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Biological activities of *Persea americana: in vitro* and *in vivo* studies

Tahani AL-OTAIBI[1], Maysar Abu HAWSAH[2], Ghada ALOJAYRI[2], Esam Mohamed Al-SHAEBI[2], Mohamed Abdelmonem DKHIL[3,4], Felwa THAGFAN[5], Manal Fawzy Elkhadragy[5], Saleh AL-QURAISHY[2], Rewaida ABDEL-GABER[2]*

Abstract

Natural resources emerge as an essential and alternative tool for controlling parasitic agents. The present study was carried out to evaluate *in vitro* and *in vivo* effect of *Persea americana* fruit extract (PAFE) on helminthiasis and coccidiosis. *In vitro,* anthelmintic activity was performed using PAFE against *Allolobophora caliginose*. PAFE elicited significant anthelmintic activity comparable to mebendazole with the lesser time taken to paralysis and death for 200 mg/mL. *In vitro* anticoccidial activity of PAFE, amprolium, phenol, Dettol™, and formalin were studied after incubation with unsporulated oocysts. PAFE was able to significantly inhibit oocyst sporulation in a dose-dependent manner. A total 35 male mice were divided into seven groups. At day 0, all groups except first and second groups were infected with 1×10^3 *E. papillata*. Group 1 served as control. Group 2 served as uninfected-treated one. Group 3 was considered an infected-untreated group. After 60 min of infection, groups 4, 5, and 6 were treated with PAFE. Group 7 was treated with amprolium. However, not *at par* with amprolium, PAFE showed a good anticoccidial activity adjudged based on a significant change in body weight gain, feed intake, oocysts output, and content of carbohydrates and protein. Therefore, PAFE exhibits powerful anthelmintic and anticoccidial effects.

Keywords: *Persea americana*; helminths; *Eimeria*; coccidiosis; anticoccidial drugs.

Practical Application: Efficacy of *Persea americana* as antihelmintic and anticoccidial effects.

1 Introduction

Parasitic infections caused by protozoans and helminths induce considerable health problems and economic losses in animals in many countries (Mehlhorn, 2014). Weakness due to malnutrition and anemia is the major complaint of helminth infections (Taylor et al., 2007; Jones & Berkley, 2014). Anthelmintic drugs are used for expelling parasitic worms from the body, however, it induces some side effects, especially for host tissue (Hong, 2018).

Coccidiosis due to *Eimeria* infection is a major health problem in different animal species (Mehlhorn, 2001; Dkhil et al., 2019). *Eimeria papillata* is a coccidian parasite that was first reported in the house mouse (*Mus musculus*). Infection with coccidian parasites causes a lot of pathological changes which can be observed in blood, urine, and feces (Jithendran & Bhat, 1996; Tambur et al., 1998; Dkhil and Al-Quraishy, 2012). Coccidiosis is mostly treated with synthetic anticoccidial drugs, but this approach is facing a serious threat of the development of resistance in *Eimeria* strains (Wunderlich et al., 2014; Grandi et al., 2016).

To control coccidiosis and helminthiasis in various animal species, different alternative options and protocols were effectively used worldwide (Liaqat et al., 2016). Among these options, different compounds obtained from natural medicinal plants or natural health alternatives found to have some therapeutic effects (Abbas et al., 2015; Habibi et al., 2016) not target only the parasites, but also have organ-protective properties in the host (Alzahrani et al., 2016). The anticoccidial activity of botanicals has generally been attributed to their antioxidant properties. The use of antioxidant-rich plant extracts has shown comparable results to synthetic drugs for coccidiosis and other diseases (Khaliq et al., 2015).

The avocado (*Persea americana*) belongs to the laurel family Lauraceae (Scora & Bergh, 1990). This plant is a source of bioactive compounds especially antioxidants and its seed extracts have been proven to possess various therapeutic activities (Dabas et al., 2013; Melgar et al., 2018; Widiyastuti et al., 2018; Bhuyan et al., 2019; Pacheco-Coello & Seijas-Perdomo, 2020). Moreover, the antihelmithic activity for avocado was reported previously via Rosa et al. (2018) against goat gastrointestinal nematodes and Soldera-Silva et al. (2018) against third-stage larvae of *Haemonchus contortus*. For this, avocado has gained worldwide recognition and its consumption has considerably increased in the last years.

This study aims to evaluate the potential role of *Persea americana* extract as an antihelmintic and anticoccidial agent against *E. papillata*- experimentally infected mice.

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[1] *Department of Science and Technology, Al-Nairiyah University College, University of Hafr Al-Batin, Hafr Al-Batin, Saudi Arabia*
[2] *Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia*
[3] *Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo, Egypt*
[4] *Applied Science Research Center, Applied Science Private University, Amman, Jordan*
[5] *Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia*
*Corresponding author: rabdelgaber.c@ksu.edu.sa

2 Materials and methods

2.1 Plant and drug collection

Fruit of *Persea americana* was purchased from the local markets in Riyadh, Saudi Arabia. A botanist from the Department of Botany, College of Science, King Saud University, Saudi Arabia authenticated the samples. Amprolium (Amproxine 20%, water-soluble powder, Gulf Veterinary Pharmacy, Riyadh, Saudi Arabia) was used as a reference drug for the treatment of coccidiosis.

