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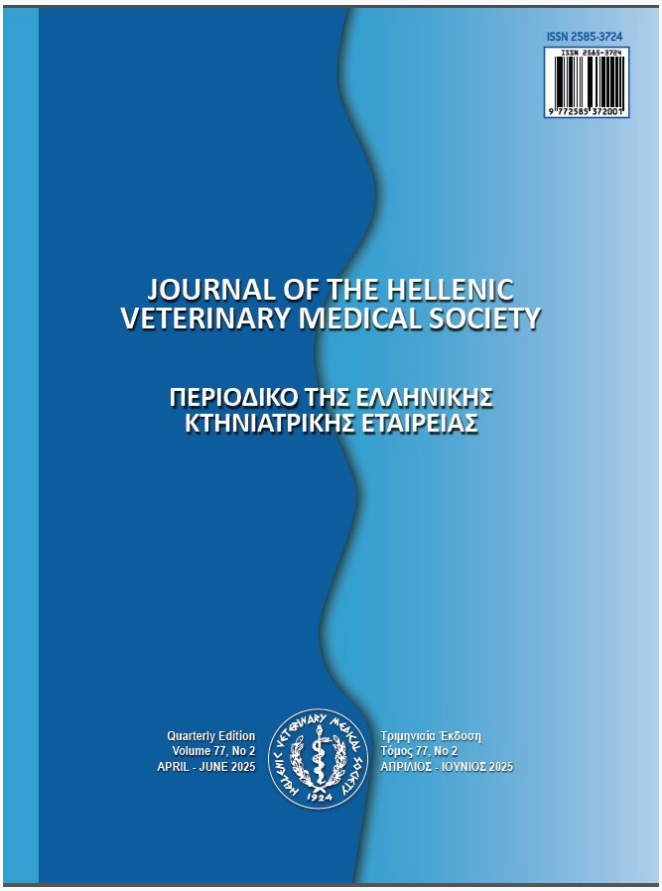

Effectiveness of Calotropis procera Leaf Extract in Treating Eimeriosis- Induced Inflammation Alteration of the Jejunum in Mice

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Research article
Ερευνητικό άρθρο

Effectiveness of *Calotropis procera* Leaf Extract in Treating Eimeriosis-Induced Inflammation Alteration of the Jejunum in Mice

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ABSTRACT: Medicinal plants that are used extensively containactive biological chemicals.*Calotropis procera*-leaf extract (CPLE) was utilized to assess the anti-oxidative status and development stages *of Eimeria papillate* infection in mice.Divided the mice into five groups, with group 1 serving as the control. Group 2, 200 mg/kg extract without infection, to determine its toxicity. We gavaged 1 x 10^3 sporulated oocysts of E. papillate in groups 3, 4, and 5. Group 3 without treatment. Group 4, 200 mg/kg of extract daily, and Group 5 received toltrazuril by gavage at a daily dosage of 25 mg/kg. On day 5 p.i., mice were slaughtered and jejunum tissues were prepared for histology and oxidative stress. GC-MS analysis revealed nine biologically active chemical compounds. The most effective dose was 200 mg/kg, significantly reducing jejunal parasite stages. Also, quantitative results showed phenolics, flavonoids, and tannin 160.22±0.652 mg GAE/g DW, 34.046±0.545 mg QE/g DW, and 65.869±0.263 mg TAE/g DW, respectively. Moreover, IC_{50} was obtained at 44.2±0.007 g/mL for Hep-G2 cell lines. The results showed that when the mice received a dose of 200 mg/kg of the extract, there were statistically significant differences compared to the infected group ($P \leq 0.05$), as the tissue injury within the jejunum decreased by 66%. The *C. procera* extract successfully reverses the effects of the *E. papillate* infection on gamma-glutamyl transferase, nitric oxide, and antioxidant enzyme catalase. It has been demonstrated that extract possesses anti-inflammatory activities, reduces the output of oocysts, and improves oxidative status.

Keyword: *Eimeria papillate*; anti-inflammation; oxidative status; C.*procera*.

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INTRODUCTION

Coccidiosis, often considered one of the deadliest diseases that may affect chickens, is also sometimes referred to as red dysentery (McDougald et al., 2020). The condition is caused by an endogenous protozoan parasite called *Eimeria*, which completes its life cycle inside the intestinal mucosa of the avian host affected by the ailment (Mesa-Pineda et al., 2021). Parasites have a single-celled life cycle and are passed from host to host through the gastrointestinal tract and feces. *Eimeria* has the potential to cause extensive damage to the epithelial cells. Consequently, the host may have symptoms such as diarrhea, malabsorption, and difficulty gaining weight (Lu C et al., 2021). In jejunum infections, *E. papillate*-mice infection has significant inflammation in the gastrointestinal. Mice are the most commonly used experimental models in biomedical research. Indeed, *E. papillate* is a great model for studying avian coccidiosis (Murshed et al., 2022). The management of coccidiosis is achieved by the use of chemical coccidiostats, which include several medications such as pyridine group (Coyden), quinolone (nequinate and decoquinate), ionophore, halofuginone and quinazoline, thiamine antagonists (amprolium), and sulphonamide. Nevertheless, apart from medication resistance, there are apprehensions about food safety and public health regarding medicine residues in animal products, hence imposing limitations on using those goods (Chapman and Rathinam 2022).

