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ORIGINAL ARTICLE

Anthelmintic efficacy of aqueous extract of *Butea monosperma* (Lam.) Kuntze against *Haemonchus contortus* of sheep and goats

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Abstract Infection with *Haemonchus contortus* is one of the most important economic problems in small ruminants worldwide. Resistance development by parasites, drug residues in meat, toxicity, non-availability and high cost limit the usefulness of currently used synthetic drugs. Therefore, the present study was undertaken to evaluate in vitro anthelmintic efficacy of aqueous extract of seeds of *Butea monosperma* (Lam.) Kuntze against *H. contortus*. Phytochemical analysis of extract showed high concentration of phenolic (11.93 ± 0.64 mg of GAE/g of extract), flavonoids (238.17 ± 19.14 mg of quercetin/g extract) and tannin (10.80 ± 0.70 mg of GAE/g of extract) content. The observations revealed that parasites were sluggish and movement was little at 4 h post exposure of 25, 50 mg/ml and very sluggish in 100 mg/ml concentration. The extract showed complete mortality of the adult *H. contortus* worms at the concentrations of 100 mg/ml at the time exposure of 6 h and with the concentration of 50 mg/ml at the post exposure of 8 h. At 25 mg/ml concentration 50 % mortality was recorded at 6 h and complete at 8 h post exposure. The LC_{50} at 6 and 8 h were 45.20 and 17.50 mg/ml respectively. Levamisole at concentration of 0.5 mg/ml caused 50 % mortality at 2 h post exposure and full mortality at 4 h post exposure. These cidal effects may be due to presence of high phenolic, flavonoids and tannin content in the extract. The results confirm the aqueous extract of *B. monosperma* (Lam.) Kuntze on adult *H. contortus* worms.

Keywords Anthelmintic · *Butea monosperma* · *Haemonchus contortus* · Levamisole

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Introduction

Haemonchosis has been identified as one of the top ten constraints to the profitable sheep and goat rearing throughout the world (Perry et al. 2002). High prevalence, ubiquitous distribution and pathological consequences of infection associated with huge economic losses to the farmer and rearing industry (Coop and Holms 1996). These parasites cause damage to the host in several ways, which include damage of gastrointestinal wall and suckling of blood and nutrients. Severe infections may result into anorexia, diarrhea, emaciation, hypoproteinemia, oedema and high mortality in all classes of affected animals (Githigia et al. 2001). In tropical countries, infestation was responsible for 23–63 % reduction in growth rate, up to 25 % pre-weaning mortality, 27 % loss in meat and 40 % loss in wool production, respectively in sheep and goat. A number of epidemiological studies have been conducted on haemonchosis of sheep and goats from different parts of universe and India. In India, haemonchosis has been reported from several states, viz. Uttarakhand (Yadav et al. 2009), Chattisgarh (Dey et al. 2008), Punjab (Kaur and Kaur 2008), Himachal Pradesh (Katoch et al. 1999) and Jammu and Kashmir (Pandit et al. 2003; Darzi et al. 2004; Khajuria 2010).

Eradication of gastrointestinal haemonchosis infections from grazing ruminants is not easy due to variation in host susceptibility to the parasite, wide distribution in nature

and the presence of wild ruminants. Therefore, control of diseases is only attractive option left behind. To control and treat haemonchosis in sheep and goats, common drugs belong to one of three drug classes: the benzimidazoles, the imidazothiazoles and the macrocyclic lactones. Benzimidazoles such as albendazole and fenbendazole are approved for use in sheep and goats, respectively (Zajac and Conboy 2006). Levamisole is a widely used imidazothiazole and is effective against adult and larval stages of *H. contortus* (Bowman 2008). Macrocyclic lactones such as ivermectin and moxidectin provide very broad spectrum activity against adult and larval nematodes (Aiello and Mays 1998). Recently, one more new drug of amino-acetonitrile derivative, monopantel has been recommended for control of haemonchosis (Sager et al. 2009).

Anthelmintic resistance in *Haemonchus* spp. has become a major practical problem in many countries. Injudicious and frequent uses are primarily responsible for the declining effectiveness (Donald 1994). In India, since the first report of anthelmintic resistance in *H. contortus* to phenothiazine and thiabendazole in sheep from Uttar Pradesh (Varshney and Singh 1976) and after that number of reports have been published from different parts of country (Das and Singh 2005; Easwaran et al. 2009), and indicated consistent increasing trend in drug resistance. The development of drug resistance in *H. contortus* has reduced production efficiency and increased the risk of environmental contamination. The resistant parasite may be more fecund and pathogenic and also has an increased establishment rate in the host and increased survivability of free living stages (Sanyal et al. 2003).