2.2 Preparation of the methanolic extract

The *P. americana* methanolic extract was prepared according to the method of Amer et al. (2015). In brief, peel and pulp were obtained from the fruits and dried, grinded by an electronic blender (Senses, MG-503T, Korea) into a powder that was extracted with 70% methanol, the collected residue used for the experiment.

2.3 Infrared spectroscopy

P. americana extract was mixed with a powder of potassium bromide powder (1:99 wt %) to obtain a translucent sample disc (see in Al-Quraishy et al., 2020). The NICOLET 6700 Fourier-transform Infrared Spectroscopy (FT-IR) from Thermo Scientific (Waltham, MA, USA) was used for the analysis. Maximum absorption was reported in the number of waves in the spectral range of 4000-400 cm^{-1}.

2.4 Anthelmintic activity of P. americana

Allolobophora caliginosa was used as a model worm, according to the method of Ajaiyeoba et al. (2001). Three doses were used (200, 100, and 50 mg/mL) to study the anthelmintic activity of *P. americana* (PAFE). The reference drug, mebendazole (Saudi Pharmaceutical Industries, Riyadh, Saudi Arabia) was used with a concentration of 10 mg/mL. Worms in distilled water were used as a control. The time to reach paralysis and death state was expressed in minutes (Dkhil, 2013).

2.5 Histological examinations

The treated and control worms were prepared for histological study immediately after paralysis and death experiment, according to Drury & Wallington (1973). Using an Olympus B×61 microscope (Tokyo, Japan), the stained sections were examined and photographed and then the cuticular thickness were measured via ImageJ 1.53e software (Wayne Rasband and contributors, National Institute of Health, USA) and expressed in micrometers.

2.6 Parasite

E. papillata was used as a model coccidial murine parasite. Oocysts were passaged in laboratory mice. Unsporulated oocysts were collected from mice feces and then washed in phosphate buffer solution (PBS). Part of these oocysts was used in the *in vitro* study and the other sporulated in 2.5% (*w/v*) potassium dichromate ($K_2Cr_2O_7$) for *in vivo* study.

2.7 In vitro oocyst sporulation

The unsporulated oocysts (1×10^5) were incubated for 72 and 96 hr at 25-29 ºC in 5 mL Dist. H_2O (negative control), 5 mL 2.5% $K_2Cr_2O_7$ (positive control) and finally in 5 mL $K_2Cr_2O_7$ containing one of the following: PAFE (100, 50, and 25 mg/mL), 8.3 mg amprolium, 109 µL Dettol ™, 25 µl phenol, and 5% formalin. Sporulation of the oocysts was monitored by examining sporocysts using an Olympus compound microscope (Olympus Co., Tokyo, Japan). Sporulation and inhibition (%) were calculated according to Thagfan et al. (2020).

2.8 Mice and coccidial infection

A total of 35 male mice of the strain C57BL/6 (10-12 weeks old, weighing 20-25 g) were obtained from the animal facility in the College of Pharmacy at King Saud University. Mice were divided into seven groups with five mice in each. The groups included an uninfected-untreated control, uninfected-treated with PAFE (500 mg/kg mice weight), and infected-untreated control with 1×10^3 *E. papillata* oocysts. The remaining groups 4, 5 and 6 were infected and then after 60 min, they were treated with PAFE 100, 300, and 500 mg/kg, respectively. Group 7 was infected and treated with Amprolium at 120 mg/kg. Treatment was applied for 5 days. Fecal pellets were collected from infected untreated and treated groups to estimate the number of oocysts per gram (OPG) of feces according to Dkhil et al. (2015).

2.9 Body weight and feed intake

The experimental mice performance indicators included change in body weight (BWG) and feed intake (FI) according to Al-Quraishy et al. (2020). All mice were weighed individually and the BWG was calculated by subtracting the initial body weight (at day 0 p.i.) from the final body weight (at day 5 p.i.). Also, FI was calculated by subtracting the weight of the residual food (at day 5 p.i.) from the weight of the food offered at the start of the experiment (at day 0 p.i.).

2.10 Sample collection

On day 5 p.i., all mice were euthanized. The jejunum was aseptically removed from all groups, cut up into small pieces, washed in physiological saline (0.9%), and then fixed in neutral buffered formalin (10%).

2.11 Histochemical studies of the jejunal tissue

The fixed jejunal specimens were dehydrated, embedded in wax, and then sectioned to 5 µm thickness. Sections were stained with periodic acid-Schiff's method for total carbohydrates demonstration according to Hotchkiss (1948), and with mercuric bromophenol blue method to demonstrate total proteins according to Mazia et al. (1953).

2.12 Statistical analysis

Data were analyzed using SigmaPlot® version 11.0 (Systat Software, Inc., Chicago, IL, USA). The statistical comparison among groups was performed using a one-way analysis of variance (ANOVA). All values were expressed as mean ± SD, at a significant level of p-value ≤ 0.01.