This indicates that treating *Eimeria* with industrial drugs is ineffective in some cases due to parasite resistance, and has secondary effects (Abdelhady et al., 2012). Amprolium is a synthetic pharmaceutical agent used for the therapeutic management of coccidiosis. Amprolium hydrochloride, a synthetic chemical compound, is classified as a quaternized pyrimidine derivative with the molecular formula $C_{14}H_{19}ClN_4$. This product belongs to the family of chemical compounds referred to as methyl pyridines, distinguished by the occurrence of a methyl group at two locations on a pyridine ring. The disruption of thiamine metabolism caused by this analog, an analog of vitamin B1 (thiamine), inhibits thiamine absorption. Consequently, this inhibition halts the synthesis of carbohydrates in coccidiosis (EFSA Panel et al., 2018). Because of the challenges of treating avian coccidiosis, researchers are motivated to study newer disease management techniques and natural products are currently being investigated.

Recent studies have shown that using natural plant sources exhibits potential advantages in treating various parasite infections while concurrently offering a side-effect-free therapeutic method. An alternate method for treating coccidiosis is developing anti-coccidiosis drugs derived from naturally occurring substances and secondary metabolites (Murshed et al., 2023). Compared to current treatments, herbal remedies have several benefits, the most notable of which are their cost-effectiveness and accessibility. These advantages encourage the creation of herbal-based drugs. On the market now are a variety of anti-coccidiosis drugs, including those that are naturally occurring, semi-synthetic, and synthetic (Mohammadi et al., 2020). Plant-derived products have the potential to provide an alternate avenue for managing coccidia, without the development of resistance (Quadros et al., 2020). *Calotropisprocera*, a plant of significant importance in traditional medicine, has a diverse range of pharmacological properties. These include antioxidant, antidiarrheal, anti-inflammatory, analgesic, antiulcer, antibacterial, hepatoprotective, antipyretic, and antiparasitic effects (Mahato, 2020).

The leaf extract of *C.Procera*, commonly referred to as Madar, is a medicinal herb that is utilized in various herbal formulations for the treatment of various body disorders and diseases (Gupta and Lutsenko 2009). This well-known tribal shrub is used in many traditional medicines to treat rheumatism, asthma, leprosy, toothaches, skin conditions, and elephantiasis (Khan AQ, Malik et al., 1989). Ayurvedic medicine also makes use of *Calotropis procera*. It is an ingredient in many polyherbal remedies for treating illnesses. One plant whose potential as an anticonvulsant has been studied is Ayurveda (Ojha et al., 2003).

Calotropis procera leaf extract (CPLE), contains a diverse range of metabolites, including glycosides and cardenolides, triterpenoids, steroids, saponins, lignans (Ojha et al., 2017), proteins, and different enzymes (Bezerra et al., 2017), hydrocarbons (Erdman M, Erdman and Erdman 1981), saturated and unsaturated fatty acids (Hunter et al., 2010). *C. procera* showed a diverse array of biological activities such as antimicrobial (Tiwari and Upmanyu 2020), antidiarrhoeal(Kumar et al., 2001), wound healing (de Figueiredo-Feitosa et al., 2017), anti-inflammatory (Ramos et al., 2020), analgesic (Pathak and Argal 2007), anthelmintic (Iqbalet al., 2005), antioxidant (Yesmin et al., 2008), and in vivo anti-hyperglycemic (Rahmatullah et al., 2010).

This study aims to evaluate the efficacy of *C. procera* leaf extract in mitigating the histopathological changes and oxidative stress induced by *E. papillae* infection in mice. It is advisable to integrate the presently accessible approaches for managing medication resistance and controlling coccidiosis.

MATERIAL AND METHODS

Ethical approval

Regulations for the use of animals in Saudi Arabia were adhered to through the research experiment (Ethic Committee King Saud University, ethical permission number: KSU-SE-21-86).

Plant collection and extract preparation

Riyadh's wild areas fresh *C. procera* leaves were obtained (Figure 1). Voucher specimens were deposited in King Saud University's Botany and Microbiology, Department herbarium to establish their botanical identity using the technique presented (Manikandan et al., 2008). The leaves that were gathered were thoroughly cleaned to eliminate any undesired particles. Subsequently, they were air-dried at ambient temperature in preparation for further analysis. Finally, the leaves were ground using an electric blender. Later, a quantity of 100 grams of powdered leaves was subjected to extraction using 400 mL of methanol as the solvent. The resulting mixture was then put in a shaker and allowed to react at ambient temperature for a duration of 24 hours. The leaf extract underwent filtration using Whatman filter sheets. Subsequently, the resulting extract was subjected to concentration and drying using a Yamato RE300 rotating vacuum evaporator (Tokyo, Japan) at a temperature of 40 °C and reduced pressure, to facilitate its further application.

Phyttochemical analysis GC-MS

Phytochemical Analysis GC-MS-Extracted *C. procera* Leaves. Trace GC-ISQ Quantum mass spectrometer system (Thermo Scientific, Austin, TX, USA) analyzed *C. procera* extract. This analysis was done according to the method Kanthal et al (2014), (Kanthal et al., 2014). The *C. procera* extract was examined using a Thermo Scientific Trace GC-ISQ Quantum mass spectrometer system (Austin, TX, USA). A flow rate of 1 mL.mn−1/min was utilized. A GC-MS outfitted with a TG–5MS column (30 m × 0.25 mm ID, 0.25 µm film thickness) was filled with approximately 1 µL of the sample. As a carrier, helium gas was utilized at a steady flow rate of 1.0 mL min^{-1}. The detected mass spectra ranged from 50 to 500 m/z. After 10 minutes at 50 ◦C, the temperature rose to 250 °C at a rate of 5 ◦C min−1, where it remained isothermal for 2 minutes at 300 °C, and then for 10 minutes at 350 °C. By comparing the recorded mass spectra of each component with the information kept in the preceding libraries, the phytochemical constituents were identified using the NIST, Adams, Terpenoids, and Volatile Organic Compounds libraries. By comparing each component's average peak area with the overall peak areas and using the Retention time index, the relative quantity for each was determined.

