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Molecular Amelioration of *Acacia arabica* Gum on Some Male Reproductive Aspects in *Schistosoma mansoni* Infected Mice

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ABSTRACT

The prospective effects of *Acacia arabica* gum (Gum Arabic, GA) and/or Praziquantel (PZQ) treatment on testes and sperms of *Schistosoma mansoni*-infected mice were studied. Sperm head abnormalities and sperm nuclear morphology using acridine orange/ethidium bromide dual fluorescent staining (AO/EB) were carried out, in addition to histopathological examination and evaluation of apoptosis by Bcl-2 immunohistochemical expression as a cytoplasmic pro-apoptotic marker which was confirmed by agarose gel electrophoresis of testes tissues. The present data recorded that combined treatment with PZQ (300mg/kg one dose, seven weeks post infection) and GA (25gm/kg/day for 14 day, seven weeks post infection) showed significant reduction ($p < 0.05$) in sperm head abnormalities in *S. mansoni*-infected mice. Additionally, GA recorded significant decrease in the mean percentage of sperm head nuclear chromatin morphology after dual fluorescent staining in *S. mansoni*-infected mice when compared with infected untreated group. The examination of hematoxylin and eosin-stained testis sections revealed the ameliorative effect of GA against histopathological changes in *S. mansoni*-infected groups with/without PZQ treatment. Moreover, a significant decrease was observed immunohisochemically in the mean value of Bcl-2 expression and total genomic DNA fragmentation in testicular tissues of *S. mansoni*-infected mice treated with GA rather than PZQ treatment. It was concluded that *Acacia arabica* gum can ameliorate the reproductive hazardous effects of *Schistosoma mansoni*-infection and/or Praziquantel treatment on reproduction in male mice and it may be useful to integrate Gum Arabic in Schistosomiasis treatment.

Keywords: *Schistosoma mansoni*, PZQ, Gum Arabic, Bcl-2, Sperm abnormalities and DNA damage.

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INTRODUCTION

schistosomiasis is a major and sever neglected tropical disease caused by parasitic flatworms of the genus *Schistosoma*, with three species (*Schistosoma mansoni, S. haematobium, and S. japonicum*). These parasites cause a chronic and often debilitating infection that impairs development and productivity, and exposure to these parasites is strongly linked to extreme poverty [1,2]. Recent estimates of the World Health Organization suggest that more than 249 million people have been infected in 78 endemic countries located in sub-Saharan Africa, the Middle East, the Caribbean, and South America resulting in approximately 200,000 deaths annually [3]. Primary infertility and several ova of *S. mansoni* in the connective tissue of the testes were associated with schistosomiasis [4]. A testicular neoplasm is presented in a case of schistosomiasis [5].

Praziquantel (PZQ) is a chemotherapy based on repeated doses is still the most effective control strategy against schistosomiasis [6]. PZQ is currently the drug of choice for the treatment of schistosomiasis [7,8]. It induces worm muscle contractions and tegumental disruption, followed by exposure of parasite surface membrane antigens to the host immunological defense mechanisms [9]. PZQ has been available for human use for over three decades, About 34 million of people have received PZQ in 2010, and seven times more (235 millions) are projected for 2018 [10]. In Egypt, schistosomiasis is traditionally one of the most important public environmental health problems leading to liver diseases [11]. Liver histopathological and immunological alterations were countered in *S. mansoni*-infected mice with/without PZQ treatment [12,13]. PZQ is genotoxic through the induction of a significant increase in chromosomal aberrations; and also induced hepatic injury in male rats [14]. Medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. Medicinal plants have a promising future because there are about half million plants around the world. Their medical activities have not investigated yet for most of these plants [15]. One of these plants is *Acacia* trees. They are spiny shrubs or small trees preferring sandy or sterile regions. Gum Arabic is a natural gum made of the hardened sap of *Acacia Arabica*. Chemically, GA is a branched chain complex mixture of polysaccharides and glycol proteins either neutral or slightly acidic, found as mixed calcium, magnesium and potassium salts of polysaccharidic acid [16]. GA is used in pharmaceutical, cosmetic and food industries as an emulsifier and stabilizer, and in some countries in the traditional treatment of patients with chronic kidney disease [17]. Additionally, *Acacia sp* gave no evidence of genotoxicity throughout chromosomal aberrations assay, and *in vivo* micronucleus/Comet assays methods [18]. Their extracts inhibited microsomal activation or directly protect DNA strands from the electrophilic metabolite of mutagens [19].

The objective of this study is to investigate the ameliorative effect of Gum Arabic against *Schistosoma mansoni* infection with/without Praziquantel treatment on some reproductive aspects.