3 Results

3.1 Infrared spectroscopy

The analysis of PAFE using FT-IR showed major bands at 3419.55 cm^{-1}, 2930.88 cm^{-1}, 1619.05 cm^{-1}, 1412.14 cm^{-1}, 1261.84 cm^{-1}, 1058.72 cm^{-1}, 901.26 cm^{-1}, 820.05 cm^{-1}, and 594.99 cm^{-1} (Figure 1, Table 1). O-H stretching was indicated by the band at 3419.55 cm^{-1} confirming the presence of alcohol. The band at 2930.88 cm^{-1} implied N-H stretching for the presence of amine salt. C=C stretching at 1619.05 cm^{-1} confirming the presence of α,β-unsaturated ketone. The band at 1412.14 cm^{-1} implied S=O stretching for the presence of sulfate. C-O stretching at the band 1261.84 cm^{-1} confirmed the presence of alkyl aryl ether. The band 1058.72 cm^{-1} (S=O stretching), 901.26 cm^{-1} (C-H stretching), 820.05 cm^{-1} (C-C stretching), and 594.99 cm^{-1} (C-I stretching) confirmed the presence of sulfoxide, 1,2,3-trisubstituted, alkene and halo compound, respectively.

3.2 Anthelmintic activity of P. americana

The methanolic PAFE extracts were able to exert greater anthelmintic activity against live adult *A. caliginosa* worms (Figure 2, Table 2). The most efficient dose, 200 mg/kg showed the time to paralysis and death were 6.17 ± 0.40 and 9.87 ± 1.50 min, respectively. However, the reference drug mebendazole (10 mg/mL) showed less effect (9.07 ± 1.35 and 16.74 ± 5.62 for paralysis and death time, respectively) compared to the 200 mg/kg PAFE (Table 2). Moreover, microscopic examinations revealed uniform normal body architecture for control worms (Figure 3) as well as a significant reduction in the cuticular thickness for treated worms with PAFE and mebendazole (Figure 3).

3.3 Effect of PAFE on oocyst sporulation in vitro

There was no change for oocysts incubation in dist. H_2O (negative control) at both 72 and 96 hr. Oocyst incubation with $K_2Cr_2O_7$ (2.5%), PAFE (100, 50, 25 mg/mL), amprolium, phenol, and Dettol™ showed different levels of sporulation (Table 3). After incubation with formalin, the unsporulated *E. papillata* oocysts showed no rate of sporulation. Incubation with PAFE (100 mg/mL) for 72 and 96 hr inhibited oocysts sporulation by 66.24% and 59.27%, respectively. PAFE (50 and 25 mg/mL), amprolium, Dettol™, and phenol induced variable inhibition levels at 96 hr of 30.04%, 7.19%, 73.18%, 84.16%, and 90.47%, respectively (Table 3).

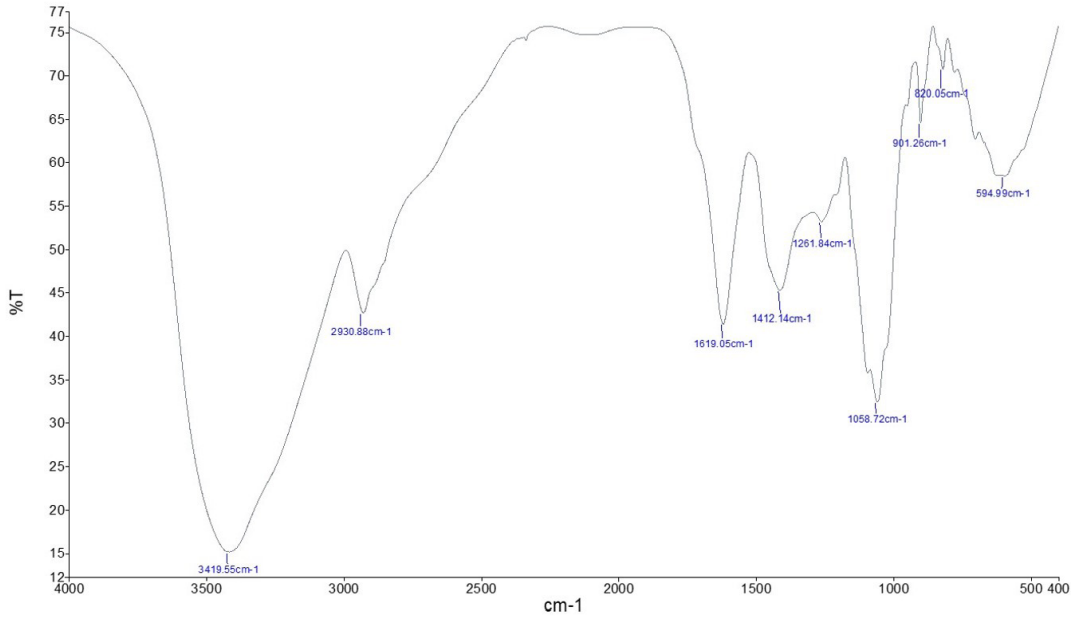

Figure 1. FTIR of *Persea americana* fruit extract in an aqueous medium showing the functional characteristic of material.

Table 1. FTIR for *Persea americana* fruit extract.

