

Treatment of natural tropical theileriosis with the extract of the plant *Peganum harmala*

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Abstract: *Theileria annulata*, a protozoan parasite of cattle and domestic buffaloes, is transmitted by ticks of the genus *Hyalomma*, and causes a disease named Mediterranean or tropical theileriosis. In this research 50 cattle naturally infected with *Theileria annulata* were treated with the extract of the plant *Peganum harmala*. The treatment was continued for 5 days, the dose of the extract being 5 mg/kg per day. After the treatment, 39 cattle responded to the treatment and recovered, but 11 did not respond to the treatment and died. The recovery rate of animals treated with the extract of the plant *Peganum harmala* was 78%.

Key words: *Theileria annulata*, cattle, tropical theileriosis, *Peganum harmala*

INTRODUCTION

Theileria annulata is a protozoan parasite of cattle and domestic buffaloes, and is transmitted by ticks of the genus *Hyalomma*. This protozoan causes a disease named Mediterranean or tropical theileriosis. The disease affects cattle in a wide strip that covers southern Europe, northern Africa, and the Middle East (Iran), and reaches the south of the former USSR, India, and China (Purnell, 1978). Tropical theileriosis represents a major threat to crossbred and purebred cattle in Iran. During the last 4 decades scientists in the Razi and other institutes throughout the world have worked to find a potent compound to cure theileriosis (Hashemi-Fesharki, 1991). Parvaquone and buparvaquone are 2 effective drugs against tropical theileriosis. The recovery rate of animals treated with parvaquone was 60.7% and with buparvaquone it was

88.7% (Hashemi-Fesharki, 1991). Parvaquone and buparvaquone are chemical drugs which infiltrate in muscles of cattle. They are not easily and quickly eliminated from the body of animals (McHardy et al., 1985), which can constitute a public health hazard if milk and meat of treated animals are consumed by humans, but the extract of *Peganum harmala* is a natural drug which does not infiltrate in muscles of cattle (Puzii and Serov, 1983).

Peganum harmala is the plant from which harmine was first isolated, as well as a source of harmaline and tetrahydroharmine. This plant grows in semi-arid conditions like Iran. It originated from Central Asia, and is held in high esteem throughout Asia Minor as a medicinal plant. The pharmacologically active compounds of *P. harmala* are several alkaloids. These include β -carbolines, such as harmine, harmaline (identical with harmidine), harmalol, and harman (Fig. 1), and quinazoline derivatives, such as vasicine and vasicinone (Kamel et al., 1970). Alkaloid compounds well illustrate the diversity of antiprotozoal compounds found in *P. harmala* plants (Wright and

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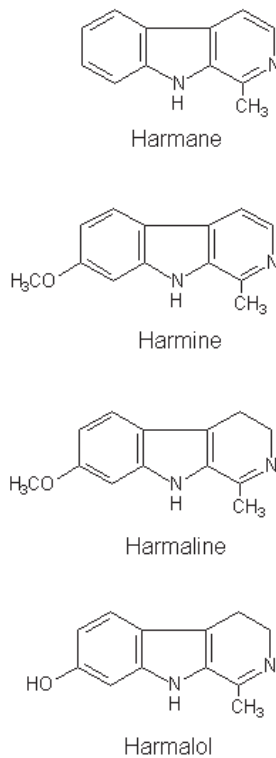


Fig. 1. Chemical structure of β -carboline alkaloids.

Phillipson, 1990), and among the several alkaloids, harmaline (harmidine, C₁₃H₁₄N₂O) has been found to be the major active alkaloid (Budavari and O'neil, 1996). In the present study, it has been tried to evaluate therapeutic effects of the extract of *P. harmala* on natural tropical theileriosis in cattle.

