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Short Communication

In vitro Efficacy of Herbal Extracts against *Eimeria tenella*

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Abstract

Effect of leaves of *Curcuma* (*C.*) *longa*, seeds of *Artemisia* (*A.*) *absinthium* and roots of *Saussurea* (*S.*) *lappa* was evaluated on sporulation of *Eimeria* (*E.*) *tenella* under laboratory conditions. The oocysts used in this study were obtained by challenging the 14th day-old broiler chicks with 75,000 sporulated oocysts of *E. tenella*. The harvested oocysts were exposed to various doses of the 70% aqueous-methanolic herbal extracts (0.244–500 µg mL^{-1}). The oocysts were provided 27.5°C temperature with 60–80% humidity and continuous aeration. The yield (w/w) of *C. longa, A. absinthium* and *S. lappa,* was 46.2, 45.9 and 47.5%, respectively. Inhibition of sporulation was criterion for evaluation of the efficacy. The herbal extracts exhibited graded dose response on sporulation of *E. tenella* as evident by their lethal concentration (LC_{50}) values. LC_{50} values of *C. longa, A. absinthium* and *S. lappa* were 173.4, 221.3 and 960.6 µg mL^{-1}, respectively. *C. longa* demonstrated the highest inhibitory effects on sporulation of *E. tenella* oocysts.

Keywords: Herbal extracts; *Eimeria tenella*; Coccidiosis; Disinfectants

Introduction

Caecal coccidioisis, caused by *Eimeria tenella*, is a major threat to poultry production. Heaping of litter near the poultry farms is customary (Islam *et al.*, 2013) especially in the developing countries. *E. tenella* oocysts remain infective for nine months in litter (Jenkins *et al.,* 2013) and, therefore, become a source of disease for other farms through air current dispersion. The drugs which can inhibit sporulation process are best choice as preventive measures. Ammonia, methyl bromide, carbon disulfide and some phenolic products are commonly used as disinfectants on soil and litter but are related to several pitfalls and public concerns (Williams, 1997). Herbal anticoccidials open new perspectives and proved suitable to serve as alternatives to conventional treatment, particularly in countries with limited economic potential (Saratsis *et al.,* 2012). This research was carried out to screen anti-sporulation activities of aqueous-methanolic extracts of leaves of *Curcuma longa*, seeds of *Artemisia absinthium* and roots of *Saussurea lappa* against *E. tenella*.

Materials and Methods

Plant Material and Extraction

Leaves, seeds and roots of *C. longa* Linn. (Zingiberaceae), *A. absinthium* (Asteraceae), and *S. lappa* (Compositae) were procured from local market (Faisalabad, Pakistan), identified and authenticated by a botanist by comparing with the specimens stored in the herbarium of Department of Botany, University of Agriculture, Faisalabad, Pakistan. Herbal materials were dried in an oven at 40°C, ground to a fine powder and stored in polythene bags at 4°C until used.

Aqueous-methanol extracts of the plant materials were prepared following Jabbar *et al.* (2007). The w/w yield of *C. longa, A. absinthium* and *S. lappa* was 46.2, 45.9 and 47.5%, respectively. These extracts were stored at 4°C until use. The extracts were found exhaustively soluble in water. The doses were prepared using distilled deionized water as per desired.

Chickens, Feed and Management

Sixty (n=60), day-old Hubbard broiler chicks (Hubbard Al-Noor Chicks, Pvt.-Pakistan) were purchased from a local hatchery. The chicks were reared in poultry shed, Department of Parasitology, University of Agriculture, Faisalabad-Pakistan, under standard conditions. Chicks were vaccinated twice (days 7 and 28) for Newcastle disease, and once each for Infectious bursal disease and for hydropericardium syndrome on 14th and 18th day of their age, respectively. All chicks were maintained free from coccidian infection. Prior to artificial infection, droppings of the chicks were examined for validation of their coccidia-free status. At 12th day of age, all chicks were inoculated with 75,000 sporulated oocysts of *E. tenella,*