Figure 1. *Calotropis procera* plant leaves.

Total phenolics, flavonoid, and tannin (TTC, TFC, TTC) content measurements

The phenolic content of CPLE was determined according to the method by Singleton et al (Singleton et al1999). 25-150 µg/mL gallic acid solutions were utilized. After mixing Folin-Ciocalteu reagent (0.1 mL), ultrapure water (Milli-Q) (1.5 mL), and gallic acid or 0.1 mL of the plant extract (1 mg/mL) for 8 min, 20% sodium carbonate (0.3 mL) solution was blended and vortexed in darkness for 2 h and incubated. The blue color absorbance was measured at 765 nm with a UV-visible spectrophotometer. Based on the curve calibration equation (y = 0.005 − x − 0.0088), the extracts' phenolic content was estimated as gallic acid equivalent (mg/g DW), where (y) is absorbance and (x) is concentration.

CPLE's total flavonoids were measured according to the method Ordonez et al (2006). 2% AlCl3 (1.0 mL) water solution was combined with 1.0 mL of plant extract (1 mg/mL). Incubation at room temperature for an hour yielded absorbance at 420 nm. The standard solution and standard curve (R2 = 0.9996) were prepared using 50-800 g/mL quercetin solution. Flavonoids in the extracts were expressed as quercetin (mg/g DW) using the calibration curve equation, y = 0.0011x + 0.0928, where y is absorbance and x is quercetin equivalent concentration (mg/g).

The total tannin content was determined by utilizing this approach for the leaf extract. An overall volume of 0.1 mL of the extract samples was put into an Eppendorf tube with a capacity of 2 mL, which already included 1.5 mL of Milli-Q water and 0.1 mL of the Folin–Ciocalteu phenol reagent. This mixture was incubated for 8 minutes. After that, to neutralize the solution, 0.3 mL of a sodium carbonate solution containing 35 % was added to the combination. After that, the ingredients were thoroughly combined and then placed in a dark, ambient temperature area for twenty minutes. The measured value for the wavelength was 700 nm. The following equation, (Y = 0.0013x+ 0.0052 with R2 = 9937) was utilized to determine the total tannin content that was present in the leaf extract. The total tannin content that was calculated was given in units of mg/g dry weight (DW).

Cytotoxicity evaluation (cell viability assay)

Hep-G2/2.2.15 Human Hepatoblastoma Cell Line were obtained from Sigma-Aldrich Chemie GmbH (Taufkirchen - Germany). The cell lines were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) in a humid environment with 5% CO2 at 37℃. The detection of cell viability and cell growth was performed using MTT Assay (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium Bromide, cat#475989-1GM, Sigma-Aldrich, Germany). Briefly, aliquots of 120 µl of the suspended cells (5 $x10^4$ mL^{-1}) were given to 60 ng/mL of a serial dilution of the *C. procera* Leaf extract, in a 96-well plate. After 3 days of incubation, 20 µl of MTT-solution was given to each well, and the cells were further cultivated for an additional two hours. Formazan crystals were dissolved in isopropanol. The intensity of the resulting color was measured at 595 nm using a microplate reader (BioTek, USA). The cell viability % was calculated as follows:

Cell Viability (%) = Mean absorbance [treated cells / untreated cells] × 100

The IC_{50} values (concentration of extract that triggered 50% inhibition) were specified from the dose-response curve of cell viability percent using OriginPro software.

Evaluation of the anticoccidial effect of different treatments

The experiment was conducted at the Laboratory of Parasitology at the College of Sciences, specifically within the Department of Zoology at King Saud University. The mice were raised in cages equipped with continuous lighting and proper ventilation. Before each experiment, the cages underwent a thorough cleaning, washing, and disinfection process. Additionally, separate feeders and waterers were installed in each cage. The fecal samples of the mice were analyzed several days before the commencement of the experiment to verify their absence of *Eimeria* infection as described by Allen et al (1997). According to a particular protocol, the mice were housed in a controlled environment. The temperature was kept at 21°C, with a light period of 12 hours and a darkness period of 12 hours. The mice were provided with standard feed and water ad libitum, ensuring they had unrestricted access to food and water. The housing conditions were pathogen-free, minimizing the risk of any infectious agents. A total of 30 adult female C57BL/6 mice, aged 9 to 11 weeks and with an average weight of 19 g per mouse, were included in the study.

A pre-test contained 50, 100, 200, and 400 mg/kg of the extract in mice infected with E. papillate. The best dose for reducing the number of oocysts was obtained (200 mg/kg). These mice were randomly assigned to five different groups. The groups were given the designations of Groups 1st, 2nd, 3rd, 4th, and 5th. Each group comprises six mice.

