Antiparasitic efficacy of *Artemisia absinthium*, toltrazuril and amprolium against intestinal coccidiosis in goats

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Abstract Various anti-protozoal drugs have been popularly used in the treatment of goat coccidiosis; however, residual effects are well noticed in host animals. The present study was undertaken with the objective to evaluate the anticoccidial efficacy of *Artemisia absinthium*, as a safe alternative in comparison to two conventional anticoccidial drugs (toltrazuril and amprolium) in goats (*Capra hircus*) naturally infected with *Eimeria* spp. (>5,000 oocyst per gram of faeces). Goat kids (1–3 month old, 10 kg body weight) were randomly allocated into five groups (eight kids each). Group A was negative for coccidiosis and was retained as uninfected and untreated (negative control). Group B was infected and was kept untreated (positive control). Group C was given a single oral dose of toltrazuril (Baycox 5 %) at 20 mg kg⁻¹ BW (IM). Group D received amprolium soluble powder 20 w/w% at 50 mg kg⁻¹ BW 5 days daily. Group E was given a single dose of ethanolic extract of *Artemisia absinthium* at 2 g kg⁻¹ BW. Clinical signs, body-weight gain (BWG) and number of oocysts per gram faeces (OPG) were monitored daily for 30 days post treatment (DPT). The OPG was highly reduced as early as 7 DPT and there was a marked improvement in body weight gain (7 DPT) and faster clinical recovery (3–6 DPT) in the toltrazuril treated kids compared to amprolium and *Artemisia absinthium* treated groups ($P \leq 0.05$). In *Artemisia absinthium* treated kids, the

oocysts continued to be in faeces up to 28th day post treatment indicating that ethanolic extract of herb was less efficacious against caprine coccidiosis as compared to amprolium and toltrazuril. From the observations of the present study it can be concluded that control of goat coccidiosis through single treatment of toltrazuril was highly effective as compared to the other two treatments. However, integrated control involving pasture management, chemical and herbal control will be a more realistic and sustainable means of controlling coccidiosis in goats. Further studies at different doses and concentrations using different solvent preparations of the plant extract are recommended to arrive at a certain conclusion about the anti-coccidial efficacy of *Artemisia absinthium*, which has been reported to be highly effective against other parasites in ruminants.

Keywords Amprolium · *Artemisia absinthium* · Coccidiosis · Control · Goat · Toltrazuril

Introduction

Coccidiosis is one of the most important infections in ruminants (Borgsteede and Dercksen 1996; Jalila et al. 1998; Faizal et al. 1999; Faizal and Rajapakse 2001; Waller 2004) and the infection is of greater economic importance in goats (Foreyt 1990). The most common clinical manifestations include inappetence, weakness, loss of weight, diarrhoea, depression and anaemia. The disease in goats is chiefly confined to females and young animals up to 6 months of age (Soliman and Zalat 2003) and has been reported from different states of India viz., Chattisgarh, Tamil Nadu, Patna (Tiwari and Roy 2004; Senthilvel et al. 2004; Kumar et al. 2005, 2007). A total of 16 species

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of *Eimeria* have been reported as cause of caprine coccidiosis (Soe and Pomroy 1992; Smith and Sherman 1994). Prevention of reduced productivity and losses from coccidiosis in young animals depends on maintenance of hygiene and effective treatment to reduce the level of environmental contamination by infective oocysts.

To control and treat coccidiosis in goats, several drugs including amprolium, decoquinate, monensin, clopidol-methyl benzoquate, sulpha drugs and lasolocid are used (Fitzimmons 1967; Marlow 1968; Shelton et al. 1982; Craig 1986; Foreyt et al. 1986; Polack et al. 1987; Hadjipanayiotou et al. 1988). It is important to establish a successful programme and to look for the development of alternative, safer and environmentally friendly anti-coccidial 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 comparative therapeutic efficacy of the conventional drugs (toltrazuril and amprolium) in comparison to *Artemisia absinthium* on natural infection of goat coccidiosis. The comparative assessment was based on the clinical signs, the number of oocysts per gram of faeces (OPG) and the body-weight gain (BWG).

Materials and methods

The comparative antiparasitic efficacy of ethanolic extracts of *Artemisia absinthium* and conventional anticoccidial drugs (toltrazuril and amprolium) were tested in goats naturally suffering from *Eimeria* spp. infection. The study was carried out at Division of Veterinary Epidemiology & Preventive Medicine, Faculty of Veterinary Sciences and Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences and Technology-Jammu.

