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

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ABSTRAC: In the current experiment, the *in vitro* anticoccidial effect of *Vitis venifera* (grape seed) extract was evaluated. For this purpose, an *in vitro* sporulation inhbition 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 ($K_2Cr_2O_7$) 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 morhology of oocysts in terms of shape, size and number of sporocysts.

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

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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 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.,* 2013). 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 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 evalaute *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 4^0C 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 concenterations (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 ($K_2Cr_2O_7$) served as control groups. Incubation of *Eimeria* oocysts was done for 48 hours at 27-29^{0}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 ($K_2Cr_2O_7$) Figure 1. Effect of *Vitis vinifera* on percent damage of *Eimeria* oocysts is shown in Figure 2. *V. vinifera* extract damaged internal and external the morphology of of *Eimeria* oocysts in concentration-dependent manner as compared to both control groups (Control 1: DMSO, Control 2: Potassium dichromate solution ($K_2Cr_2O_7$). Among all tested *Eimeri*a species *V. vinifera* extract at higher dose significantly reduced sporulation process and damaged occysts 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 follwing method as decribed 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.

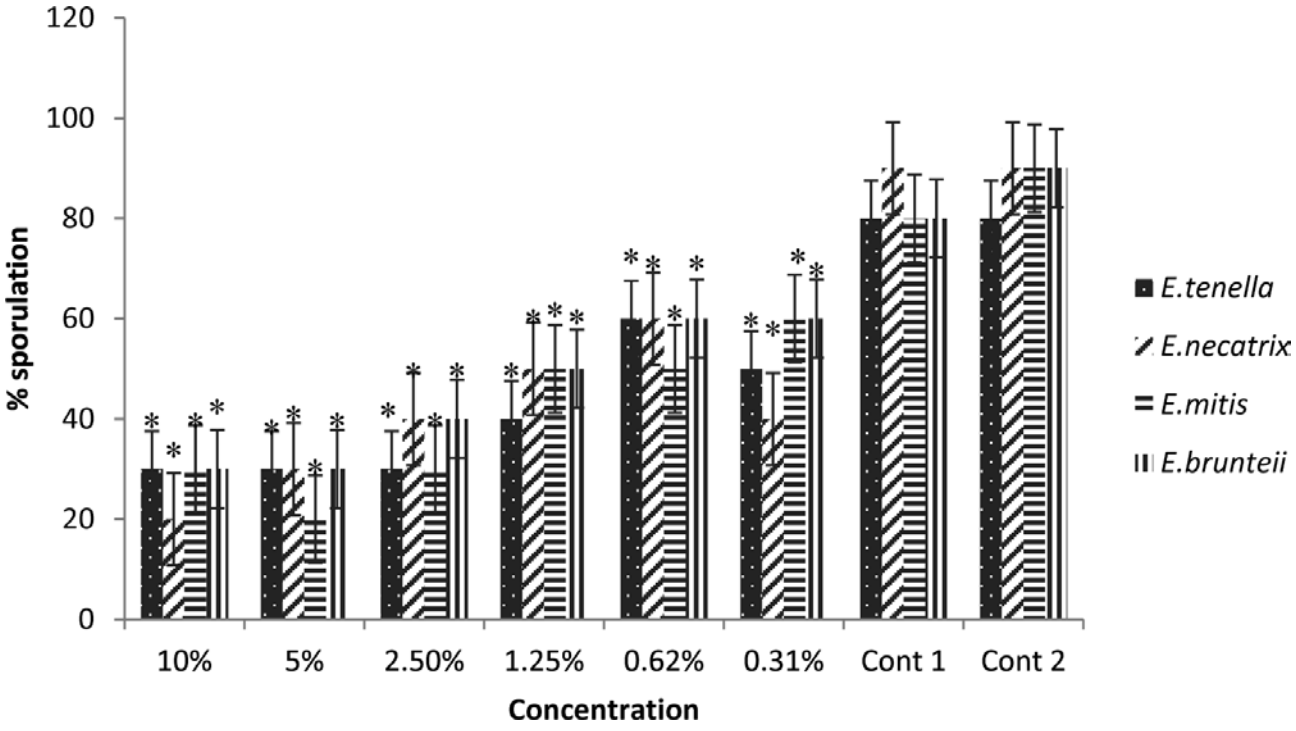

Figure 1: Effect of *V. vinifera* extract on % sporulation of oocysts of four *Eimeria* species Control-1 (DMSO) and Control-2 ($K_2Cr_2O_7$). Asterisks (*) indicate a difference from the control groups

