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PROTECTIVE ROLE OF LACTOBACILLUS SPOROGENES (PROBIOTIC) ON CHROMOSOMAL ABERRATIONS AND DNA FRAGMENTATION IN SCHISTOSOMA MANSONI INFECTED MICE

ABSTRACT:
Effects of Lactobacillus sporogenes (probiotic material) oral administration (12.5 million spore/mice/week for eight weeks from the first day of infection) and/or Praziquantel (PZQ) treatment (300mg/Kg one dose seven weeks post infection) on chromosomal aberrations and DNA fragmentation in Schistosoma mansoni infected mice were studied. The present data showed a significant induction ($P<0.05$) in both numerical and structural chromosomal aberrations (chromatid deletion, fragmentation, chromosomal ring, centric fusion, centromeric attenuation, end to end association, break and chromatid gap) in Schistosoma mansoni infected group, and PZQ treated infected or non-infected groups when compared with normal mice. Administration of Lactobacillus sporogenes showed a significant reduction in chromosomal aberrations induced by infection or PZQ treatment. Using gel electrophoresis, both liver and spleen, DNA fragmentation (apoptosis) increased in mice infected with Schistosoma mansoni and non-infected mice treated with PZQ. Lactobacillus sporogenes administration decreased DNA damage induced by infection. It can be concluded that administration of Lactobacillus sporogenes accompanied with PZQ treatment reduces the chromosomal aberrations and DNA damage caused by schistosomiasis and PZQ treatment.

KEY WORDS:
Schistosoma mansoni, PZQ, Lactobacillus sporogenes, Chromosomal aberrations, DNA damage and Apoptosis.

INTRODUCTION:
Schistosomes are trematode worms that live in the blood stream of humans and animals. There are 5 species of Schistosoma; the most common are Schistosoma mansoni, S. japonicum, and S. haematobium. Schistosomiasis occurs in at least 74 countries, where 200 million individuals are infected and 600 million are at risk (Pearce, 2003 and Emam et al., 2009).

Praziquantel is the current drug of choice for the control of schistosomiasis. It is highly effective against all species of schistosomes and shows minimal adverse effects. Though it introduced for the treatment of schistosomiasis more than 20 years ago, its mode of action remains to be elucidated (Greenberg, 2005). However, Melhorn et al. (1981); Chaiworaporn et al. (2005); Ebeid et al. (2005) and Emam et al. (2009) demonstrated that PZQ cause
paralysis, uncoupling and shifting of adult worms from the mesenteric vein to the liver where they were finally destroyed by the phagocytic system.

Probiotics are live microorganisms which by administration in adequate amounts confer a health benefits on host. Nowadays, probiotics are also becoming an integral part of the aquaculture practices to obtain high production (Nayak, 2010). They have been used in aquaculture as a means of controlling disease, enhancing the immune response supplementing or even in some cases replacing the use of antimicrobial compounds, providing nutrients and enzymatic contributions, and improving water quality (Balcázar et al., 2006).

*Bacillus coagulans* is a species of lactic acid forming *Bacillus* bacteria, which can contaminate canned food and gives it a flat sour taste. This includes foods that are normally too acidic for most bacteria. It is also sometimes marketed as *Lactobacillus sporogenes* (De Vecchi and Drago, 2006).

Chromosome is an organized structure of DNA and protein that is found in cells. The DNA molecule may be circular or linear, and composed of nucleotides in a long chain (Paux et al., 2008). Changes in the number, size and organization of chromosome are known as chromosomal mutations, abnormalities or aberrations (Ayala and Kiger, 1984). Chromosomal aberrations can be classified into structural and numerical aberrations.

Apoptosis or programmed cell death is defined as genetically encoded cell death program, which is morphologically and biochemically distinct from necrosis or accidental cell death. The characteristic morphological signs of apoptosis (cellular shrinkage, membrane blebbing, nuclear condensation and fragmentation) are the final results of a complex biochemical cascade of events which is an integral part of physiological homeostasis.

Thus, the aim of the present work is to study the possible effect of *Lactobacillus sporogenes* (probiotic material) and/or praziquantel treatment on chromosomal aberrations and DNA fragmentation in *Schistosoma mansoni* infected mice.

