

Effect of *Peganum harmala* (wild rue) extract on experimental ovine malignant theileriosis: pathological and parasitological findings

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ABSTRACT

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Malignant theileriosis of sheep is a highly fatal, acute or subacute disease is caused by the tick-borne protozoan parasite, Theileria hirci. In this investigation ten healthy male lambs aged 5-6 months were randomly divided into two groups, A and B and were kept in isolated tick-proof pens. They were treated for internal and external parasite before commencement of the experiment. The lambs were experimentally infected with T. hirci by placing ticks Hyalomma anatolicum anatolicum infected with T. hirci on them. The ticks used in this survey had originally been isolated from sheep and colonies of them were established in an insectarium. Before and after infection rectal temperatures and clinical signs of the lambs were recorded, blood and prescapular lymph node smears were prepared and examined to determine the extent of the parasitaemia, and blood samples were analyzed to evaluate their haemoglobin (Hb) and packed cell volume (PCV) rates. Three days after the commencement of a febrile reaction and appearance of the schizonts in the lymph node smears, treatment of the lambs in Group A with an extract containing the alkaloids of Peganum harmala (wild rue) was commenced. Group B lambs were kept untreated controls. Before treatment there were no significant differences in the rectal temperature, parasitaemia rate, and the Hb and PCV values between animals in the two groups but after treatment significant differences in these values was detected (P < 0.05). After treatment, the clinical signs and parasites in the lymph node smears of the animals in Group A disappeared and they all animals recovered. These parameters in the animals of Group B progressed until their death. Pathological studies showed the characteristic lesions of theileriosis in lambs in Group B, but not in Group A. The results indicate a therapeutic effect of the alkaloids of P. harmala for treatment of ovine malignant theileriosis.

Keywords: *Hyalomma anatolicum anatolicum, Peganum harmala*, sheep, *Theileria hirci*, theileriosis, wild rue

INTRODUCTION

Malignant theileriosis of sheep is a highly fatal, acute or subacute disease caused by the tick-borne proto-

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zoan parasite, *Theileria hirci* (Syn. *T. lestoquardi*) which is transmitted by ticks of the family Ixodidae particularly the species *Hyalomma anatolicum anatolicum* (Hooshmand-Rad & Hawa 1973; Morel & Uilenberg 1981). The chemotherapeutic efficacy of a number of compounds including parvaquone (Clexon, CCS Chem. Co. Ltd) and buparvaquone (Butalex, Hangzhou Onicon Chemical Co. Ltd) has been tested for the treatment of the disease. Tests have shown that these two compounds are effective (Hooshmand-Rad 1989) but they are not easily and rapidly eliminated from the body of animals (McHardy, Wekesa, Hudson & Randall 1985) which can consti-

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tute a public health hazard if milk and meat of treated animals are consumed by humans.

Peganum harmala L. (wild rue) is a species of family Zygophyllaceae. This is the plant from which harmine was first isolated, and in addition, is a source of harmaline and tetrahydroharmine. Wild rue grows in semi-arid conditions like Iran. It originated in 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, as well as the guinazoline derivatives vasicine and vasicinone (Kamel, Ibrahim, Afifi & Hamza 1970). Alkaloid compounds well illustrate the diversity of antiprotozoal compounds found in P. harmala (Wright & Phillipson 1990). Among the several alkaloids, harmaline (harmidine, C₁₃H₁₄N₂O) has been found to be the major active alkaloid which can cause cell death due to non-specific membrane damage (Budavari & O'Neil 1996; Lala, Pramanick, Mukhopadhyay, Bandyopadhyay & Basu 2004). The therapeutic effect of the alkaloids of the plant Peganum harmala has been investigated for the treatment of tropical theileriosis in cattle, which is caused by *Theileria annulata*, and in sheep naturally infected with Theileria hirci; it was shown that they are effective (Fan, Liang, Men, Gao, Li, Zhao, Hu, Dang, Zhang, Preston & Yin 1997; Hu, Fan, Liang, Zhao, Dang, Gao, Dong, Preston & Yin 1997; Mirzaiedehaghi 2006). In addition, it has been shown that these alkaloids, when inoculated intramuscularly. do not infiltrate the muscular tissue surrounding the inoculation site but are rapidly eliminated from the body (Puzii & Serov 1983). The object of this investigation was to study the effect of all the alkaloids of P. harmala when used for the treatment of experimentally infected cases of ovine malignant theileriosis.

