Original Article

Anti-schistosomal activity of colostral and mature camel milk on Schistosoma mansoni infected mice

Amany S Maghraby PhD1, Mahmoud A Mohamed PhD2 and Ahmed M Abdel-Salam PhD3

1Therapeutical Chemistry Department, National Research Centre, Dokki, Egypt.
2Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt.
3Department of Food Science and Human Nutrition College of Agriculture and Veterinary Medicine, Qassim University, Buraidah, PO Box 1482, Saudi Arabia

The aim of the present study was to investigate the anti-schistosomal activity of colostral and mature camel milk on Schistosoma mansoni infected mice. Six weeks post infection, mean percentage of protection was detected through the hepatic portal vein. Glutathione-s-transferase (GST), alanine, aspartate transaminase (ALT and AST) and immunoglobulin G (IgG) levels were detected in sera of treated mice before and after infection. Antischistosomal activity of colostral and mature camel milk on Schistosoma mansoni infected mice were 12.81% and 31.60% respectively. The results showed that GST levels in sera of mice fed on colostral and mature camel milk were increased with mean values of 0.070, 0.108, 0.128 and 0.120 in colostral milk groups and 0.072, 0.085, 0.166 and 0.20 in mature camel milk groups compared with the mice fed on basal diet with means values of 0.070, 0.085, 0.078 and 0.069 before infection and after two, four and six weeks of infection, respectively. On the other hand, there were slight differences on ALT and AST activities. Mice treated with colostral and mature milk (200 µl/day) showed an immunostimulatory effect by inducing IgG titers against soluble worm antigen preparation (SWAP) compared with control. Nevertheless, the difference was not considered significant (0.31 ± 0.1) for colostrum (0.34 ± 0.1) and for mature milk, as compared to normal control (0.2 ± 0.04). Two, four and six weeks post infection, IgG level showed no significant change in sera from mice treated with colostral and mature milk as compared to control. In conclusion, colostral and mature camel milk showed an immunomodulatory effect in normal healthy mice by inducing IgG and GST levels before and after infection with Schistosoma mansoni. Colostral and mature camel milk have a protective response against schistosomiasis.

Key Words: colostrum, camel milk, parasites, Schistosoma mansoni, schistosomiasis, lactoferrin, GST, ALT, AST.

Introduction
Camel milk is given to the sick, the elderly and the very young because of the belief that it is not only healthier, but it works especially well in bone formation. The belief among the Bedouin of the Sinai Peninsula is that drinking camel milk can cure any internal disease.1 Camel colostrum has total solids of 50.4%. Postpartum solids decrease to 18.4% after the two days of lactation. This decline was due to the decrease in protein and minerals rather than its fat content.2 Compared to cow, buffalo and ewe milk fat, camel milk fat contained less short-chained fatty acids, but the same long-chained fatty acids can be found. Compared with cow's milk, colostral and mature camel milk contain a high quantity of protective milk proteins, especially lactoferrin, which has multifunction properties in clinical nutrition.3 Schistosomiasis is one of the most widespread parasitic infections of man, next to malaria. The major infectious species for human are Schistosoma mansoni, Schistosoma haematobium and Schistosoma japonicum. Five to fifteen percent of subjects infected by Schistosoma mansoni develop a severe hepatosplenic disease that may be fatal if left untreated.4 Although infections can be cured by schistosomicides, chemotherapy is not appropriate for the long-term control of these infections and a major World Health Organisation objective is the develop-ment of a vaccine against schistosomiasis.5 The host is exposed to antigens produced by several stages of the parasite and its products, all of which elicit a very intense humoral response. In terms of antibodies, IgG1, IgG2, IgG3, IgG4, IgA, IgE and IgM have all been shown to be stimulated by schistosome infection.6 Immuno-modulators of natural origin can modulate the immune response by utilizing the host's endogenous substances or by using exogenous products of various sources. Immuno-modulators include any agent or substance that has a stimulatory, suppressive or regulatory effect. However, the differentiation between immunoregulators and immunostimulants is not always clearly defined.7

Colostral and camel milk contain a very high quantity of protective and bioactive proteins that play an important role in the immune system response and in protecting the body.