**MATERIAL AND METHODS:**

Experimental animals and their mode of infection:

Seventy male albino CD-1 mice (weighing 20±2 g) were obtained from Schistosoma Biological Supply Program (SBSP) unit at the Theodor Bilharz Research Institute (TBRI) Giza, Egypt. The animals were housed under standard caging conditions, i.e., temperature of 21 ± 1°C and permitted *ad libitum* consumption of water and pellet chow. All experiments were done in compliance with the guide lines for the Egyptian care and use of laboratory animals.

For mice infection, the animals were injected subcutaneously with 65 ± 5 *S. mansoni* cercariae (Peters and Warren, 1969). The cercariae were shed from *Biomphalaria alexandrina* snails infected with miracidia of Egyptian strain of *S. mansoni* which purchased from SBSP Unit at TBRI.

**Experimental materials:**

*Lactobacillus sporogenes*:

It is sporolac powder manufactured in India by UNI – SANKYO LTD Plate No. B - 4, MIDC, Lote parashuram MAHARASHTRA – 415722. One gram sporolac powder, containing not less than 150 million spores of *Lactobacillus sporogenes*, was dissolved in 3.0 ml distilled water. Each mouse was administrated orally with 12.5 million spores of *Lactobacillus sporogenes* per week for eight weeks to obtain the total dose 100 million spores/mice (Son et al., 2009).

**Praziquantel:**

Praziquantel (PZQ) was produced by SEDICO pharmaceutical Co. 6 October City – Egypt. Each tablet, containing 600 mg, was ground into white powder and suspended in 4.8 ml distilled water. The drug was freshly prepared and orally administered to mice using a stainless steel oral cannula. One dose (300 mg/Kg of body weight) was given seven weeks post infection (Chaiworaporn et al., 2005).

**Experimental mice groups:**

Mice were divided into seven groups (10 mice each) as follows: group (I) non-infected control mice. Group (II) non-infected mice treated with PZQ. Group (III) non-infected mice treated with *Lactobacillus sporogenes*. Group (IV) infected control mice. Group (V) infected mice treated with PZQ. Group (VI) infected mice treated with *lactobacillus sporogenes*. Group (VII) infected mice treated with PZQ and *Lactobacillus sporogenes*.

**Cytogenetic method:**

After one week of treatment with PZQ and/or *Lactobacillus sporogenes*, 5 mice from each group were selected randomly and bone marrow cells were used to study the chromosomal abnormalities. Metaphase spreads were prepared according to Zowail (1997). Each mouse was injected interperitoneally with 0.5ml of 0.1% solution of colchicine for each 20 gm of body weight then the mice anesthetized by ether after 2 hours. Both femurs of each animal were dissected out and their bone marrow cells were flushed out of the bone into centrifuge tube by using a syringe with 5ml of 0.9 % sodium chloride. After centrifugation, the supernatant was removed and 5 ml of hypotonic solution (0.56% KCl) were added...
to the cells pellet by using Pasteur pipette. The cells were resuspended and incubated at 37°C for 20 minutes. For fixation, 5 ml of freshly prepared methyl alcohol and glacial acetic acid (3:1) were smoothly added drop by drop on the wall of the tube and mixed very gently, the cells were left for 30 minutes in the refrigerator without disturbance. Then they were centrifuged for 10 minutes at 1000 rpm. This step was repeated three times with 5 ml of the fixative to complete fixation.

After the last step, the supernatant was discarded and the cells were suspended in one ml of fixative; the suspension should have milky appearance. Two or three drops of the suspension were spread by Pasteur pipette on the surface of a clean, cold slide dipped in 70% cold ethyle alcohol. The slides were flamed on a Bunsen burner, and left to dry completely. The slides were then stained in 10% Giemsa stain at PH 6.8 for 45 minutes, washed in phosphate buffer for five minutes, allowed to dry at room temperature, and mounted in DPX. Fifty well spread and well stained metaphases from each animal were examined by light microscope. Micrographs were taken under a Carl Zeiss microscope provided with automatic camera using Kodak gold 200 films. The type and frequency of numerical (polyploidy, aneuploidy and stickiness) and structural aberrations (gaps, break, deletion, ring, centric fusion, end to end, centromeric attenuation and fragmentation) were recorded. The numbers of cells with sticky chromosomes were scored in at least 1000 cells per animal.

