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In vivo Anticoccidial Activity of *Salvadora persica* Root Extracts

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ABSTRACT

Salvadora persica is one of the commonly used plants in Islamic countries.The root extracts of *S. persica* has been tested for its anticoccidial activity against *Eimeria paillata* induced infection in mice jejunum. Three different doses, 300, 600 and 900 mg/kg were used after infection of mice with sporulated oocysts. A dose of 300 mg/kg reduced the number of oocyst output in mice faeces by about 56.8 % and also improved the body weight. In addition, the root extract decreased the number of parasitic stages and the number of goblet cells in the jejunal villi in the infected mice. Moreover, the expression of the goblet cell mucin gene, muc2 was upregulated after treatment with the root extract. Based on our results, it could be concluded that, *S. persica* root extracts acts as anticoccidial agent.

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Authors' Contributions
MAD and SAQ designed the experiments, FAT and MAD performed the experiments,. All the authors worked together in analysing data and writing the manuscript.

Key words
***Salvadora persica*, *Eimeria papillata* infection, Goblet cell response**

INTRODUCTION

Intestinal eimeriosis caused by the parasite, *Eimeria* spp. are considered to be one of the major threats affecting animal health and production (Mehlhorn, 2014). Such parasite has a great economic importance as it causes severe reduction in meat and milk production and death of domestic agriculture animals (Schito and Barta, 1997). There are many anticoccidial agents used against eimeriosis. However these agents have many side effects (Mehlhorn, 2014; Wunderlich *et al*., 2014). Researchers efforts are now directed towards finding natural agents with reduced or with no side effects on the host infected with *Eimeria*. Extracts of pomegranate, neem, palm date, garlic and others were found to be good agents against *E. papillata* induced infection in mice jejunum (Wunderlich *et al*., 2014).

In this study, we used *Salvadora persica*, commonly known as miswak, and used most extensively against microbes (Elvin-Lewis, 1982). The propagation and use of miswaks is due to the spread of Islamic culture in many countries (Bos, 1993).

It was reported that, *S. persica*, has antimicrobial activity against numerous oral pathogens (Poureslami *et al*., 2007). Recently, Abdel-Baki *et al*. (2016) investigated the antiparasitic activity of *S. persica* against against *Echinococcus granulosus*. *S. persica* extracts contain important phytochemicals like vitamin C, salvadorine, salvadourea, alkaloids, trimethylamine, cyanogenic glycosides, tannins, saponins and salts mostly as chlorides (Alali *et al*., 2005).

Miswak was harvested from the root of *S. persica* and methanolic extract was prepared for studying its potential against *E. papillata* infection in mice.

MATERIALS AND METHODS

Collection and extraction of Salvadora persica *roots*

Fresh roots of *Salvadora persica* were harvested from Jizan region, Kingdom of Saudi Arabia. A botanist, Department of Botany, College of Science, King Saud University, Saudi Arabia authenticated the samples. *S. persica* root methanolic extracts were prepared according to Amer *et al*. (2013). Briefly, the roots were dried, grinded by an electronic blender into a powder that extracted by 70% methanol. The used collected residue was dissolved in water for the inoculation in mice.

Infection and experimental design

Adult male C57BL/6 mice were obtained from the Department of Comparative Medicine, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia. The mice were bred under specified pathogen-free conditions and fed a standard diet and water *ad libitum*. The experiments were approved by the state authorities and followed Saudi Arabian rules on animal protection.

To prepare *Eimeria papillata* oocysts for infection, the stored sporulated *E. papillata* were inoculated into mice using an epigastric tube. Every mouse received 100 µl saline contained 1000 sporulated oocysts.

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Animals were divided into 5 groups of 5 mice per group. The first group was the non-infected control group. Mice of this group were daily inoculated with 100 µl saline for 5 days. The second, third, fourth and fifth groups group were infected with 1000 sporulated *E. papillata* oocysts. One hour later, the third, fourth and fifth groups were daily treated with 300, 600 and 900 mg/kg, respectively.

The faeces were daily collected from each mouse, weighed and prepared for counting using McMaster chamber (Dkhil *et al*., 2011). To investigate the change in weight, all mice were weighed at the beginning and at the end of the experiment.

Samples collection and counting of parasitic stages of E. papillata

Jejuna of mice were collected after 5 days postinfection with *E. papillata*. Each jejunum was washed and then fixed in 10% formalin. Tissue sections were prepared for histological examination (Adam and Caihak, 1964). To calculate the number of parasitic stages, the sections were stained with hematoxylin and eosin.

Goblet cell response

To calculate the number of goblet cells in non-infected, infected and infected-treated mice, jejunum tissue sections were stained with Alcian blue and the number of goblet cells was scored in 10 well orinted jejuna villi (Allen *et al*., 1986).