Absorption (cm^{-1})	Transmittance (%)	Appearance	Group	Compound class
3419.55	24.39	strong, broad	O-H stretching	alcohol
2930.88	20.90	strong, broad	N-H stretching	amine salt
1619.05	11.54	strong	C=C stretching	α,β-unsaturated ketone
1412.14	10.07	strong	S=O stretching	sulfate
1261.84	9.00	strong	C-O stretching	alkyl aryl ether
1058.72	7.55	strong	S=O stretching	Sulfoxide
901.26	6.42	strong	C-H bending	1,2,4-trisubstituted
820.05	5.84	medium	C=C bending	alkene
594.99	4.24	strong	C-I stretching	halo compound

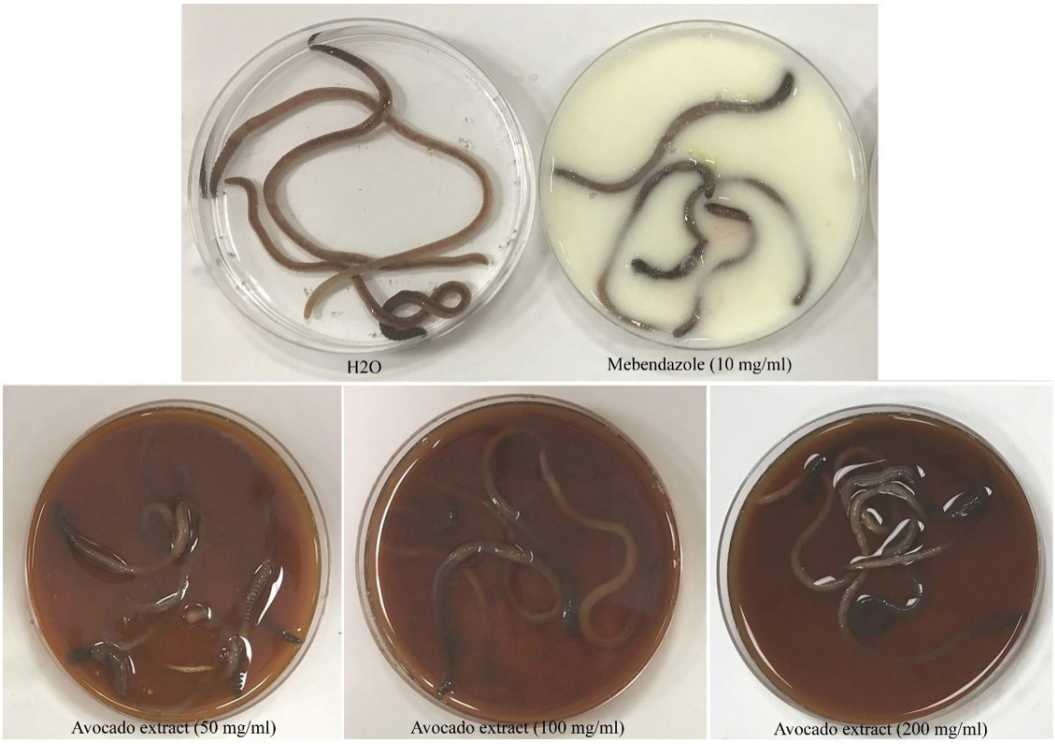

Figure 2. *In vitro* anthelmintic effect of methanolic extract of fruit of *Persea americana* against *Allolobophora caliginosa* in comparison to the reference drug (mebendazole).

Table 2. *In vitro* anthelminthic activity of *Persea americana* fruit extract.

Test samples	Concentration (mg/mL)	Time taken for paralysis (min.)	Time taken for death (min.)
Control (H_2O)	--	--	--
PAFE	200 mg/mL	6.17 ± 0.40 ^{acd}	9.87 ± 1.50 ^{acd}
	100 mg/mL	38.62 ± 1.94 ^{abce}	42.58 ± 5.96 ^{abce}
	50 mg/mL	133.00 ± 0.09 ^{abde}	137.00 ± 0.11 ^{abde}
Mebendazole	10 mg/mL	9.07 ± 1.35 ^{acd}	16.74 ± 5.62 ^{acd}

Values are mean ± SD. All superscripts indicate significance at $P \leq 0.05$. [a]compared to untreated (H_2O); [b]compared to mebendazole; [c]compared to the lowest concentration of methanolic extract of *P. americana* fruit; [d]compared to the moderate concentration of methanolic extracts of *P. americana* fruit; [e]compared to the highest concentration of methanolic extract of *P. americana* fruit.