Therefore, it is important to establish a successful programme and to look for the development of alternative, safer and environmentally friendly antinematodal agents. There has been resurgence in the use of medicinal plants to treat cases of parasitism in ruminants and much of the success has been achieved in this direction against a variety of parasites. The present study was drafted taking the above view into consideration with the objective to evaluate the anthelmintic efficacy of the aqueous extract of seeds of *B. monosperma* (Lam.) Kuntze, a widely available plant, in an in vitro trial with conventional drugs levamisole a reference drug. The comparative assessment was based on the mortality and motility of *H. contortus* of sheep and goats.

Materials and methods

In vitro trials were conducted to determine anthelmintic activity of aqueous extract of seeds of *B. monosperma* (Lam.) Kuntze against *H. contortus* of sheep and goat origin. The levamisole was used as reference drug for the trial. The study was carried out at Division of Veterinary Medicine, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu.

Description of plant material and proximate analysis

Plant materials are used by the farmers as dewormer in man and animals. Based on the relevant ethno-botanical literature, we choose the seeds of *B. monosperma* (Lam.) Kuntze with promising anthelmintic property especially for roundworms and tapeworms (Prashant et al. 2001; Iqbal et al. 2006). The plant is highly used in indigenous systems of medicine as astringent, anticonvulsant, antioxidant, antistress, memory and behaviour stimulant, antigout, diuretic, antileprotic, antiulcer, antihepatotoxic, aphrodisiac, antibacterial and antihyperglycemic (Somani et al. 2006; Bavarva and Narasimhacharya 2008; Sehrawat and Kumar 2012). The seeds are used to treat skin and eye diseases, bleeding piles, urinary stones, abdominal troubles, intestinal worms and tumour. When seeds are pounded with lemon juice and applied to the skin, they act as a rubefacient (Das et al. 2011). The plant belongs to the family Fabaceae, grown wildly in many parts of India and commonly known as Palash, Dhak, Palah, Flame of the Forest, Tesu and Keshu. It is an erect, medium-sized, 12–15 m high, deciduous tree with a crooked trunk and irregular branches. The leaves are compound, with three leaflets. The leaves fall off by December and reappear during spring. When the tree is leafless, it bears flaming orange to red-coloured flower. The fruit is a flat legume pod and seeds are flat, reddish brown in colour. The plant is widely available throughout of our country. The seeds of *B. monosperma* (Lam.) Kuntze were purchased from the local shop, cleaned of adulterants, shed dried and milled to a fine powder using an electric mixer grinder. The powdered plant material was stored in an airtight container at 4 °C until extraction.

Determination of crude protein and minerals

The powdered plant sample was analyzed for the crude protein and minerals, calcium, phosphorus as per the standard protocol described in AOAC (1995). The trace minerals zinc and coppers were analysis in 1.0 g plant sample in Polarized Zeeman Atomic Absorption Spectrophotometer (Z-2300, HITACHI) as per method described by Kolmer et al. (1951).

Preparation of aqueous extract

Aqueous extract was prepared as per method of Singh et al. (2012). The 100 g of powder was placed in a conical glass percolator to which 500 ml of distilled water was added.

Plant material was allowed to macerate for 72 h at room temperature and the percolate was collected by filtering through non-absorbent cotton wool. The solvent was removed in a rotary vacuum evaporator under reduced temperature below 60 °C at a rotation speed of 20 rpm yielded aqueous extract. The extract was scrapped off, transferred to an air tight container and stored in a deep freezer at −20 °C till subsequent uses.

Phytochemical analysis of extract

1 % of plant extract solution was prepared in methanol and the amount of total phenolic and flavonoids content in extract was determined by the methods of Savitree et al. (2004) and Zhishen et al. (1999) respectively. Non-tannin content was determined using insoluble polyvinyl-polypyrrolidone as described by Makkar et al. (1993) and tannin content was calculated by subtracting non-tannin content from the total phenolic content in the extract (Velioglu et al. 1998).

Reference drug

Levamisole HCl tablet (Dicaris Adults) of Janssen Cilag Pharmaceuticals made in India by Johnson & Johnson, Mumbai was used at the rate of 0.5 mg/ml as reference drug.