**Molecular method:**

**DNA extraction and apoptosis detection in tissues (liver and spleen):**

Nucleic acids extraction and detection of apoptosis were done according to "salting out extraction method" of Aljanabi and Martinez (1997) and the modification introduced by Hassab El-Nabi (2004b). Protein was precipitated by saturated solution of NaCl (5M).

For DNA extraction, 10 mg of tissues (0.01 gm) were lysed in Eppendorf tubes with 600 microlitres of lysing buffer (50 mM NaCl, 1mM Na2EDTA, 0.5% SDS, pH 8.3) and gently shaken. The mixture was incubated overnight at 37°C then, 200 microlitres of saturated NaCl were added to the samples, shook and centrifuged at 12000 rpm for 10 min. The supernatant was transferred to new Eppendorf tubes and then DNA precipitated by 600 microlitres cold isopropanol. The mix was inverted several times till fine fibers appear, and then centrifuged for 5 min at 12000 rpm. The supernatant was removed and the pellets were washed with 500 microlitres 70% ethyl alcohol and centrifuged at 12000 rpm for 5 min. After centrifugation, the alcohol was decanted or tipped out and the tubes blotted on whatman paper to be dry. The pellets were resuspended in 50 microlitres or appropriate volume of TE buffer (10 mM Tris, 1 mM EDTA, pH8) supplemented with 5% glycerol. The resuspended DNA was incubated for 30-60 min with loading mix (RNase + loading buffer) and then loaded into the gell wells for detection of apoptosis.

**Gel Preparation:**

Gel was prepared using 1.8% electrophoretic grade agarose. The agarose was boiled with tris borate EDTA buffer (1 X TBE buffer ; 89 mM tris, 89 mM boric acid, 2 mM EDTA, pH 8.3), and then 0.5 microgram/ml ethidium bromide was added to agarose mixture at 40°C. Gel was poured and allowed to solidify at room temperature for 1 hour before samples were loaded. Gel was photographed using digital camera while the DNA was visualized using a 312 nm UV light under a transilluminator.

**Apoptosis analysis:**

Apoptotic bands were appeared and located at 200 bp and it's multiplies like 400 bp, 600 bp, and 800 bp etc. The intensities of apoptotic bands were measured by software gel pro program as maximum optical density values (Hassab El-Nabi, 2004a) comparing by Fermentas O’ Gene Ruler 100 bp Plus DNA Ladder.

**Statistical analysis:**

Data are 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 10.0. P values less than 0.05 were considered significant.

**RESULTS:**

**Effect of S. mansoni infection on Chromosomal aberrations:**

Data in table 1 and figure 1 show the mean values of chromosomal aberrations in 50 metaphase spreads of male mice infected with S. mansoni and treated with Lactobacillus sporogenes and / or PZQ. data represent a significant increase in structural aberrations such as chromatid deletion, fragmentation, chromosomal ring, centric fusion, centromeric attenuation, end to end association, break and chromatid gap in infected control group and infected group treated with PZQ or treated with Lactobacillus sporogenes. Also, normal group treated with PZQ showed induction in these aberrations when compared with normal controls.
Table 1. Average of chromosomal abnormalities observed in bone marrow cells of male mice infected with *Schistosoma mansoni* and treated with PZQ and/or *Lactobacillus sporogenes*.

