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Efficacy of *Vitis vinifera* Leaves Extracts against *Eimeria stiedae* Infections in New Zealand Rabbit

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ABSTRACT

Background: Coccidiosis is considered one of the most serious diseases affecting rabbits and various other animal species, leading to substantial economic losses worldwide. Plant-derived extracts have emerged as promising alternatives to conventional chemotherapeutic agents, particularly in light of increasing drug resistance, as well as their advantage of not causing harmful tissue residues.

Methods: This study was conducted to determine the effectiveness of *Vitis vinifera* leaf extract (VVLE) against *Eimeria stiedae* in rabbits. The extract was subjected to phytochemical analysis by GC-MS, revealing the existence of 34 biologically active compounds. A total of 35 male New Zealand rabbits were divided into seven groups. Group 1 served as the uninfected, untreated (negative control) group. Group 2 uninfected and treated at 400 mg/kg of extract to test for toxicity. Group 3 represented the infected, non-treated (positive control) group. While, groups 4, 5 and 6 comprised infected treated with at 100 mg/kg, 200 mg/kg and 400 mg/kg of extracts, respectively. Finally, group 7 consisted of infected rabbits treated with Toltrazuril at a dosage of 5 mg/kg.

Result: The findings indicate that 400 mg/kg concentration of VVLE has a significant protective effect against *Eimeria stiedae*. It relieves clinical symptoms, reduces oocyst production, improves biochemical parameters and decreases the level of iron (Fe), copper (Cu), magnesium (Mg) and zinc (Zn) in the liver of *Eimeria stiedae*-infected rabbits. This refers to the effectiveness of *Vitis vinifera* leaf extracts (VVLE) against coccidiosis. Further investigations are needed to identify the pharmacological ingredients responsible for these effects and their potential as a natural alternative for coccidia prevention in animal feed.

Key words: *Eimeria stiedae*, Hepatic coccidiosis, New Zealand rabbits, Oocysts, *Vitis vinifera* leaves.

INTRODUCTION

Rabbit farming for meat has made a significant contribution to addressing the global meat shortage, playing an important role in supplying substantial quantities of meat alongside beef and poultry (Trocino *et al*., 2019). Moreover, rabbit meat is highly nutritious, with abundant protein, low fat and cholesterol and a rich source of calcium and phosphorus. Additionally, it contains high levels of linoleic acid, which is beneficial for overall health (Nistor *et al*., 2013). In recent years, its production has increased significantly and it has become one of the major animal protein sources in the Kingdom of Saudi Arabia (Al-Mathal, 2008). Coccidiosis, a highly transmissible disease, significantly affects the global domestic rabbit industry, leading to substantial financial losses (Geru *et al*., 2017). Coccidiosis in rabbits is caused by approximately 15 species of the genus *Eimeria* that affect the intestinal tract, including *E. magna*, *E. matsubayashi*, *E. neoleporis*, *E. nagpurensis*, *E. irresidua*, *E. flavescens*, *E. piriformis*, *E. intestinalis*, *E. exigua*, *E. elongata*, *E. perforans*, *E. vejdovskyi*, *E. coecicola* and *E. media* (Soulsby, 1968). Among these species, *Eimeria stiedae* is specifically responsible for hepatic and bile duct infections (Li and Ooi, 2009). The disease affects the intestines, liver and bile ducts of animals and is associated with digestive disorders (Manjunatha *et al*., 2019). The disease significantly affects growth performance parameters, leading to elevated morbidity and mortality rates and resulting in a reduction of carcass weight by over 23% (Lebas *et al*., 1997). Young rabbits, especially those in the first four months of life, are more susceptible to the disease, while older rabbits tend to show greater resistance to infection (Sivajothi *et al*., 2014). The treatment of hepatic coccidiosis poses challenges and the disease may persist throughout an animal's lifetime (Karaer, 2001). The challenges outlined above underscore the critical necessity of discovering natural alternatives to drugs and disinfectants that can effectively inhibit *Eimeria* growth, enhance rabbit immunity and boost animal productivity (Ogolla *et al*., 2018). Recently, several plants have been tested for their potential to control coccidiosis (Tipu *et al*., 2006). *Vitis vinifera* (L.) is a perennial woody vine in the Vitaceae family that has been studied for potential medicinal use. Leaves are used externally to treat wounds and abscesses (Kallel *et al*., 2008). Traditional medicine derived from grape leaves has historical use in treating

