of the host. Results showed that both infection and DM provoked hepatocytic nucleic acid damage, which was not observed after EXT. Indeed, Hulstijn *et al.* (2002) observed that DNA was damaged by the induction of DM using STZ in mice. This damage could be secondary to the development of oxidative

stress due to either schistosomiasis or DM. DNA damage has been reported to be one of the depleting consequences of oxidative stress (Jornot *et al*., 1998).

In conclusion, host environmental changes caused by alloxan-induced DM influenced the biological characteristics of *Schistosoma* worms and enhanced the aspects of schistosomiasis. The induction of diabetes augmented the effect of infection by enhancing the hypothyroidism, hypoinsulism and hypoproteinemia which could be secondary to the observed enhancement of oxidative stress. Based on these assumptions the beneficial effects of EXT on thyroid and pancreatic hormones status seems to be contradictory to its beneficial anti-schistosomiasis effects. This contradiction may suggest that EXT exerts its beneficial effects through its direct effects on the parasite, not secondary to its effect on the host. The current results showing differential effects of both diabetes and extract on schistosomiasis, through both are beneficial, serve to corroborate this hypothesis. The present study emphasizes the necessity of further investigations to delineate the precise mechanism (s) of EXT protection.

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عن التفاعل بين مرض السكر المستحث والبلهارسيا : الميكانيكية والحماية

نهلة سليمان محمد الشناوى ـ   مها فريد محمد سليمان
قسم علم الحيوان- كلية العلوم – جامعة قناة السويس- الإسماعيلية – جمهورية مصر العربية

فى هـذه الدراسـة تمت عدوى فئـرانِ التجـارب بشيستوسوما مانسونى و بعد سبعة أسابيع من الإصابة تم تقسيم الحيوانات إلى ثلاثة مجمـوعـات : (1) مجموعـة ضـابطة موجبـة، (2) مجموعة تم حقنها بجرعة واحدة من الألوكسان فى التجويف البريتونى (200 مجم/كم) لاستحداث السكر بها، (3) مجموعة تم استحداث السكر بها بالإضـافة إلى علاجها يوميـاً و لمدة 18 يوم بالمستخلص الكحولى لنبات السموه (0.3 جم/كجم) بعد ثلاث أيام من المعالجـة بالألوكسان. و هذا بالأضافة إلى مجموعة ضـابطة سالبة من الفئران. اوضحت الدراسة ان استحداث الإصابة بالسكر قد أثر على بعض النواحى الخاصه بالطفيل فيما يخص التغيير الملحوظة فى نسب الأطوار المختلفة للبويضات فى الأنسجة و خاصـه ارتفاع نسبة الطور الميت و كذلك إنخفاض اعداد البويضـات فى البـراز. و مـن ناحيـة اخرى فإن المعالجـة بالمستخلص النباتى قد أثرت على السمات المذكورة سابقا بالأضافة إلى تأثيرهـا الواضح على عدد الديدان المسترجعة و تقليص حجم الأورام الحبيبية الكبديـة وكذلك تقليص نسبة الأنواع المتقدمة منها. وقد أشارت النتائج ايضاً بـأنّ الإصابه بالسكر قد زاد من مضاعفات الإصابة بالبلهارسيا والذى تمثل فى الزيادة المضاعفة لمستوى الجلوكوز فى الدم والذى كان مصحوبا بنقص حاد فى كل من محتوى الجليكوجين فى الكبد وهورمونات الغدة الدرقّية (ثلاثى يوديد الثيرونين ؛ T3 ، هرمون الثيروكسين T4) و الأنسيولين فى المصل و كذلك الحامض النووى فى الخلايا الكبدية. و ذلك بالإضافة إلى أن نقص الجلوتاثيون فى الكبد و الذى كان مصحوباً بزيادة في مستوى حامض اليوريك. وقد لوحظ نقص تركيز البروتين الكلى فى المصل وكذلك نقص أو أختفاء بعض أنواع البروتينات فى كل من الفئران المصابة بالبلهارسيا أو المصابة بالبلهارسيا و السكر معاً ماعدا جزئية الجاما التى زادت فى نفس المجمـوعتين. نجح المستخلص النباتى بشكل جيد فى تخفيف حدة التغييرات السابق ذكرها. و من الملاحظ ان التأثيرات المفيدة للمستخلص النباتى على هورمونات كلاً من الغده الدرقّية والبنكرياس تبدو مناقضه لتأثيره المفيد ضد مضاعفات البلهارسيا. و من هذا التناقض يقترح بأنّ التأثير النافع للمستخلص النباتى قد يكون من خلال تأثيره المباشر على الطفيل و ليس نتيجه تأثيره على العائل. و تؤيد نتائج هذه الدراسة و توضح أوجه أختلاف فى التأثيرات المفيده لكل من مرض السكّر المستحث و المستخلص النباتى على مرض البلهارسيا.