BF-1618
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RESEARCH ARTICLE Indian Journal of Animal Research

In vitro Anthelmintic Activity of *Vitis vinifera* Leaf Extract on *Dicrocoelium dendriticum*

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ABSTRACT

Background: Helminth parasites of ruminants are a major factor limiting animal production in many parts of the world. *Dicrocoelium dendriticum*, causes liver fluke disease in ruminants and is of zoonotic and economic importance. *D. dendriticum* lives in the adult stage, in the bile ducts and gallbladders of cattle, sheep, goats and pigs. Dicrocoeliasis causes severe pathological changes in the liver and bile system such as abscesses, granulomas and fibrosis. The objective of this study was to identify the anthelmintic activity of *Vitis vinifera* leaf extract against *D. dendriticum* and comparison with some drugs used against internal parasites.

Methods: A study was performed to evaluate the anthelmintic activity of the methanolic extract of *V. vinifera* leaf against *D. dendriticum* using an adult worm motility test. Six graduated concentrations of extract (25, 50, 75, 100, 125 and 150 mg/ml), were tested at different periods and changes over time in the viability of worms were registered for 2, 4 and 6 hr. Normal RPMI-1640 medium and Albendazole were used as negative and positive control, respectively.

Result: A significant elevation in mean inhibition of adult worm motility beginning 2 h post-exposure with 150 mg/ml of *V. vinifera* leaf extract and Albendazole. After 4 h of the exposure time, Albendazole and concentrations of 125 and 150 mg/ml of the extract resulted in significantly higher inhibited motility compared to the remaining concentrations below 125 mg/ml. The lower concentration (25 mg/ml) was significantly more lethal than the negative control (RPMI-1640 medium) at 6 h of exposure. Within 6 h after exposure, concentrations of 100, 125 and 150 mg/ml of *V. vinifera* leaf extract were more effective against adult worms than the remaining concentrations below 100 mg/ml. As the concentration and duration of exposure increased, the mortality of adult worms and also increased death for all parasites ($P<0.05$). Each concentration damages the tegument and muscles of *D. dendriticum*. The increase in concentration is proportional to the increase in damage to the integument. The results of this investigation demonstrated the anthelmintic action of *V. vinifera* leaf extract.

Key words: Albendazole, *Dicrocoelium dendriticum*, Gallbladder, *Vitis vinifera*.

INTRODUCTION

Helminth parasites of ruminants are a major factor limiting animal production in many parts of the world (Sangster, 1999). *Dicrocoelium dendriticum* is a hepatic parasitic of clinical and financial significance in ruminant breeding, which causes direct losses due to the confiscation of parasitized livers. It is prevalent in many regions of the world it has been identified in America, Asia, North Africa and Europe (Otranto and Traversa, 2002; Majidi *et al*., 2018). *D. dendriticum* lives in the bile ducts and gallbladders of Cattle, Sheep and other ruminants are the primary hosts of this parasite and humans and other animals are substitutional hosts (Beck *et al*., 2015). Most *D. dendriticum* infections cause no symptoms or only minor ones, hence remain undetected. The clinical infection of dicrocoeliasis is normally resulting in mild symptoms, but heavy infections can lead to serious animal health problems (Arbabi *et al*., 2018). Dicrocoeliasis causes severe pathological changes including pale or hardened liver, tension and inflammation of bile ducts, presence of parasites in bile ducts and gallbladder, whitish foci on the Liver, scarring and fibrosis may occur depending on the severity of the infection (Hilbe *et al*., 2015). The control of helminths is mainly based on the use of commercial anthelmintics; such as albendazole, benzimidazoles, levamisole, pyrantel, monepantel, tribendimidine and ivermectin (Gasser and Samson, 2016). Albendazole, which can be used to treat dicrocoeliasis, has been reported to be toxic in camelids (Gruntman *et al*., 2009). The use of synthetic anthelmintics is thought to cause resistance if used for a long period with inappropriate doses (Haryuningtyas, 2008). The use of herbal anthelmintics can be an alternative that is cheap, safe and can overcome the problem of resistance with the presence of multitarget compounds. *Vitis vinifiera* is an Asian native perennial woody vine. From different parts of this plant essentially fruits, several preparations used in folk medicine have been derived (Bombardelli and Morazzoni, 1995). It is rich in various useful

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How to cite this article: Mares, M.M., Abdel-Gaber, R., Al-Quraishy, S. and Ibrahim, K.E. (2023). *In vitro* Anthelmintic Activity of *Vitis vinifera* Leaf Extract on *Dicrocoelium dendriticum*. Indian Journal of Animal Research. doi: 10.18805/IJAR.BF-1618.