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conditions such as diarrhea, hepatitis and abdominal pain (Felicio *et al*., 2001). The main compounds in this plant are resveratrol, quercetin, catechin, flavones, flavanols, anthocyanins, gallic acid and epicatechin (Aouey *et al*., 2016). The extract from the leaves of *V. vinifera* has antioxidant, anti-inflammatory, analgesic and antipyretic activities (Aouey *et al*., 2016). The aqueous extract obtained from the leaves of *Vitis vinifera* exhibits antidiabetic and antioxidant properties, as well as antibacterial activity against *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Vibrio alginolyticus* (Orhan *et al*., 2006; Mansour *et al*., 2011). Furthermore, it was found that the organic Zn-glycine complex and grape seed powder effectively alleviated the negative effects on growth performance, lesion scores and oocyst shedding in broilers infected with *Eimeria tenella* (Trocino *et al*., 2019). Herbal remedies have proven to be effective and cost-efficient alternatives to preventive anticoccidial medications (Tipu *et al*., 2006). However, their application against coccidiosis in production rabbits remains limited. Therefore, this study was conducted to evaluate the ameliorative effects of *Vitis vinifera* leaf extracts on *Eimeria stiedae* infection in New Zealand rabbits.

MATERIALS AND METHODS

Ethical approval

The research experiment was conducted in compliance with Saudi Arabian regulations governing the use of animals. Ethical approval for the study was granted by the King Saud University Research Ethics Committee (REC) under approval number KSU-SE-22-38.

Preparation of the *Vitis vinifera* extract

Vitis vinifera leaves were obtained from local markets in Riyadh, Saudi Arabia. The leaves were air-dried and then ground into a fine powder using an electric blender. Subsequently, cold extraction was carried out using 70% methanol, with continuous agitation on a shaker for 24 hours (Amer *et al*., 2015). The dissolved contents obtained were homogenized, filtered using filter paper and evaporated at 40°C in a rotary evaporator (IKA, Germany). The solvent was evaporated to obtain extractions (Chikoto and Eloff, 2005). The extract obtained was kept at -20°C until use.

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS technique was employed to identify the active components in the extract from *Vitis vinifera* leaves. GC-MS analysis of this extract was performed using Thermo Scientific, Trace GC Ultra/ISQ single Quadrupole MS, TG 5MS fused silica capillary column (length 30 mL, inner diameter 0.251 mm and film thickness 0.1 mm). For GC/MS detection employed an electron ionization system with an ionization energy of 70 eV, utilizing helium gas as the carrier gas at a constant flow rate of 1 ml/min. The injector and MS transfer line were both maintained at a temperature of 280°C. The oven temperature was ramped up from an initial 40°C (held for 3 minutes) to a final temperature of 280°C, increasing at a rate of 5°C per minute (with a 5-minute hold at the final temperature). The relative peak area percentages were used to quantify all identified components. Additionally, we attempted to identify the compounds by comparing their relative retention times and mass spectra with data from the NIST WILLY library using a GC/MS system (Hussein *et al*., 2016).

Experimental animals

A total of 35 male New Zealand White rabbits (*Oryctolagus cuniculus*) were obtained from a farm in Riyadh, Saudi Arabia. Upon arrival, they were immediately housed individually in disinfected wire cages of uniform size under hygienic conditions at the animal facility of the Department of Zoology, King Saud University. All animals were provided with ad libitum access to feed and water and the diet was free of anticoccidial drugs. The rabbits were acclimatized for one week, during which fecal samples were examined daily for coccidia using a concentrated flotation technique (Heelan and Ingersoll, 2002).