Goblet cell mucin gene

To determine the expression of MUC2-mRNA, we used a quantitative real-time PCR. In brief, the total RNA was isolated from mice jejuna using Trizol (Invitrogen). RNA samples were treated with DNase (Applied Biosystems, Darmstadt, Germany) and then converted into cDNA using the reverse transcription kit (Qiagen, Hilden, Germany). Quantitative real-time PCR (qRT-PCR) was performed using the ABI Prism 7500HT sequence detection system (Applied Biosystems, Darmstadt, Germany) with SYBR green PCR master mix from Qiagen (Hilden, Germany). MUC2 primer was obtained from Qiagen (Dkhil *et al*., 2013). mRNA levels were normalized to 18S rRNA. The fold induction of mRNA expression upon infection with *E. papillata* was determined using the 2−ΔΔCT method (Livak and Schmittgen, 2001).

Statistical analysis

Statistical analysis was carried out using one-way ANOVA, and Duncan's test was used for comparison between groups.

RESULTS

On day 5 *p.i.* the expelled oocysts of *E. papillata* were at its maximum level being about 6242.7 ± 731.5 oocysts / g faeces in infected group. *S. persica* root extracts was significantly able to suppress the oocyst output by 56.8, 22.8 and 32.2 %, respectively (Table I).

Table 1.- *S. persica* induced reduction in oocyst output.

Group	Oocyst output/g faeces	Suppression of oocyst shedding (%)
Infected	6242.7 ± 731.5	0%
Infected (300 mg/kg)	2696.7 ± 441*	56.8%
Infected (600 mg/kg)	4820.7 ± 2650	22.8%
Infected (900 mg/kg)	4230.6 ± 2531	32.2%

Values are means ± SD.
*Significance at P≤0.001.

It is clear now that the dose 300 mg/kg is the best dose for suppression of oocyst output. So, we used only 300 mg/kg root extracts to investigate the histological changes as well as the goblet cell response to infection and/or treatment.

Control non-infected mice grew normally resulting in an average weight gain of about 5.8 %. Whereas experimentally infected mice with *E. papillata* had lost their original weight by about 2.8 %. After treatment with 300 mg/kg *S. persica* root extracts, the loss of the original weight was reduced to -1.89 % (Fig. 1).

Table II shows the change in the number of parasitic stages on day 5 p.i. due to treatment of mice with 300 mg/kg *S. persica* root extracts. The developmental stages of *E. papillata* clearly appeared inside the intestinal epithelial of the villi and we found no parasites inside goblet cells.

Examination of Alcian blue stained sections showed that the infection induced a significant decrease in the number of goblet cells inside the jejuna villi (Table II).

Table II.- *S. persica* induced reduction in the number of parasitic stages, goblet cells and weight change of mice infected with *Eimeria papillata*.

Group	Uninfected	Infected	Infected with extract (300 mg/kg)
Number of parasitic stages	-	116.75±3.5	75.25 ± 13.8
Number of goblet cells/10 VCU	73.25±8.5	35.75±5.3	53± 5.6*
Weight change (%)	5.8±1.10	−2.8±1.2	- 1.8±3.08*

Values are means ± SD.
*Significance at P≤0.001.

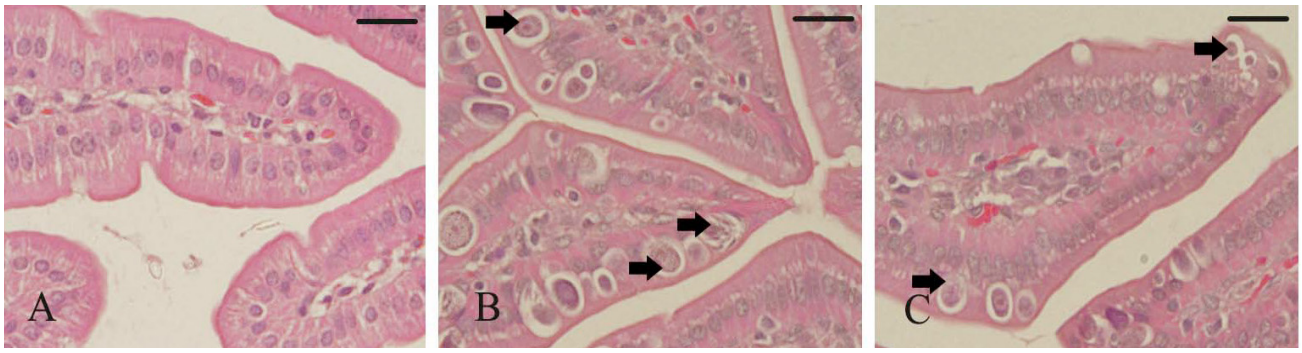

Fig. 1. *S. persica* reduced change in E. papillata parasitic stages. A, uninfected control jejunum; B, Iinfected jejunum containing developmental stages (arrows); C, Infected treated mouse jejunum (300 mg/Kg) with reduced numbers of developmental stages. Sections were stained with hematoxylin and eosin. Bar=20 um.

