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In vivo Anti-Eimeria and *in vitro* Anthelmintic Activity of *Ziziphus spina-christi* Leaf Extracts

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ABSTRACT

Intestinal parasitic infection by *Eimeria* and helminthes in poultry are responsible for worldwide economic losses. This study was conducted to investigate the Anti-*Eimeria* and anthelmintic activity of *Ziziphus Spina-christi* leaves extract (ZLE). Experimental mice were divided into 5 groups. The first group is the non-infected control group. The second, third, fourth and fifth groups were orally infected with 1.2×10^3 *E. papillata*-sporulated oocysts. The third, fourth and fifth groups were orally inoculated with ZLE at a dose of 100, 200 and 300 mg/kg, respectively. The anthelmintic potential effect of ZLE was investigated on adult earthworm, *Allolobophora caliginosa*. ZLE could significantly decrease the shedding of oocysts to about 10.7×10^3, 28.3×10^3 and 23.8×10^3 oocysts/g faeces in the third, fourth and fifth group of mice respectively. Also, ZLE was able to improve the induced jejunal injury by *E. papillata* infection. In addition, Treatment of infected mice with 100 and 300 mg ZLE/Kg could significantly elevate the number of goblet cells in the jejuna villi. Our in vivo study revealed that the time taken to induce paralysis and death of worms is dose dependant. The time consumed to induce paralysis and death decreased with the increased ZLE dose. Based on our results we can conclude that, *Z. Spina-christi* possesses anticoccidial as well as antihelmintic activity against *E. papillata* induced infection.

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MAD and SAQ designed the experiments, FA and EMAS performed the experiments, FA, EMAS and MAD analyzed the data, MAD, SAQ and FA wrote the paper.

Key words:
***Ziziphus Spina-christi*, Eimeria papillata, jejunum, anthelminthic activity.**

INTRODUCTION

Eimeriosis is an intestinal disease caused by various species of protozoan parasites within the genus *Eimeria* (Mehlhorn, 2014) and considered to be a disease of major economic importance affecting many species of farm and domestic animals. Gastrointestinal helminths also affect farming systems worldwide. Economic importance of eimeriosis and helminthiasis is due to production losses and high mortality rates of animals (Dkhil, 2013).

Most of the parasite control research programs are based on the use of anti-parasitic drugs are no longer considered sustainable because of an increased prevalence of parasite resistance, high costs and concerns regarding residues in the food and environment. Now, researchers used plant-based natural products against parasites because they have promising sources for novel anti-parasite candidate agents. These agents do not target only the parasites, but may also have organ-protective properties in the parasite-infected target hosts (Wunderlich *et al*., 2014). Recently, we used several plant extracts as pomegranate (Amer *et al.*, 2015), neem (Dkhil *et al*., 2013), date palm (Metwaly *et al*., 2012) and garlic (Al-Quraishy *et al*., 2011) against *E. papillata* induced infection in mice. hese extracts were not only anti-Eimeria but also protect the jejunum from the parasite induced injury.

Zizyphus spina-christi Willd belongs to the family Rhamnaceae. In Arab countries, it has a common name ''Nabka". In Bedouin, it is commonly used to treat ulcers and wounds. Nafisy (1993) reported that the plant leaves could be used as antiseptic, anti-fungal and anti-inflammatory agent. In addition, *Z. spina-christi* is also used to relief digestive disorders, obesity, urinary troubles and as a potent anti-microbial agent (Shahat *et al*., 2001; Nazif, 2002; Adzu *et al*., 2001). Furthermore, Abdel-Zahar *et al*. (2005) reported that *Z. spina-christi* leaves are used in folk medicine for the treatment of diabetes mellitus. The biological and pharmacological tests have shown antibacterial, antiviral activities of *Z. spina-christi* (Shahat *et al*., 2001). Furthermore, Kadir *et al*. (2008) used *Z. spina-christi* leaves ethanolic extract against cryptosporediosis induced in mice.

Our study was aimed at investigating the protective role of *Z. spina-christi* leaves extract on *Eimeria papillata* induced jejunal damage in the experimental animal, *Mus musculus*.