Description of plant material and preparation of extracts

Based on the relevant ethnobotanical literature, we chose the *Artemisia absinthium* Linn. (Asteraceae) with promising antiparasitic properties and is locally known as Tethwen. It is used in indigenous systems of medicine as a vermifuge, an insecticide, in the treatment of chronic fevers and for inflammation of the liver, as an antispasmodic and antiseptic (Koul 1997). Its essential oil has antimicrobial (Juteau et al. 2003), antifungal (Saban et al. 2005) and its aerial parts show anthelmintic activity (Tariq et al. 2009).

It is found throughout the Kashmir valley usually on open mountainous slopes. It is an erect, herbaceous, perennial, 0.9–1.9 m tall with many branching stems that arise from a woody base. Leaves are small, pinnately

compound and silvery in colour with deeply dissected leaflets (Koul 1997). The plant material for the study was collected from the Aharbal area of southern Kashmir valley; the plants collected were processed for shade drying in a well ventilated room. The dried plant parts were milled to a fine powder using an electric blender. The powdered plant material was then stored in an airtight container at 4 °C until extraction.

The powdered plant material at 200 mg/1,000 ml was exhaustively extracted with 95 % ethanol in a percolator. The extraction was carried out by a combination of maceration (at night) and percolation (during the day) at room temperature. The final percolate was collected by filtering through cotton wool (non-absorbent). The process of maceration/percolation was repeated three times (at 1,000 ml). The extraction process lasted 48 h. Removal of the solvents at 50 °C in a vacuum rotary evaporator yielded the respective extract. The extract was scraped off and transferred to a container and kept air tight; it was stored at 4 °C until further use. Stability of the extract during storage was checked by thin layer chromatography.

Reference drugs and dosage

Toltrazuril 5 % (Bycox) solution (Bayer Animal Health, Pharmaceuticals Industries, Mumbai, India) was given orally once at 20 mg kg⁻¹ BW according to McKenna (1988).

Amprolium 20 % (Merind Ltd) was given orally daily at 50 mg kg⁻¹ BW for five successive days according to Swarup et al. (1982).

Experimental design and treatment of animals

The in vivo studies were performed in 32 beetle kids of 1–3 months of age of mixed sex harbouring a naturally acquired coccidial infection reared in organised goat farm. The animals were pre adapted to the pen conditions for 10 days prior to the start of the study. Water, hay and feed were provided regularly to all of the study animals. The study continued for a period of 28 days post treatments (DPTs). On the basis of clinical signs and the presence of coccidian oocysts in faeces (mean ± SE of eggs per gram), the kids were randomly assigned to four treatment groups of eight animals each. Group A was negative for coccidiosis and was kept as uninfected and untreated (negative control). Group B was infected untreated (positive control). Group C was given a single oral dose of toltrazuril (Baycox 5 %) at 20 mg kg⁻¹ BW (IM). Group D received amprolium soluble powder 20 % w/w at 50 mg kg⁻¹ BW 5 days daily. Group E was given a single dose of ethanolic extract of *Artemisia absinthium* at 2 g kg⁻¹ BW.

Each group was isolated from other groups and no physical contact was possible between animals from different treatment groups. The clinical signs and faecal oocysts counts were monitored weekly till 28 DPT. The therapeutic efficacies of plant extract and drugs were assessed on the basis of faecal oocyst count, clinical signs and growth rate.

BWG, collection of faecal samples and oocyst counts

Weekly weight gain was recorded up to 1 month PT with the help of spring balance. Each kid was weighed three times at each occasion and mean was used in data analysis.

The faecal samples were collected in morning on weekly basis directly from the rectum of all goat kids over a period of 30 days (the period of experiment). The samples were immediately analysed by Stoll's technique (Soulsby 1982) to estimate the number of OPG.

Statistical analysis

Sigma stat software (SPSS) was used to conduct a two-way analysis of variance (ANOVA) and Student's *t* test to calculate the significance level between and among different treatment groups to test the effects of drug on the OPG and on body weight. Results were expressed as mean $\pm$ standard error at $P = 0.05$.

Results

The kids in the three treatment groups (*A. absinthium*, toltrazuril and amprolium) had initial body weights

(Mean $\pm$ SE) 9 kg (8–11 kg), 9 kg (9–11 kg) and 9 kg (7–11 kg), respectively. All infected animals showed various degrees of diarrhoea manifested by soiling of anus and hind quarter with sometimes tenesmus, loss of appetite and dehydration. The clinical signs were ameliorated at different periods after onset of treatment with *A. absinthium*, toltrazuril and amprolium.