<table>
<thead>
<tr>
<th>Observation</th>
<th>Deletion Mean± SE</th>
<th>Chromatid Fragment Mean ± SE</th>
<th>Chromosomal ring Mean ± SE</th>
<th>Centric fusion Mean ± SE</th>
<th>Centromeric attenuation Mean ± SE</th>
<th>End to end association Mean ± SE</th>
<th>Gaps Mean ± SE</th>
<th>Breaks Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>13.5 ± 0.92</td>
<td>3 ± 0.31</td>
<td>2.8 ± 0.37</td>
<td>5.4 ± 0.74</td>
<td>5 ± 0.44</td>
<td>2.2 ± 0.37</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Normal treated with PZQ</td>
<td>29.8 ± 1.65</td>
<td>9 ± 0.66</td>
<td>10.2 ± 0.67</td>
<td>10.4 ± 0.74</td>
<td>19.4 ± 1.36</td>
<td>15 ± 1.41</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Normal treated with <em>L. sporogenes</em></td>
<td>11.4 ± 0.5</td>
<td>2.8 ± 0.66</td>
<td>4 ± 0.66</td>
<td>7.8 ± 0.66</td>
<td>5.2 ± 0.73</td>
<td>2.6 ± 0.24</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Infected control</td>
<td>20.8 ± 2.05</td>
<td>6.8 ± 1.11</td>
<td>8 ± 1.04</td>
<td>12.6 ± 1.04</td>
<td>21.2 ± 1.93</td>
<td>11.8 ± 1.06</td>
<td>1.6 ± 0.37</td>
<td>1.2 ± 0.37</td>
</tr>
<tr>
<td>Infected treated with PZQ</td>
<td>31.4 ± 2.78</td>
<td>11.8 ± 1.24</td>
<td>16.4 ± 1.69</td>
<td>13.8 ± 1.15</td>
<td>18.6 ± 1.86</td>
<td>11.6 ± 1.07</td>
<td>2.2 ± 0.40</td>
<td>1.6 ± 0.40</td>
</tr>
<tr>
<td>Infected treated with <em>L. sporogenes</em></td>
<td>21.4 ± 1.02</td>
<td>4.6 ± 0.37</td>
<td>3.6 ± 0.5</td>
<td>7.2 ± 0.58</td>
<td>9.6 ± 0.81</td>
<td>6.6 ± 0.24</td>
<td>0.8 ± 0.37</td>
<td>0.6 ± 0.37</td>
</tr>
<tr>
<td>Infected treated with PZQ&amp; <em>L. sporogenes</em></td>
<td>14.2 ± 0.86</td>
<td>3.2 ± 0.37</td>
<td>3.2 ± 0.48</td>
<td>8.2 ± 0.86</td>
<td>6.6 ± 0.92</td>
<td>2.8 ± 0.37</td>
<td>0* ± 0*</td>
<td>0* ± 0*</td>
</tr>
</tbody>
</table>

The number of mice/group = 5. The mice were infected with 65 ± 5 *S. mansoni* cercariae/mouse.

The treatment with *Lactobacillus sporogenes* started from the first day of infection in weekly oral dose for 8 weeks, while PZQ treatment started 49 Days post infection in one only oral dose (300 mg/kg).

All animals were sacrificed 8 weeks post infection. Data are expressed as Mean ± SE.

† Significant difference compared to non-infected control group at P < 0.05. * Significant difference compared to infected control group at P < 0.05.

Fig. 1. Metaphase spread showing (A) normal spread, (B) deletion D, (C) fragment F, (D) centromeric attenuation C. att, (E) centric fusion CF, (F) end to end association E to E, (G) gap G, (H) break B, (I) Chromosomal ring R and (J) stickness S in bone marrow cells of mice (Giemsa stain X 1000).
On the other hand, infected mice treated with PZQ only for one week exhibited an increase in chromosomal aberrations compared with untreated infected group, but this value reduced and showed a significant decrease in mice treated with PZQ accompanied with \textit{Lactobacillus sporogenes}.

Besides, normal group treated with \textit{Lactobacillus sporogenes} or infected group treated with PZQ accompanied with \textit{Lactobacillus sporogenes} showed no significant differences when compared with normal control mice.

\textbf{Effect of \textit{S. mansoni} infection on deoxyribonucleic acid (DNA):}

Figure 2 shows the effect of \textit{S. mansoni} infection on DNA liver and spleen of mice treated with PZQ and / or \textit{Lactobacillus sporogenes}. Lane 1 shows 100 pb DNA ladder and lanes 2, 3, 4, 5, 6, 7, and 8 represent the groups non-infected control, non-infected treated with PZQ, non-infected treated with \textit{Lactobacillus sporogenes}, infected control, infected treated with PZQ, infected treated with \textit{Lactobacillus sporogenes} and infected treated with PZQ accompanied with \textit{Lactobacillus sporogenes}, respectively.

