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Research Article
Assessment of Anticoccidial and Antioxidant Efficacy of Methanolic Extract of *Pentaclethra macrophylla* on Rabbits

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Abstract

Background and Objective: Coccidiosis caused by protozoans of the *Eimeria* genus is a serious problem for the health of rabbits (*Oryctolagus cuniculus*) and their production. This study was therefore carried out in order to scientifically validate the use of *Pentaclethra macrophylla* in fighting against Coccidiosis. **Materials and Methods:** A total of 48 domestic rabbits (60 days old and about 1.5-2 kg b.wt.) and free from coccidia infection were used. All groups except group 6 were infected with 1000 sporulated *Eimeria intestinalis* oocysts. Faecal samples were collected and examined starting on day 1 post-inoculation until Day 9 post-inoculation, during which oocysts appeared in faeces. For quantitative analysis or determination of the number of oocysts per gram (OPG) of faeces, the Mc Master technique was used. Serum was used for determination of biochemical parameters related to oxidative stress such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), malondialdehyde (MDA), nitric oxide (NO) and glutathione using standardized diagnostic kits and a spectrophotometer. **Results:** The highest oocyst count reduction rate was 95% in the group which received the standard anti-coccidial drug. Among the groups that received the plant extract, the highest oocyst reduction rate was 69.41% at a dose of 500 mg kg^{-1} and reduced in a dose-dependent manner from 58.92% (250 mg kg^{-1}) and then 55.01% (125 mg kg^{-1}). The result showed a significant decrease in tissues and serum catalase and peroxidase and a significant increase in NO, MDA and GLU levels in negative control animals. Treatment resulted in a significant normalization of the levels of the above markers when compared with the neutral control group. **Conclusion:** Our findings, therefore scientifically validate the use of *P. macrophylla* in fighting against Coccidiosis.

Key words: *Pentaclethra macrophylla*, anticoccidial, antioxidant, *Eimeria intestinalis*, coccidiosis

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Coccidiosis constitute a major parasitic disease in poultry and other domestic animals, including rabbits. Coccidial infection is initiated by oral ingestion of sporulated oocysts by the susceptible host and the infection can lead to clinical coccidiosis primarily in kits, whereas adults are mostly healthy carriers[1]. Eleven distinct *Eimeria* sp. have been identified in rabbits (*Oryctolagus cuniculus*), with 10 species colonizing the intestinal tract and one species (*Eimeria stiedae*) infecting the biliary ducts of the liver[2]. This disease occurs in two forms, hepatic and intestinal, the latter being more common than the former[3]. According to their pathogenicity, species responsible for rabbit intestinal coccidiosis can be classified into four types. These are non-pathogenic (*Eimeria coecicola*), slightly pathogenic (*Eimeria exigua*, *Eimeria perforans*, *Eimeria vejdovskyi*), mildly pathogenic or pathogenic (*Eimeria irresidua*, *Eimeria magna*, *Eimeria media*, *Eimeria piriformis*) and highly pathogenic (*Eimeria intestinalis*, *Eimeria flavescens*)[4]. Hepatic coccidiosis (*Eimeria stiedae*) is one of the most pathogenic coccidian protozoans in domestic rabbits causing severe coccidiosis and increased mortality[5]. Most of these *Eimeria* sp. affect rabbit production and, according to their level of pathogenicity can cause reduced growth rate, feed conversion and increased mortality. Coccidiosis remains one of the most important infectious causes of digestive disorders in rabbits[6]. According to Chapman[7], coccidiosis may cost the United States rabbit industry about $127 million annually. Thus, coccidiosis is probably the most expensive and wide spread infectious disease in commercial rabbit systems. Conventional disease control strategies have relied mainly on chemoprophylaxis, which is very expensive[8]. Furthermore, the continuous use and misuse of anticoccidial drugs has led to the emergence of drug resistant *Eimeria* parasites[9]. The use of safe and effective medicinal plants can reduce farmer input costs, preserve the resource base and protect animal health[10]. Scientists all over the world are shifting towards alternative approaches for the control of parasitic problems[11].

There is a close relationship between oxidative stress and coccidiosis. According to Cedric *et al.*[12,13], when a healthy animal ingest sporulated oocysts these parasites goes at the level of the intestine or liver (in the case of hepatic coccidiosis) and causes inflammations. The inflammation process lead to the over production of free radicals. These free radicals are not only toxic to the parasites they are equally toxic to the host by causing lipids peroxidations, damaging DNA and proteins. Today, the use of antioxidants as anticoccidial remedies, therefore, holds promise as an alternative in the control of coccidioses[12,13]. Then, in fighting coccidiosis, a drug with both anticoccidial effect and antioxidant activity can be more efficient, which is not the case with currently used anticoccidial drugs.

It is in this context that the Research Unit of Biology and Applied Ecology of the Department of Animal Biology at the University of Dschang has set up a research programme for several years, in which one of the aims is to promote medicinal plants used in the treatment of coccidiosis. Among the plants already identified, several have been the subject of anticoccidial studies. These include the leaf of *Kalanchoe crenata*[14], *Carica papaya*[15] and *Psiduim guajava*[12,13].