In vitro anthelmintic test

Mature live *H. contortus* from sheep and goats were used to determine the effect of aqueous extracts of *B. monosperma* (Lam.) Kuntze as per method described by Tariq et al. (2009). Briefly, the mature worms were collected from the abomasa of freshly slaughtered sheep and goats. The worms were collected, washed and finally suspended in Hank's balanced salt solution (HBSS). The 20–25 adult actively moving *H. contortus* worms constituting approximately 100 mg of weight were exposed in triplicate in each petri dish containing 10, 25, 50 and 100 mg/ml concentrations of the aqueous extract prepared in 5 ml of HBSS and HBSS alone for the negative control group. The levamisole at the rate of 0.5 mg/ml was used as reference drug of positive control. The petri dishes were kept in an incubator at 37 °C. The inhibition of motility, activeness and mortality of the worms was observed at an interval of 30 min, 1, 2, 4, 6, 8 h. The number of motile (alive) and non motile (dead) worms were counted and recorded for each concentration. Death of worms was ascertained by absence of motility for an observation period of 30 s in the lukewarm fresh HBSS.

Statistical analysis

The phytochemical determinations were conducted in triplicate and results were expressed as mean ± SE. Statistical analyses were done by one-way ANOVA followed by Dunnett's test with $P < 0.05$ as a limit of significance. LC_{50} were calculated by the method of probits using the programme SPSS 8.0 for windows at different hour intervals of each extract.

Results

Proximate analysis of seeds of *B. monosperma* (Lam.) Kuntze

The proximate composition of analyzed seeds revealed crude protein content of 18.68 ± 0.35 %. The calcium was 1.07 ± 0.07 % and phosphorus was 0.52 ± 0.11 %. The copper content was 9.45 ± 0.55 ppm whereas zinc content was 25.38 ± 3.50 ppm (Table 1).

Phytochemical analysis of aqueous extract of *B. monosperma* (Lam.) Kuntze

The phytochemical analysis of the aqueous extract of *B. monosperma* (Lam.) Kuntze revealed total phenolic content of 11.93 ± 0.64 mg of GAE/g of extract. The flavonoids content was 238.17 ± 19.14 mg of quercetin/g of extract. The tannin and non tannin content in the extract were 10.80 ± 0.70 and 1.14 ± 0.35 mg of GAE/g of extract respectively (Table 1).

Effect of aqueous extract of *B. monosperma* (Lam.) Kuntze and reference drug levamisole on mortality and mortality of adult *H. contortus*

The % mortality along with the observations on activeness, motility in each concentration of aqueous extract was recorded at the intervals of 0, 30 min, 1, 2, 4, 6 and 8 h, respectively and results have been presented in Table 2. The results revealed that parasites were sluggish and movement was little at 4 h post exposure of 25, 50 mg/ml concentration and very sluggish in 100 mg/ml concentration. The aqueous extract of *B. monosperma* (Lam.) Kuntze showed complete mortality of the adult *H. contortus* worms at the concentrations of 100 mg/ml at the time exposure of 6 h and with the concentration of 50 mg/ml at the post exposure of 8 h. At 25 mg/ml concentration 50 % mortality was recorded at 6 h and complete at 8 h post exposure. The LC_{50} at 6 and 8 h were 45.20 and 17.50 mg/ml, respectively. Levamisole at concentration of 0.5 mg/ml caused 50 % mortality at 2 h post exposure and full

Table 1 The bioactive components and mineral composition in the aqueous extract of *B. monosperma*

Total phenolic content	11.93 ± 0.64 mg of GAE/g of extract
Total flavonoids content	238.17 ± 19.14 mg quercetin/g of extract
Tannin content	10.80 ± 0.70 mg of GAE/g of extract
Non-tannin content	1.14 ± 0.35 mg of GAE/g of extract
Crude protein[a]	18.68 ± 0.35
Calcium (Ca)[a]	1.07 ± 0.07
Phosphorus (P)[a]	0.52 ± 0.11
Zinc (Zn)	25.38 ± 3.50 ppm
Copper (Cu)	9.45 ± 0.55 ppm

Values are expressed as mean ± SE of three replicates. Values of copper and zinc are expressed as ppm

[a] Crude protein, calcium, phosphorus are expressed on dry matter basis

mortality at 4 h post exposure. The results confirm the cidal effect of aqueous extract of *B. monosperma* (Lam.) Kuntze on adult *H. contortus* worms.