Collection and preparation of *Eimeria* oocysts

Oocysts of *Eimeria stiedae* were collected from the gallbladders of naturally infected rabbits obtained from a bird market in Riyadh, Kingdom of Saudi Arabia. The oocysts were suspended in 2.5% potassium dichromate solution and incubated at 25°C with continuous aeration. Daily monitoring was performed to assess sporulation (Soulsby, 1986). After complete sporulation, the oocysts were repeatedly washed by centrifugation until a clear supernatant was obtained and then stored at 4°C for subsequent use.

In vivo infection and experimental design

A total of 35 male New Zealand rabbits (*Oryctolagus cuniculus*) aged 12 weeks were individually weighed and assigned to 7 groups of 5 rabbits per group as the following:

Group 1: Non-treated (negative control) (n=5).

Group 2: Non-infected and treated (n=5) with the plant extracts at 400 mg/kg of body weight.

Group 3: Infected-non-treated (positive control) (n=5).

Group 4: Infected and treated (n=5) with the plant extracts at 100 mg/kg of body weight.

Group 5: Infected and treated (n=5) with the plant extracts at 200 mg/kg of body weight.

Group 6: Infected and treated (n=5) with the plant extracts at 400 mg/kg of body weight.

Group 7: Infected and treated (n=5) with Toltrazuril at 5 mg/kg body weight.

All groups except groups 1 and 2 were inoculated with 5×10^4 sporulated *Eimeria stiedae* oocysts (Mohammed *et al*., 2021). After one hour of infection, the three doses of *Vitis vinifera* leaf extracts and the reference drug was injected orally into all rabbits according to Aouey *et al*. (2016) and El-Ghoneimy and El-Shahawy (2017), respectively.

Oocyst number in gallbladder

Fresh gallbladder samples were collected on day 18 post-infection with *Eimeria stiediae* after sacrificing rabbits to compare the oocyst rate in gall bladder from both infected and infected treated groups. To determine the average of oocysts number. The Contents gallbladders were suspended in a tube then mixed well and pipetting 10 µL into a glass slide was done and then covered by a coverslip. After that, the total number of oocysts within 10 µL was counted by using the light microscope per Contents gallbladders.

Macroscopic examination and lesion scoring

On day 18 post-infection, rabbits from each group were euthanized and their livers were subjected to macroscopic examination. Liver lesion scores were evaluated and compared among the infected untreated group, the group treated with VVLE (400 mg/kg) and the control group.

Histochemical studies

Liver samples were immediately excised and cut into small sections, then fixed in 10% neutral buffered formalin. After fixation, the tissues were processed through dehydration, embedded in paraffin wax and sectioned at a thickness of 5 µm. The sections were subsequently stained using the periodic acid-Schiff (PAS) technique for the detection of total carbohydrates (Hotchkiss, 1948), while mercury bromophenol blue staining was applied to demonstrate total protein content (Maize *et al*., 1953).

Biochemical study

In rabbit plasma, the liver enzymes alkaline phosphatase (ALP) and alanine aminotransferase (ALT) were measured using kits provided by Biodiagnostic Co. (Giza, Egypt). ALP and ALT activities were determined according to the methods described by Belfield and Goldberg (1971) and Bergmeyer *et al*. (1978), respectively.

Metal ions in the liver

The concentrations of metal ions (Cu, Fe, Zn and Mg) in liver samples from both infected and non-infected rabbits were determined using concentrated nitric acid, following the methodology outlined by UNEP/FAO/IAEA/IOC, (1984). The concentrations of metal ions were analyzed using the atomic emission spectrometer iCAP-6500 Duo, which utilizes inductively coupled plasma (Thermo Scientific, U.K.).

Statistical analysis

Statistical significance was assessed using one-way analysis of variance (ANOVA) and multiple comparisons among groups were conducted using Duncan's test with SPSS software (version 17.0). All data are presented as mean ± standard error of the mean (SEM). A two-tailed p-value of ≤ 0.05 was considered statistically significant for all analyses.

RESULTS AND DISCUSSION

Gas chromatography-mass spectrometry (GC-MS) analysis of (VVLE).

