In vitro anticoccidial activity of Vitis vinifera extract on oocysts of different Eimeria species of Broiler Chicken

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ABSTRACT: In the current experiment, the in vitro anticoccidial effect of Vitis vinifera (grape seed) extract was evaluated. For this purpose, an in vitro sporulation inhibition assay was used. Collected oocysts of four Eimeria species (E. tenella, E. necatrix, E. brunetti and E. mitis) were exposed to six different concentrations (w/v) of Vitis vinifera extract (VVE) in 10% Dimethylsulphoxide solution (DMSO), while Dimethylsulphoxide (DMSO) and Potassium dichromate solution (K₂Cr₂O₇) served as control groups. The results of the present study revealed that V. vinifera extract showed inhibitory effect on sporulation (%) and damage (%) of Eimeria oocysts in a dose dependent manner as compared to both control groups. V. vinifera extract also damaged the morphology of oocysts in terms of shape, size and number of sporocysts.

Keywords: Vitis vinifera, in vitro, Eimeria, oocysts
INTRODUCTION

Coccidiosis is an important disease infecting the intestine of chicken, which is caused by *Eimeria* (protozoa) species (Alzahrani et al., 2016; Abbas et al., 2019a). Coccidiosis causes heavy economic losses to poultry farming in different parts of the world (Bachaya et al., 2015; Abbas et al., 2017). Disease has various clinical features such as poor weight gain, high mortality and bloody feces (Masood et al., 2016). Oocysts of *Eimeria* sporulate rapidly in soil having high multiplication rate due to which its prevention is difficult once its outbreak has occurred at poultry farm (Zaman et al., 2012). Poultry coccidiosis has been controlled by using synthetic anticoccidial drugs but, due to their frequent and irrationale use resistance has been developed due to which this method is ineffective in controlling of disease (Alzahrani et al., 2016; Abbas et al., 2017).

So, in matter of achieving success in controlling this severe disease other options and protocols are effectively used in different countries of the world (Zhang et al., 2018; Bakr et al., 2019; Sarwar et al., 2019). Among other options plant-derived compounds and their products have shown better anticoccidial effects (Gadelhaq et al., 2018; Farieha et al., 2019; Zhang et al., 2020). Botanicals such as *Camellia sinensis* (Abbas et al., 2017), *Ageratum conyzoides* (Nweze and Obiwulu, 2009), *Vitis vinifera* (Wang et al., 2008), *Saccharum officinarum* (Abbas et al., 2015) are reported to have excellent anticoccidial and immunomodulatory activity against coccidiosis.

*Vitis vinifera* commonly known as grape is one of the most abundant cultivated plants all over the world and is rich with various usefull antioxidant compounds including flavanoids, anthocyanins, catechin and epicatechins. These antioxidant compounds are well known for their therapeutic, health beneficial and immunomodulatory effects in poultry and other livestock (Kara et al., 2016; Kasapidou et al., 2016). Due to its availibilty in large quantity and its content in bioactive compounds contained in it is a suitable candidate for improving poultry production.

Based on the various therapeutic and health beneficial effects of *V. vinifera* and very limited published research on its effect against *Eimeria*, the current experiment was conducted to evaluate in vitro anticoccidial potential of *V. vinifera* extract a on oocysts of four *Eimeria* species of broiler chicken.

MATERIALS AND METHODS

Preparation of *Vitis vinifera* extract

Seeds of *V. vinifera* were obtained from local market in Faisalabad, Pakistan. The plant material were authenticated by botanist of University of Agriculture Faisalabad and were dried and converted in powder form using an electric grinder. Aqueous methanolic extract of *V. vinifera* (seeds) was prepared using using Soxhlet’s apparatus (Velp Italy) following method described by Abbas et al. (2015). Prepared *V. vinifera* extract (VVE), was stored at 4°C untill further use.

Collection of *Eimeria* oocysts

Oocysts of four *Eimeria* species were collected from the caeca of infected broilers from different reported cases in Faisalabad. Collected oocysts were preserved in potassium dichromate solution (2.5%) following the procedure as described by Ryley et al. (1976).

Experimental design

The experiment was reviewed and approved by Research and Ethics Committee of Department of Parasitology, University of Agriculture Faisalabad, Pakistan. In vitro efficacy of *V. vinifera* extract (VVE) was evaluated by a sporulation inhibition assay. For this purpose, unsporulated oocysts (100 oocysts/5ml) of four *Eimeria* species (*E. tenella*, *E. brunetti*, *E. necatrix* and *E. mitis*) were exposed to different concentrations (w/v; 10, 5, 2.5, 1.25, 0.625 and 0.31%) of VVE in 10% Dimethylsulfoxide (DMSO) solution in 5cm petri dishes by making two fold serial dilutions. DMSO and potassium dichromate solution (K$_2$Cr$_2$O$_7$) 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. A total of 40 oocysts for each *Eimeria* species were counted in all treatment and control groups. The oocysts with 4 sporocysts was considered sporulated regardless the shape and size of the sporocysts. *Eimeria* oocysts having damaged wall and misshapen were considered damaged. The Percentage of sporulation and damaged of each *Eimeria* species was determined out of total counted oocysts. Sporulation inhibition (SI) and damage of *Eimeria* oocysts was determined in percentage by following the method by You (2014).