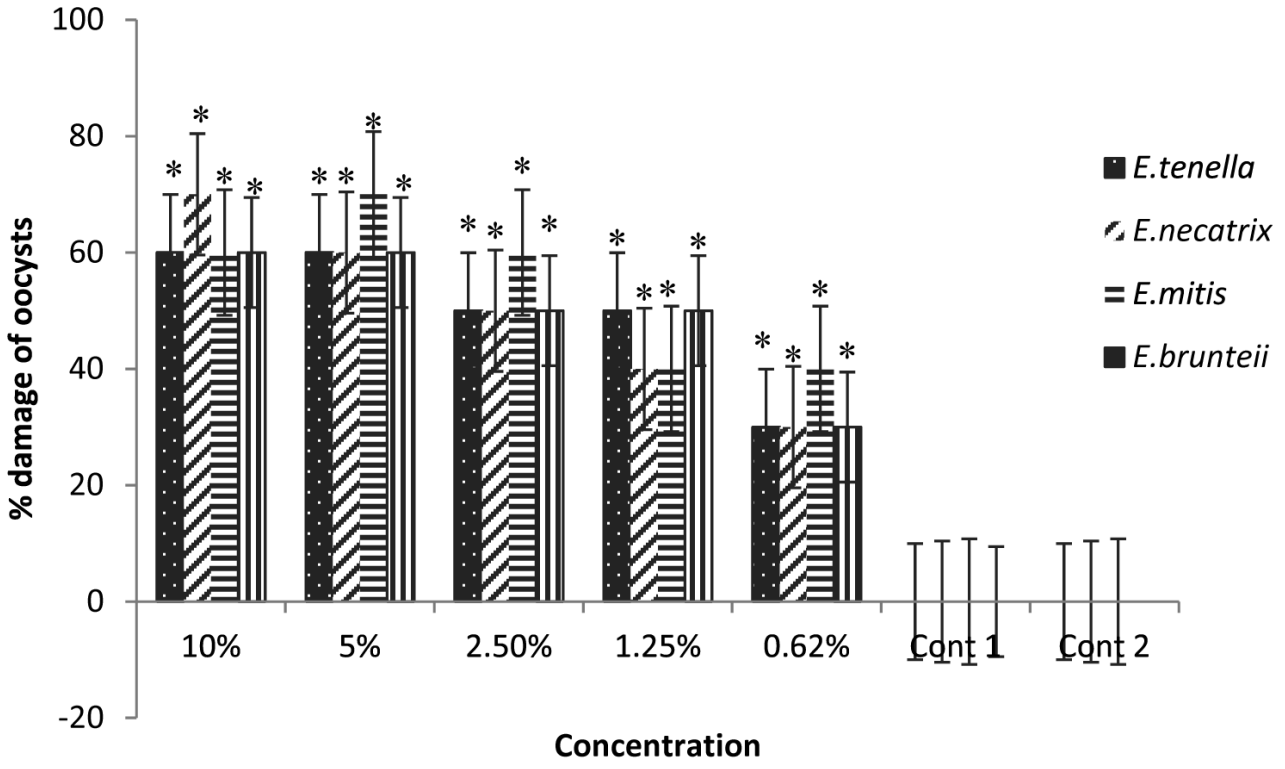

Figure 2: Effect of *Vitis vinifera* extract on percent damage of oocysts of four *Eimeria* species. Control-1 (DMSO) and Control-2 ($K_2Cr_2O_7$). Asterisks (*) indicate a difference from the control groups

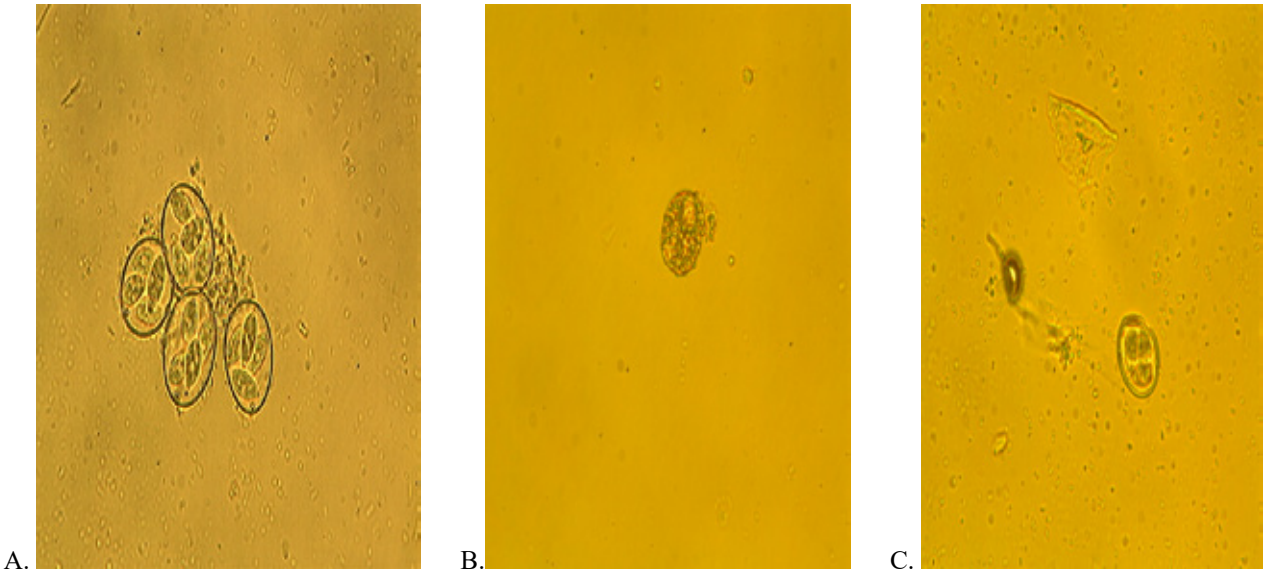

Figure 3: Photomicrographs of *Eimeria* oocysts

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 *Trachsypermum 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. officiarum* (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 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 t 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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