which were harvested from the chicks which had not been exposed to any anticoccidial drug. To ensure resistant free strain, a pilot project was executed in which 25, day-old chicks were infected and treated with the reference drugs. The drugs efficiently controlled coccidiosis. The experimental chickens were offered broiler standard feed except anticoccidial additives. Feed and water was provided *ad libitum*. Temperature, during the first week of age was maintained at 29.44–32.22°C; however, it was reduced on weekly basis by -15°C. Light was provided for 24 h throughout the experimental period.

The experimental chicks were sacrificed at 20th day of their age. Oocysts of *E. tenella* were obtained from the caeca of the broiler chick. The confirmation of the oocysts species was done with the help of keys given by Hofstad (1984). It was further validated by propagation in broiler chickens and the oocysts were preserved in potassium dichromate solution to induce sporulation.

Test Procedure

The oocysts suspension was prepared by serial dilution with 2.5% Potassium dichromate to contain concentration of 900 oocysts mL^{-1}. One mL of this suspension was distributed in each well of 24-flat bottomed microtitre plate and mixed with same volume of different concentrations (0.244–500 µg/mL) of each herbal extract. The control plates contained the diluents and the oocyst suspension. The oocyst suspension in these mixtures was incubated for 48 h at 27.5°C with 60–80% humidity and continuous aeration. Test for every dilutions were repeated thrice. The oocysts, after the initial exposure to herbal extracts, were washed clean and reincubated to establish whether any inhibition of sporulation was a true -cidal (lethal) effect rather than a reversible -stasis (growth).

Oocyst Counting

The oocyst counting in each well was done following Williams *et al.* (2006). The suspension in a well was thoroughly mixed, 1 mL of this sample diluted with 9 mL distilled and deionized water, which was later subjected to centrifugation at 400 g for 10 min. Most part of the supernatant (9 mL) was removed using a pipette and 1 mL remaining supernatant was resuspended with the sediment. A 100 µL subsample was immediately removed, deposited onto clean, grease free-standard glass microscope slide and the drop was covered with no. 1 glass coverslip measuring 64×22 mm. The slide was examined under inverted microscope using low and high magnifications. Both sporulated and unsporulated oocysts were counted. Each oocyst seen under the coverslips represented one oocyst mL^{-1} of the suspension in the well. This procedure was repeated for remaining of the suspension in the well. The average count of two subsamples was considered for total number of oocysts (sporulated and unsporulated) of suspension of the well.

Statistical analysis

LC_{50} and probit analysis were carried out following Manage and Petrikovics (2013). Duncan's multiple range test and significant difference between groups means were considered at $P < 0.05$.

Results

Dose-dependent inhibition of the sporulation of *E. tenella* was observed. Most effective was *C. longa* (LC_{50}= 173.4 µg/mL) followed by *A. absinthium* (LC_{50}= 221.3 µg/mL) and *S. lappa* (LC_{50}= 960.6 µg/mL) (Table 1). The confidence intervals (95%) and the fitness of the model based on chi square analysis were 435.4 to 587.2 and P 0.663, respectively.

Discussion

This is first study focused on these herbs as far as their anti-sporulation properties are concerned. *Curcuma longa*, *A. absinthium* and *S. lappa* are commonly known as Haldi,

Table 1: Comparative values of sporulation inhibition efficacy of *Eimeria tenella* oocysts at various concentrations of *Curcuma longa*, *Atremisia absinthium* and *Saussuria lappa*

