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ANTHELMINTIC, ANTICOCIDIAL AND ANTIOXIDANT ACTIVITY OF SALVADORA PERSICA ROOT EXTRACTS

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ABSTRACT

Human infection with parasites is still one of the big problems worldwide. Medicinal plants succeeded to overcome a variety of protozoan and helminthic parasites. In this study,

Salvadora persica root extracts (SE) were used to treat helminthosis and coccidiosis. Three doses were used (200, 100 and 50 mg/ml) to study the anthelmintic activity of *S. persica*. *Allolobophora caliginosa* was used as a model worm. Also, Albendazole was used as a reference drug. In order to study the anticoccidial activity of SE, a group of mice were infected with *Eimeria papillata* sporulated oocysts. Experimental mice were treated with SE (300 mg/Kg) for 5 days. The extract was able to decrease the number of meronts and gamonts of the parasite in jejunum. Also, it regulates the level of glutathione and malondialdehyde and the activity of catalase as well. We conclude that *S. persica* possesses a powerful Anthelmintic, anticoccidial and antioxidant activity.

KEYWORDS : *Salvadora persica*, helminths, *Eimeria*, antioxidant, Mice, Jejunum.

INTRODUCTION

Parasitic infections caused by protozoa and helminths induce considerable death and led to economic loss in many countries (Mehlhorn, 2014). Weakness due to malnutrition and anemia is the major complains of the infection with worms (Jones and Berkley, 2014). The currently used Anthelmintic drugs induce some problems in human body especially in liver and kidney (Tripathi, 2008; Hong, 2018). Also, coccidiosis due to *Eimeria* infection is a major health problem in poultry animals (Mehlhorn, 2014). The oocyst of *Eimeria* present in the feces of the infected animals could induced upon engulfment of a new host to these oocysts and induce a severe injuries to the target organ. *Eimeria papillata* infect the mouse jejunum causing a lot of pathological changes (Dkhil and Al-Quraishy, 2012). Anticoccidial drugs (e.g. dindamycin, narasin and decoquinate) against eimeriosis are harmful to the host tissues due to several side effects (Wunderlich et al., 2014). Recently, herbal medications are proven to be effective against complains of eimeriosis (Habibi et al., 2016). *Salvadora persica* belongs to family *Salvadoraceae*. The plant roots are commonly used in Islamic countries due to their excellent biological activities (Eid et al., 1990; Sher et al., 2010). It is considered to be a plant with medicinal value because it contains many active chemical components with antibacterial (Almas and Majeed, 2011).

In this study, *S. persica* root extracts are used as anthelmintic, anticoccidial and antioxidant agent.

2. Methods

2.1. Collection of roots

Roots of *Salvadora persica* were obtained from Jazan city, Saudi Arabia. Methanolic extracts from the plant root were prepared based on Amer et al. (2015) method. In brief, *S. Persica* dry roots were grinded then extracted by methanol (70%). For both the in vivo and the in vitro studies, the extract powder was dissolved in water.

2.2. Extract analysis

Concentration of phenolic and flavonoid compounds in SE were determined as gallic acid equivalents per ml (Kim et al., 2003) and quercetin equivalents per ml (Dewanto et al., 2002), respectively.

2.3. Anthelmintic activity of *S. persica*.

Used as a model worm. Three doses were used (200, 100 and 50 mg/ml) to study the anthelmintic activity of *S. Persica*. We used a reference drug, Albendazole (Saudi Pharmaceutical Industries, Riyadh, Saudi Arabia) with a concentration of 10 mg/ml (Murugamani et al., 2012). Worms in distilled water were used as a control. In this experiment, the time to reach paralysis and death state

was expressed in minutes (Dkhil, 2013).

2.4. Mice and coccidial infection

Male mice of the strain C57Bl/6 (9-12 weeks old) were used as experimental animals. We obtained mice from the animal facility of Zoology Department at King Saud University and we followed the ethical rules for animal protection. *E. papillata* was used as a model coccidial parasite. Oocysts of *E. papillata* were passaged in laboratory mice. Unsporulated oocysts were collected from mice faeces, sporulated in 2.5% potassium dichromate, and then washed in buffered phosphate solution (Schito et al., 1996). Eight mice were served as a vehicle control. These animals received only saline. Sixteen mice were orally infected with 1000 sporulated oocysts. After 60 min, eight mice from this group were orally treated with *S. Persica* extract (300 mg/Kg) mice were killed and part of the jejunum was isolated and stored at -80 C for the oxidative stress study while the other part was fixed in 10% formalin to prepare paraffin sections for counting the parasitic stages.

2.5. Parasitic stages

Tissue paraffin sections were prepared according to Adam and Caihak (1964). To differentiate the different parasitic stages in mice jejunum, the sections were stained with hematoxylin and eosin then examined by microscope then we counted meronts, gamonts and developing oocysts in infected and infected- treated groups. Values were expressed in 10 villous crypt units (VCU).

2.6. Oxidative damage in jejunum

Mice jejunum were prepared from the control, infected and infected-treated groups to determine the change in oxidative status in mice jejunum (Dkhi et al., 2015a).

The level of glutathione in jejunal homogenate was estimated by the fluorometric method as reported in Hissin and Hilf (1976). It is expressed in mg/g. Also, catalase activity (U/g) was determined spectrophotometrically by following the Aebi method (Aebi, 1984). In addition, the level of malonaldehyde was assayed by the method of Satoh (1978) and finally evaluated as nmol/g tissue. significance between groups ($P \leq 0.5$) was compared using one-way ANOVA, and Duncan's test.