Correspondence address: Dr. Ahmed M. Abdel-Salam, Department of Food Science and Human Nutrition College of Agriculture and Veterinary Medicine, Qassim University, Buraidah, PO Box 1482, Saudi Arabia
Tel.: +966557840301; Fax: +96663801360
E-mail: anssalam68@hotmail.com

Accepted 31st May 2005
against infections. Besides the stimulation of the immune system, scientific studies have revealed that lactoferrin also prevents the growth of pathogens, exerts antibacterial and antiviral properties, controls cell and tissue damage caused by oxidation, and facilitates iron transport. In addition, these bioactive proteins contribute to the primary defense system against invading pathogenic organisms, they stimulate the immune system and the growth of various cell lines, help regulate iron status in the body and also serve as natural antioxidants.

The aim of the present study is to determine whether camel milk modulates the immune responses of *Schistosoma mansoni* infected mice. This study was conducted in accordance with the internationally agreed ethical principles for the conduct of medical research.

**Materials and methods**

**Colostral and mature camel milk**

Camel milk and colostrum were obtained from a camel local farm (Egyptian Hegin Co.).

**Diets and animals**

The composition of basal diets used in this study are milk protein (12%), sucrose (5%), fat (10%), vitamin mixtures (1%), salt mixtures (4%), fiber (4%) and starch (64%). Thirty female Swiss albino mice (18-20 gram) were divided randomly into three test groups (each containing 10 mice). Animals were placed in cages and were given the diets containing colostral and mature camel milk for 45 days. The first group (G1) was fed on the basal diet + Colostral camel milk injected orally (200µl/mouse/day). The second group (G2) was fed on basal diet + mature camel milk injected orally (200µl/mouse/day). The third group (G3) was fed on basal diet only.

**Infection and experimental groups**

After forty-five days post feeding, each mouse was infected subcutaneously (S.C.) with 100 *Schistosoma mansoni* cercariae.

**Assessment of worms burden**

Perfusion and recovery of adult worms were performed at 6 weeks post infection through hepatic portal vein by the perfusion method. The total number of worms in the liver and intestine were determined. Protection was assessed as the percentage reduction in worm counts in liver and intestine according to the formula:

$$P = \frac{C - T}{C} \times 100$$

Where, P: percentage reduction of worms. C: mean worm burdens in control infected animals. T: mean worm burdens in pre-treated infected animals

**Preparation of sera from feeding mice before and after infection with *Schistosoma mansoni***

After forty-five days post feeding the blood was collected from colostral, mature as well as basal diet feeding mice. Six weeks post-infection, the blood was allowed to coagulate at room temperature for 15 min. Sera were obtained by centrifugation at 3000 r.p.m and kept in aliquots at -80°C until used.

Detection of IgG levels against soluble worm antigen preparation (SWAP) using enzyme-linked immunoassay (ELISA)

Enzyme linked immunosorbent assay was performed according to Hiller et al. Plates were coated with cer-carial soluble worm and soluble egg antigens SWAP. The antigens were diluted to 10 µg/ml in 50mM carbonate buffer, pH 9.6 (100 µL/well) and incubated overnight at 4°C. Plates were washed with the working buffer (PBS-T20) and blocked for sites free of antigen against non-specific binding using 200 µL/well of 1% Bovine serum albumin in the blocking buffer (PBS-T20-BSA), for one hour at room temperature. Sera from *S. mansoni* infected control and treated infected mice were used at dilution 1:100 in PBS (100 µL/well) and the plates were incubated at room temperature for 2 hrs then washed three times using working buffer (PBS-T20). 100µl of anti-mouse IgG and IgM peroxidase conjugate were used at dilution of 1:3000 and 1:10000 in PBS-T20-BSA respectively for one hour at room temperature. Bound antibody was detected by the addition of O-phenylene diamine dihydrochloride (OPD) as a substrate for visualization of the enzymatic reaction. The reaction was developed for 30 min. at room temperature and stopped using 50 µL/well of 4 M H₂SO₄. The change in optical densities was recorded at λmax 490 nm by the aid of an automatic Titertek multiskan Reader model ELX 800 UV, INC, USA for reading ELISA plated.

