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In vitro anticoccidial activity of *Trachyspermum ammi* (Ajwain) extract on oocysts of *Eimeria* species of Chicken

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Abstract

Background: Due to development of synthetic anticoccidial drug resistance there is improvement of anticoccidial medicines due to which exploration of diverse therapeutic agents is attractive now a days. Amongst original agents' miscellaneous botanicals have shown encouraging properties against coccidiosis. Present study is also a part of probing innovative therapeutic drugs against coccidiosis which can provide replacement solution to treat coccidiosis.

Methods: In current experiment *in vitro* anticoccidial effect of *Trachyspermum ammi* (seeds) extract was evaluated. For this purpose, an *in vitro* sporulation inhibition assay was used. Collected oocysts of four *Eimeria* species were exposed to six different concentrations (w/v) of *T. ammi* in 10% Dimethyl sulphoxide solution (DMSO), while Dimethyl sulphoxide and Potassium dichromate solution (K₂Cr₂O₇) served as control groups.

Results: Results of study revealed that *T. ammi* extract showed *in vitro* anticoccidial effect by affecting on sporulation (%) and damaging (%) *Eimeria* oocysts in dose dependent manner. *T. ammi* extract also damaged the morphology of oocysts in terms of shape, size and number of sporocysts.

Conclusion: The results strongly support the botanicals applications of *T. ammi* extract and also demonstrate its potential for use in Poultry coccidiosis control strategies.



Introduction

In Pakistan, Poultry industry is expanding with very promising results and uplifting the socio-economic status of sectors related to this industry. However, the poultry production systems are facing serious constraints of infectious diseases as hindrance in getting desirable economic benefits [1,2]. Among all infectious diseases, parasitic diseases like coccidiosis cause serious threat to poultry industry. Coccidiosis is the most severe and devastating disease infecting gastrointestinal tract of chicken which is caused by *Eimeria* (protozoa) having various species [3]. Coccidiosis causes huge economic losses to poultry industry in different parts of world. Disease has various clinical features such as poor weight gain, high mortality and bloody feces [4,5]. Oocysts of *Eimeria* sporulate rapidly in soil having higher multiplication rate due to which its prevention is difficult once its outbreak has occurred at poultry farm. This disease is mostly controlled by using anticoccidial drugs but, their efficacy has been lowered in field due to resistance problems to these drugs and now it is not reliable and effective protocol for its control [6].

So, in matter of achieving success in controlling this severe disease other options and protocols are effectively used in different countries of world [7,8]. Among other options plant driven compounds and their products have shown better therapeutic effects against different parasitic [9], viral and bacterial diseases of poultry [10,11]. Botanicals such as *Pinus radiata* [4], *Beta vulgaris* [6], and *Saccharum officinarum* [9] are reported to have excellent anticoccidial and immunomodulatory activity against coccidiosis. *Trachyspermum ammi* commonly known as "Ajwain" has been traditionally used to treat many diseases which cause serious threat to human and animals [12]. Based on the various therapeutic effects of *T. ammi*, current experiment was conducted to check its *in vitro* anticoccidial activity against oocysts of *Eimeria* species of chicken.

Methods

Preparation of *Trachyspermum ammi* extract

T. ammi seeds were obtained from Faisalabad local market, authenticated by a botanist and aqueous methanol extract of seeds was prepared using Soxhlet apparatus (Velp Italy) following method as described previously [4]. Prepared *T. ammi* extract was stored at 4°C until further use.

Collection of *Eimeria* oocysts

Eimeria oocysts of four species were collected from the caeca of infected intestine which were collected from different poultry sale shops and reported cases in Faisalabad. Identification of oocysts of different *Eimeria* species was done on the basis of morphology of *Eimeria*

oocysts and isolation site in the gastrointestinal tract of infected chickens. Collected oocysts were preserved and sporulated in potassium dichromate solution (2.5%) following the procedure as described previously [13].