3. Results

The methanolic extracts from the root of *S. persica* were able to exert greater anthelmintic activity against live adult *A. caliginosa* worms (Table 1). The most efficient dose, 200 mg/kg showed the time to paralysis and death at about 5 and 6 min, respectively. However, the reference drug albendazole (10 mg/ml) showed less effect compared to the 200 mg/kg *S. persica* root extract.

The total concentration of flavonoids and phenolics in the *S. persica* root methanolic extracts were found to be 37 ± 1.7 as mg quercetin equivalents/ g of the sample and, 78 ± 2.2 mg gallic acid equivalents/ g of the sample, respectively (Fig.

1). Oocysts output were at its highest level on the fifth day post infection being about 6242.7 ± 731.5 oocysts / g faeces in infected animals. After treatment with *S. persica* extract, a significantly ($p < 0.01$) reduced the oocyst output by 2696.7 ± 441.3 was observed (Thagfan et al., 2017).

Oocysts in jejunal villi (Fig. 2). Remarkably, the number of meronts and male and female gamonts were significantly ($p < 0.01$) decreased after treatment by 37 ± 8 and 12 ± 2 , respectively (Fig. 3).

Glutathione level was significantly decreased from 79.8 ± 12 in non-infected group to 51.6 ± 11.2 mg/g in infected group. While, the level of glutathione of mice treated with the extract was increased to 68 ± 24.1 mg/g, (Fig. 4).

The activity of catalase enzyme was diminished from 8.3 ± 1.3 to 5 ± 1.9 U/g (Fig. 4). Upon oral administration of infected mice with 300 mg/kg *S. persica* root, an improvement in the antioxidant system within infected jejunum tissue occurred. Here, the antioxidant activity of catalase was significantly raised in treated mice to 7.9 ± 2.2 U/g. Also, malondialdehyde level was significantly ($p < 0.01$) increased from 249.3 ± 53.5 in non-infected group to 304 ± 45.8 nmol/g in infected group. While, the level of malondialdehyde of mice treated with *S. persica* was significantly ($p < 0.01$) decreased to 206.3 ± 11.2 nmol/g (Fig. 4).

4. Discussion

Several studies have reported the anthelmintic role of certain herbal extracts (Klimpel et al., 2011; Mehlhorn et al., 2011; Yadav, 2012). The earth worms have been chosen as a model for the anthelmintic activity experiment due to the (Awad, 2004). *S. persica* could perfectly kill worms in a short time compared to Albendazole, probably owing to the presence of active phytochemical constituents in the root extract.

Coccidiosis in poultry animals caused by *Eimeria* spp is responsible for economic losses a cross the world (Schito and Barta, 1997; Mehlhorn, 2014; Wunderlich et al., 2014). Previous studies have attempted to determine a solution for this issue.

This study investigated the anthelmintic, anticoccidial and antioxidant activity of *S. persica*. The used extracts exhibited adequate anticoccidial properties, probably attributed to the extract composition. Khan et al. (2010) reported that of *S. persica* extracts contain flavonoids, alkaloids, glycosides, steroids, carbohydrates, tannins and saponins. *E. papillata* persisting in the intestinal epithelia are associated with infiltration of inflammatory cells as macrophages, neutrophils, mast cells and T-cells (Laurent et al., 2001). This lead to initiation of cytotoxic and oxidative damage within infected mucosal tissue leading to their destruction via reactive oxygen production and nitrogen intermediates, and severe disturbance in the protective antioxidant systems (Allen, 1997; Georgieva et al., 2006). response and oxidative damage to the mice jejunum (Dkhil et al., 2015b).

Figure 1. Concentration of phenolics (mg) and flavenoids (mg) in root extract of *Salvadora persica*.

Figure 2. Parasitic stages of *E. papillata* in jejunum of mice. Meronts (white arrow), male (black arrow head) and female (black arrow) gamonts and developing oocysts (white arrow head). Sections stained with eosin and hematoxylin. Bar=10 μ m

Figure 3. Number of meronts, gamonts and developing oocysts per 10 villous crypt units (VCU) in mouse jejunum ifected with *E. papillata*. *, Significance against infected group.

Figure 4. Effect of *S. persica* on glutathione level, malondialdehyde levels and catalase activity in mouse jejunum infected with *E. papillata*. Data expressed as mean and standard deviation. a, significant change against control animals. b, significant change against infected animals.

CONCLUSION

Treatment of the infected animals with SE showed an excellent modulation of oxidative damage and enhancing antioxidant capability of mice jejunum tissue. The pronounced potential effect of *S. persica* results from the antioxidant (Mohamed and Khan 2013) and anti-inflammatory (Ezmirly et al., 1979) activities of the components of the plant extract.

The oxidation of lipid peroxides finally yields numerous carbonyl compounds production, such as malondialdehyde (Shinmoto et al., 1992) which in turn increase after treatment with SE. This reflects the potential role of SE as antioxidant. Previous studies have reported that SE contained active compounds as flavonoids and other derivatives (et al., 2003; Sher et al., 2010). In this study, the presence of flavonoids in SE reflecting its biological role (Duh et al., 2001).

Based on the presented results, we conclude that *Salvadora persica* possesses a powerful anthelmintic, anticoccidial and antioxidant activity. Future studies are needed to know the mechanism of *S. persica* action on both of the parasite and the host.

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