**Determination of GST**

The enzyme activity of GST was determined according to Habig et al. Method is based on the fact that the GST enzyme catalyzes the conjugation of glutathione with 1-chlor 2,4 dinitrobenzene (CDNB) and forms a complex which has an absorbance at UV region (340 nm). The mean decrease of absorbance per min was calculated. To 100µl serum, 100µl glutathione solution, 10 µl fresh CDNB and 1290µl phosphate buffer were added and gently mixed. The contents were incubated at room temperature for 1 hour. The U.V absorbance was measured using UV spectrophotometer at 340nm. The U.V absorbance of blank was obtained using assay mixture without serum. Enzyme activity was defined as the amount of enzyme catalyzing the formation of 1mol of products per min under condition of assay.

Activity = A 340 nm/(9.6) x 1000 = M/min.

**Determination of alanine and aspartate transaminase (ALT& AST) activities**

Alanine and aspartate transaminase (ALT& AST) activities were determined according to the method described by Reitman and Frankel.

**Statistical analysis**

Mean and standard error of the obtained data from each different experimental group were calculated. One-way Analysis of Variance (ANOVA) was applied to the data versus the corresponding values of the control. Differences were higher than the theoretical one at P ≤ 0.05. Statistical analysis of variance (t-test) of glutathione s-transferase, alanine and aspartate transaminase (ALT& AST) activities within groups and between groups was conducted as described by Miller and Miller.
Results

Anti-schistosomal activity of colostral and mature camel milk

Figure 1 and 2 showed that the level of protection against schistosomiasis with 12.8% and 31.6% reduction in total worm burden in Colostral Camel milk (G1) and mature camel milk (G2), respectively as compared to positive control (Control) infected.

Glutathione S-transferase (GST) and transaminases liver enzymes (ALT and AST) activities in sera

Table (1) shows the glutathione S-transferase (GST) levels in mice fed on the experimental diets. GST levels in mice fed on colostral and mature camel milk were the highest compared with mice fed on basal diet. The results showed that glutathione S-transferase (GST) levels in mice fed on colostral and mature camel milk were increased with means values of 0.070, 0.108, 0.128 and 0.120 in colostral milk groups and 0.072, 0.085, 0.166 and 0.20 in mature camel milk groups compared with the mice fed on basal diet with means values of 0.070, 0.085, 0.078 and 0.069 before infection and after two, four and six weeks of infection with Cercariae of Schistosoma mansoni respectively (Table 1).

Detection of IgG level in sera from mice treated with colostral and mature milk of camel before and after infection with Schistosoma mansoni

One-way analysis of variance (ANOVA) showed positive reactivity for IgG levels in sera from mice treated with colostral (0.32 ± 0.08) and mature milk (0.35 ± 0.08), forty five days post feeding, in comparison to sera from untreated mice (0.22 ± 0.03) (Fig.3). Post infection with S. mansoni cercariae, sera from colostral (0.28± 0.05, 0.27± 0.04 and 0.32± 0.09) and mature milk (0.12± 0.01, 0.36± 0.05 and 0.34± 0.05) treated infected mice showed

Table 1. Glutathione S-transferase levels in sera of mice fed on colostral and mature camel milk