Effect of *A. absinthium* extracts and conventional drugs on faecal oocyst counts and body weight gains

In comparison to the control, highly significant reduction ($P < 0.05$) of faecal oocyst counts were obtained in goat kids for toltrazuril treatment kids (Group C) on day 7 post treatment compared to amprolium and *A. absinthium* treatment groups (Table 1). The toltrazuril treated kids showed complete reduction of oocyst in faeces at 28 DPT while as amprolium treated groups showed complete reduction on 21 DPT. In *A. absinthium* treatment group, the animals passed oocysts continuously up to 28 of post treatment and there was little reduction of oocysts count in faeces as compared to toltrazuril and amprolium treatments. Therefore, ethanolic extract of *A. absinthium* was not effective in reducing the oocyst output of the infected goat kids. The overall reduction of oocysts during the period of experimentation in treated groups was highly significant ($P < 0.05$) when compared to that of control groups. The animals showed signs of clinical recovery viz., improvement in feed intake and normal consistency of faeces from 22–27 DPT in *A. absinthium* group, 3–6 DPT in toltrazuril group and 15–20 DPT in amprolium group.

Table 1 Effectiveness of different medications on faecal oocyst counts (mean $\pm$ SE) in different treatment groups of goat kids

Groups and treatment regimen	Mean faecal oocyst per gram of faeces pre and post treatment with different drugs				
	Pre treatment	Post treatment			
	0 day	7 days	14 days	21 days	28 days
Uninfected un-medicated (negative control)	–	–	–	–	–
Infected un-medicated (positive control)	15,025.38 $\pm$ 838.29	28,536.13 $\pm$ 177.26	31,221.62 $\pm$ 1,352.77	29,747.00 $\pm$ 420.04	19,403.25 $\pm$ 1,127.99
Toltrazuril @ 20 mg kg ⁻¹ BW	16,289.00 $\pm$ 185.63	–	–	–	–
Amprolium @ 50 mg kg ⁻¹ BW	16,518.00 $\pm$ 1,219.83	219.00 $\pm$ 23.47	114.12 $\pm$ 19.45	–	–
<i>A. absinthium</i> @ 2 g kg ⁻¹ BW	16,731 $\pm$ 1,046.22	11,107.00 $\pm$ 156.47	6,038.25 $\pm$ 198.11	4,201.50 $\pm$ 140.69	2,097.25 $\pm$ 102.29
Values	F-1.89 ^{NS} CD-2,963.23	F-8.13** CD-8.13	F-437.78** CD-24.06	T-19.42** $P < 0.0001$	T-13.37** $P < 0.0001$

^{NS} non-significant difference within the column ($P < 0.05$)

** Values shows highly significant difference within the column ($P < 0.05$ and $P < 0.0001$)

Table 2 Observations on weekly body weight gains (mean $\pm$ SE in Kg) in goat kids infected with coccidiosis

Groups and treatment regimen	Mean body weight gains pre and post treatment with different drugs				
	Pre treatment	Post treatment			
	0 day	7 days	14 days	21 days	28 days
Uninfected un-medicated (negative control)	9.14 $\pm$ 0.19 ^a	9.54 $\pm$ 0.20 ^a	9.92 $\pm$ 0.20 ^a	10.39 $\pm$ 0.25 ^a	11.82 $\pm$ 0.17 ^b
Infected un-medicated (positive control)	8.09 $\pm$ 0.13 ^a	7.71 $\pm$ 0.15 ^{ab}	7.66 $\pm$ 0.14 ^{b,c}	7.20 $\pm$ 0.12 ^c	7.88 $\pm$ 0.15
Toltrazuril @ 20 mg kg ⁻¹ BW	8.13 $\pm$ 0.14 ^a	8.74 $\pm$ 0.15 ^b	8.93 $\pm$ 0.13 ^c	9.48 $\pm$ 0.14 ^{c,d}	10.80 $\pm$ 0.15 ^d
Amprolium @ 50 mg kg ⁻¹ BW	8.29 $\pm$ 0.12 ^a	8.66 $\pm$ 0.13 ^a	8.83 $\pm$ 0.14 ^a	9.20 $\pm$ 0.10 ^b	10.16 $\pm$ 0.12 ^c
<i>A. absinthium</i> @ 2 g kg ⁻¹ BW	8.11 $\pm$ 0.11 ^a	8.21 $\pm$ 0.15 ^a	8.42 $\pm$ 0.15 ^a	8.85 $\pm$ 0.13 ^{a,b}	9.07 $\pm$ 0.13 ^c

Values bearing different superscripts differ significantly ($P < 0.05$)