Treatment of mice with *S. persica* root extracts (Table II) could significantly increase the number of goblet cells compared to the infected group.

The expression of the jejunal goblet cell gene, MUC2 was downregulated due to infection of mice with *E. papillata* (Fig. 2). *S. persica* root extracts could significantly upregulate MUC2 in the infected jejuna (Fig. 2).

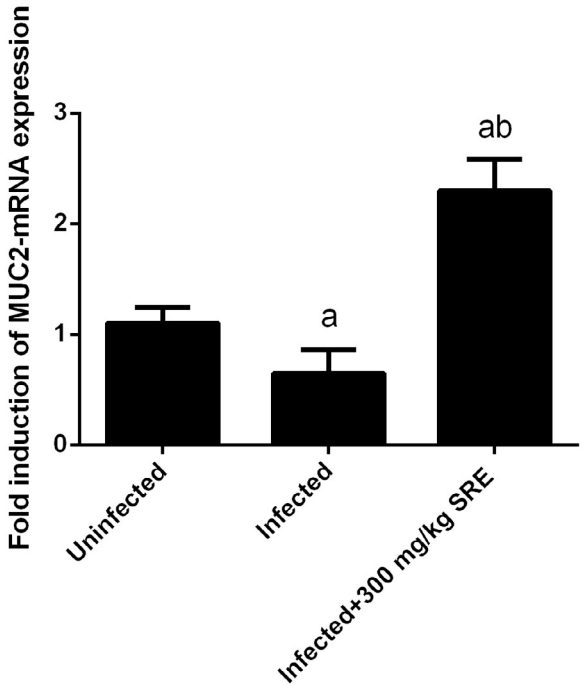

Fig. 2. MUC2 gene expression in the jejunum of mice. Expression was normalized to 18S rRNA signals, and relative expression is given as fold increase compared to the non-infected control mice. Values are means±SD. *Significant change at $P < 0.01$ with respect to infected mice.

DISCUSSION

Recently, several studies used plant extracts as antiparasitic agents (Klimpel *et al*., 2011; Metwaly *et al*., 2015; Alzahrani *et al*., 2016). The results of the current study indicated that, *S. persica* root extracts exhibit good anticoccidial properties. This effect may be due to the content of the extract. Khan *et al*. (2010) found that the root extracts contain flavonoids, alkaloids, carbohydrates, glycosides, saponins, steroids and tannins.

S. persica was able to improve the weight loss due to infection with *E. papillata*. This weight loss was previously studied by others (Anwar *et al*.,2008; Metwaly *et al*., 2013; Dkhil *et al*., 2014) and explained this loss due to decrease in water and food intake (Anwar *et al*., 2008). Also, Metwaly *et al*. (2013) related the weight loss due to the consumption of the parasitic stages to the carbohydrate content inside the intestinal villi and also led to structural alterations of the villi. The most structural change is the disruption of the host epithelium due to the discharge of the developed oocysts to the intestinal lumen.

Through the examination of the infected jejunum tissue sections, we observed that, different stages of *E. papillata* develop in the crypt region. Also, Cheng (1974) reported that, crypt region contains the stem cells producing goblet cells. These cells were invaded by the parasite leading to the decrease in the number of produced goblet cells in the jejunal villi. These observations were also documented by Dkhil (2013) during the investigation of the anticoccidial action of neem extract and its ability to increase the reduced goblet cell number due to *E. papillata* infection.

Dkhil *et al*. (2013) reported that, MUC2 gene was downregulated in the mice jejuna due to the infection with *E. papillata*. This gene is responsible for the immune response against pathogen-induced injury. *S. persica*

root extract could significantly downregulate the MUC2 expression due to infection however, this mechanism of downregulation has not been studied before.

The high potential effect of *S. persica* is due to the antioxidant (Mohamed and Khan 2013) and anti-inflammatory (Ezmirly *et al*., 1979) activities of the plant extract constituents. The dose 300 mg/kg was also proved to have a significant anti-inflammatory activity on rat induced paw oedema (Ahmed *et al*., 2011) but until now there was no anti-coccidial study about this and our study was the first one dealing with the anti-eimerial properties of *S. persica* extract.

CONCLUSION

Based on these findings, we conclude that *S. persica* root extract exhibits antieimerial activities and could be used with the normal food of animals as an additive.

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Conflict of interest statement

We declare that we have no conflict of interest.

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