MATERIALS AND METHODS

Animals and infection

Forty male albino CD-1 mice (weighing 20±2 g) were obtained from Schistosome Biological Supply Program (SBSP) unit at the Theodor Bilharz Research Institute (TBRI) Giza, Egypt. The animals were housed under standard caging conditions and permitted ad libitum consumption of water and pellet chow. All experiments were done in compliance with the guide lines for the care and use of laboratory animals. For mice infection, the animals were injected subcutaneously with 70±5 *Schistosoma mansoni* cercariae/mouse [20]. The cercariae were shed from *Biomphalaria alexandrina* snails infected with miracidiae of Egyptian strain of *S. mansoni* which purchased from SBSP Unit at TBRI.

Experimental materials

Gum Arabic (*Acacia arabica*)

It is a soluble dietary fiber obtained naturally from the stems and branches of *Acacia arabica* trees (family: legume). It was purchased for this study from local markets as crystals and was grounded to fine powder. Aqueous solution of Gum Arabic was prepared freshly every day and administered orally to the mice at a dose of (25gm/kg/day) using stomach tube for consecutive 14 days according to [21].

Praziquantel

Praziquantel (PZQ) was produced by SEDICO pharmaceutical Co. 6th October City, Egypt. PZQ tablet (600 mg) was ground into fine powder and suspended in 4.8 ml distilled water. The drug was freshly prepared and orally administered to mice using stomach tube. A single dose (300 mg/Kg of body weight) was given seven weeks post infection [22].

Experimental animals

Mice were divided into five groups; each group consisted of 8 male mice as follow:

Group (I) none infected control mice.
Group (II) *S. mansoni*-infected control mice.
Group (III) *S. mansoni*-infected mice treated with Praziquantel (PZQ).
Group (IV) *S. mansoni*-infected mice treated with Gum Arabic (GA).
Group (V) *S. mansoni*-infected mice treated simultaneously with PZQ and GA.

Sperm head abnormalities

Mice were sacrificed and the caudal epididymis was excised. Both epididymides of each mouse were minced together with small scissors in isotonic saline solution and filtered to exclude the large tissue fragments. The suspended sperms were smeared on clean glass slide and air dried. The slides were stained with 1 % eosin for 15 minutes and examined using light microscope (Olympus BX 41, Japan). One thousand of sperms per animal were evaluated under the oil immersion lens and the abnormal sperms were recorded and digitally photographed [23].

Acridine orange/ ethidium bromide (AO/EB) dual fluorescent staining for sperm head nuclear morphology

Nuclear staining for sperm head was performed according to the method of [24]. Briefly, sperms from the control and treated groups were smeared on a glass slide and air-dried. Smears were fixed with methanol/acetic glacial acid (3:1) for 5 minutes then they were hydrated with PBS for 1 minute and stained with a mixture (1:1) of acridine orange (50 µg/ml)/ethidium bromide (5 µg/ml) solutions for 10 minutes. Sperms were immediately washed with PBS and examination was done using fluorescent microscope (Olympus BX 41, Japan). Five hundred of sperms per animal were evaluated under the oil immersion lens and the damaged (apoptotic and necrotic) sperms were recorded according to the affinity and pattern of fluorescent staining, and then representative photos were digitally photographed.

Histopathological study

For light microscopic studies, animals were dissected and their testes were excised and fixed in 10% neutral formalin for 24 hr, washed in running tap water for 24 hr, transferred to 70% ethyl alcohol, and then dehydrated in ascending series of ethyl alcohol, cleared in two changes of xylene, and embedded in paraplast. Section of 5 micrometers thickness were cut using rotary microtome and mounted on clean slides without using any adhesive medium. Sections were stained with Ehrlich's haematoxylin and counter stained with eosin [25]. Sections were examined (400 X) using light microscope (Olympus BX 41, Japan) and digitally photographed.

Immunohistochemical study

Immunohistochemical reaction was performed using an avidin biotin complex immunoperoxidase technique on paraffin sections [26]. Bcl-2 as a cytoplasmic marker for apoptosis was detected using an antimouse Bcl- 2 and monoclonal antibody (Glostrup). The mean percentage of Bcl-2 positive cells in all groups of mice spermatogoneal cells was used as immunohistochemical scoring system. Sections were examined (400 X) using light microscope (Olympus BX 41, Japan) and digitally photographed.