Significantly higher ($P < 0.05$) gain in body weight was recorded in toltrazuril (8.13 $\pm$ 0.14 to 10.80 $\pm$ 0.15 in Kg) and amprolium (8.13 $\pm$ 0.14 to 10.80 $\pm$ 0.15 in Kg) treated group compared to infected un treated (8.09 $\pm$ 0.13 to 7.88 $\pm$ 0.15 in Kg) group (Table 2). Amongst the treated groups the maximum weight gain was recorded with toltrazuril treatment and it was very much close to the healthy control (9.14 $\pm$ 0.19 to 11.82 $\pm$ 0.17 in Kg) whereas, *A. absinthium* treated kids showed significant reduction in body weight (8.11 $\pm$ 0.11 to 9.07 $\pm$ 0.13 in Kg) compared to healthy control. Highly significant ($P < 0.05$) decrease in body weight was noticed in infected untreated group compared to that of healthy control. Significantly higher increase ($P < 0.05$) of gain body weight was recorded in toltrazuril on 7 day of post treatment compared to amprolium on 21 DPT and *A. absinthium* was observed from 28 DPT.

Discussion and conclusion

The faecal oocyst reduction assay and body weight gains revealed good anticoccidial efficacies for toltrazuril at day 7, amprolium at day 21 and *A. absinthium* at day 28 post treatment. The effectiveness of the plant extract was not similar to the reference drugs. The signs and symptoms of the infected kids (dullness, anorexia, diarrhoea, dehydration and loss of body weight starting) matched well with the earlier findings (Dubey 1992; Senthilvel et al. 2004). Diarrhoea and anorexia were the main clinical signs in all groups treated with different preparations and these signs appeared later (by inspection) than in kids with delayed treatment. The association between the delayed appearances of signs to treatment from first day is unexplained and therefore, further investigations are required to establish this association. Radostitis et al. (1994) stated that coccidiostat must be given early in the life cycle of the coccidia to suppress the development of life cycle of the parasite. Earlier the treatments with toltrazuril was also

effective against coccidia in poultry (Haberkorn and Stolfuss 1987; calves (Staschen 2004) and lambs (Le Sueur et al. 2009). Our findings suggest that toltrazuril is more effective than amprolium and *A. absinthium* in reducing the number of excreted oocyst in naturally infected goat kids. These findings are in agreement with earlier observations (Tauseef et al. 2011). In the present study the amprolium was also more effective than *A. absinthium* in suppressing the *Eimeria* oocyst production. In the earlier studies on intestinal nematodes of sheep *A. absinthium* was very effective in reducing the faecal egg counts (Tariq et al. 2009). However, it was not found effective against coccidian infection in goats. However, further studies with different dosages and concentrations of *Artemisia* extracts are recommended to arrive at a certain conclusion about its efficacy against goat coccidian. Moreover, Radostitis et al. (1994) stated that the susceptibility to coccidiostats varied with the different stages of life cycle of *Eimeria*. When the drug is given during the drug-susceptible stage (before 13–15 days) it produces its desirable effect. However, drugs given after 16–17 days appear to be less effective. Given the significant early interaction between the drug and timing of the treatment, we suggest that treatment with toltrazuril (20 mg kg⁻¹ BW orally once at a 1-week interval) on first day of treatment is the most effective way to overcome *Eimeriosis* in goat kids as demonstrated by reduction of the OPG. Mundt et al. (2003) reported treatment of coccidiosis in calves by toltrazuril during the prepatent period (metaphylactic treatment) produced high anticoccidial effect. The effectiveness of toltrazuril may be attributed to its effect against all intracellular stages (schizogony and gamogony) of *Eimeriosis* (Balicka-Ramisz 1999). Treatment from the first day of infection produced higher body weight gain than treatment given after observing signs or oocysts shedding on the 28th day post infection, because the duration of diarrhoea and oocyst shedding was longer and more severe than first day of treatment. Because diarrhoea hinders the absorption of food from coccidia-induced damaged intestinal mucosa

(Greiner et al. 1984; Mundt et al. 2003), this diarrhoea can lead to weight loss and emaciation (Greiner et al. 1984). On the other hand, comparison between the three drugs in regard to the body weight gain reveals a significant change in the oocysts per gram among the three drugs.

In conclusion, the present study revealed that toltrazuril was highly effective anticoccidial chemotherapeutic agent in terms of early reduction of oocysts, faster clinical recovery and improvement in body weight gain compared to *A. absinthium* and amprolium treatments in goat kids. We recommend treatment on the first day of suspected exposure rather than waiting for clinical signs or oocyst shedding, because treatment from the first day of infection lowered oocyst shedding and improved BWG 28 days after infection. Further studies at different doses and using different solvent preparations of the plant extract are recommended to arrive at a certain conclusion about the anti-coccidial efficacy of *A. absinthium*.

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