GC-MS analysis was used to examine the phytochemical composition of *Vitis vinifera* leaf extract (VVLE). The analysis revealed 34 phytochemical components present at varying peak areas and retention times (Table 1).

During necropsy, a detailed macroscopic examination of the control group rabbits revealed no detectable lesions. In contrast, the infected untreated group showed marked enlargement of the liver, along with dilation of the bile ducts. On the surface of the liver in this group, nodules of different sizes, ranging in color from yellowish to white, were observed. The cross section of the nodules displayed a fluid that ranged in color from yellowish to cream. No nodules were yellowish in the liver of rabbits in the group infected and treated with Toltrazuril. The macroscopic liver findings in rabbits from the infected group treated with 400 of VVLE were comparable to those observed in the group treated with toltrazuril (Fig 1). These changes were particularly pronounced in the untreated infected group, consistent with previous reports on *E. stiedae* infection (Abed and Yakoob, 2013). The observed changes may be due to the proliferation and dilation of the bile ducts, which lead to the formation of raised nodules on the liver surface. This is subsequently associated with an increased secretion of yellowish-white caseous material (Al-Naimi *et al*., 2012). It has been suggested that the toxic effects of protozoa may play a role in the development of coccidian nodules on the liver surface (Barriga and Arnoni, 1981).

During the examination of gall bladders from rabbits infected with *Eimeria stiedae*, day 18 post-infection was identified as the peak of infection. The percentage reduction in oocyst output was higher in animals treated with VVLE at a dose of 400 mg/kg compared with those treated with 100 and 200 mg/kg, as well as the infected untreated group. However, the highest suppression of oocyst shedding was observed in the toltrazuril-treated group, which exceeded all VVLE-treated groups (Fig 2). Furthermore, this extract may enhance both innate and adaptive immune responses in rabbits, which is consistent with previous reports by Brisbin *et al*. (2008) and Chand *et al*. (2021). The findings of the present study suggest that *Vitis vinifera* leaf extract, similar to toltrazuril, effectively inhibits the development of the sexual stages of *Eimeria stiedae* in the liver, thereby reducing oocyst production in the gall bladder. This effect may be partly attributed to the potent antioxidant compounds present in the extract, which could suppress the growth and development of *E. stiedae* stages, ultimately

decreasing oocyst formation and accumulation. In addition, *Vitis vinifera* leaf extract may induce oxidative stress through the generation of highly reactive oxygen species via the reductive cleavage of endoperoxide bridges mediated by iron complexes or iron-induced free radicals (Levander *et al*., 1989; Mares *et al*., 2024). These reactive species are capable of alkylating parasite proteins, leading to parasite death. This proposed mechanism is in agreement with the findings of Levander *et al*. (1989); Zhang and Gerhard (2008); del Cacho *et al*. (2010); Murshed *et al*. (2025) and Mares *et al*. (2023).

In histochemical studies, *Eimeria stiedae* infection resulted in a reduction in carbohydrate and protein content within hepatic cells, along with an altered distribution in all infected groups on day 18 post-infection compared with the control group. However, treatment of infected rabbits with VVLE (400 mg/kg) led to a restoration and increase of carbohydrate and protein levels in liver cells, approaching those observed in the toltrazuril-treated group (Fig 3 and 4). The reduction in carbohydrate content and its distribution within hepatic cells may be attributed to the increased metabolic burden on the liver resulting from parasitic

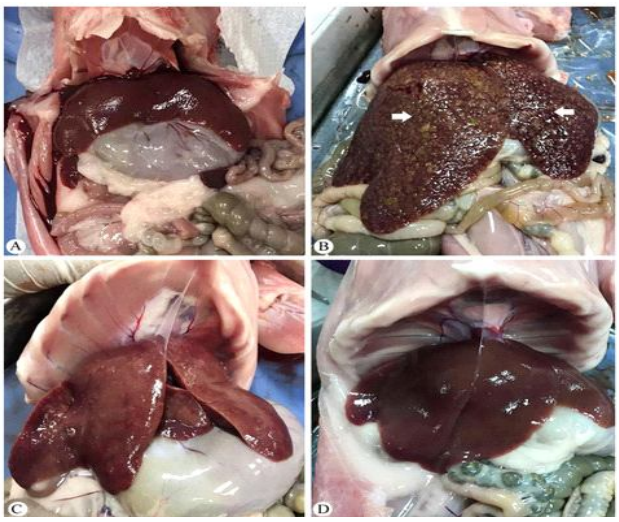
Table 1: The phytochemical compounds present in *Vitis vinifera* leaves extract (VVLE) identified using GC-MS.