Experimental design

In vitro efficacy of *T. ammi* extract was evaluated by sporulation inhibition assay. For this purpose, unsporulated oocysts of four *Eimeria* species (*E. tenella*, *E. brunetti*, *E. necatrix* and *E. mitis*) were subjected to different concentrations (w/v; 10, 5, 2.5, 1.25, 0.625 and 0.31%) of *T. ammi* in 10% DMSO in 5cm petri dishes by making two fold serial dilutions. DMSO and potassium dichromate solution ($K_2Cr_2O_7$) served as control groups. Incubation of *Eimeria* oocysts was done for 48 hours at 27-29°C and 60% humidity. Three replications were made for each concentration. The sporulation process of *Eimeria* oocysts was checked under light microscope at 40x. Sporulation inhibition (SI) and damage of *Eimeria* oocysts was determined in percentage by following method as described previously [14].

Statistical analysis

Statistical analysis was done by ANOVA technique and significance among groups was determined at ($P < 0.05$).

Results

Results of study showed that *T. ammi* extract significantly affected the sporulation process of *Eimeria* oocysts of different species as compared to both control groups (Control 1: DMSO, Control 2: Potassium dichromate solution ($K_2Cr_2O_7$)) ($P < 0.05$) as shown in Figure 1. *T. ammi* significantly reduced the sporulation of *Eimeria* oocysts, maximum sporulation reduction was observed in group treated with highest concentration of *T. ammi* extract (10%) followed by lower concentrations and the minimum sporulation reduction was observed in the group treated with lowest concentration of *T. ammi* extract (0.31%) which is suggestive of dose dependent sporulation inhibition activity of *T. ammi* extract against oocysts of all four *Eimeria* species.

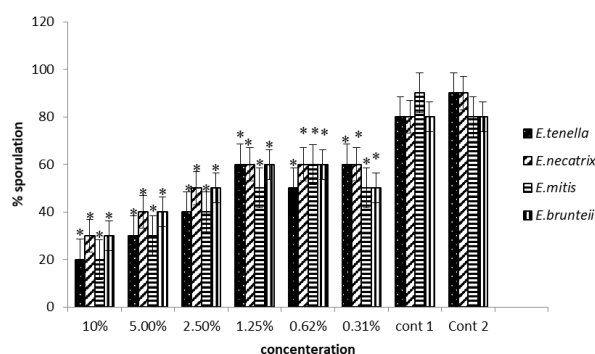


Figure 1: Effect of *T. ammi* extract on % sporulation of *Eimeria* oocysts (mean±SEM) Control-1 (DMSO); Control-2 ($K_2Cr_2O_7$)

T. ammi extract damaged morphology of *Eimeria* oocysts observed internally and externally as compared to both control groups ($P < 0.05$). Like that of sporulation inhibition activity of various concentrations of *T. ammi* extract, almost same trend was observed in terms of *Eimeria* oocysts damage. The detailed percent damage of oocysts caused by various concentrations of *T. ammi* extract is shown in Figure 2.

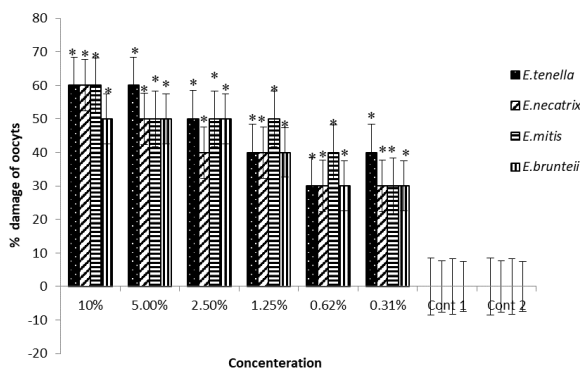


Figure 2: Effect of *T. ammi* extract on % damage of *Eimeria* oocysts (mean±SEM) Control-1 (DMSO); Control-2 (K2Cr2O7)

Figure 3A shows the normal sporulated oocysts containing four sporocysts. While Figures 3B, C & D show the unsporulated and damaged oocysts.