MATERIALS AND METHODS

Materials used in this study were the Kazeroon isolate of *T. hirci*, susceptible male sheep, ticks as vector of the parasite and alkaloids of *P. harmala*.

The pathogenic Kazeroon isolate of *T. hirci* had been maintained for several generations by passage in sheep and ticks. Ten healthy male lambs aged 5–6 months were chosen, randomly divided into Groups A and B and kept in isolated tick-proof pens. Before the experiment commenced the lambs were treated for internal and external parasites. They were experimentally infected with *T. hirci* by

the ticks which were carriers of *T. hirci. Theileria*-free nymphal *Hyalomma anatolicum* anatolicum were fed on one infected sheep and the resultant adult ticks were fed on the experimental lambs. The infected ticks used in this study were originally isolated from field sheep and identified as *Hyalomma anatolicum anatolicum*, by using the taxonomy keys of Hoogstraal & Kaiser (1958) and clean colonies of them were established and maintained in our insectarium (Hosie & Walker 1979).

The aerial parts of P. harmala were collected in Isfahan Province, Iran. The plant was positively identified by botanists in the Department of Biology, Shiraz University, Shiraz, Iran. A concentrated extract of P. harmala was prepared from the seeds of the plant according to the method for alkaloid extraction described by Manske & Holmes (1952). Briefly, the crushed seeds of P. harmala are covered with three times their mass of water containing 30 g of acetic acid per liter of water. The seeds swell as they absorb the liquid and form a thick dough which is pressed after 3 days. The pressed seeds are once more treated as above with twice their mass of dilute acetic acid and, after maceration, the liquid is again pressed out. The concentrated extract was subsequently sterilized in ultraviolet light for 12 h then dried below 70 °C in an oven. Ten grams of the dried alkaloid was dissolved in 90 mℓ distilled water and injected to sheep at dose of 5 mg/kg.

All the lambs were infected by placing infected adult ticks in bags made of a cotton material which were then attached to the ears of the sheep. For 5 days before the ticks had been placed on the animals and every day thereafter until either recovery or death, their rectal temperatures and clinical signs were recorded twice a day. In addition, peripheral blood and prescapular lymph node smears were made on a daily basis to determine the extent of the parasite infection after the ticks had been placed on the animals. Blood samples from the jugular vein were taken every three days after the ticks had been placed on the animals in order to evaluate their haemoglobin (Hb) and packed cell volume values.

The blood and prescapular lymph node smears were air-dried, fixed in methanol, stained with Giemsa stain and microscopically examined for the presence or absence of piroplasms and schizonts of T. hirci. Three days after a febrile reaction had developed and the detection of schizonts in the prescapular lymph node smears, treatment of the lambs in Group A with the alkaloids of P. harmala was commenced intramuscularly at a dose of 5 mg of the dried material in 0.1 m ℓ normal saline/kg body

mass (Mirzaiedehaghi, 2006) once daily for 7 days. The lambs in Group B were untreated and served as controls.

Fifty days after infection (at the end of the experiment) all surviving animals in Group A were euthanized and subjected to a complete macroscopic and microscopic post mortem examination.

Anova and Duncan statistic methods with P < 0.05 were used for analyzing of the results.