Experimental challenge

Eimeria papillate sporulated oocysts were acquired from the laboratory of parasitology, by periodic passing of infection-free mice. Un-sporulated oocysts were collected from the feces of mice, specifically 4-5 days post-infection. Subsequently, the fecal pellets obtained from each mouse were suspended in a solution consisting of 2.5% (w/v) potassium dichromate in saturated sodium chloride (NaCl) by a flotation process, in preparation for their use in the experiment. Following the washing process to eliminate the solution, the quantity of these recently obtained oocysts was modified to ensure that each mouse received 1×10^3 sporulated oocysts in 100 µl of saline by oral gavage. The faecal oocyst was quantified between the third and fifth day after infection. Feces were collected from each subgroup of infected individuals, and subsequently, the average count of oocysts was determined.

$$\text{Reduction in oocyst rate\%} = \frac{\text{(OPG oocysts control infected – OPG oocysts treatment infected)}}{\text{OPG control infected}} \times 100$$

Group 1st mice were given a conventional control

diet devoid of any infection. In Group 2nd, the infected mice were given a dosage of 1x10^3*E. papillate* oocysts without any therapy. Group 3rd was administered a daily dose of 200 mg/kg of *C. procera* leaf methanolic extract by gavage without subjecting them to any challenge to assess toxicity. The members of Groups 4th and 5th were subjected to oral infection with a dosage of 1×10^3*E. papillate* oocysts. One hour after the infection. The 4th group was administered a dosage of 200 mg/kg of *C. procera* leaf, whilst the 5th group was given a daily dosage of 25 mg/kg of Toltrazuril) Veterinary Agriculture Products Company – VAP-CO) for five days.

Sample collection, count of oocysts in the jejunum, and histological examination

On the fifth day of the experiment, the mice were euthanized, and sections of the jejunum were collected to assess parasite developmental stages, do histological examination, and examine oxidative status. The specimens of the jejunum were afterward immersed in a 10% neutral formalin solution for fixation, followed by dehydration in ethanol. They were then embedded in paraffin wax and sliced into slices with a thickness of 5 micrometers. The sections underwent a staining procedure called hematoxylin and eosin (H&E), (Carleton ET A., 1980).

Oxidative stress

Following the determination of the weight of the jejunum extracted from each mouse, it was subjected to homogenization in phosphate-buffered saline (PBS) using a buffer solution with a pH value not exceeding 7.4. Subsequently, centrifugation was performed at a temperature of 4°C for 15 minutes at a force of 5000 g. Following the supernatant collection, a portion equivalent to 10% of the total volume was used to assess the levels of oxidative stress markers. The quantification of glutathione (GSH), according to Ellman, (1959),and catalase activity was determined according to the method of Aebi (1984), and the estimation of nitrite oxide (NO) carried out according to the mode of Berkels et al (2004), in the jejunum was performed.

STATISTICAL ANALYSIS

The data was inputted using Microsoft Excel version 2020, and further statistical analysis was conducted using Minitab Statistical Software version 20. Descriptive statistics, including measures of central tendency such as means and frequency tables and charts, were computed to present the current state of *Eimeria* spp. Analysis of variance (ANOVA) tests were conducted, followed by Duncan's multiple range tests. The results are presented as means with standard error (±SE). Significance was attributed to values with a probability less than or equal to ($p \leq 0.05$).

RESULTS

The GC-MS investigation uncovered in the *C. procera* leaf methanolic extract a large number of bioactive compounds associated with plant secondary metabolites with their retention time (RT), and peak area percentage. The components most available and potentially effective are as follows: high Monounsaturated Oleic Acid (47.39%), Linoleic acid (18.44%), Pyranose (10.7), diterpene (9.39%), Fatty acid (4.5%). They may serve a role as an anti-inflammatory, an antioxidant, or an antibacterial agent (Table 1 and Figures 2).

Figure 3 shows the results of the phenolics TPC, flavonoids TFC, and tannin TTC measurements taken of the extract. The total potency concentration of the extract was determined to be 160.22±0.652 mg GAE/g DW, 34.046±0.545 mg QE/g DW, and 65.869±0.263 mg TAE/g DW, respectively.

The findings regarding the cytotoxicity of the extract are presented in Figure 3, which shows the results of testing it at varying concentrations. The experiment included exposing Hep-G2 (hepatoblastoma cell line) cells to various concentrations of an extract, namely 1, 12.5, 25, 50, 100, 200, 400, and 800 ng/mL. The survival rates of the cells at these concentrations were found to be 99.76, 84.00, 73.19, 62.27, 53.11, 35.29, 18.81, and 5.19%, respectively. The IC50 value for Hep-G2 was calculated to be 44.2±0.007 ng/mL. As can be shown in Figure 3, the percentage of cancer cells that managed to survive was reduced as the concentration of the extract was raised.

Oocyst output on day 5 post-infection, the infected group had a peak stool oocyst output of 5787 ± 172.33 oocysts/g faeces. In the groups treated with CPLE suspension, oocyst output was reduced by 63 and 66%, in extract and Toltrazuril respectively (Figure 5). It was so clear that the 200 mg/kg dose was the most efficient at suppressing faecal oocyst production oocyst output.

The overall count of intracellular *E. papillate* stages, including meronts, gamonts, and developing oocysts, exhibited a notable reduction in infected mice administered with different dosages of CPLE, particularly at a dosage of 200 mg/kg CPLE, as

Table 1. GC-MS analysis of phytochemical compounds of methanolic leaf extracts.