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>S.D.</th>
<th>A/9.6*1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet before infection</td>
<td>0.0678</td>
<td>0.0721</td>
<td>0.0702</td>
<td>0.0022</td>
<td>7.316</td>
</tr>
<tr>
<td>Colostral camel milk before infection</td>
<td>0.070</td>
<td>0.0702</td>
<td>0.0702</td>
<td>0.0001</td>
<td>7.302</td>
</tr>
<tr>
<td>Camel milk before infection</td>
<td>0.071</td>
<td>0.072</td>
<td>0.0725</td>
<td>0.0007</td>
<td>7.444</td>
</tr>
<tr>
<td>Basal diet after two weeks of infection</td>
<td>0.0778</td>
<td>0.0986</td>
<td>0.0851</td>
<td>0.0117</td>
<td>8.861</td>
</tr>
<tr>
<td>Colostral camel milk after two weeks of infection</td>
<td>0.1085</td>
<td>0.1097</td>
<td>0.1089</td>
<td>0.0007</td>
<td>11.344</td>
</tr>
<tr>
<td>Camel milk after two weeks of infection</td>
<td>0.080</td>
<td>0.091</td>
<td>0.0855</td>
<td>0.0078</td>
<td>8.906</td>
</tr>
<tr>
<td>Basal diet after four weeks of infection</td>
<td>0.0738</td>
<td>0.0837</td>
<td>0.0787</td>
<td>0.00625</td>
<td>8.301</td>
</tr>
<tr>
<td>Colostral camel milk after four weeks of infection</td>
<td>0.1260</td>
<td>0.1338</td>
<td>0.1282</td>
<td>0.0049</td>
<td>13.351</td>
</tr>
<tr>
<td>Camel milk after four weeks of infection</td>
<td>0.133</td>
<td>0.20</td>
<td>0.1665</td>
<td>0.0474</td>
<td>17.344</td>
</tr>
<tr>
<td>Basal diet after six weeks of infection</td>
<td>0.0688</td>
<td>0.070</td>
<td>0.0691</td>
<td>0.0008</td>
<td>7.201</td>
</tr>
<tr>
<td>Colostral camel milk after six weeks of infection</td>
<td>0.120</td>
<td>0.121</td>
<td>0.120</td>
<td>0.0007</td>
<td>12.490</td>
</tr>
<tr>
<td>Camel milk after six weeks of infection</td>
<td>0.199</td>
<td>0.21</td>
<td>0.200</td>
<td>0.0007</td>
<td>20.781</td>
</tr>
</tbody>
</table>

Min: Minimum concentration; Max: Maximum concentration; SD: Standard Deviation; C.V.: Coefficient of Variation
Table 2. ALT and AST (U/ml) levels in mice fed on colostral and mature camel milk

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>sAST (U/L)</th>
<th>sALT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Basal diet before infection</td>
<td>25.1</td>
<td>29.50</td>
</tr>
<tr>
<td>Colostral camel milk before infection</td>
<td>26.10</td>
<td>28.22</td>
</tr>
<tr>
<td>Camel milk before infection</td>
<td>23.90</td>
<td>28.90</td>
</tr>
<tr>
<td>Basal diet after two weeks of infection</td>
<td>24.10</td>
<td>27.20</td>
</tr>
<tr>
<td>Colostral camel after two weeks of infection</td>
<td>24.50</td>
<td>25.90</td>
</tr>
<tr>
<td>Camel milk after two weeks of infection</td>
<td>25.70</td>
<td>25.90</td>
</tr>
<tr>
<td>Basal diet after four weeks of infection</td>
<td>24.50</td>
<td>25.90</td>
</tr>
<tr>
<td>Colostral camel after four weeks of infection</td>
<td>23.92</td>
<td>28.90</td>
</tr>
<tr>
<td>Camel milk after four weeks of infection</td>
<td>25.70</td>
<td>25.90</td>
</tr>
<tr>
<td>Basal diet after six weeks of infection</td>
<td>28.10</td>
<td>29.22</td>
</tr>
<tr>
<td>Colostral camel milk after six weeks of infection</td>
<td>19.32</td>
<td>28.99</td>
</tr>
<tr>
<td>Camel milk after six weeks of infection</td>
<td>24.10</td>
<td>26.90</td>
</tr>
</tbody>
</table>

Min: Minimum concentration; Max: Maximum concentration; SD: Standard Deviation; CV: Coefficient of Variation.

Figure 3. Detection of IgG level in sera from mice injected orally by PBS, colostral and mature camel milk (A, B, C column) respectively.

Figure 4. Detection of IgG level in sera from mice injected orally by PBS, colostral and mature camel milk (A, B, C column) respectively at 2 weeks post infection with Schistosoma mansoni cercariae.