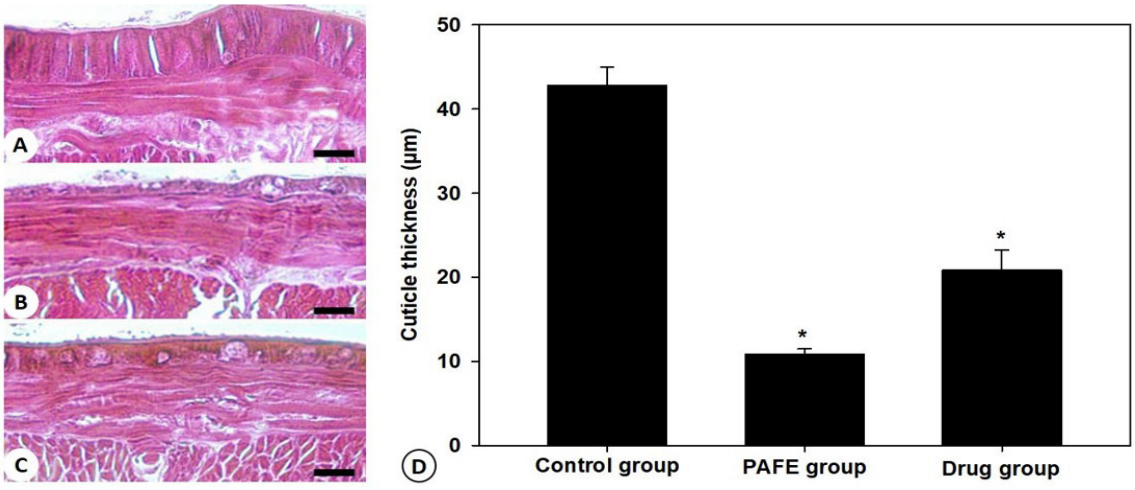

Figure 3. Cuticle thickness of *A. caliginose* with various treatments. (A) worms in dist. H_2O (control). (B) worms in 200 mg/mL PAFE leaf extracts. (C) worms in the reference drug (mebendazole). (D) Bar chart is thickness of worm cuticle (µm) among three groups, control group, PAFE-treated group, and drug-treated group. Each group represents an average of five different fields of cuticle sections stained with hematoxylin and eosin, Scale bar = 25 µm. *significance change with respect to control group.

Table 3. *In vitro* anti-coccidial effects of the methanolic extract of *Persea americana* fruit on the sporulation percentage of *Eimeria papillata* oocysts.

Groups	Time	Sporulation of oocyst (%)	Inhibition of sporulation (%)	P-value
Distilled H_2O	72 h	84.33 ± 1	0	-
	96 h	88.47 ± 1	0	-
Potassium dichromate (2.5%)	72 h	89.21 ± 1	0	-
	96 h	93.92 ± 1	0	-
PAFE (100 mg/mL)	72 h	30.11 ± 1	66.24 ± 1	0.01
	96 h	38.25 ± 1	59.27 ± 1	0.01
PAFE (50 mg/mL)	72 h	57.03 ± 1	36.07 ± 1	0.01
	96 h	65.71 ± 1	30.04 ± 1	0.01
PAFE (25 mg/mL)	72 h	73.34 ± 1	17.78 ± 1	0.01
	96 h	87.16 ± 1	7.19 ± 1	0.01
Amprolium	72 h	17.6 ± 1	80.27 ± 1	0.01
	96 h	25.18 ± 1	73.18 ± 1	0.01
Dettol™	72 h	6.28 ± 1	92.96 ± 1	0.01
	96 h	14.87 ± 1	84.16 ± 1	0.01
Phenol	72 h	3.06 ± 1	96.56 ± 1	0.01
	96 h	8.95 ± 1	90.47 ± 1	0.01
Formalin	72 h	0	100 ± 0.1	0.01
	96 h	0	100 ± 0.1	0.01

3.4 Oocysts per gram feces

Oocysts output reached the highest peak on the 5th-day p.i. being about $4075 \times 10^6 \pm 129.7 \times 10^6$ oocysts/g feces in the infected group (Table 4). All treated groups showed lower OPG as compared to the infected-untreated group. Among PAFE-treated groups, a significant reduction of the oocyst output by $594.3 \times 10^6 \pm 89.4 \times 10^6$ oocysts/g feces was observed in the group treated with 500 mg/kg followed in increasing order by the groups treated with 300 and 100 mg/kg. This dose was considered the best dose for lowering the oocysts output in comparison to $677 \times 10^6 \pm 89.3 \times 10^6$ oocysts/g feces with the reference drug (Table 4).

Table 4. PAFE induced reduction in oocyst output.

Group	Oocyst output /g feces
Uninfected-untreated (-ve control)	-
Uninfected-treated (+ve control)	-
Infected-untreated	$4075 \times 10^6 \pm 129.7 \times 10^6$
Infected-treated PAFE (100 mg/kg)	$2613 \times 10^6 \pm 126.9 \times 10^6$
Infected-treated PAFE (300 mg/kg)	$2456 \times 10^6 \pm 136.2 \times 10^6$
Infected-treated PAFE (500 mg/kg)	$594.3 \times 10^6 \pm 89.4 \times 10^6$*
Infected-treated drug (120 mg/kg)	$677 \times 10^6 \pm 89.3 \times 10^6$

*The most effective dose of PAFE.

3.5 Body weight and feed intake

On the 5th-day, the uninfected-untreated group have a significant increase in BW by an average percent of 1.96%, while the *E. papillata* infection caused a great significant diminish in BW of the infected group by an average percent of 2.54% (Figure 4). The infected-treated groups showed a significant increase in BW by 1.36% compared to the infected one, especially at the 500 mg/kg PAFE dose showing more BWG than the reference drug 2.03% (Figure 4). The minimum FI was recorded in 5th-day p.i. to be 50.29 ± 1.33 for the infected-untreated group (Figure 5). The treated groups showed enhancement of FI as compared to the infected group. Among PAFE-treated groups, a maximum FI (56.59 ± 1.25) was reported in mice treated with 500 mg/kg PAFE, this dose is considered as good as the amprolium-treated group (50.84 ±1.30) (Figure 5).