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Detection of DNA fragmentation by agarose gel electrophoresis for testis tissues

DNA extraction and detection of fragmentation were carried out according to "salting out extraction method" of [27], with some modifications [28]. Twenty milligrams of tissues were lysed in lysing buffer (10 mM Tris base, 10 mM NaCl, 10 mM Na_2 EDTA, 0.5% SDS, pH 8.3) overnight at 37°C then; 4M NaCl was added to the samples. Centrifuge the mixture at 10,000 rpm for 10 minutes. The supernatant was transferred to a new tube then DNA was precipitated by 1 ml cold isopropanol by centrifugation for 5 minutes at 12.000 rpm. The pellets were washed with 70% ethanol and were resuspended in TE buffer (10 mM Tris, 1mM EDTA, pH 8). Then they were Incubated for 60 minutes with loading mix (0.1% RNase + loading buffer), and they were loaded directly into the gel-wells. Gels were prepared using 1.8% normal melting electrophoretic grade agarose in 1X Tris borate EDTA buffer (89 mM Tris, 89 mM boric acid, 2mM EDTA, pH 8.3) for 1 hour at 50 volts. The apoptotic bands of DNA fragmentation appeared and located at 180 bp and its multiples 360, 540 and 720 bp against DNA marker (100–3000 bp). The intensity of DNA fragmentation were measured by (ImageJ software) as a mean optical density values.

Statistical analysis

Data were presented as mean ± standard error (M ± SE). Comparisons were made between the infected, untreated and treated groups. All numerical data were statistically analyzed using Statistical Program of Social Sciences (SPSS) software for windows, version 10. ($p< 0.05$) were considered positive.

RESULTS

Effect of Gum Arabic and/or PZQ on sperm head morphology of *S. mansoni*-infected mice

The results indicated that treatment with GA in combination with PZQ caused a significant decrease in sperm head abnormalities and ameliorated the adverse effects of *S. mansoni* infection and PZQ treatment as it resulted in the reduction of sperm head abnormalities more than the records of untreated control group among all types of evaluated abnormalities (Table: 1).

Table 1. The mean values of sperm head abnormalities for *Schistosoma mansoni*-infected mice showing the effect of Gum Arabic and / or PZQ treatments.

	Sperm head abnormalities			
	Without hock	Banana shape	Amorphous	Hummer shape
Control	8 ± 2.9	2.8 ± 1.6	1.8 ± 1.3	0.2 ± 0.4
Infected	9.6 ± 0.9	7.2 ± 3.2*	8 ± 3.3*	1.6 ± 0.9*
PZQ post-infection treatment	24 ± 6.2*#	5.2 ± 1.3	6.4 ± 4.0*	0.4 ± 0.9#
GA post-infection treatment	12.4 ± 3.3	1.4 ± 0.5#	2 ± 1.5#	0.2 ± 0.4#
PZQ + GA post-infection treatment	7.6 ± 1.8	0.8 ± 0.8*#	1.4 ± 1.1#	ND

* Significant difference at ($p< 0.05$) compared with control group. # Significant difference at ($p< 0.05$) compared with infected group. ND: not detected values.

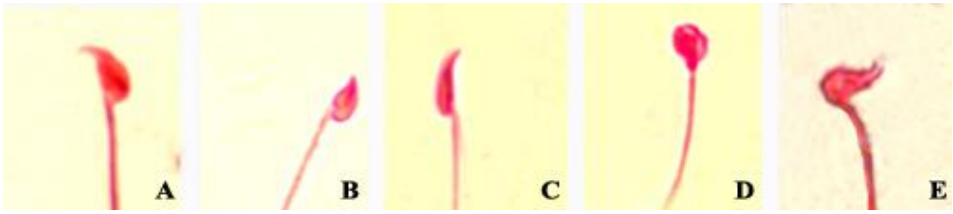

Figure 1. Shows the representative examples of normal and abnormal sperm head morphology (1000X). Where A: normal, B: without hock, C: banana shape, D: amorphous and E: hummer shape.

Figure (1) Shows sperms with normal morphology and different types of sperm head abnormalities (without hock, banana shape, amorphous and hummer shape).

Effect of Gum Arabic and/or PZQ on sperm head nuclear chromatin of *S. mansoni*-infected mice

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Table (2) Shows damage in chromatin of sperm head using acridine orange & ethidium bromide technique (Figure: 2). *S. mansoni* infection with/without PZQ treatment caused significant increase ($p< 0.05$) in both apoptotic and necrotic types of damaged sperm chromatin percentage when compared with control group. Treatment with Gum Arabic decreased both types of chromatin damage when compared with control and infected groups.