RT (min)	Compounds	Molecular weight	[M-H]-(m/z)	Formula	Peak area %
3.66	1,3-Cyclohexadiene, 5-ethyl-	108.18	107.18	C_8H_{12}	10.15
3.99	Mepivacaine	246.35	245.35	$C_{15}H_{22}N_{2O}$	7.63
4.38	2-Methoxy-6-methylaniline	137.18	136.18	$C_8H_{11}NO$	9.55
4.76	Butanoic acid, 4-[4-methylimidazol-5-yl]-,ethyl ester	196.25	195.25	$C_{10}H_{16}N_2O_2$	4.65
5.24	N, N-Dimethyl-4-t-butylbenzeneamine	177.29	176.29	$C_{12}H_{19}N$	4.540
5.79	N-(1-(3-ethoxyphenyl) propan-2-y)-N-ethyl 2,2,2-trifluoroactamide	303.32	302.32	$C_{15}H_{20}F_3NO_2$	2.83
6.09	Quinoxaline, 2-methoxy-, 4-oxide	176.17	175.17	$C_9H_8N_2O_2$	5.16
6.68	Trimethylboroxine	125.5	124.5	$C_3H_9B_3O_3$	5.015
7.29	4-(But-3-en-1-yl)-9-fluoro-2-(furan-2- ylmethyl)-3, 4-dihydro-2H-benzo[b] [1,4,5] oxathazepine 1,1-dioxide	351.4	350.4	C_{17}H18FNO4S	11.30
7.61	Benzenamine, 4-(phenylazo)-	197.24	196.24	$C_{12}H_{11}N_3$	2.123
8.03	(3-Methylphenyl) methanol, neopentyl ether	192.30	191.3	$C_{13}H_{20}O$	0.846
8.39	Fumaric acid, 6-ethyloct-3-yl heptyl ester	354.5	353.5	$C_{21}H_{38}O_4$	0.848
8.82	1-Azaspiro [4.4] nonane	125.21	124.21	$C_8H_{15}N$	2.068
9.26	6-Methyl-bicycl [4.2.0] octan-7-ol	140.22	139.22	$C_9H_{16}O$	2.344
10.10	Hexanoic acid	116.16	115.16	$C_6H_{12}O_2$	2.858
10.65	2-Amino-3-hydroxypyridine	110.12	109.114	$C_5H_6N_2O$	0.678
11.26	3,5-Dinitro-2-methoxybenzoic acid	242.14	241.14	$C_8H_6N_2O_7$	1.510
11.72	Formic acid, undecyl ester	200.32	199.32	$C_{12}H_{24}O_2$	1.177
12.08	Dimethyl sulfone	94.133	93.133	$C_2H_6O_2S$	15.08
12.59	Lenacil	234.29	233.29	$C_{13}H_{18}N_2O_2$	0.68
13.20	5-Methoxysatin, TBDMS derivative	291.42	290.42	$C_{15}H_{21}NO_3Si$	0.251
13.53	1H-Indole-2,3-dione, 1-methyl-, 3-(Obenzoyloxime)	280.28	279.28	$C_{16}H_{12}N_2O_3$	0.066
13.93	Benzimidazo[2,1-a] isoquinoline	218.25	217.25	$C_{15}H_{10}N_2$	1.79
14.30	Heptanoic acid, octyl ester	242.40	241.398	$C_{15}H_{30}O_2$	3.39
14.76	Octadecane, 1-isocyanato-	295.5	294.50	$C_{19}H_{37}NO$	0.563
15.87	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	278.43	277.43	$C_{18}H_{30}O_2$	1.035
16.25	Octylamine, N, N-di(allyl)-	209.37	208.37	$C_{14}H_{27}N$	0.166
16.73	Phosphine, [2-(6-methyl-2-pyridyl) ethyl]- [2-(diphenylphosphino)ethyl]-(phenyl)-	441.5	440.5	$C_{28}H_{29}NP_2$	0.012
17.23	2-Thiophenecarboxylic acid, 2-ethylcyclohexyl ester	238.35	237.35	$C_{13}H_{18}O_2S$	0.152
17.73	Phosphinic acid, diphenyl-	218.19	217.19	$C_{12}H_{11}O_2P$	0.065
18.17	Phenol, 4,4'-(1-methylethylidene) bis-	228.29	227.29	$C_{15}H_{16}O_2$	0.260
18.68	Silane, methylvinyl(1-cyclopentyl ethoxy methyl vinyl silyloxy) ethoxy-	314.17	313.17	$C_{15}H_{30}O_3Si_2$	1.127
18.9	Chloroxine	214.05	213.05	$C_9H_5Cl_2NO$	0.013
19.42	Butylphosphonic acid, 2-chlorophenylundecyl ester	402.21	401.21	$C_{21}H_{36}ClO_3P$	0.095