Submitted: 05-12-2022 **Accepted:** 08-02-2023 **Online:** 09-03-2023

antioxidant compounds including flavonoids, anthocyanins, catechin and epicatechins (Kara *et al*., 2016). It was also mentioned that the aqueous extract of *V. vinifera* leaves shows antibacterial activity against *Escherichia coli, Enterococcus feacalis and Staphylococcus aureus* (Mansour *et al*., 2011). The grape seed extract is used to treat *Ostertagia (Teladorsagia) circumcincta* and *Trichostrongylus colubriformis* in sheep (Waghorn *et al*., 2006). The *V. venifera* extract are used anticoccidial (Abbas *et al*., 2020). Considering the preceding rationale, the goal of this work was to determine the *in vitro* anthelmintic activity of *V. venifera* methanolic extract against *D. dendriticum*.

MATERIALS AND METHODS

Preparation of extracts

The *V. vinifera* leaves were collected from a local market in Riyadh, Saudi Arabia. A total of 500 gm of powder from the plant was extracted with 70% methanol as follows: 100 g of dry powder was added to 400 ml of 70% methanol and mixed gently for 1 h using a magnetic stirrer. The obtained solution was left at room temperature for 24 h, then stirred again and filtered. The solvent was then evaporated on a rotary evaporator (Chikoto and Eloff, 2005).

Adult worm collection

Adult fresh *D. dendriticum* were collected from the liver of slaughtered sheep from Al-Kharj abattoir, Saudi Arabia. Worms are collected using a small paintbrush. Parasites were collected into containers with physiological saline solution (0.9%) and transferred to the laboratory of Parasitology (Department of Zoology, College of Science, King Saud University). After washing the worms several times with saline, healthy ones with normal microscopic structure and good motility were selected. They were kept in RPMI 1640 medium (nutritious and growth medium) until the experiment began (Sambodo *et al*., 2018).

Adult worm motility test

Solutions of V. Vinifera extract were prepared at six different concentrations (25, 50, 75, 100, 125 and 150 mg/ml). Ten actively moving adult worm was then placed into each petri dish at room temperature. Normal RPMI-1640 medium and Albendazole 400 mg were also prepared and used as negative and positive controls. The test was repeated three times for all treatments. After treatment, observations were made by recording the death time for worms at 2, 4 and 6 h. Worms are considered dead if the worms do not move for 30 sec after the worm's body parts are touched using a surgical needle and shaking the petri dish. Dead worms were fixed in 10% formalin and stored in the refrigerator until used.

Histological preparation

For the histological study, the integumentary tissue of *D. dendriticum* was performed according to Jeyathilakan *et al*. (2012). In brief, integument tissues were fixed in 10% formalin for 24 h, dehydrated with graded alcohol concentrations and then cleared in xylol. Worms were embedded in paraffin, then sections were sliced at 5-7 µm in the transverse plane using a rotary microtome. Tissue staining was performed with hematoxylin and eosin (H&E) stain.

Statistical analysis

Data were analyzed via the Statistical Package for the Social Sciences (SPSS for Windows (IBM), version 22, Chicago, USA) and presented as averages and $p<0.05$ is considered a significant value.

RESULTS AND DISCUSSION

A significant elevation in mean inhibition of adult worm motility beginning 2 h post-exposure with 150 mg/ml of *V. vinifera* leaf extract and Albendazole. After 4 h of the exposure time, Albendazole and concentrations of 125 and 150 mg/ml of the extract resulted in significantly higher inhibited motility compared to the remaining concentrations below 125 mg/ml. The lower concentration (25 mg/ml) was significantly more lethal than the negative control (RPMI-1640 medium) at 6 h of exposure. Within 6 h after exposure, concentrations of 100, 125 and 150 mg/ml of *V. vinifera* leaf extract were more effective against adult worms than the remaining concentrations below 100 mg/ml. As the concentration and duration of exposure increased, the mortality of adult worms also increased causing death for all parasites ($P<0.05$) (Fig 1, Table 1). The problem of resistance to anthelmintic drugs, their toxicity and growing concern about the presence of drug residues in animal products has led to a renewed interest in the use of herbal medicines. The *in vitro* tests using free-living stages of parasitic nematodes offer a means of evaluating the anthelmintic activity of new plant compounds (Asase *et al*., 2005). *V. vinifera* leaf extract showed good *in vitro* on *D. dendriticum* lethal effect. As the concentration and duration of exposure increased. Within 6 h after exposure, a concentration of 100, 125 and 150 mg/ml of *V. vinifera* leaf extract was more effective against adult worms than the remaining concentrations below 100 mg/ml ($p<0.05$). The present result is comparable to those obtained utilizing different kinds of parasites reported by some researchers.