Figure 5. Detection of IgG level in sera from mice injected orally by colostral and mature camel milk (A, B, C column) respectively at 4 weeks post infection with Schistosoma mansoni cercariae.

Figure 6. Detection of IgG reactivity in sera of mice fed on mature and colostral milk of camel and infected six weeks with Schistosoma mansoni (A, B & C column).
no significant change in IgG levels at regular time intervals 2, 4, and 6 weeks respectively when compared with that in sera of infected untreated groups (0.25 ± 0.01, 0.44 0.08 and 0.30 ± 0.1) respectively (Fig.4-6).

**Discussion**

Our results showed that anti-schistosomal activity of colostral and mature camel milk on *Schistosoma mansoni* infected mice were 12.8 % and 31.60 % respectively. Rey *et al.*, used milk with oltipraz in the urinary schistosomiasis patients. They were treated with a single dose of 35mg/kg oltipraz, which was given under surveillance together with either whole milk or herring in oil. The tolerance of the product was very good as only 3% of the patients reported vomiting and 3% parasthesias of the fingers. Sixty-six percent of the patients examined on day 30 and/or on day 90 were egg-negative and egg excretion was reduced by at least 90% in 22 other subjects, bringing the percentage of good results to 74%. On day 90, the mean egg excretion was reduced by 82.5% in the overall population and by 80.9% in the 5 to 14 year-olds.

Our data are in agreement with data from a large number of epidemiological studies which have assessed the influence of milk intake on infectious diseases. Studies have shown that milk proteins such as casein, whey proteins and membrane structures, might all exert a stimulatory effect on the immune system and a preventive effect on many diseases such as cancer and infectious diseases. Also, whey proteins were found to be protective relative to other protein sources, this being associated with an increase in the intracellular levels of glutathione (GSH), where whey is a prime source of precursors. When liver glutathione levels rise, the liver is able to more effectively detoxify the body also; undenatured whey protein optimizes serum and liver glutathione levels.

So, there is a relationship between *Schistosoma GST* and immune response. Whereas, GST is one from multi-epitope *Schistosoma* vaccine candidates tested for protective immunogenicity in mice. Several promising candidate vaccine antigens including the glycolytic enzymes triose-phosphate isomerase (*Sm TPI*), a 28 kDa glutathione-S-transferase (*Sm 28*), the myofibrilar protein para-triose-phosphate isomerase (*Sm TPI*), a 28 kDa glutathione vaccine antigens including the glycolytic enzymes

Schistosome bovis 28 kD GST. Mice immunized with 14-3-3GST led to protection ranging from 25-46% as determined by reduction of adult worm burden after challenging with *Schistosoma mansoni*. The cellular and humoral acquired immune responses to *Schistosoma haemtobium* 28 GST (*Sh28 GST*) antigens were evaluated in a Senegalese population chronically infected with *Schistosoma haemtobium* parasite. Intra-dermal injection of *Sm 28 GST* showed a significant reduced parasitemia and a decreased egg-induced inflammatory response in the liver and intestine. Rezende *et al.*, showed that GM-CSF and TNF-alfa synergize to increase in vitro granuloma size of peripheral blood induced by mononuclear cells (PBMC) from human intestinal *Schistosomiasis* recombinant 28-kDa GST. There is a persistence of the protective immunity (30%) to *Schistosoma japonicum* in yellow cattle induced be recombinant 26 kDa GST (resjc 26 GST).

There was a slightly insignificant difference on transaminases liver enzymes (ALT and AST) activities (Table 2). The activities of aspartate transferase (AST) and alanine transferase (ALT) are considerably increased following the administration of various hepatotoxic compounds that lead to acute hepatocellular damage and/or extra-hepatic obstructions. Therefore, feeding with collostral and mature milk of camel stimulates a specific immune response that protects against *Schistosoma mansoni* infection. The immuno-protective response results in an increased level of GST which is able to more effectively detoxify the body.