[M-H]-(m/z) molecular weight[1].

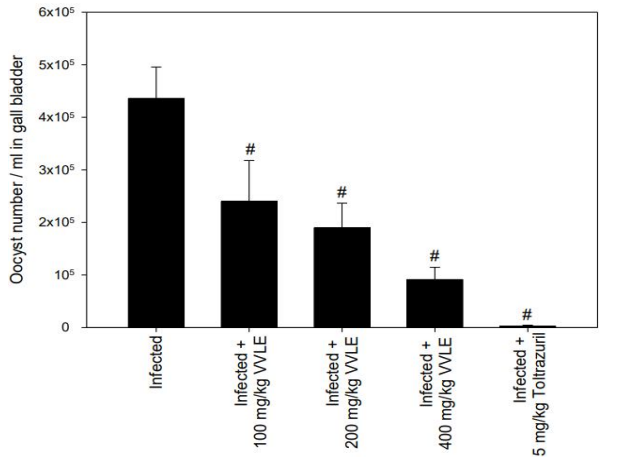
infection and the presence of intracellular developmental stages, which require substantial energy consumption (Ibrahim, 1999). It may also reflect the maintenance of normal blood glucose levels through enhanced catabolism of carbohydrate reserves stored in tissues (Chapman *et al*., 1982); (Mares *et al*., 2023).

Similarly, the observed decrease in protein content within liver cells may be due to the disruption of lysosomal membranes under the influence of various deleterious factors, leading to the release of proteolytic enzymes (Awasthi *et al*., 1984). Comparable findings have been reported in previous studies, where reduced hepatic protein



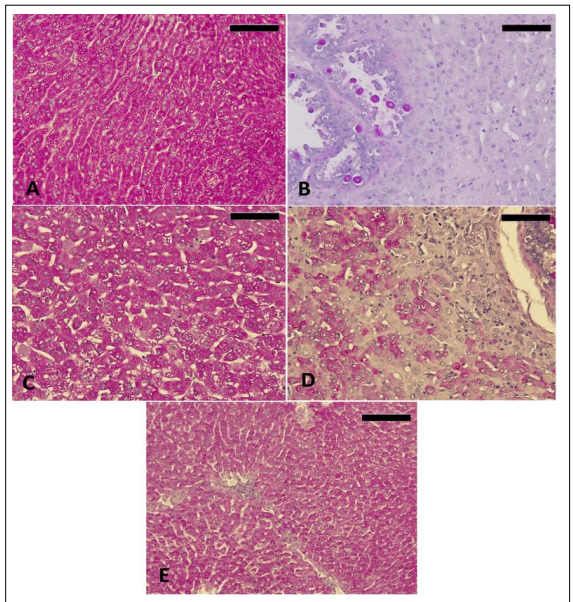
(A) Control-untreated group. (B) Infected-untreated group with yellowish nodules (white arrows). (C) Infected-treated group with 400 mg/kg VVLE. (D) Infected-treated group with 5 mg/kg toltrazuril.