The optical density of DNA is shown in figure 3a&b. Gel proanalyzer chart illustrates a reduction in the intensity of intact DNA in liver and spleen of mice infected with \textit{S. mansoni} as well as mice administered PZQ for one week. Treatment with \textit{Lactobacillus sporogenes} for eight weeks from the first day of infection showed an increase in the intensity of intact DNA when compared with infected controls. On the other hand, the intensity of released DNA of infected mice with \textit{S. mansoni} showed increases to 55.7, 255, 251.8 and 253.8 for liver and 243.56, 250.8, 199.97 and 78.61 for spleen at 200, 400, 600 and 800pb, respectively.

Treatment with PZQ for one week increased the intensity of the released DNA in liver showing apoptotic bands at 200, 400,
600 and 800 pb with values 84.3, 110, 100 and 138.2, respectively. Mice infected for eight weeks and treated with PZQ for one week showed a decrease in the intact and an increase in the released DNA at 200, 400, 600 and 800 pb when compared with DNA ladder.

Administration of Lactobacillus sporogenes for eight weeks from the first day of infection with S. mansoni increased the intensity of intact DNA that reduced by infection and decreased in the optical density of released DNA with values 32, 91, 90 and 177 in liver and 80.73, 111.62, 137.53 and 182.32 in spleen at 200, 400, 600 and 800 pb, respectively. Also, treatment with Lactobacillus sporogenes accompanied with PZQ reduced the released DNA optical density in both liver and spleen.

Fig. 3a. Computerized gel pro analyzing charts for DNA apoptotic fragments of S. mansoni infected mice liver treated with PZQ and / or Lactobacillus sporogenes. Lane (1) DNA ladder; lane (2) non-infected control; lane (3) non-infected treated with PZQ; lane (4) non-infected treated with Lactobacillus sporogenes; Lane (5) infected control; lane (6) infected treated with PZQ; lane (7) infected treated with Lactobacillus sporogenes and lane (8) infected treated with PZQ and Lactobacillus sporogenes.
Fig. 3b. Computerized gel pro analyzing charts for DNA apoptotic fragments of *S. mansoni* infected mice spleen treated with PZQ and / or *Lactobacillus sporogenes*. Lane (1) DNA ladder; lane (2) non-infected control; lane (3) non-infected treated with PZQ; lane (4) non-infected treated with *Lactobacillus sporogenes*; Lane (5) infected control; lane (6) infected treated with PZQ; lane (7) infected treated with *Lactobacillus sporogenes* and lane (8) infected treated with PZQ and *Lactobacillus sporogenes*. 
DISCUSSION:

Data in the present study recorded a significant increase in both numerical and structural chromosomal aberrations in *S. mansoni* infected mice. Also, data illustrated that PZQ induced an increase in chromosomal aberrations either in infected or non-infected mice. This result agrees with that of Shubber and Salih (1987) who reported that the mean frequencies of chromatid and chromosome breaks in patients infected with *S. mansoni* were significantly higher than the means scored for 20 healthy controls. They also observed reductions in the lymphocyte divisions and replications in the patients and indicated that infection with *S. mansoni* could have *in vivo* mutagenic effects on human chromosomes. Moreover, El-Sharkawy et al., (2003) studied the antimitotic effect of schistosomiasis and demonstrated that although schistosomiasis did not show a direct clastogenic effect, it can be considered as a comutagen when there is concomitant exposure, especially chronic ones, to radiomimetic agents. Schistosomiasis may amplify their cytogenetic damage. Besides, Khaled et al., (2011) reported that spontaneous human lymphocyte cultures of *S. mansoni* patients, showed a significant increase in the number of metaphases with chromosomal aberration.