$RT_{(min)}$	Phytochemicals	Chemical group	Formula	[M-H] (m/z)	Peak area %
5.76	1-Amino-2,6-dimethylpiperidine	Heterocyclic compounds	$C_7H_{16}N_2$	128	2.09
7.47	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Heterocyclic compounds	$C_6H_8O_4$	144	2.82
8.88	7-Ethyl-4-decen-6-one	Organic compound	$C_{12}H_{22}O$	182	10.7
12.63	β-D-Glucopyranose, 1,6-anhydro-	Monosaccharides	$C_6H_{10}O_5$	162	4.5
17.88	n-Hexadecanoic acid	Palmitic acid (fatty acid)	$C_{16}H_{32}O_2$	256	9.39
19.61	Linoleic acid	fatty acids	$C_{18}H_{32}O_2$	280	18.44
19.66	Oleic Acid	fatty acids	$C_{18}H_{34}O_2$	282	47.39
19.61	Linoleic acid	fatty acids	$C_{18}H_{32}O_2$	280	18.44
19.66	Oleic Acid	fatty acids	$C_{18}H_{34}O_2$	282	47.39
19.85	Octadecanoic acid	fatty acids	$C_{18}H_{36}O_2$	284	3.43
22.61	9,12-Octadecadienoyl chloride, (Z, Z)-	fatty acids	$C_{18}H_{31}ClO$	298	1.24

shown in Figure 6, which displays hematoxylin and eosin-stained sections of the jejunal villi. This was especially apparent in mice that had received extract treatment. Mice contracted *E. papillae* after being given the bacteria. Various stages of parasites were seen in the jejunum of mice that were intentionally infected with *E. papillate* oocysts. The administration of CPLE resulted in a substantial decrease of 61% in the number of parasite stages seen per ten villous-crypt units, in comparison to the infected group ($P \leq 0.05$).

There has been a remarkable change in the infected and treated mice in the glutathione (GSH), nitric oxide (NO), and the antioxidant enzyme catalase (CAT).

As a consequence of comparing the non-infected group to the infected group, it was discovered that infection with *E. papillate* in the infected group led to a significant decrease in the level of glutathione (GSH). There was a statistically significant rise in the level of glutathione (GSH) after the administration of 200 mg/mL of *C. procera* through gavage ($p < 0.001$). On the other hand, we observed that the injection of 200 mg/kg of *C. procera*into mice that

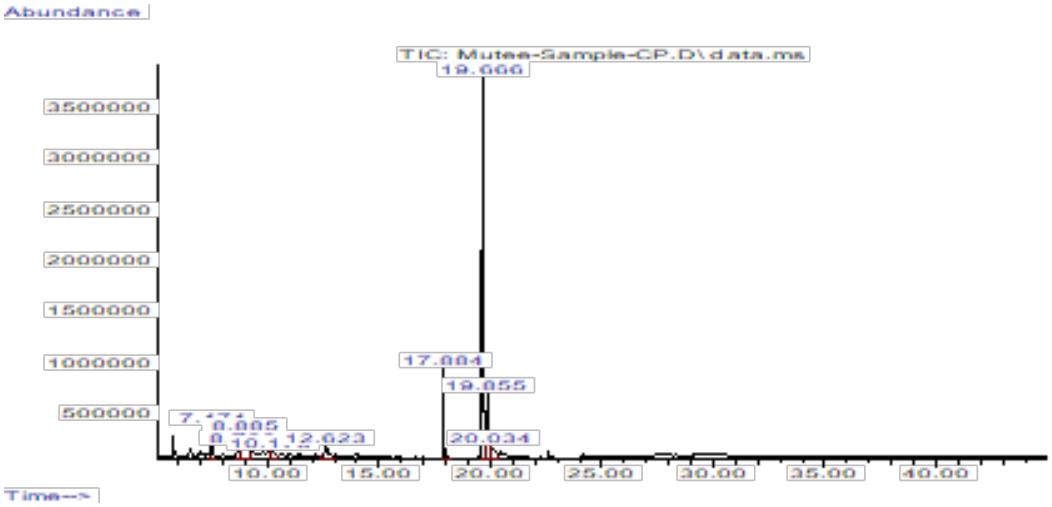

Figure 2. GC-MS analysis reveals the presence of some phytochemical substances in *C. procera*leaf extracts.

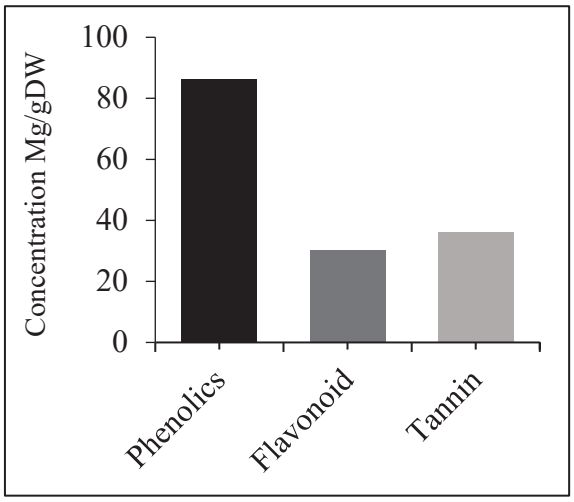

Figure 3. Total of phenols, flavonoids, and tannin in methanolic extract of *C. procera* leaf.

were without any infection increased GSH (Figure 7).

In Figure 8, *E. papillate* infection caused a significant increase (P<001) in the level of nitric oxide (NO) in the jejunum. This increase reached was ameliorated after treatment of the infected mice with *E. papillate*, the level of nitric oxide was altered to nearly the same as in the non-infected control groups. Compared to the reference drug, toltrazuril, the level of nitric oxide was ameliorated after treatment with the *C. procera* extract (Figure 8).