MATERIALS AND METHODS

Preparation of the plant extract

Ziziphus spina-christi leaves were collected from Riyadh, Kingdom of Saudi Arabia. The plant identification was confirmed by Dr. Pandalayil Department of Botany and Microbiology, College of

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Science, King Saud University. The plant leaves were dried then ground into powder. This powder was extracted with 70% methanol. In brief, the powder was incubated at 4 ºC for 24 h with mixing from time to time. *Z. spina-christi* leaves extract (ZLE) was filtered and then evaporated to dryness in vacuum evaporator (Heidolph, Germany). The residue was dissolved in distilled water and used in this experiment.

Animals

Forty male Swiss albino mice 9–11 weeks old and weighing 20-25 g, were obtained from the animal facilities of King Saud University, 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 state authorities and followed Saudi Arabian rules for animal protection.

Infection of mice

Mice were orally inoculated with *E. papillata* sporulated oocysts. Every 24 h, fresh faecal pellets were collected from each mouse then weighed and the bedding was changed to eliminate reinfection. Faecal pellets were suspended in potassium dichromate and diluted in saturated sodium chloride for oocysts flotation. The number of oocysts was counted in a McMaster chamber according to Schito *et al*. (1996).

Experimental design

A total of 32 adult female Swiss albino mice were divided into 5 groups, each of 8 animals. The first group (non-infected) served as a vehicle control. This group gavaged only with 100 µl distilled water. The second, third, fourth and fifth groups were orally infected with 1.2×10^3 *E. papillata*-sporulated oocysts. Then, after 60 min, mice of the third, fourth and fifth groups were gavaged with 100 µl of 100, 200 and 300 mg ZLE/Kg, respectively, once daily for 5 days.

Number of goblet cells

Pieces of jejunum were freshly prepared, fixed in 10% neutral buffered formalin, and then embedded in paraffin. Sections were cut and then stained with hematoxylin and eosin for parasite detection. Also, other sections were stained with Alcian blue for the determination of the goblet cells. For each animal, the number of goblet cells in the jejunum was counted on at least ten villi. Results were expressed as the mean number of goblet cells per ten VCU (Allen *et al*., 1986).

Evaluation of anthelmintic activity of Z. spina-christi

The anthelmintic study was carried out using three doses (100, 200 and 300 mg/ml) of methanolic ZLE against the earthworm (*Allolobophora caliginosa*) by following the method of Ajaiyeoba *et al*. (2001). Five worms of nearly the same size per dose were used. Time for paralysis in minutes was noted when no movement of any sort could be observed, except when the worm was shaken vigorously, while the time of death in minutes was recorded after ascertaining the worms neither moved when shaken vigorously nor when they were dipped in warm water (50°C) (Ajaiyeoba *et al*., 2001) and followed by fading away of their body colours. Albendazole suspension (10 mg/ml) was used as the reference drug (Murugamani *et al*., 2012; Dkhil, 2013). Distilled water was used as negative control. All the extracts and drug solution were freshly prepared before starting the experiment.

Statistical analysis

One-way ANOVA was carried out, and the statistical comparisons among the groups were performed with Duncan's t-test using a statistical package program (SPSS version 17.0). $P \leq 0.05$ was considered as significant for all statistical analysis in this study.

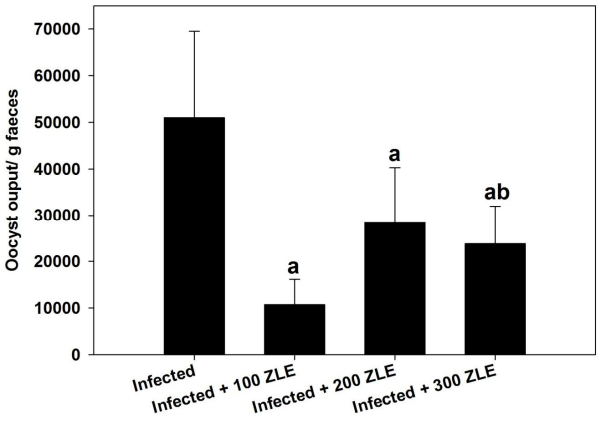

Fig. 1. *Ziziphus spina-christi* leaves extract induced changes in mice oocyst output on day 5 p.i. with *E. papillata* oocysts. All values are means±SD.
[a]Significant against non-infected group at P≤0.05.
[b]Significant against infected group at P≤0.05