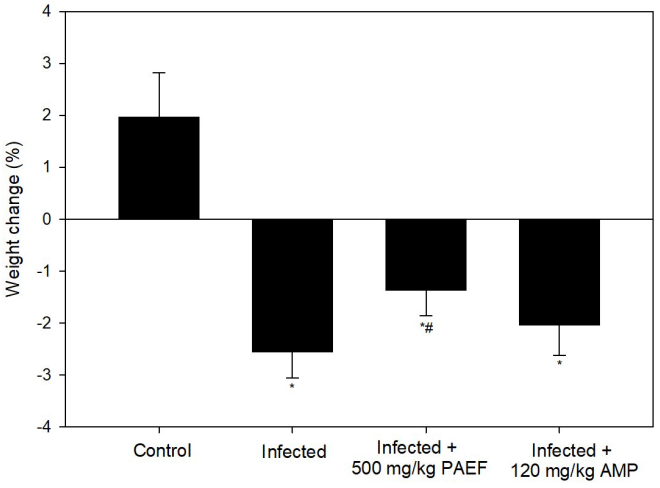

Figure 4. PAFE improved weight loss due to infection with *Eimeria papillata* in mice. Values are means ± SEM. *significance ($P \leq 0.05$) against non-infected control group, #significance ($P \leq 0.05$) against infected group.

3.6 Histochemical studies of the jejunal tissue

Qualitative analysis of carbohydrate content revealed depletion in the jejunal tissue of the infected group in comparison to the control one which refers to an increased carbohydrate content (Figure 6). After treatment of PAFE, the carbohydrate status showed a significant change compared to the infected group (Figure 6). Additionally, the *E. papillata* infection caused depletion in the soluble protein content in the jejunal tissue in comparison to the control group (Figure 7). Treatment with PAEF restored the jejunal soluble proteins compared to the infected group (Figure 7).

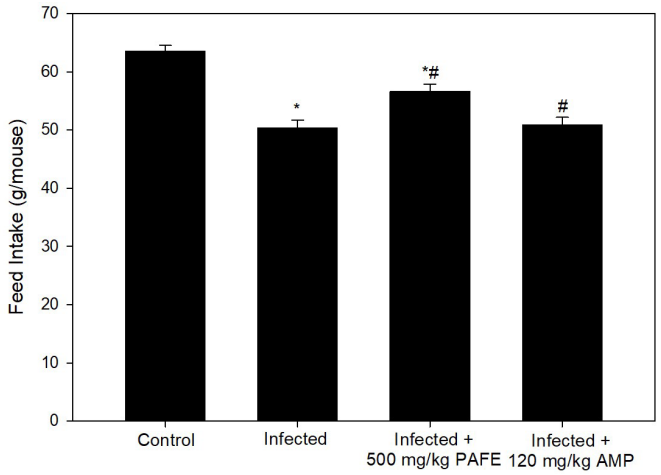

Figure 5. PAFE improved food intake due to infection with *Eimeria papillata* in mice. *significance ($P \leq 0.05$) against non-infected control group, #significance ($P \leq 0.05$) against infected group.

4 Discussion

Murine coccidiosis caused by *E. papillata* began with the oral uptake of the sporulated oocysts that invade the intestinal caecum destroying the epithelium due to the multiplication of *E. papillata* stages and finally, the developed oocysts were released in feces (Al-Quraishy et al., 2011). Previous studies have attempted to determine a solution for this issue. To avoid adverse effects on animal performance, there's a need to develop new agents with minimum side effects against coccidiosis. This study aimed to evaluate the anthelmintic and anticoccidial activities of *P. americana*. Previous studies reported the effective role of other fruit extracts like *Puniga granotum* (Amer et al., 2015; Khorrami et al., 2022; Yang et al., 2022), *Citrus x paradise* (Pérez-Fonseca et al., 2016), *Tribulus terrestris* (Hong et al., 2018), *Ananas comosus* (Daiba et al., 2022).

Several studies have reported the anthelmintic role of certain fruit extracts (Yadav & Temjenmongla, 2012; Dkhil et al., 2019;