In the infected mice jejunum, the enzyme catalase activity was reduced significantly (P<001) after infection of mice with *E. papillate* (Figure 9). *C. procera* was able to increase the activity of catalase to reach that of the control.

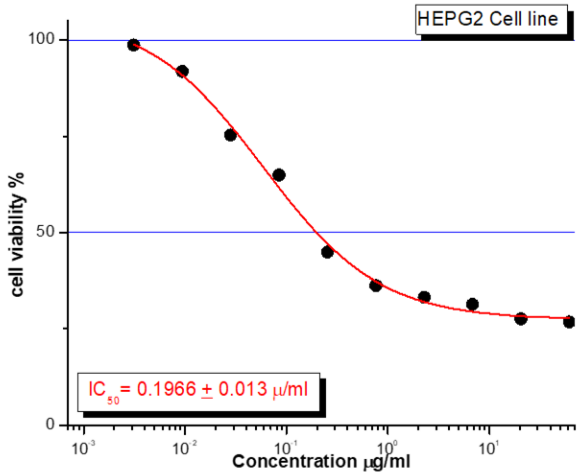

Figure 4. The effect of different doses of *C. procera* extract on the cytotoxicity and subsequent survival of the HT29 cell line. HT-29: Human Colorectal Adenocarcinoma Cell Line.

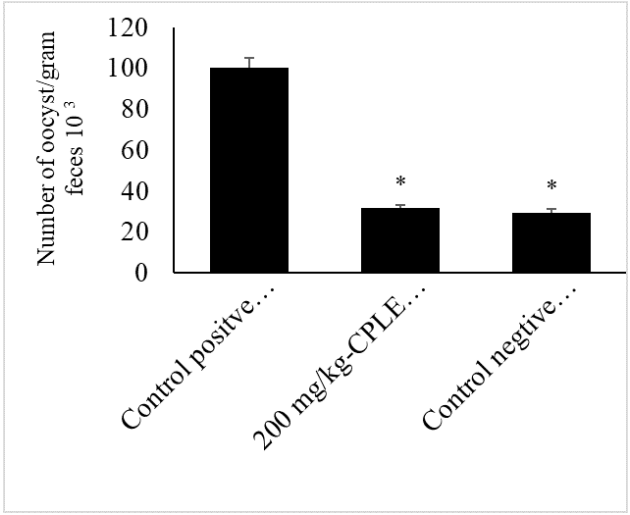

Figure 5. Oocyst output patterns in mice infected with *E. papillate* that were treated with varied dosages of *C. procera* Leaf extract on day 5 of being treated (all values provided as Mean±SE). * statistically significant (P≤ 0.05).

DISCUSSION

Although many anticoccidial medicines have been utilized, there have numerous cases of these interventions having negative side effects. Concerns over drug residues among consumers have redirected research efforts toward alternative molecules that are natural, risk-free, and effective (Kalia et al., 2017). One of these natural alternatives, utilizing medicinal phyto-product extracts that have attracted attention as potential anticoccidial (Kola et al., 2022). In the chicken industry, the prevention and control of coccidian infections are one of the main pillars of the success of poultry projects (Orengo et al., 2012). This is because coccidian infections contribute to production losses, treatment expenses, and disease prevention (Blake et al., 2020). So, in addition to the risk of coccidiosis. It can increase the risk of other contagious diseases and parasitic disorders, such as pneumonia and helminthiasis agents (Głuchowska et al., 2021). This research aimed to evaluate, *in vivo*, the anticoccidial efficacy of CPLE. on oxidative status and parasitic stages.

According to (Ghule and Jagtap, 2022), the transport of medicine across the intestinal epithelial cells is reduced when its lipophilicity is increased. Phe-

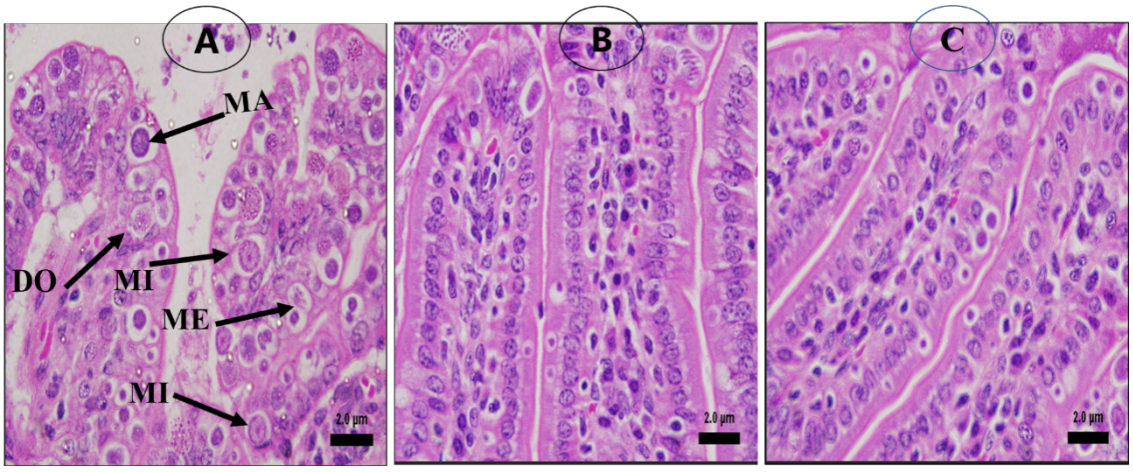

Figure 6. Comparison of the stages of development of oocysts in the jejunum of the infected group (A) with the extract (B) and the reference drug (C), different development phases were observed. Meronts (ME). Both the macrogamont (MA) and the microgamont (MI) were present. Macrogamont (MA), microgaming (MI), and developing oocyst (DO) are present. Staining the sections with hematoxylin and eosin, more commonly referred to as H&E, was accomplished.