Table 1: *In vitro* worm lethal effect of *Vitis vinifiera* leaf extract on *D. dendriticum.*

Extract	Average number of worm dead concentration (average of mortality±SD) after (mg/ml) exposure		
	2 hr	4 hr	6 hr
150	5.66±0.57	7.66±0.00	9.00±0.57
125	3.33±0.00	5.00±0.57	7.66±0.00
100	2.33±0.57	3.66±0.57	5.33±0.57
75	1.66±0.57	3.00±0.57	3.66±0.57
50	0.66±0.57	1.66±0.57	2.33±0.57
25	0.00±0.00	0.00±0.00	0.33±0.57
Albendazole	7.00±0.00	10.00±0.00	10.00±0.00
RPMI-1640 medium	0.00±0.00	0.00±0.00	0.00±0.00

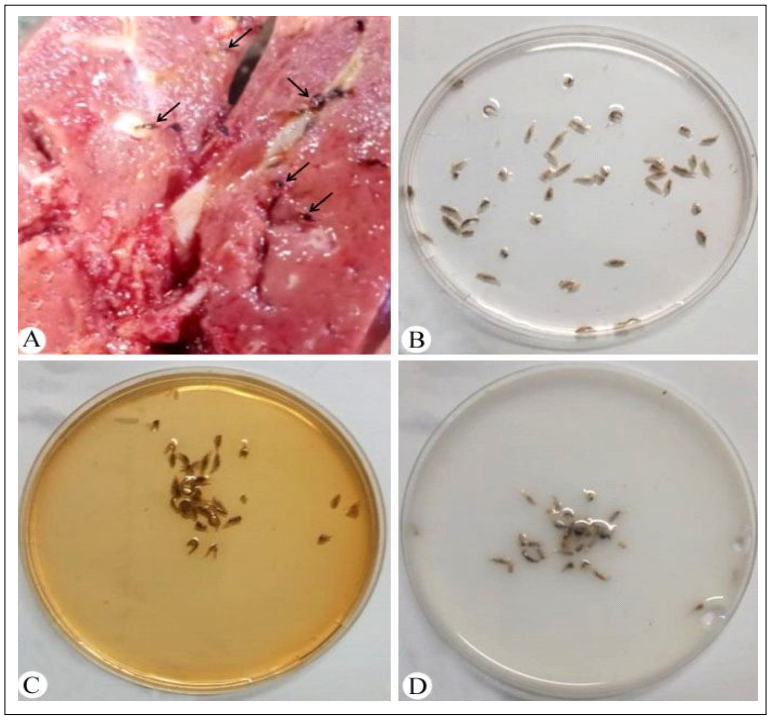

Fig 1: (a) *D. dendriticum* in the liver (arrows). (b) worms with RPMI 1640 medium. (c) worms treated with *V. vinifera* leaf extract (150 mg/ml) after 6 hr. (d) worms treated with Albendazole after 6 hr.

Fig 2: Transverse section of *D. dendriticum* at normal RPMI-1640 showing the tegument layer (TG), ridges of the tegument (R) and muscular layer (ML). Scale bar = 20µm.