	Curcuma longa			*Atremesia absinthium*			*Saussuria lappa*		
Log dose	Probit	Regression	LC_{50}	Probit	Regression	LC_{50}	Probit	Regression	LC_{50}
2.38	5.62	4.28		3.62	4.16		5.41	5.35	
2.68	4.41	3.95		3.56	4.04		3.61	5.23	
2.98	3.35	3.62		3.52	3.92		3.57	4.99	
3.29	3.11	3.29		3.5	3.79		3.53	4.50	
3.59	3.04	2.96		3.5	3.67		3.44	3.52	
3.89	2.96	2.63	173.44	3.52	3.55	221.30	3.4	1.55	960.65
4.19	2.97	2.30	µg/mL	3.53	3.43	µg/mL	3.41	3.37	µg/mL
4.49	2.52	1.97		3.5	3.30		3.36	3.22	
4.79	0	1.64		3.35	3.18		3.2	3.07	
5.09	0	1.31		3.35	3.06		3.06	2.92	
5.39	0	0.98		3.2	2.94		2.46	2.77	
5.69	2.42	0.65		2.94	2.82		2.96	2.62	

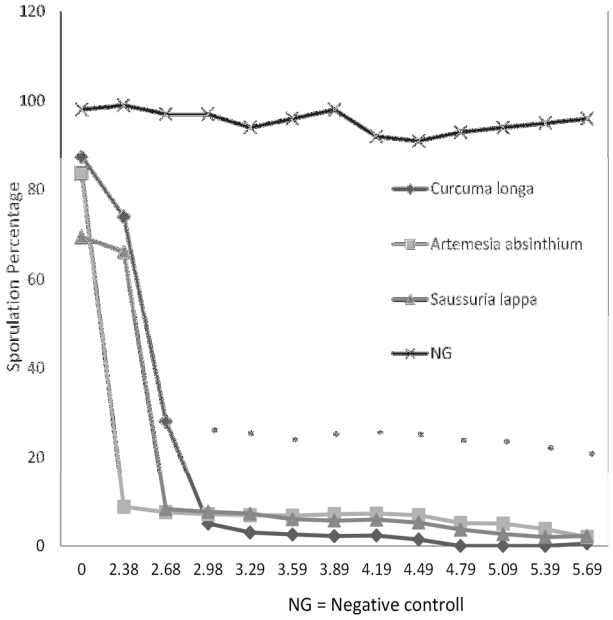

Fig. 1: Line graph showing dose-dependent anticoccidial effect of herbal extracts on sporulation of *Eimeria tenella* oocysts; asterisk (*) indicates difference ($P \leq 0.05$) in sporulation percentage compared with negative control

Afsantheen and Kust, respectively in Pakistan. These herbs are enriched in biological active chemicals like *C. longa*, *A. absinthium* and *S. lappa* reported for curcumin, thujone and germacra-1,4,11-trien-12-oic acid, respectively (de Kraker *et al.,* 2011; Liu *et al.,* 2012; Riahi *et al.*, 2013).

Sporulation inhibition is a common criterion to assess the anticoccidial properties (Molan *et al.*, 2009). However, most of the workers investigated *in vitro* effect by using cell culture method (Burt *et al.*, 2013) and effect on oocyst shedding after administration of anticoccidials (Zaman *et al.*, 2012a). However, the method used in this study is cost effective and time efficient. In addition, this method is equally valid for assessment of efficacy of inactivation measures performed for botanicals and synthetic agents to disinfect the environment. This method was based on the effect of some herbal extracts on the sporulation of oocysts.

It can be speculated that the phytochemicals exhibited anti-sporulation effect by interfering in the physiological process necessary for sporulation process like preventing access of oxygen and inhibition of the enzyme responsible for sporulation as in helminth eggs (Zaman *et al.*, 2012b). Minor differences shown in the results of the botanicals may be attributable to various factors. For example, ability of the phytochemicals in crossing the resistant oocyst wall and variation in molecular size, might be smaller in those herbs that hinders/ceased the sporulation process more significantly.

Conclusion

The herbal extracts tested in this study are effective disinfectant agents for prophylactic control of caecal coccidiosis. However, further investigation through challenging broiler chicken using the sporulated oocysts treated with herbal extracts is recommended.

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