Fig 1: Rabbit livers from different groups.



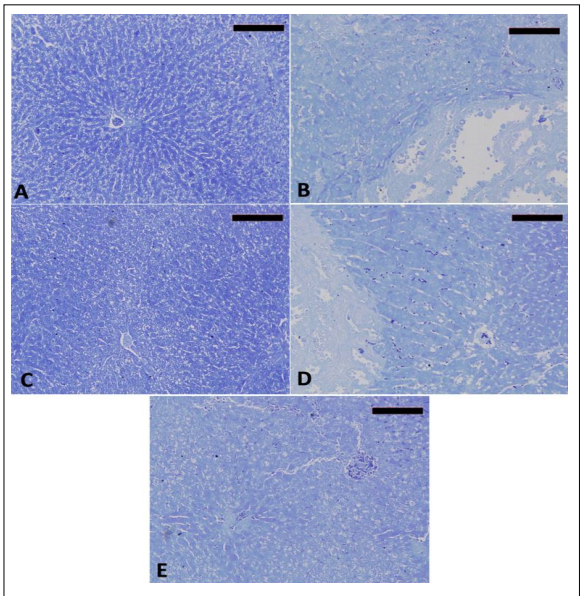
significance (P≤0.05) was assessed against the infected group.

Fig 2: Oocyst number in gall bladder of rabbits infected with *Eimeria stiedae* and for infected treated groups with 100 mg/kg, 200 mg/kg and 400 mg/kg VVLE on day 18 p.i.



Liver in control group (A), Uninfected-treated group with 400 mg/kg VVLE (C) Treated with toltrazuril (E) Contained more carbohydrates than infected group without treated. Infected-untreated group (B) Appeared with the low carbohydrates store while Infected-treated group with 400 mg/kg VVLE (D) restore the carbohydrates in the liver. Bar = 50 µm.

Fig 3: Periodic acid schiff's-stained sections showing carbohydrates stores in the liver for the rabbits in the infected groups and infected-treated group with toltrazuril.

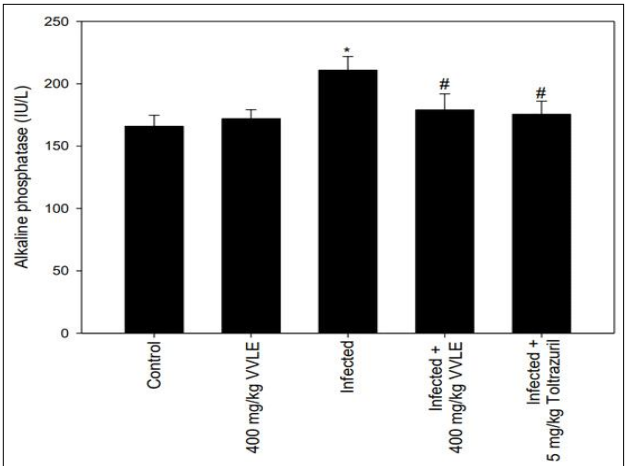


Liver in control group (A), Uninfected-treated group with 400 mg/kg VVLE (C) Treated with toltrazuril (E) Contained more proteins than infected group without treated. Infected-untreated group (B) Appeared with the low proteins store while Infected-treated group with 400 mg/kg VVLE (D) restore the proteins in the liver. Bar = 50 µm.

Fig 4: Mercuric bromophenol blue -stained sections showing proteins stores in the liver for the rabbits in the infected groups and Infected-treated group with toltrazuril.