Gholami *et al*. (2012) concluded that *in vitro* the methanolic extract of Vitis vinifera was tested as anti-leech *Limnatis nilotica* and found the methanol extract of grape could be presented as a complementary treatment against leech *L. nilotica* in future. Waghorn *et al*. (2006) reported that the methanolic extract of the grape seed extract is used to treat *Ostertagia* (*Teladorsagia*) *circumcincta* and *Trichostrongylus colubriformis* in sheep. Abbas *et al*. (2020) reported the *V. venifera* seeds extract are showed an inhibitory effect on sporulation and damage of *Eimeria* oocysts in chickens, as the morphology of oocysts in terms of shape, size and number of sporocysts, so used anticoccidial. It was also mentioned that the aqueous extract of *V. vinifera* leaves shows antibacterial activity against *Escherichia coli, Enterococcus feacalis, Staphylococcus aureus* (Mansour *et al*., 2011). Histological preparations showed changes in *D. dendriticum* after *in vitro* exposure to *V. vinifiera* leaf extract at a concentration of 150 mg/ml, RPMI-1640 medium and Albendazole. Histological observations of *D. dendriticum* at RPMI-1640 medium, the tegument was seen covering the outer surface of the body of the worm that the tegument layer ridges were intact and thick. The muscular layer was intact. The nucleus and most of the cytoplasm reside in a massive, bulging cell body (Fig 2). while, in histological observations of *D. dendriticum* at a concentration of 150 mg/ml (Fig 3), there was a similarity with Albendazole (Fig 4). The disruption of the apical tegumental layer was eroded so that it looked thinner than the tegumental layer of *D. dendriticum* at RPMI-1640 medium and blebbing of the tegument layer. The muscular layer looks stringy and wrinkled. These situations made the muscular layer of *D. dendriticum* at a concentration of 150 mg/ml looked shorter than the negative control. Histological observations of *D. dendriticum* at a concentration of 150 mg/ml and Albendazole caused damage to the integumentary structure of *D. dendriticum*, especially in the tegument layer and muscular layer. The increase in the level of concentration is directly proportional to the increase in damage to the tegument layer and muscular layer compared to the RPMI-1640 medium these results are similar to Becker *et al*. (1980). Waghorn *et al*. (2006) reported that all the grape

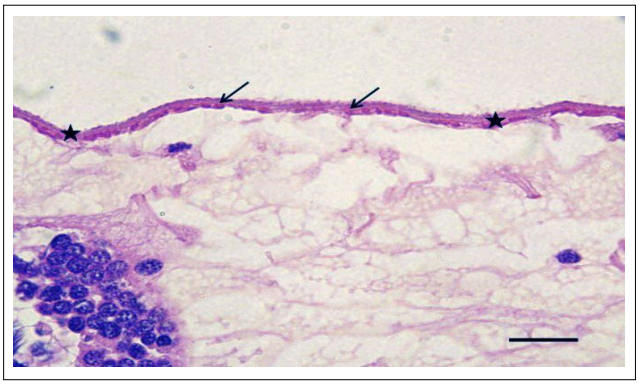

Fig 3: Transverse section of *D. dendriticum* at 150 mg/ml of *V. vinifera* leaf extract showing the detachment of ridges of the tegument (arrows) and the muscular layer stringy and wrinkled (asterisk). Scale bar = 20µm.

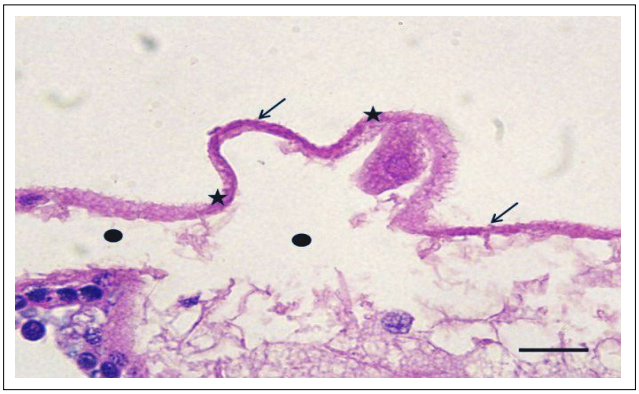

Fig 4: Transverse section of *D. dendriticum* at Albendazole showing the detachment of ridges of the tegument (arrows), the muscular layer stringy and wrinkled (asterisk) and blebbing of the muscular layer (circle). Scale bar = 20µm.

seed extracts have *in vivo* anthelmintic potential on *Ostertagia* (*Teladorsagia*) *circumcincta* and *Trichostrongylus colubriformis* in sheep. Mansour *et al*. (2011) found that the ethanolic and the aqueous extracts of *Vitis vinifera* promoted the destruction of cytoplasmic and nuclear membranes of *Leishmania infantum* promastigotes and altered the overall shape of the cell. The bioactive compounds responsible for these activities could be antioxidant compounds including flavonoids, anthocyanins, catechin and epicatechins (Kara *et al*., 2016). However further studies and *in vivo* trials are needed to understand its anthelmintic effect in sheep.

CONCLUSION

The current study concluded that the medicinal plant tested showed a promising lethal effect against *D. dendriticum* worms that could be utilized as a possible alternative to replace commercially available drugs. More *in vivo* and *in vitro* studies are needed to better evaluate the possibility of these extracts.

ACKNOWLEDGEMENT

This work was supported by the Researcher supporting project (RSP2023R3), King Saud University.

Data availability statement

All the datasets generated or analyzed during this study are included in this published article.

Conflict of interest

The author(s) declare that they have no conflict of interest regarding the content of this article.

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