RESULTS

Figure 1 shows the effect of ZLE on the outcome of *E. papillata* infections. On day 5 p.i., the output differed between ZLE-treated and nontreated infected mice. The number of excreted oocysts in the infected mice reached approximately 51×10^3/g faeces (Fig. 1). However, on day 5 p.i., the ZLE treatment significantly lowered the shedding of oocysts to about 10.7×10^3, 28.3×10^3 and

23.8×10^3 oocysts/g faeces in the third, fourth and fifth group of mice, respectively (Fig. 1).

ZLE was able to improve the injured jejunum induced by *E. papillata* infection (Fig. 2). Also, the jejuna villi contained less number of parasitic stages after treatment (Fig. 2). Also, the examination of Alcian blue stained sections showed the decreased number of goblet cells in jejuna villi of mice infected with *E. papillata* sporulated oocysts (Fig. 3). Treatment of infected mice with 100 and 300 mg ZLE/Kg could significantly increase the number of goblet cells (Fig. 3).

Our experiment showed that the anthelminthic activity of ZLE to induce paralysis and that to induce death of worms is dose dependant. i.e. when we increased the ZLE dose, the time taken to induce paralysis and death decrease (Table I).

Table I.- Anthelminthic action of *Z. spina-christi* leaves extract.

Treatment	Time taken for paralysis (min)	Time taken for death (min)
Vehicle control	-	-
Z. spina-christi (100 mg/ml)	40.4±6	65.4±10
Z. spina-christi (200 mg/ml)	22.2±4.4	37.8±12.8
Z. spina-christi (300 mg/ml)	20.6±2.6	32.4±6.5
Albendazole (10 mg/ml)	21±2.8	40.4±2.5

Values are means±SD. N=5 in each group.

DISCUSSION

It was reported that toltrazuril, the commonly used anticoccidial drug has many toxic effects on the host (Wunderlich *et al*., 2014). In this study, we showed that *Z. spina-christi* exhibits anticoccidial activity, as is evidenced by a significant decrease in the output of *E. papillata* non-sporulated oocysts with the faeces of the infected mice. This decreased output of oocysts reflects that *Z. spina-christi* impairs the development of *E. papillata* in the host before oocysts are finally released in faeces of mice. The fact that *Z. spina-christi* possesses anticoccidial activity has also been reported in mice infected with *Cryptosporedium* spp. (Kadir *et al*., 2008). These anticoccidial properties caused by *Z. spina-christi* are also known to occur with most anticoccidial drugs (Wunderlich *et al*., 2014).

