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FULL LENGTH ARTICLE

In vitro and *in vivo* anthelmintic activities of *Iris kashmiriana* Linn.

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Abstract In search of a natural antiparasitic, *in vitro* and *in vivo* anthelmintic activities of methanol and aqueous extracts of *Iris kashmiriana* Linn. rhizome were tested against gastrointestinal nematodes of sheep. A worm motility inhibition assay was used for *in vitro* study and a faecal egg count reduction assay was used for *in vivo* study. Crude aqueous extracts of *I. kashmiriana* exhibited greater anthelmintic activity against *Haemonchus contortus* than crude methanolic extract ($P < 0.05$). The aqueous extracts of *I. kashmiriana* resulted in a mean worm motility inhibition of 85.0%, while methanolic extracts resulted in a mean worm motility inhibition of 100.0%. The *in vivo* anthelmintic activity of aqueous and methanolic extracts of *I. kashmiriana* in sheep naturally infected with mixed gastrointestinal nematodes species demonstrated a maximum (70.27%) egg count reduction in sheep treated with aqueous extracts at 2 g kg^{-1} body weight on day 15 after treatment, closely followed by methanolic extracts at 1 g kg^{-1} body weight on day 15 after treatment (33.17% egg count reduction) while as lethal concentration (LC_{50}) values of aqueous and methanolic extracts of *I. kashmiriana* on adult worms of gastrointestinal nematodes of sheep *H. contortus* are 18.50 mg/ml and 16.66 mg/ml respectively. Thus aqueous extracts exhibited greater anthelmintic activity under both *in vitro* and *in vivo* conditions; this could be due to the presence of water soluble active ingredients in *I. kashmiriana*. From the present study it can be suggested that *I. kashmiriana* rhizome exhibited significant anthelmintic activity against gastrointestinal nematodes of sheep and has the potential to contribute to the control of gastrointestinal nematode parasites of small ruminants.

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1. Introduction

Gastrointestinal parasitism is a significant obstacle in the breeding of sheep, goat and other ruminants (Anderson and May, 1979; Daszak et al., 2000; Pessoa et al., 2002). Parasitism, especially by helminth species, impairs health by causing lack of appetite, diarrhoea, anaemia and, in severe cases, death (Athanasiadou and Kyriazakis, 2004). Synthetic anthelmintics have been used throughout the world for decades to minimize the losses caused by helminth infection. However, anthelmintic resistance in nematodes has become a major practical problem in many countries (Varady and Corba, 1999). Parasite resistance increases costs, reduces production efficiency along with the risk of contamination of the animal products (Waller, 1994; Dewanjee et al., 2007; Saddiqi et al., 2010) and increases the risk of environmental contamination (Hammond et al., 1997). Frequent use, increased dosage and increased application rate all correlate with declining effectiveness of synthetic anthelmintics (Donald, 1994). These disadvantages have stimulated a search for alternative control methods such as the use of traditional medicinal plants. Screening and proper evaluation of medicinal plants could reveal bioactive compounds that may be sustainable and environmentally acceptable (Eguale et al., 2007; Nisa et al., 2013).

Even today, plants play an important role in the health care of about 80% of the world population and is estimated that more than half of the drugs under clinical use at present owe their origin to plants (Sarin, 1996). Plants are utilized as therapeutic agents since time immemorial in both organized (Ayurveda, Unani) and unorganized (folk, tribal and native) forms (Girach et al., 2003). Recently, there has been an increasing interest in ethnomedical and ethnoveterinary practices around the world, especially as they pertain to the use of medicinal plants in treating various ailments (Bizimenyera et al., 2006). For acceptance of medicinal plants into scientific veterinary medicine, it is necessary that their effectiveness and safety be evaluated and confirmed through *in vitro* and *in vivo* testings (Rates, 2001).

Considering the vast potentiality of plants as sources of anthelmintic agents the present study was undertaken to screen the *in vitro* and *in vivo* anthelmintic activities of *Iris kashmiriana* commonly used in ethno-veterinary medicine by many tribes of Kashmir valley (Kaul, 1997; Khan et al., 2004). There is, however, no published scientific evidence for the anthelmintic and/or antimicrobial effects of *I. kashmiriana*.

2. Materials and methods

All animal proceedings were approved by the Ethical Committee of University of Kashmir (Number: F(Ethical Com. Animal) KU/2012/419.

2.1. Collection of plant materials

I. kashmiriana Foster (Iridaceae), locally called mazarmund, is a small herb with long stem, slender rhizome, short perianth tube and flowers white with darker blotches, found on open grassy slopes at 2400–3300 feet (730–1000 m) above sea level. The plant material was collected from Ganderbal, Kashmir (34°17′04″N, 75°13′46″E Altitude 10,068 ft), during May–August 2011. The mature plant at peak of flowering was collected in polythene bags and was processed by standard technique adopted by KASH (Kashmir University Herbarium). The plant was identified and authenticated by Plant Taxonomist Prof. Irshad Ahmad Nawchoo, Department of Botany, University of Kashmir, Srinagar, India. A voucher specimen (voucher No. 1701) was deposited in KASH (Kashmir University Herbarium). The collected plants were processed for shade drying at the environmental temperatures (25–30 °C) in a well-ventilated room (Drying room at Centre of Research for Development, University of Kashmir, Srinagar). The dried plant parts were milled to a fine powder using an electric stainless steel blender. The powdered plant material was stored in an airtight container/cellophane bags at 4 °C until extraction.

2.2. Preparations of extracts

Methanolic extracts were prepared by dissolving 200 g of the powdered plant material in a conical glass percolator to which 1000 ml (Qualigens) of methanol was added. The plant material was allowed to macerate for 16 h at room temperature and the percolate was collected by filtering through non-absorbent cotton wool. The process of maceration/percolation was repeated three times. The combined filtrate was evaporated in a vacuum rotary evaporator (R-201, Shanghai Shenshen) under reduced pressure of 22–26 mmHg at 40 °C. The final crude methanol extract (8.43 g) extract was scrapped off and transferred to a container and kept airtight for storage at 4 °C until further use.

Aqueous extracts were prepared by dissolving 200 g of the powdered plant material in 500 ml of distilled water in a glass percolator. It was allowed to macerate for 24 h at room temperature and the brew was filtered using Whatman #1 filter paper. The process of percolation was repeated three times. The combined filtrate was evaporated in a vacuum rotary evaporator (R-201, Shanghai Shenshen) under reduced pressure of 22–26 mmHg at 40 °C. The final crude aqueous extract (6.5 g) was scrapped off and transferred to a container and kept airtight for storage at 4 °C until further use.

2.3. In vitro anthelmintic activity by adult motility assay (AMA)

In vitro anthelmintic activity of the plant materials was evaluated by exposing the adult *Haemonchus contortus* worms to aqueous and methanolic extracts of *I. kashmiriana* as described in our earlier work (Lone et al., 2012). Adult live and motile *H. contortus* nematodes were collected from the gastrointestinal tract of slaughtered sheep at a local abattoir. Briefly, a minimum of 20 female *H. contortus* worms was exposed in 3 replicates to each of the treatments in separate petri dishes at room temperature (25–30 °C) and two petri dishes were also set for controls (Levamisole 0.5 mg ml^{-1} positive and for 0.95% phosphate buffer saline as negative control). The inhibition of motility and/or mortality of the worms kept in the above treatments was used as a criterion for anthelmintic activity. The motility was observed after 0, 1, 2, 5 and 8 h intervals and post-treatment revival of motility (if any) was observed by keeping the treated worms in the lukewarm fresh PBS for 30 min. The number of worms found dead at 8 h

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