In our study we detected anti-SWAP IgG in sera from mice treated with collostral and mature milk before and after infection with *Schistosoma mansoni*. Our results showed that collostral and mature milk have immunostimulatory effects by increasing the levels of IgG in mice fed with two types of milk. After infection with *S. mansoni* (2, 4, and 6 weeks) the levels of IgG in sera from mice treated with collostral or mature milks showed no significant change as compared with untreated infected mice.

The data indicated that collostral and mature milk of camel play a role in enhancing the immune system and increasing resistant factors against *Schistosoma mansoni*. Also, our results are in agreement with Nassr *et al.*, who showed that anti-SWAP IgG1 and IgG4 are useful in diagnosis and cure. Anti-SWAP IgG1 and IgG4 can be used as parameters for evaluating cure. Follow-up of anti-schistosomal IgG1 and IgG4 is useful for assessment of treatment. In addition, Feng *et al.*, showed that no obvious changes were found in the AWA-IgG4 positive rates of 27 schistosomiasis cases before and after treatment, whereas the antibody level of specific IgG4 was decreased. Nessim and Demerdash showed that praziquantel achieved better cure rates in mice with heavy infection (infected with 120 *Schistosoma mansoni* cercariae) than in less intensely infected animals (infected with 60 *Schistosoma mansoni* cercariae). Finally, collostral and mature milk of camel have an immuno-protective response against *Schistosoma mansoni*. This study suggested that camel milk can be used with anti-chistosomal drugs in Schistosomiasis patients.
Acknowledgement
Authors thank Prof. Dr. M. H. Abd El-Salam, Professor of Dairy Science and head of the Egyptian Society of Dairy Science for his advising, suggestion and promotion.

References
3. Abdel-Salam AM. Studies on lactoferrin of colo-strum and milk from different species of animals. MSc. Thesis in Dairy Science, Faculty of Agri-culture, Cairo University, 1996.
Anti-schistosomal activity of colostral and mature camel milk on Schistosoma mansoni infected mice
骆驼初乳和成熟乳对感染曼氏血吸虫的鼠具有抗血吸虫的能力

S Maghraby PhD¹, M A Mohamed PhD² and AM Abdel-Salam PhD³

¹Therapeutical Chemistry Department, National Research Centre, Dokki, Egypt.
²Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt.
³Dairy Science Department, National Research Centre, Dokki, Egypt.

本研究的目的在于调查骆驼初乳和成熟乳对感染曼氏血吸虫的鼠具有的抗血吸虫能力。感染后 6 星期，通过肝门静脉检测了保护作用的平均百分率。在感染前和后测定了血清中谷胱甘肽-s-转移酶 (GST)、丙氨酸转氨酶 (ALT)、天冬酰胺转氨酶 (AST) 和免疫球蛋白 G (IgG) 的水平。骆驼初乳和成熟乳对于感染曼氏血吸虫的鼠，其抗血吸虫的能力分别为 12.81％和 31.60％。结果表明，在感染前和后 2、4 及 6 个星期，对于鼠血清中 GST 的平均增加值，初乳组、成熟乳组和基础饮食组分别为 0.070、0.108、0.128、0.120、0.072、0.085、0.166、0.20、0.070、0.085、0.078、0.069。此外，ALT 和 AST 的活性有轻微但不显著的变化。和正常健康鼠相比，饲喂初乳和成熟乳 (200 µl/day) 具有免疫刺激作用，可诱导抗可溶性虫抗原制剂的 IgG 效价。然而，与正常控制组 (0.2±0.04) 相比，初乳组 (0.31±0.1) 和成熟乳组 (0.34±0.1) 的差别不显著。与控制组相比，在感染 2、4、6 星期后，初乳组和成熟乳组血清 IgG 的水平没有显著变化。总之，通过在感染前后诱导 IgG 和 GST 的水平，骆驼初乳和成熟乳在正常健康鼠中具有免疫调节作用。骆驼初乳和成熟乳具有抗血吸虫病的作用。

关键词: 初乳，骆驼乳，寄生虫，曼氏血吸虫，血吸虫病，乳铁传递蛋白，谷胱甘肽-s-转移酶，丙氨酸转氨酶，天冬酰胺转氨酶