Table 2. The mean percentage of sperm head chromatin changes, optical densities (OD) of total genomic DNA fragmentation and immunohistochemical evaluation of apoptosis by Bcl-2 expression for *Schistosoma mansoni*-infected mice showing the effect of Gum Arabic and / or PZQ treatments.

	% damaged chromatin (AO/EB) sperm head nuclear staining	OD of fragmented total genomic DNA	% Bcl-2 Immunohistochemically +ve stained cells
Control	24	4.4 ± 1.2	16.6 ± 1.2
Infected	50	10.4 ± 1.1*	38.9 ± 4.0*
PZQ post infection treatment	51	28.7 ± 12.1*#	52.5 ± 0.8*#
G A post infection treatment	41	12.2 ± 1.6*	24.5 ± 3.4*#
PZQ + GA post infection treatment	25	7.8 ± 0.8*#	22.1 ± 3.7*#

***** Significant difference at ($p< 0.05$) compared with control group. **#** Significant difference at ($p< 0.05$) compared with infected group. **(AO/EB)**: acridine orange/ ethidium bromide dual fluorescent staining

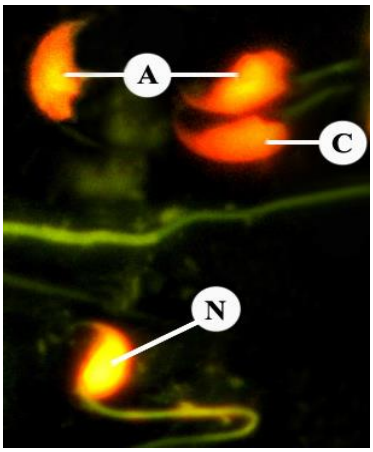

Figure 2. Shows the representative examples of control (C) and damaged (A: apoptotic & N: necrotic) sperms head chromatin with increased affinity to fluorescent staining (1000X).

Histopathological observation of Gum Arabic and/or PZQ effect on testis tissues of *S. mansoni*-infected mice

Figure (3A) for examination of testis of control mice showed typical features of normal seminiferous tubules, spermatogenic cells, interstitial tissue and spermatozoa. *S. mansoni* infection and PZQ treatment exhibited a distinct histological difference when compared with the control one. Marked degeneration of spermatogenic cells and damaged sperm bundles appeared (Figure: 5B). The seminiferous tubules were degenerated with irregular boundaries, degenerated spermatogenic cells were exfoliated in the lumen of the tubules and the sperm bundles were less abundant or completely absent (Figure: 5C). Administration of Gum Arabic ameliorated the adverse effect caused by infection with/ without PZQ treatment.

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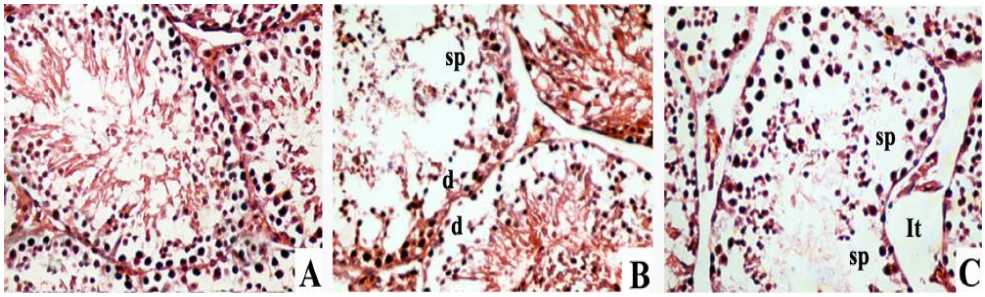

Figure 3. Representative photomicrographs of testes sections (400 X) showing: A) normal histological aspects of control mice, histopathological changes of treated groups as B) degenerative spermatogenic cells (d) with less and damaged sperms scattered randomly in the tubules (sp) and C) tubules with lack of sperms (sp) and irregular boundaries with wide interstitial spaces (It).

Immunohisochemical evaluation of Gum Arabic and/or PZQ effect on testis tissues of *S. mansoni*-infected mice among Bcl-2 expression:

Table (2) and Figure (4) shows that *S. mansoni* infection and PZQ treatment caused significant increase in Bcl-2 expression in spermatogonial cells. On the other hand, treatment with Gum Arabic ameliorated this effect and caused significant decrease in the percentage of positive cells when compared with infected with/without PZQ treatment groups.