MATERIALS AND METHODS

Fifty naturally infected cattle with tropical theileriosis were selected. These cattle were from different ages and breeds (crossbreed and native breed). Rectal temperatures and schizont parasitosis values were scored on a 3-point scale (Table 1) as described by Mbwanbo et al. (2002). Only animals which were at the primary phase of their disease were used. Animals

that attended to at an early stage of the infection were those whose lymph node biopsy smears showed fairly numerous to numerous schizonts, and only rare piroplasm parasitemia was observed. In addition, only one or both prescapular lymph nodes were slightly to moderately enlarged with temperature reaction between 39.5 and 40.9°C.

Animals were totally managed by the farmers, and our responsibility was to screen the disease-suspected animals and to conduct treatments accordingly. Rectal temperatures of cattle were measured daily in the morning until dropped to normal values. Vigor, appetite, visible mucous membranes and other signs were observed clinically every day. Indicative signs of a possible *T. annulata* infection were enlargement of the prescapular glands, accompanied by rectal temperatures above 39.5°C. The peripheral ear vein was punctured for preparation of thin blood smears. Lymph node biopsy smears from enlarged prescapular glands were collected using a 16-gauge needle, 1 inch long. Thin blood and lymph node biopsy smears were air-dried, fixed in absolute methanol, stained with Giemsa's stain for 45 min and examined under microscope for presence or absence of piroplasms and schizonts of *T. annulata*.

The aerial parts of *P. harmala* were collected around Isfahan province, Iran. The plant was taxonomically identified by botanists in the Department of Biology (Shiraz University, Shiraz, Iran). An extract of *P. harmala* was prepared from the seeds of the plant according to the method described by Manske and Holmes (1952). The finally resulting concentrated extract was sterilized by ultraviolet then dried below 70°C in an oven.

Treatment with the extract containing the alkaloids of *P. harmala* was performed intramuscularly at a dose of 5 mg/kg body weight once daily for 5 days (Mirzaiedehghi, 2006). All cattle with rectal tempera-

Table 1. Scores of rectal temperature and *Theileria annulata* schizonts in cattle

Rectal temperature range (°C)	Score	Schizont parasitosis	Score
39.5-40.0	1	Fairly numerous	1
40.1-40.9	2	Numerous	2
> 41.0	3	Very numerous	3

tures $\geq 39.5^{\circ}\text{C}$ and diagnosed positive for *T. annulata* schizonts in lymph node biopsy smears were treated with the extract of *P. harmala*.

RESULTS

Lymph node biopsy smears from enlarged prescapular glands showed schizonts of *T. annulata* (Fig. 2) before treatment but 15 ± 3 days after beginning of the treatment most of cattle (39 cases) did not show schizonts of *T. annulata*.

The disease symptoms and parasite existence in most of cattle (39 cases) disappeared, and all animals recovered 15 ± 3 days after beginning of the treatment. The parasitemia and the body temperature in most of cattle (39 cases) were reduced after the treatment, and finally they didn't show any parasitemia and had normal body temperature 15 ± 3 days after beginning of the treatment. Clinical symptoms in 11 cattle became severe and resulted in their death. Also parasitemia, the body temperature, and schizonts of *T. annulata* in these cattle were raised and finally caused their death.

Thirty-nine (78%) out of 50 infected cattle responded to the treatment with the extract of *P. harmala*, and 11 (22%) of 50 infected cattle did not respond to the treatment with the extract of *P. harmala* and died (Table 2).

DISCUSSION

Therapeutic evaluations for medicinal plants are essential because of the growing interests in alternative therapies and the therapeutic use of natural products. Natural products have potential in the search for new and selective agents for the treatment of important tropical diseases caused by protozoans (Wright and Phillipson, 1990). Natural products can be lead to

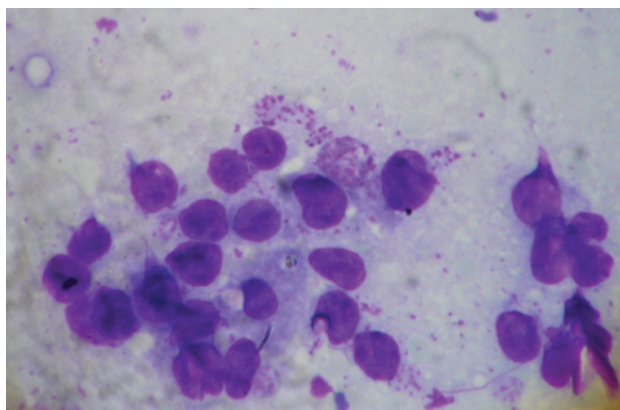


Fig. 2. A lymph node smear showing *Theileria annulata* schizonts in lymphoblastoid cells. Giemsa stain. $\times 1,000$.

compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development, and discovery of new therapeutic properties that are not yet attributed to known compounds (Hamburger and Hostettmann, 1991).

In the present study, we report the antitheilerial activity of *P. harmala* extract on *T. annulata*. This activity represents an exciting advance in the search for antitheilerial agents from natural sources, since significant effects against the protozoan were demonstrated. Cattle treated with the extract of *P. harmala* were considered as "cured" when their body temperature returned to normal and schizonts were not detectable for 3 consecutive days. The results indicate therapeutic effects of the extract of *P. harmala* for the treatment of tropical theileriosis. Though researches have been rarely performed on the treatment of tropical theileriosis with the extract of *P. harmala* in cattle, the results of the present study were approximately in agreement with the results of other researches which were done on the effects of the total alkaloids of *P. harmala* for the treatment of tropical theileriosis (Vecherkin et al., 1977; Puzii et al., 1982; Hu et al., 1997; Fan et al., 1997).

The recovery rate of cattle from theileriosis was

Table 2. Number of cattle treated with *Peganum harmala* extract at an early stage of *Theileria annulata* infection, doses used and outcome of treatment

Drug	Dosage per day	Duration of treatment	No. of cattle cured (%)	No. of cattle not cured (%)	Total number of cattle
<i>Peganum harmala</i> extract	5 mg/kg	5 days	39 (78)	11 (22)	50

78%, but in our previous study on natural malignant theileriosis in sheep, it was 65% (Mirzaiedehaghi, 2006). The reason for this discrepancy in the success rate of these 2 studies could be attributed to the different extracts of *P. harmala*. In the present study, the extract of seeds was used, whereas, in the previous research, a chloroform extract of the stem and leaves were used. The extract of seeds contains more alkaloids. Also other researches expressed different recovery rates. For example, in a research by Puzii et al. (1982), the recovery rate of total alkaloids of *P. harmala* on tropical theileriosis was 93%. This difference may have been caused by different application methods, because in the present study the extract of *P. harmala* was injected to cattle intramuscularly, but other researchers including Puzii et al. (1982) administered it intravenously. Also in the treatment of cattle in the present study, additional treatments were not applied and this can explain the lower cure rate in comparison with the other researchers. Additional treatments were necessary for more cattle to recover and of great importance for the protection of animals from hepatic and renal damage induced by poisonous substances. The poisonous substances were mainly produced by metabolism of the parasites.

Though the antiprotozoan mechanisms of *P. harmala* extract on *T. annulata* have been unknown yet, alkaloid compounds well illustrate the diversity of antiprotozoal compounds found in *P. harmala* plants (Wright and Phillipson, 1990), and among the several alkaloids (harmine, harmaline, harmalol, harman, vasicine and vasicinon) derived from *P. harmala* extracts, harmaline (harmidine, C₁₃H₁₄N₂O) has been found to be the major active alkaloid and quite soluble in dilute acids (Budavari and O'neil, 1996). In the present study, diluted acetic acid was used to extract the alkaloids (Manske and Holmes, 1952), and although not specifically tested by us, it is probable that our extract contained this specific alkaloid, and must have been effective against *T. annulata*, also we demonstrated that *P. harmala* extracts showed excellent antitheilerial activities. A cell cycle analysis using flow cytometry suggested that, although harmine interferes with the cell division, it does not induce apoptosis in

Leishmania donovani promastigotes. The results using a confocal microscopy supported that the cell death could be attributed to necrosis due to non-specific membrane damage (Lala et al., 2004).

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