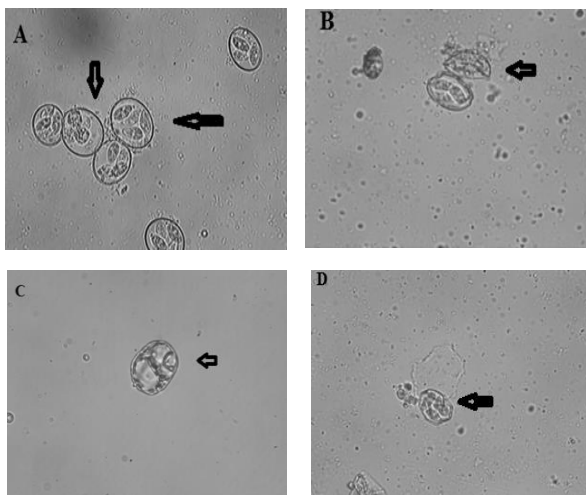


Figure 3: A) Normal sporulated oocysts from control groups. B, C and D showed abnormal and unsporulated oocysts in *T. ammi* treated groups

Discussion

Plants have many antioxidant compounds such as flavonoids and phenols to which they bear diverse therapeutic effects [15,16]. Many botanicals and their products are reported to have excellent anticoccidial and therapeutic effects as proven by different *in vitro* and *in vivo* studies [17,18]. In present study like that of previous studies [9], *in vitro* anticoccidial effect of *Trachyspermum ammi* extract was measured in terms of

sporulation inhibition and damage of *Eimeria* oocysts. The results showed that *T. ammi* stopped the sporulation process and damaged the morphology of *Eimeria* oocysts in dose dependent manner. Such higher *in vitro* anticoccidial potential of *T. ammi* extract might be due to action of its antioxidant compounds of *T. ammi* against *Eimeria*. In addition, *T. ammi* extract also affected morphology of *Eimeria* oocysts in terms of abnormal shape of oocysts and sporocysts. Such higher *in vitro* anticoccidial potential of *T. ammi* extract might be due to action of its antioxidant compounds against *Eimeria*.

Likewise, aqueous extract of pine bark extract also showed same effect on sporulation inhibition of *Eimeria* oocysts [19]. In most recent study *in vitro* anticoccidial effects of chemicals and natural products were evaluated. It was concluded from study that among tested commonly used disinfectants formalin and ethanol (70%) were proven effective against different *Eimeria* species [20]. Similar type of *in vitro* anticoccidial effects of *Sacharrum officinarum* (sugar cane) extract on inhibition sporulation and damage of *Eimeria* oocysts have already been reported [9].

Almost similar *in vitro* effects of some botanical driven essential oils (thymol, carvacol, eugenol) on various *Eimeria* species have been reported previously and results of study concluded that essential oils caused significant reduction in sporulation rate of *Eimeria* oocysts was observed after exposure to botanical driven essential oils. There was *in vitro* inhibition and destruction of *Eimeria* oocysts by plants based essential oils [21].

In a recent study, *in vitro* anticoccidial effects of *Psidium guajava* (guava) extract were evaluated against *Eimeria* species of rabbits. Results of study revealed that *Psidium guajava* extract inhibited sporulation process of *Eimeria* oocysts and also effected the internal and external morphology of oocysts [22]. In another study *in vitro* anticoccidial effects of some herbal extracts have been reported against oocysts of *Eimeria tenella*. Among all tested extracts *Curcuma longa* exhibited highest inhibitory effects against sporulation of oocysts [23].

It was concluded from the results of study that *T. ammi* extract inhibited the sporulation process of *Eimeria* oocysts. *T. ammi* extract also damaged *Eimeria* oocysts. *In vitro* results of this study suggest to conduct further *in vivo* trials in formulation of herbal drug from *T. ammi* extract for treating poultry coccidiosis.

Conflict of Interest Statement

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Authors' Contribution

Asghar Abbas, Rao Zahid Abbas performed and collected data of experiment. Muhammad Asif Raza and Muhammad Kasib Khan assisted in execution of experiment. Muhammad Kashif Saleemi and Zohaib Saeed helped in statistical analysis and final proof reading of manuscript.

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