Discussion

The World Health Organization recognized that 85 % of people in developing countries still rely on traditional medicine for human and animal health care. Plant materials are rich source of nutrients and naturally occurring bioactive molecules especially phenolic, flavonoids and tannin contents. Dietary supplementation of these phytomedicine boost immune system to destroy invading microorganisms and reduces the oxidative damage to cell membrane lipid, protein and nucleic acid due strong quenching property of free radicals (Han et al. 2007; Verma et al. 2011). The requirement of quality feed with ingredients having diversified activities against pathogenic microbes and parasites of gastrointestinal tract has now necessitated the exploration of alternate unconventional plants/plant parts having medicinal value for their incorporation in livestock ration. This needs the detailed analysis of plant for bioactive molecules, proximate contents and minerals having different role in restoring normal physiology and boosting defense mechanism through their nutritional potential. Therefore, the local herbs were analyzed for proximate composition and also for mineral estimation. The findings of proximate analysis revealed that the *B. monosperma* (Lam.) Kuntze has considerable amount of crude protein (18.68 ± 0.35) and have potential of replacing protein ingredients of ration. However, in view of paucity of reports on this aspect of local herbs detailed comparison cannot be made with the work of other workers. The mineral therapy has a direct role through wormicidal activity as well as indirect roles through strengthening of host defense, improving blood parameters and minimizing economic losses due to parasitism. The mineral profiling of the seeds revealed high content of copper (9.45 ± 0.55 ppm) and zinc (25.38 ± 3.50 ppm). Both the minerals have role in blood formation as well as in body defense through activation of various enzymes of synthetic and defense activity. The recent work has shown that copper administered orally to sheep and goats has an anthelmintic effect against *H. contortus* with extended protection up to 8 weeks (Chartier et al. 2001; Waruiru et al. 2004; Burke et al. 2005). In a study of this kind Martinez Ortis-de Montellano et al. (2007) concluded that copper treatment or supplementation reduced female worm length, number of eggs in utero and prolificacy of *H. contortus* resulting into reduced pasture infectivity. The copper also have effect on other gastrointestinal nematodes of sheep and goats and can provide additional benefit of improvement in losses of growth rate and blood parameters in parasitic enzootic areas.

The efficacy of the aqueous extract of seeds of *B. monosperma* (Lam.) Kuntze in different dose dependent concentrations revealed that the aqueous extract has good efficacy at the concentrations of 100, 50 and 25 mg/ml at

Table 2 Mean % of dead parasites ± SD and LC_{50} of different concentrations of *B. monosperma* (Lam.) Kuntze aqueous extract in in vitro trial on *H. contortus* mortality at different time intervals

Conc. (mg/ml)	Time					
	30 min(s)	1 h	2 h	4 h	6 h	8 h
LC_{50}	–	–	–	–	45.20 mg/ml	17.50 mg/ml
100	00.00 ± 0.00	00.00 ± 0.00	05.00 ± 5.00	30.00 ± 5.00	100.00 ± 0.00	100.00 ± 0.00
50	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	05.00 ± 5.00	70.00 ± 10.00	100.00 ± 0.00
25	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	05.00 ± 5.00	50.00 ± 10.00	100.00 ± 0.00
10	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
HBSS −ve	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00	00.00 ± 0.00
Levamisol 0.5 mg/ml	00.00 ± 0.00	20.00 ± 4.00	50.00 ± 4.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00

Observations: Parasites were sluggish and movement was little at 4 h post exposure of 25, 50 mg/ml and very sluggish in 100 mg/ml concentration

the exposure time of 6 and 8 h against the *H. contortus* of sheep and goat origin in the in vitro trials. Though the efficacy was not comparable with the levamisole, the reference drug used for positive anthelmintic control for the control of the haemonchosis of sheep and goat in field conditions but research out is of considerable importance. The present finding is in agreement with the previous published research work (Prashant et al. 2001; Iqbal et al. 2006). The phytochemical analysis revealed the high concentration of total phenolic and flavonoids content in the plant suggesting it as a good candidate for anthelmintic activity. The high nutritional value as indicated in proximate analysis, the presence of rational concentration of copper and zinc, which have direct wormicidal and indirect immune boosting results and gastro-protective effect of plant make it suitable for a package based control of haemonchosis in sheep and goats. The presence of activity in aqueous extract additionally allows widespread application of research outcome of the study.

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