RESULTS

All the lambs in Groups A and B developed the typical clinical signs of ovine malignant theileriosis including enlargement of peripheral lymph nodes, high



FIG. 1 Schizont of *Theileria hirci* was in a smear prepared from a prescapular lymph node of sheep. x 1000, Giemsa stain

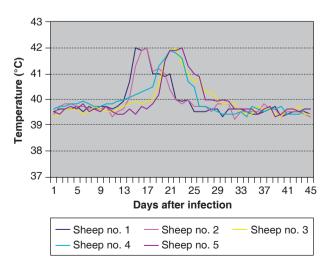


FIG. 2 Thermal reactions in the five lambs in Group A (treated with wild rue extract)

fever, anorexia and nasal and ocular discharges. The clinical signs appeared after a 15±3 days incubation period and schizonts of T. hirci were found in the smears prepared from their prescapular lymph nodes (Fig. 1). All lambs in Group A responded to the treatment regimen, the clinical signs of the disease and the presence of parasites in the lymph node and blood smears gradually disappearing over a period of 9 days. The clinical signs of the animals in Group B, however, progressed until death supervened. Before termination of experiment, on Days 34, 36, 40, 43 and 44 after infection, all five sheep in Group B died. The parasitaemia and the rectal temperature of the lambs in Group A progressively decreased; on the 9th day no parasites were detected in their blood and lymph node smears and rectal temperatures had returned to normal 9 days after treatment commenced. The parasitaemia, schizont existence in lymph node smears and the rectal temperatures of the lambs in Group B, however, progressively increased, the mean of their parasitaemia and rectal temperature being 6+4% and 41.9+ 0.05 °C, respectively (Fig. 2 and 3). After treatment, the amounts of Hb and PCV among Group A increased and then reached normal levels but the Hb and PCV among Group B were reduced to very low levels before death (Fig. 4-7).

Macroscopic findings in Group B included, emaciation, pallor of mucous membranes and yellowish discolouration of tissues, severe pulmonary oedema, hyperaemia and emphysema of the lungs, hydrothorax and hydropericardium. Large amounts of froth were present in the trachea and bronchi. Haemorrhages, from petechiae to ecchymoses, were evident in many tissues and organs. There was enlarge-

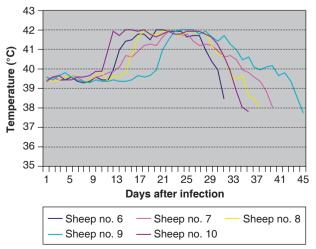


FIG. 3 Thermal reactions in the five lambs in Group B (untreated)

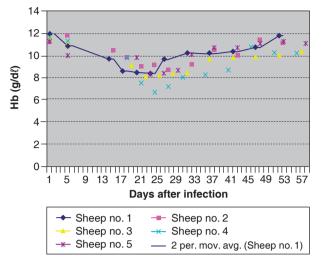


FIG. 4 Haemoglobin change in the five lambs in Group A (treated with wild rue extract)

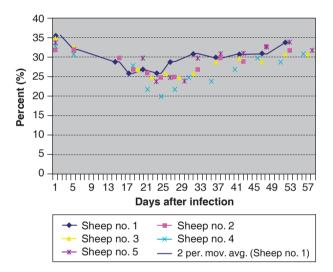
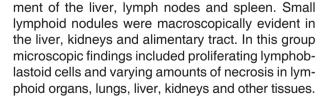


FIG. 6 Packed cell volume change in the five lambs in Group A (treated with wild rue extract)



No macroscopic or microscopic lesions of note were detected in the tissues and organs of the lambs in Group A.

Statistical analysis of the results showed that before treatment there was no significant difference in the rectal temperature, degree of parasitaemia, levels of Hb and PCV between the lambs in Groups A and B but after treatment there was a significant difference in these indices (P < 0.05).