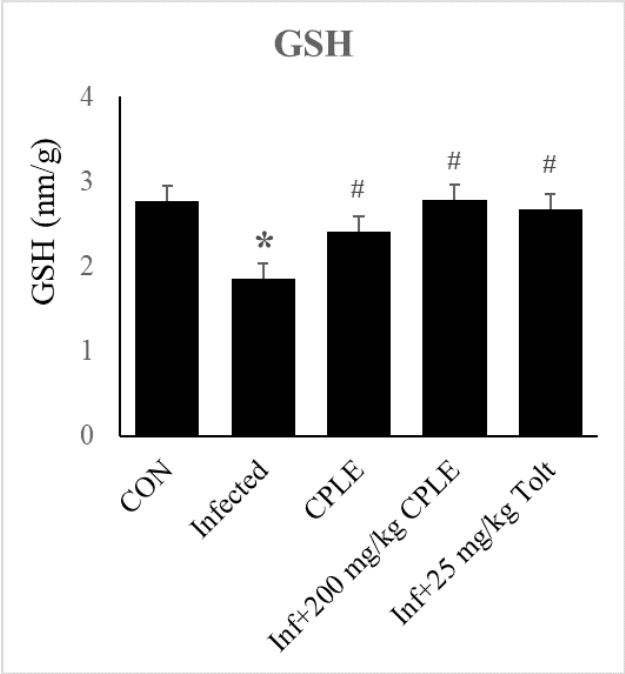

Figure 7. The effect of *C. procera* leaf extracts on the glutathione (GSH) in mice jejunum infected with *E. papillate*. * Significant difference when compared to the control group ($p \leq 0.01$). # Significant difference when compared to the infected group ($p \leq 0.05$). CPLE: *Calotropis procera* leaf extract; CON: control.

nolic ingredients their antioxidant properties,and flavonoids form compounds with metal ions to scavenge free radicals (Kiokias et al., 2020). Gholamshahi and Salehi Sardoei observed that the leaf had the highest Total Phenolic Content (TPC) value, measuring at 9.8 mg GAE/g of dry weight (DW)

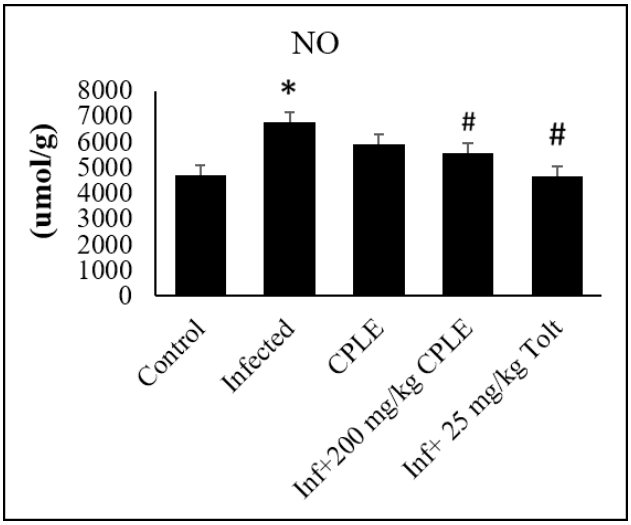

Figure 8. The impact of *C. procera* leaf extracts on nitric oxide (NO) in mice jejunum infected with E. papillate. * Significant difference when compared to the control group ($p \leq 0.01$). # Significant anther groups compared to the infected group ($p \leq 0.05$). CPLE: *Calotropis procera* leaf extract.

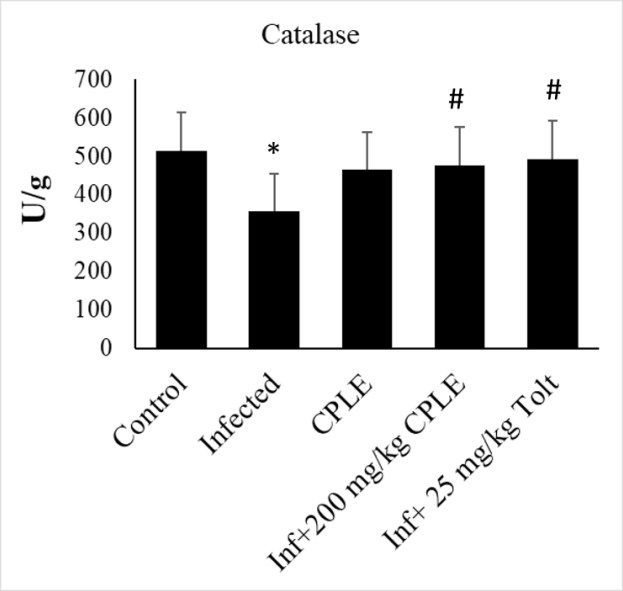

Figure 9. The effect of *C. procera* leaf extracts on the catalase in mice jejunum infected with *E. papillate*. * Significant difference when compared to the control group ($p \leq 0.01$). # Significant difference when compared to the infected group ($p \leq 0.05$ CPLE: *Calotropis procera* leaf extract; Inf: infected.