Data in the present work also reported that mice infected with *S. mansoni* and treated with PZQ showed no improvement in chromosomal aberration when compared with infected controls, while non-infected mice administered PZQ showed a significant increase in chromosomal aberration as compared with non-infected controls. This result is in agreement with Montero and Ostrosky (1997) who illustrated that in humans and pigs, PZQ induces a greater frequency of hyperploid lymphocytes as well as structural chromosomal aberrations, but not in all the individuals treated. From their, *In vitro* studies they demonstrated that PZQ can induce micronuclei in syrian hamster embryonic (SHE) cells and in lymphocytes of some individuals. In addition, Omar et al., (2005) found that PZQ induced a significant increase in the incidence of chromosomal aberrations as polyploidy, fragment, deletion and ring chromosome as compared with control group and concluded that, PZQ is considered to be a hepatotoxic, genotoxic and carcinogenic drug.

On the other hand, data in the present study showed that *Lactobacillus sporogenes* administration improved the abnormalities of chromosomes induced by *S. mansoni* infection or PZQ treatment. Vorob'eva and Abilev (2002) reported that lactic acid and propionic acid bacteria, bifidobacteria, and fecal enterococci associated with the activity of humans and animals caused antmutagenic effects (AME) on many test systems designed for detecting point mutations and chromosomal aberrations. Bacterial cells and some of their metabolites attenuated the mutagenic action of several genotoxic agents, and this effect is mediated by the mechanism of dysmutagenesis and/or bioantimutagenesis.

Apoptosis was a tightly regulated process by which cells establish an inducible non-necrotic cellular death process, and it has a major role in balancing cell proliferation and remodelling tissue activity in many organisms. The mechanism for apoptosis in multicellular organisms includes the activation of caspases via mitochondrial-mediated mechanisms and death receptor-mediated mechanisms, resulting in apoptotic DNA fragmentation, nuclear chromatin condensation and the formation of apoptotic bodies and a distinctive apoptotic cellular phenotype (Elmore, 2007). The present study showed that infection with *S. mansoni* increased the percent of apoptosis and DNA fragmentation in using DNA extraction when compared with non infected control group. This result is in agreement with Ghoneim et al., (2008) who said that cell death data were correlated to the degree of lymphoproliferative responses to phytohemagglutinin (PHA) as well as to the serum anti-schistosomal antibody titers and recorded a markedly significant increase in PHA-induced apoptosis in lymphocytes isolated from *S. mansoni*-infected patients when compared to the corresponding healthy controls. Also, their study supported the hypothesis that activation-induced cell death (AICD) is a potentially contributing factor in T helper (Th) cell regulation during chronic stages of schistosomiasis, which represented a critically determinant factor in the host-parasite interaction and might influence the destiny of parasitic infections either towards establishment of chronic infection or towards host death. Moreover, this result is in match with Zhou et al., (2009) who suggested, according to genomic studies, that schistosomes have an apoptotic pathway that is similar to that of higher organisms. Also, Chen et al., (2002) reported that the mechanism of schistosomes to induce apoptosis is not fully understood and there are several ways that can mediate apoptosis in schistosomiasis. They found that caspase-3 which is a key executioner of apoptosis was found in schistosomal eggs in infected mice.
REFERENCES:


الدور الوظيفي للالاكباوسيلس سيروجين (بادية حيوية) على الشروخات الكروموسومية وتكرير الحامض النووي الديوكسي ريبوزي في الفقار البيضاء المصاحبة ببلهارسيا المستقيم.

محمود السيد رويل*، حمالة يوسف عثمان**، عزة حسن محمد***، هناء محمد الميسوى***

تم في هذا البحث دراسة تأثير المحمول للألكباوسيلس سيروجين (بادية حيوية) على الشروخات الكروموسومية وتكرير الحامض النووي الديوكسي ريبوزي في الفقار البيضاء المصاحبة ببلهارسيا المستقيم. والنتائج الملاحقة تثبت أن الفقار البيضاء المصاب ببلهارسيا المستقيم، سيتحسن في الشروخات الكروموسومية وتكرير الحامض النووي الديوكسي ريبوزي، الناجة عن الإصابة ببلهارسيا المستقيم.

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