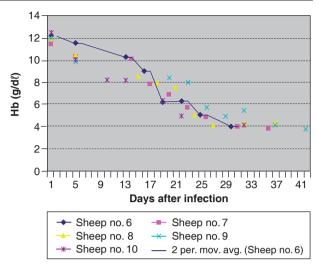


FIG. 5 Haemoglobin change in the five lambs in Group B (untreated)

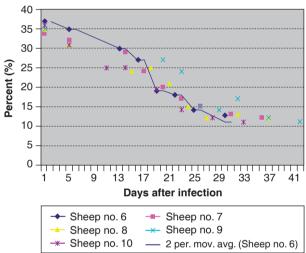


FIG. 7 Packed cell volume change in the five lambs in Group B (untreated)

DISCUSSION

Therapeutic evaluations of medicinal plants are essential because of the growing interest in alternative therapies and the therapeutic use of natural products. Natural products can function as lead compounds, allowing the design and rational planning of new drugs, biomimetic synthesis development, and the discovery of new therapeutic properties not yet attributed to known compounds (Hamburger & Hostettmann 1991). Although, *P. harmala* induced toxicity has been reported previously (Puzii, Vecherkin, Tribunskii & Romakhov 1980; Lamchouri, Settaf, Cherrah, El Hamidi, Tligui, Lyoussi & Hassar 2002), pathological findings in the lambs in Group A revealed not any sign of toxicity. In this study we report the effect of *P. harmala* extract on *T. hirci* infection

in sheep. This activity represents an important advance in the search for antitheilerial agents from natural sources, as a significant and important effect against the protozoan was demonstrated. In this study all the infected sheep treated with the alkaloids of *P. harmala* are considered "cured" whereas all the untreated sheep died of the disease.

In this study 100% of the sheep recovered after treatment but in another experiment reported by Mirzaiedehaghi (2006), the recovery rate was 65%. The reasons for this apparent discrepancy in the success rate of the treatment, could be the different environmental conditions, and the small numbers of sheep we used.

Our results are in agreement with those of other experiments in which the alkaloids of *P. harmala* were used for the treatment of tropical theileriosis in cattle (Agaeve, Mirzabekov, Gumbatov & Mirzabekov 1977; Vecherkin, Puzii, Romakhov & Tribunskii 1977; Charyev & Khudaiberdyev 1978; Levchenko 1978, 1979; Puzii, Vetchorkin, Romakhov & Vecherkin 1979; Puzii, Vecherkin, Toptaev, Tsyganova & Duisheev 1982; Fan *et al.* 1997; Hu *et al.* 1997). The pathologic findings of the lambs in Group B confirmed that they had died of malignant theileriosis (Radostits, Gay, Blood & Hinchcliff 2000). These studies confirmed the beneficial effect of *P. harmala* extract against *T. hirci* induced tissue injury in sheep.

Natural products have potential in the search for new and selective agents for the treatment of important tropical diseases caused by protozoans (Wright & Phillipson 1990). Cell cycle analysis studies using flow cytometry suggested that, although *P. harmala* extract interferes in the cell division stage, it does not induce apoptosis in *Leishmania donovani* promastigotes. Results using confocal microscopy supported the postulation that the death of this parasite in the cell could be attributed to necrosis due to nonspecific membrane damage (Lala *et al.* 2004).

Although the antiprotozoan mechanism of *P. harmala* extract on *T. hirci* is as yet unknown, a diversity of alkaloid compounds were found in the plant (Wright & Phillipson 1990), and among the several alkaloids (harmine, harmaline, harmalol, harman, vasicine and vasicinon) harmaline (harmidine, C₁₃H₁₄N₂O) has been found to be the major active alkaloid. It is quite soluble in dilute acids (Budavari & O'Neil 1996). In our study diluted acetic acid was used to extract the alkaloids (Manske & Holmes 1952) and although not specifically tested for by us, it is probable that, amongst others, our extract con-

tained this specific alkaloid. We demonstrated that our *P. harmala* extract showed excellent antitheilerial activity against *T. hirci*.

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