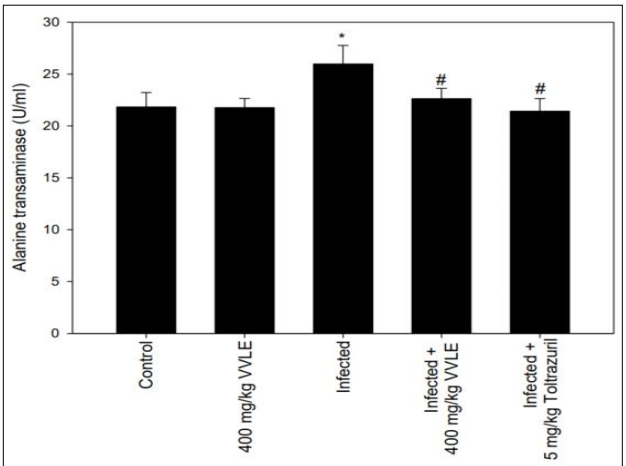
Table 2: Heavy metal concentrations in rabbits after *Eimeria stiedae* infection.

Groups	Fe	Cu	Mg	Zn
Control group	127.28±18.62	4.68±0.72	292.08±16.96	29.99±0.76
Uninfected-treated group (400 mg/kg VVLE)	136.64±19.06	5.90±1.89	303.05±20.39	31.69±2.34
Infected-untreated group	221.72±29.99	16.26±3.15	586.83±89.61	57.84±5.69
Infected-treated group (400 mg/kg VVLE)	139.28±27.43	7.43±2.60	380.42±47.26	36.40±2.19
Infected-treated group (5 mg/kg toltrazuril)	141.58±11.40	5.30±0.99	402.14±33.26	34.43±2.24



*Significance changes with respect to control group, # significance changes with respect to infected group.

Fig 5: Plasma alkaline phosphatase level after treatment of *Eimeria stiedae*-infected rabbits with *Vitis vinifera*.



*Significance changes with respect to control group, #Significance changes with respect to infected group.

Fig 6: Plasma alkaline transaminase level after treatment of *Eimeria stiedae* -infected rabbits with *Vitis vinifera*.

levels were observed in chickens infected with *Eimeria mitis* and *Eimeria tenella* (Musajev and Surkova, 1970).

Eimeria stiedae infection led to a significant increase (P≤0.05) in plasma levels of ALP and ALT in rabbits (Fig 5 and 6). However, treatment of infected rabbits with VVLE (400 mg/kg) reduced ALP and ALT levels to values nearly comparable to those of the control group. Similar effects were observed when compared with the reference drug, toltrazuril. The improvement in plasma enzyme levels may be attributed to the regeneration of hepatic cells and epithelium, as well as the inhibition of the parasite's sexual stage development. During the recovery phase, marked restoration of damaged liver tissues and bile ducts was observed. In addition, several biochemical parameters, including liver enzymes and plasma proteins, returned to normal values. These findings are consistent with those reported by Çam *et al*. (2008) and Abdel-Maged *et al*. (2013).

Rabbits infected with *Eimeria stiedae* showed a statistically significant increase (P≤0.05) in hepatic concentrations of heavy metals, including iron (Fe), copper (Cu), magnesium (Mg) and zinc (Zn) (Table 2). However, treatment with VVLE (400 mg/kg) reduced the levels of Fe, Cu, Mg and Zn to values comparable to those of the control group, with effects similar to those observed in the toltrazuril-treated group. The observed changes may be attributed to the fact that elevated heavy metal concentrations are often associated with inflammatory hepatic lesions. Inflammatory processes can induce increased ferritin synthesis. Although ferritin is generally regarded as a reliable marker of total iron stores, its role is altered under inflammatory conditions, where it becomes involved in the immune response and may affect lymphocyte function (Kernan and Carcillo, 2017). These findings are in agreement with Schultheiss *et al*. (2002) and Thornburg (2000), who reported increased hepatic copper and zinc levels in dogs with inflammatory liver lesions.

CONCLUSION

The methanolic extract of *Vitis vinifera* leaves appears to be a safe therapeutic candidate for the management of coccidiosis in rabbit production. It has shown efficacy in mitigating the adverse effects of hepatic coccidiosis in rabbits, likely due to its phytochemical constituents with anticoccidial activity. Nevertheless, further investigations are required to evaluate its potential as a prophylactic natural alternative to conventional coccidiostats in animal feed.

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Conflict of interest

The authors declare that they have no conflict of interest.

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