Statistical analysis

Data were analyzed by Analysis of Variance...
(ANOVA) and significance among groups was determined at $P<0.05$. For comparisons of means Duncan’s multiple range test was used.

RESULTS

The statistical analysis showed that all dilutions of *V. vinifera* extract (VVE) significantly affected the sporulation process of *Eimeria* oocysts of all the four species (*E. tenella*, *E. necatrix*, *E. brunetti* and *E. mitis*) as compared to both control groups (Control 1: DMSO, Control 2: Potassium dichromate solution ($K_2Cr_2O_7$)). Among all tested *Eimeria* species *V. vinifera* extract at higher dose significantly reduced sporulation process and damaged oocysts of *E. tenella* and *E. necatrix*. Oocysts of four *Eimeria* species (*E. tenella*, *E. necatrix*, *E. brunetti* and *E. mitis*) were differentiated on the basis of their morphology following method as described Abbas et al. (2019b). Photomicrographs of normal *Eimeria* oocysts and damaged *Eimeria* oocysts by *V. vinifera* extract in terms shape and wall are shown in Figure 3. It can be clearly seen in Figure 3 that *V. vinifera* extract inhibited sporulation and also damaged the morphology of *Eimeria* oocysts.

![Figure 1: Effect of *V. vinifera* extract on % sporulation of oocysts of four *Eimeria* species Control-1 (DMSO) and Control-2 ($K_2Cr_2O_7$). Asterisks (*) indicate a difference from the control groups](image-url)

*E. tenella*  
*E. necatrix*  
*E. mitis*  
*E. brunetti*
Figure 2: Effect of *Vitis vinifera* extract on percent damage of oocysts of four *Eimeria* species. Control-1 (DMSO) and Control-2 (K₂Cr₂O₇). Asterisks (*) indicate a difference from the control groups.

Figure 3: Photomicrographs of *Eimeria* oocysts
A: Normal sporulated oocysts of *Eimeria*.
B & C: Damaged *Eimeria* oocysts by *V. Vinifera* extract in terms shape and wall.
DISCUSSION

Many botanicals and their products are reported to have excellent anticoccidial activity as proven by different in vitro and in vivo studies (Abbas et al., 2015, 2017). In the present, as well as in previous studies (Zaman et al., 2012; Gadelhaq et al., 2018; Mujahid et al., 2019), the in vitro anticoccidial effect of V. vinifera extract was measured in terms of percent sporulation inhibition and damage of Eimeria oocysts. The results showed an inhibitory effect on sporulation and damage of Eimeria oocysts in dose dependent manner.

In addition, V. vinifera extract also affected the morphology of Eimeria oocysts in terms of abnormal shape of oocysts and sporocysts. Likewise, an aqueous extract of pine bark also showed a similar effect on sporulation of Eimeria oocysts (Molan et al., 2009). In a recent study, Gadelhaq et al. (2018) have reported the in vitro anticoccidial effects of chemicals and natural products. They concluded that commonly used disinfectants such as formalin and ethanol (70%) are the most effective in inhibition of sporulation process of different Eimeria species.

Abbas et al. (2019b) has reported in vitro anticoccidial effects of Trachyspermum ammi extract on oocysts of four Eimeria species of chickens. T. ammi inhibited sporulation of Eimeria oocysts and also damaged them. T. ammi effect was in dose dependent manner against Eimeria oocysts.

Abbas et al. (2015) have reported a similar, dose-dependent, in vitro anticoccidial effect of S. officinarum (sugar cane) extract on inhibition sporulation of Eimeria oocysts in dose dependent manner. Such high in vitro anticoccidial potential of V. vinifera extract might be due to action of its antioxidant compounds against Eimeria.

Somewhat similar in vitro effects of Camellia sinensis on the sporulation of various Eimeria species has been reported previously and a significant reduction in sporulation rate of Eimeria oocysts was observed after exposure to C. sinensis extract (Molan and Thomas, 2007).

In another study, in vitro destruction of Eimeria oocysts by essential oils has been reported (Remmal et al., 2013).

In the present study V. vinifera showed an in vitro anticoccidial potential against Eimeria oocysts which might be due to the action of its various flavonoids so-called antioxidant compounds including anthocyanins, catechin and epicatechins. However further studies and in vivo trials are needed to understand its anticoccidial effect in poultry.

CONCLUSION

It was concluded from the results of the present study that V. vinifera (grape seed) extract have in vitro anticoccidial potential against four Eimeria species. V. vinifera extract damaged the morphology and inhibited the sporulation process of Eimeria oocysts of all tested species. In vitro results of this study can help in future to explore and develop effective herbal remedy based on antioxidant compounds of V. vinifera by conducting in vivo trials for treating poultry coccidiosis.

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CONFLICT OF INTEREST

The authors have no conflict of interest with this publication.
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