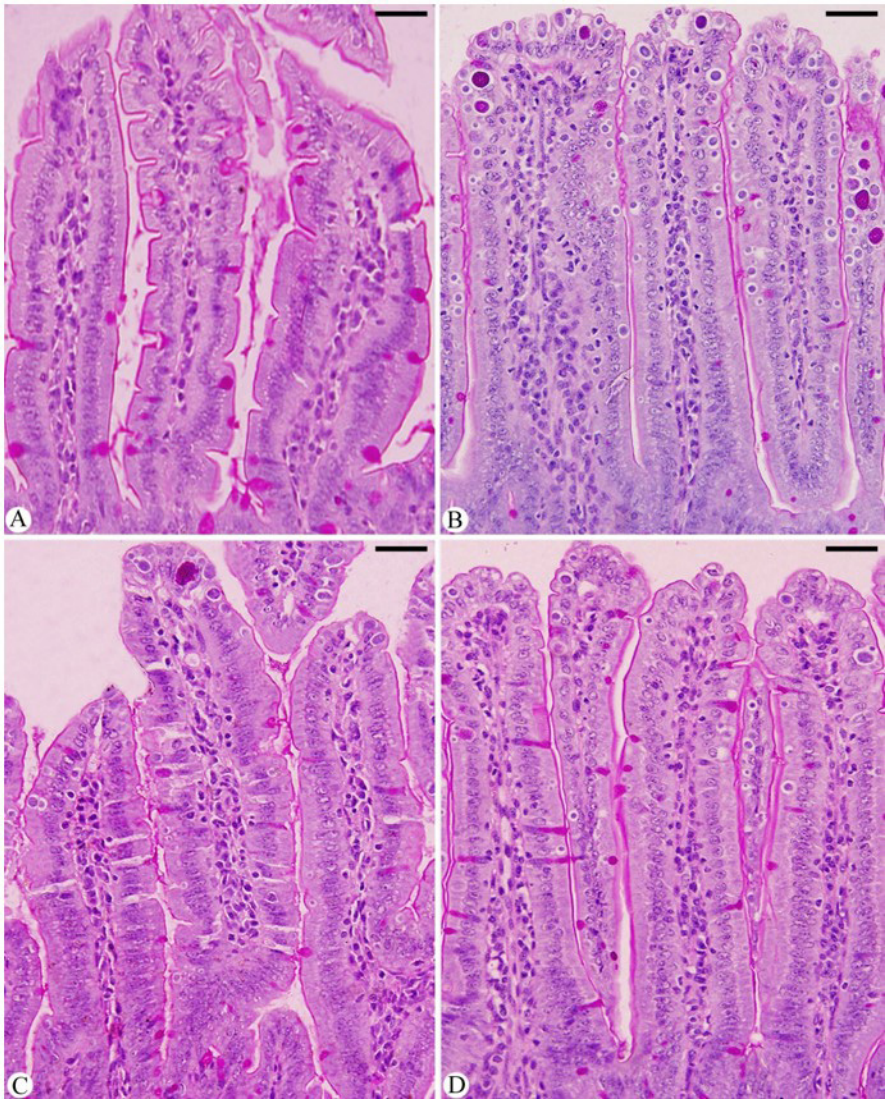

Figure 6. Carbohydrate content in jejunum sections stained with periodic Schiff's method. (A) control non-infected jejunum with normal content. (B) *E. papillata* infected jejunum with depletion in their carbohydrate content. (C and D) infected treated mice (500 mg/kg PAFE and 120 mg/kg AMP, respectively) with improvement in their level. Scale bar = 100 µm.

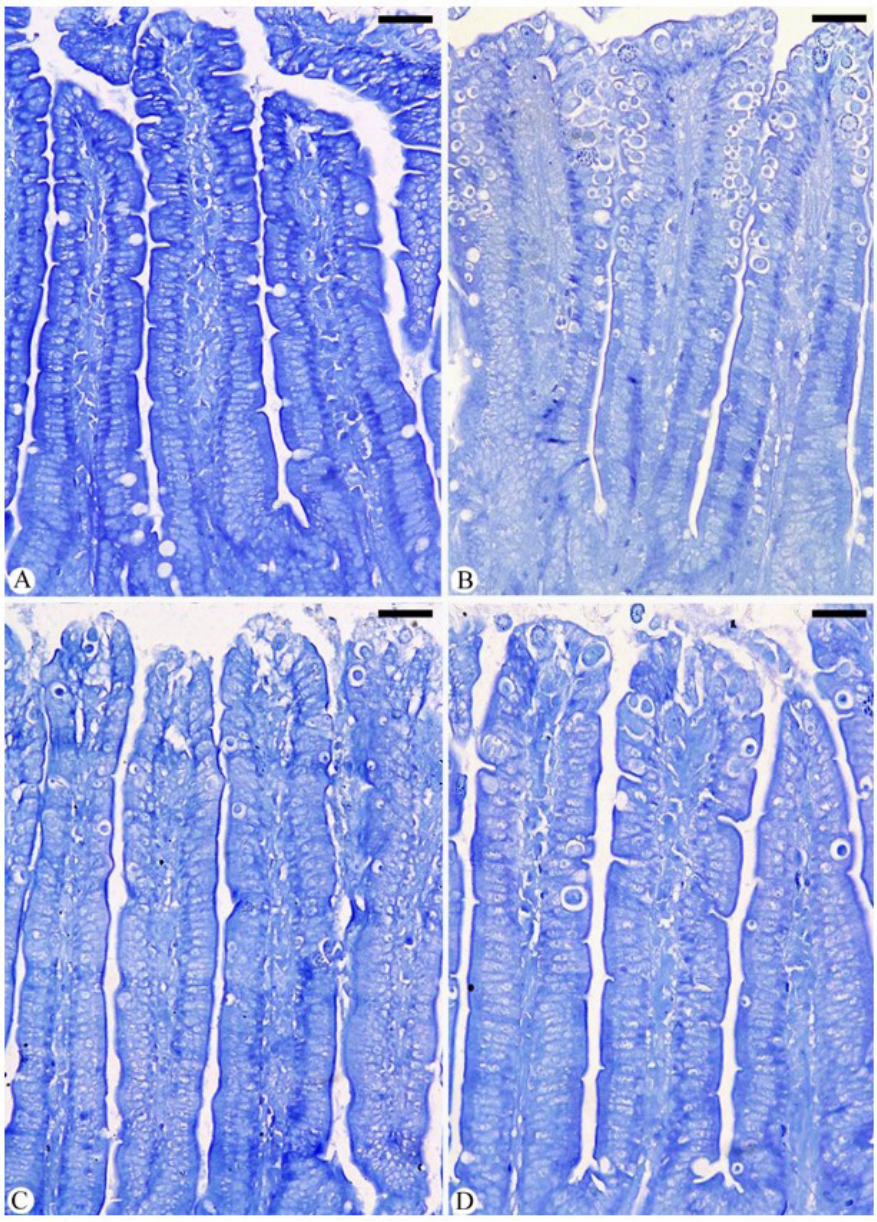

Figure 7. Protein content in jejunum sections stained with mercuric bromophenol blue method. (A) control non-infected jejunum with normal content. (B) *E. papillata* infected jejunum with depletion in their protein content. (C and D) infected treated mice (500 mg/kg PAFE and 120 mg/kg AMP, respectively) with improvement in their level. Scale bar = 100 μm.