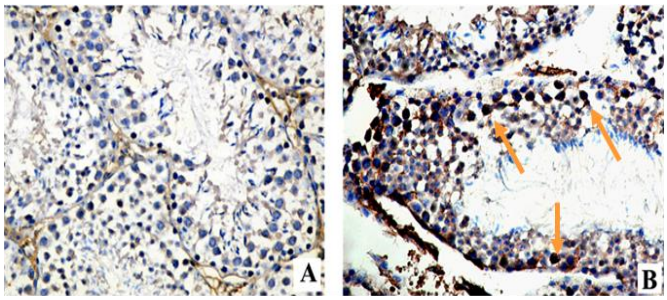

Figure 4. Representative examples of Bcl-2 expression in testicular cells. Where, A: Normal control testis; B: +ve over expressed spermatogonial cells (arrows) (400 X).

Effect of Gum Arabic and/or PZQ on total genomic DNA fragmentation of *S. mansoni*-infected mice testis tissues

Figure (5) shows the effect on testis total genomic DNA in *S. mansoni* -infected mice treated with PZQ and / or Gum Arabic. *S. mansoni* infection caused a little DNA damage. Moreover, PZQ treatment showed sever DNA damage appeared as apoptotic laddering pattern at 200, 400, 600, 800 bp. While the treatment with Gum Arabic showed an improvement without DNA fragmentation when compared with *S. mansoni*-infected with/without PZQ treatment and non infected control groups.

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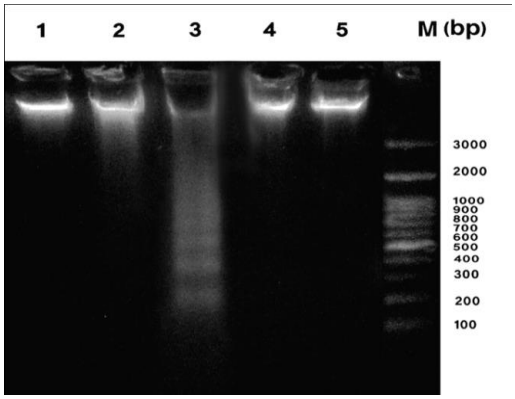

Figure 5. DNA fragmentation in testis tissues of *S. mansoni*-infected mice treated with PZQ and/or Gum Arabic. Where, lane 1: non infected control; lane 2: infected control; lane 3: infected and treated with PZQ; lane 4: infected and treated with GA; lane 5: infected and treated with PZQ & GA and M: DNA marker.

DISCUSSION

In this study, PZQ treatment of *S. mansoni*-infected mice caused a significant increase in number of sperm abnormalities, Bcl-2 expression as an apoptotic marker and DNA damage in testicular tissues beside histopathological alterations in testes. This may be due to side effects of PZQ such as micronucleus induction and alterations of hematological and biochemical parameters [29]. Moreover, total genomic DNA fragmentation was increased in PZQ-treated mice in both liver and spleen [30]. PZQ is also considered to be a hepatotoxic, genotoxic and carcinogenic drug [14] opposing its safety to testicular tissues histology finding [31]. The results of this study supported the previous findings which evaluated the metabolic effect of schistosomiasis on testicular lipids and the serum levels of testosterone [32]. Oligospermia in sperm count was also associated with schistosomiasis [4]. Combined administration of other natural products with PZQ treatment scheme is also reported to improve the histopathological changes of *S. mansoni*-infected mice livers [33,34]. GA has significant systemic effect and not only local action on the *S. mansoni* worm in the host gut [35]. In this study, treatment with GA ameliorated the testicular histological injury and total genomic DNA damage, in addition to morphological and nuclear sperm abnormalities. Moreover, GA modulated Bcl-2 regulatory protein expression in testes tissue that is responsible for apoptosis regulation [36] which induced by schistosomiasis and PZQ treatment. Although Bcl-2 protein can be considered as oncogene [37], its elevation in *S. mansoni*-infected mice and / or PZQ treated mice may leads to cancer development.

These ameliorative effects of GA were illustrated by many investigations and may be due to the antioxidant effect and possibly by inhibiting the free radical mediated process [38]. GA also exerts anti-inflammatory and antioxidant actions [17]. It also ameliorated the pictures of liver and reducing malondialdihyde in addition to ALT in serum of mice [39]. Additionally, GA exerts anti-malarial potential and extended the life span of infected mice [40], and has a strong immune-modulatory action in mice [41]. Other *Acacia sp.* extracts may inhibit several metabolic intermediates and reactive oxygen species [19]. GA protected mice from acetaminophen-induced hepatotoxicity. The protection is not through the change in metabolism of acetaminophen but may be due to reduction of oxidative stress [21].

These observations suggest that Gum Arabic may find clinical application in a variety of conditions where cellular damage is a consequence of oxidative stress.

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