(Gholamshahi et al., 2019). The quantities of total phenolic compounds (TPC) and total flavonoid compounds (TFC) may be influenced by several factors, including the technique of extraction, type of solvent used, climatic conditions, farming practices, the kind of plant, and the specific portions of the plant used (Mutlu-Ingok et al., 2020). The presence of an elevated ratio of biological substances such as phenolic compounds and flavonoids confers significant industrial and medicinal advantages, facilitating the attainment of economies of scale and enhancing the competitiveness of the agricultural sector (Sarker et al., 2020).

Given the significance of medicinal plants in mitigating cytotoxicity and their potential impact on reducing toxicity and carcinogenesis. The MTT method was used to evaluate the detoxification efficacy of the *C. procera* extract. The MTT technique was utilized by (Mathur et al., 2009). To investigate the cytotoxic effects of *C. procera* extracts derived from methanol on Hep2 cells, a study was conducted. No cytotoxic effects were seen from the *C. procera* extract, as supported by the evidence (Ibrahim et al., 2019).

Compounds with phenolic groups are the most abundant phytochemical components with antioxidant capabilities (Yu et al., 2021). Phenolic compounds, encompassing phenols and flavonoids, are well recognized for their antioxidant properties and possess several significant uses concerning human nutrition and health. Additionally, these compounds have potential in the prevention and treatment of diverse illnesses (Rahman et al., 2021). Several studies have shown that CPLE can treat a wide spectrum of body ailments (Amin et al., 2019). It is generally known that most anti-coccidian therapies reduce intracellular *Eimeria* stages and oocyst shedding. High polyphenolic chemical concentrations in plant extract may have anticoccidial actions (Blake et al., 2020). Because they alter microbe cell walls and membranes' permeability to cations and water, these chemicals are highly antibacterial. Membrane dysfunction and cell component leaking cause cell death (Amin et al., 2019). Previous studies have linked the loss to intestinal epithelial cell carbohydrate content and severe structural defects caused by *Eimeria* parasite stages (Murshed et al., 2022).

These effects were achieved because the plant extract included bioactive substances that improved jejunal histological architecture after therapy. *E. papillate* infection causes significant jejunum inflammation and oxidative damage in mice. CPLE effectively reduced jejunum tissue oxidative damage in sick mice (Huang et al., 2018). Murshed et al, (2023), chronic toxicity experiments on *Vitis vinifera*extract show no negative effects at 200 mg/kg (Murshed et al., 2023). This research indicates that administering a 200 mg/kg dosage had the most efficacy as an anticoccidial treatment. The administration of *C. procera* leaf extract to mice treated with phenols resulted in enhanced immune response (Amer et al., 2022). Additionally, the extract showed significant effectiveness in mitigating cholinesterase activity and oxidative stress in animals with retrograde amnesia (Simonetti et al., 2020). The results of this research indicate that the administration of a dosage of 200 mg/kg had the most efficacy as an anticoccidial treatment. We also found that CPLE disrupted *E. papillate* life cycle and oocyst sporulation at all stages. The jejunum parasite developmental stages and fecal oocyst excretion decreased significantly. Moreover, the abundance of Polyphenols in CPLE has been shown to impede the growth of protozoa by its interaction with cholesterol present on the cell membrane of the parasite. This interaction restricts the parasite's life cycle, ultimately leading to its demise (Zaman et al., 2012). Antioxidant and

anti-inflammatory plant extract components provide CPEL with significant potential.

Coccidian infection disrupts endogenous antioxidant defense and free radical generation (Abdel-Moneim et al., 2022). *E. papillate* infection produces oxidative damage to the mouse jejunum, depleting antioxidant enzymes lowering NO levels, and high GSH, and catalase levels. The oxidative parameters are essential for the animal body to mitigate free radical damage during an *Eimeria* infection. Antioxidant enzymes use the active sites of plant extracts to reduce the harmful effects of reactive oxygen species on the epithelial cells of the jejunum (Elmahallawy et al., 2022). The acronym CPLE refers to the concept or entity being discussed. Further context or information is the intervention significantly mitigated the decline in these indicators caused by infection-induced factors and enhanced their activity, which is typically suppressed in the presence of infection-induced reactive oxygen species (ROS). The process of lipid peroxide oxidation results in the generation of carbonyl compounds, such as nitric oxide (NO) (Hassan et al., 2020). We employed plant extracts to fight E. papillate infection in this investigation. Found that CPLE inhibits parasite stages development. According to Forder, mulberry extract treatment is efficient against Eimeria (Forder et al., 2012). Based on the aforementioned, CPLE is effective against coccidian infections. A reduction in oocyst excretion and sporulation and a decrease in the number of parasite stages present in the jejunum were seen in our study.

CONCLUSIONS

The elimination of parasites is critical to the health of organisms, which in turn can contribute to the development of sustainable production methods that maintain the health of consumers. The study demonstrated that the extract possesses anti-inflammatory properties by reducing parasite eggs during the growth stages within the jejunum. In addition, significant improvements in histological profiles and oxidative status were seen in the jejunum of mice. Further experimental and clinical studies are needed to understand the pharmacological and therapeutic properties of the plant to isolate active compounds. The creation of significant therapeutic medications that are based on the active phytochemical components of *C. procera* will be facilitated.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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