Castagna et al., 2020). The earthworms have been chosen as a model for the antihelmintic activity experiment due to the physiological similarity between intestinal roundworms infecting humans (Das et al., 2011). *In vitro* antihelmintic activity exhibited by PAFE at 200 mg/mL the lesser time is taken to paralysis and death (6.17 ± 0.40 and 9.87 ± 1.50 min, respectively) compared to mebendazole, probably owing to the extract composition as agreed with Rivai et al. (2019), and Subba et al. (2019) reported that *P. americana* extracts that contained phenols, flavonoids, alkaloids, tannins, terpenoids, saponins, steroids, carbohydrates, and proteins. Paralysis and death of worms may also due to the mucopolysaccharide membrane damage by saponins and tannins. Vidyadhar et al. (2010) stated that the direct action of tannins on the worm cuticle causes skin stiffness, resulting in paralysis and the death of worms. Wang et al. (2010) reported that saponins increase membrane permeability and pores formation which is important for the anthelminthic effect.

Our study demonstrated *in vitro* anticoccidial activity of PAFE on the oocyst's sporulation in a dose-dependent manner, which is attributable to numerous bioactive phytochemical constituents studied by Enwa et al. (2016) and Noorul et al. (2017). Also, formalin (5%) completely inhibited the sporulation of *E. papillata*, which agreed with Thagfan et al. (2020) stated that this highly reactive chemical interacts with proteins *in vitro* and inhibits sporulation. In addition, phenol and Dettol™ have been reported to inhibit sporulation at 96 hr by 90.47% and 84.16%, respectively, which is consistent with Mai et al. (2009)

that the oocyst wall is impermeable to water-soluble substances and resistant to proteolysis.

The PAFE extract exhibit adequate anticoccidial properties in a dose-dependent manner, evidenced by a significant lowering in the oocysts output in the treated group reflecting that PAFE impairs the development of parasites in the epithelial cells of the host jejunum before the relatively inert oocysts are formed and finally released. The anti-coccidial properties of PAFE may be due to its high amounts of polyphenolic compounds (Kosińska et al., 2012; Di Stefano et al., 2017; Melgar et al., 2018; Castro-López et al., 2019; Rosero et al., 2019; Abdelkader et al., 2022), which exert a strong antimicrobial activity via interaction and disruption of microbial cell walls (Chia & Dykes, 2010; Rodríguez-Carpena et al., 2011; Cardoso et al., 2016; Fernández-Castañeda et al., 2018). Such an impairment and even inhibition of the intracellular development of parasites by PAFE are also known to occur with most anticoccidial drugs.

Our finding demonstrated the weight loss and feed intake following the infection with *E. papillata*. Previous studies explained this loss due to the consumption of the parasitic *Eimeria* stages to the carbohydrate content inside the intestinal epithelium and lead to severe structural intestinal epithelium alterations (Ghanem & Abdel Raof, 2005; Anwar et al., 2008; Ogbe et al., 2009; Gilbert et al., 2011; Metwaly et al., 2013; Dkhil et al., 2014; Hamid et al., 2019; Al-Quraishy et al., 2020; Qaid et al., 2021). Upon treatment, a significant improvement in weight and food consumption was observed due to the presence of bioactive compounds in PAFE helping in the improvement of the jejunal histological architecture that restored the nutritional status of mice.

Host cell metabolism is the most affected process during coccidiosis (Al-Quraishy et al., 2014) and *Eimeria* parasite has a great capacity to manipulate host cells for their benefit via scavenging available host nutrients (Hermosilla et al., 2012). Our result demonstrated a disturbance in both carbohydrate and protein status. Metwaly et al. (2013) mentioned a possible reason for the lower carbohydrate levels related to the excessive consumption of parasitic stages to stored carbohydrate content in the jejunal tissues. Upon treatment, PAFE restored jejunal carbohydrate content regarding the bioactive components of PAFE that caused a reduction in the activity of the glucose-6-phosphatase enzyme which probably resulted in the restoration of tissue glycogen content. Intestinal coccidial infections have been classified as protein-losing enteropathy (Kouwenhoven, 1971). Many studies proved that there is a lower amount of total proteins in the infected tissues and an increased rate of protein escaping into the intestinal lumen via the ruptured intestinal wall and excreted within feces (Bangoura & Daugschies, 2007). Upon treatment, the jejunal protein content improved because of PAFE for the reduction of the catalytic processes of tissue protein and nucleic acid degradation.

Based on the presented data, it could be concluded that *P. americana* possesses powerful anthelminthic and anticoccidial activities. Future studies are needed to know the mechanism of *P. americana* action on both parasite and the host.

Ethical approval

This research was approved by the Research Ethics Committee (REC) at King Saud University (approval number KSU-SE-22-40).

Conflict of interest

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

Data Availability Statement

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

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