Z. spina-christi leaves extract could improve the histological damage done by *E. papillata*. Our previous

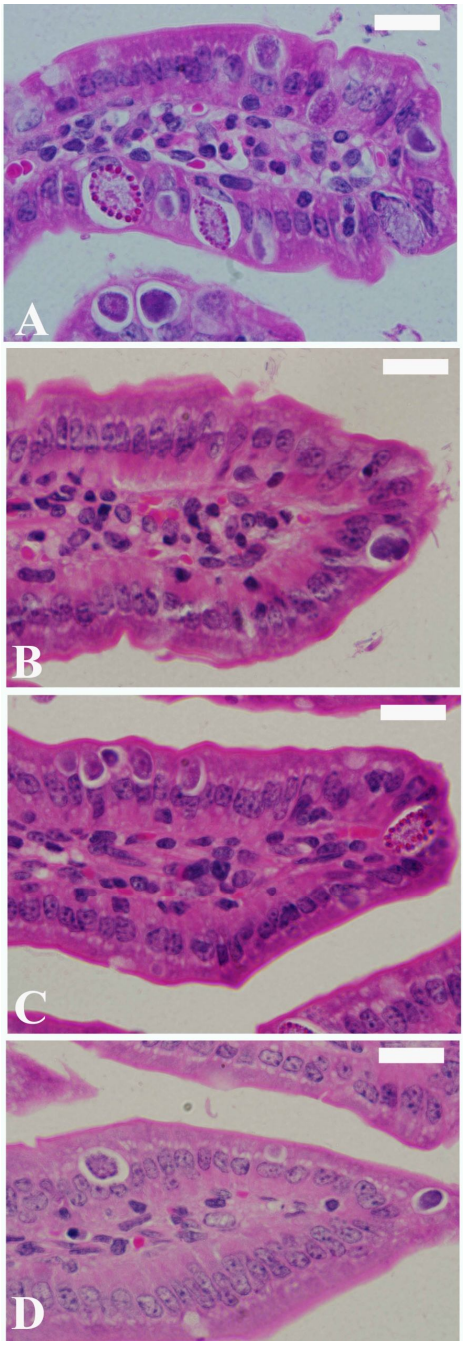

Fig. 2. Effect of ZLE on *E. papillata*-induced jejunum injury on day 5. A, Infected jejunum with some pathological changes, sections contained developmental stages appearing in the absorptive epithelia. B, Infected treated mouse (100 mg/Kg) with less parasites. C, Infected treated mouse (200 mg/Kg), D, Infected treated mouse (300 mg/Kg). Sections are stained with haematoxylin and eosin. Bar=25 µm.

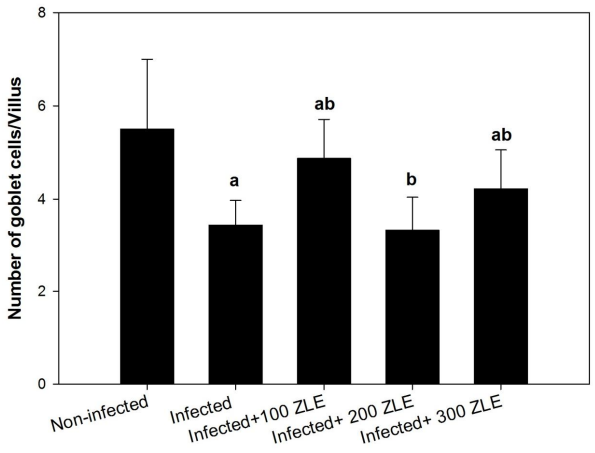

Fig. 3. ZLE induced changes in goblet cell number in jejunum of mice infected with *E. papillata* on day 5 p.i.. Values are means ± SD. Values are means ± SD.
[a]Significant against non-infected group at P≤0.05.
[b]Significant against infected group at P≤0.05

studies demonstrated that, active compounds present in plant extracts could successfully exert such improvement in the jejunal histological architecture (Metwally *et al*., 2012; Dkhil *et al*., 2013; Amer *et al*., 2015).

Goblet cells are considered to be one of the major intestinal immunocompetent cells (Dkhil, 2013) releasing mucous that can function as a defensive barrier (Deplancke and Gaskins, 2001; Linh *et al*., 2009).

The jejunal villi contain the multipotential stem cells (Cheng, 1974). The decreased number of goblet cells may reflect damage to stem cell population by the parasite (Cheng, 1974). The alteration in goblet cells could affect the susceptibility of the Eimeria-infected host to limit the capacity of pathogen from increasing or penetrating the local epithelium (Yunus *et al*., 2005). ZLE was able to increase the number of jejunal goblet cells infected with *E. papillata.*

It was reported that the administration of intestinal anthelmintic drugs could induce severe effects to the host (Gulani *et al*., 2009). Recently, several medicinal plants have been tested for their anthelmintic activity (Mehlhorn *et al*., 2011; Yadav, 2012; Dkhil, 2013). Our experiment was performed on adult earthworms due to its physiological resemblance with the intestinal worm parasite infecting human (Awad, 2004). The curative effects of the extract may be due to the present active components present in the plant like alkaloids, flavonoids, saponins, proteins and lipids (Adzu *et al*., 2003).

Collectively, *Z. spina-christi* possesses anticoccidial as well as antihelmintic activity against *E. papillata* induced infection. Further studies are required to know the mechanism of ZLE action.

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