In Dschang (West Region of Cameroon), the stem bark of *P. macrophylla* is used by farmers to treat bacterial infections as gonorrhoea, syphilis and typhoid, protozoan diseases such as coccidiosis and malaria. Antimicrobial property and the oil extracted from the seeds are used in the preparation of formulations against pruritus, intestinal worms and dysentery[16]. It is on the basis of the traditional use of the stem bark of *Pentaclethra macrophylla* as an anticoccidial agent that we found it necessary to scientifically validate the use of *P. macrophylla* in fighting against Coccidiosis.

MATERIALS AND METHODS

Plant material: The stem bark of *Pentaclethra macrophylla* was collected (March 2014) in Melong Littoral Region of Cameroon and identified by Mr. NGANSOP Eric, a Botanist at the Cameroon National Herbarium (Yaoundé) using a voucher specimen registered under the Reference No. 2328/SRF Cam.

Preparation of plant extracts: Methanol extract was obtained using the procedure described by Josue *et al.*[17]. The stem bark of *P. macrophylla* was air-dried at room temperature under shade in the Research Unit of Biology and Applied Ecology. The dried stem bark were pulverized using an electrical grinder under strict hygienic conditions. One hundred grams of plant powder were macerated in 1.5 L of the organic solvent. The mixture was stirred daily and 72 h later, the resulting solutions were then filtered using Whatman Paper N°3. The filtrate was concentrated by evaporating the solvent using a rotatory evaporator (Buchi R-200) to obtain the extract.

***In vivo* anti-coccidial evaluation of extracts:** The methanol extract of *P. macrophylla* was used to carry out the *in vivo* test. A total of 48 domestic rabbits (60 days old and about 1.5-2 kg b.wt.) and free from coccidian *Eimeriaintestinalis* oocysts, infection were used. The experimental groups were arranged as follows:

Group 1: Infected and treated with the extract of *P. macrophylla* at 500 mg kg^{-1} of body weight
Group 2: Infected and treated with the extract of *P. macrophylla* at 250 mg kg^{-1} of body weight
Group 3: Infected and treated with the extract of *P. macrophylla* at 125 mg kg^{-1} of body weight
Group 4: Infected and treated with amprocox at 5 mg kg^{-1} body weight
Group 5: Infected and treated with 2% DMSO
Group 6: Non infected-non treated (negative control)

All groups except group 6 were inoculated with 1000 sporulated *Eimeria intestinalis* oocysts.

Parasitological examination: Faecal samples were collected from each of the above groups and examined starting on day one post-inoculation until day 9 post-inoculation, during which oocysts appeared in faeces. For quantitative analysis or determination of the number of oocysts per gram (OPG) of faeces, the Mc Master technique described by Thienpont *et al.*[18] was used.

Parameters studied

Oocysts reduction rate: Oocysts reduction (%) was determined as follows:

$$\text{Ocyst reductionrate } (\%) = \frac{\text{Initialmean OPG - Finalmean OPG}}{\text{Initial mean OPG}} \times 100$$

Feed consumption: Feed consumption of the animals was determined every day by making the difference between the weight of initial food and that of remaining food.

Average daily weight gain: The daily weight gained of each animal was measured using a balance:

$$\text{AWG} = \frac{\text{Weight gain}}{\text{No. of animals}}$$

Feed conversion ratio: The feed conversion ratio was determined using the formula:

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Mean total feed consumed}}{\text{Total weight gain for a particular period}}$$

where, Total feed consumed is cumulative feed from day 1 to day 7, mean total feed consumed is total feed consumed /No. of animals, weight gain is body weight on day 7 b.wt., on day 1 and growth rate (from day 1-7) is AWG/ 7 days.

Mortality: Mortality (%) was recorded as follows.

$$\text{Mortality (\%)} = \left(\frac{\text{Number of death rabbits at the end of the experiment}}{\text{Total numberof rabbits}}\right) \times 100$$

***In vivo* antioxidant activity:** At the end of the treatment which lasted for seven days, animals were subjected to a 12 h food fasting and their blood samples were collected by cardiac puncture from chloroform vapors anaesthetized rabbits into sterilized tubes. The tubes were allowed to clot and centrifuged at 3000 rpm for 10 min and the supernatant (serum) was collected. Blood samples were collected in heparinised vacutainer tubes for hematological analysis. The animals were further dissected and intestines removed. The harvested tissue (intestine for therapeutic efficacy) was rinsed with phosphate buffered saline (PBS) and blotted with filter paper and weighed. Fifteen percent homogenate of the intestine was prepared in 0.1 M phosphate buffer, pH 7.4 and then centrifuged at 3000 rpm for 15 min. The supernatant and sera were used for the determination of biochemical parameters related to oxidative stress such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), malondialdehyde (MDA), nitric oxide (NO) and Glutathione.

Evaluation of biochemical antioxidant parameters

Enzymatic parameters

CAT (catalase, EC 1.11.1.6) activity: Catalase activity was determined in tissue and serum using the method described by Fodouop *et al.*[19] method with some modifications. A total of 25 µL of the homogenate were added to tubes containing 375 µL of phosphate buffer (pH 7.4) and 100 µL of 50 mmol L^{-1} H_2O_2. After 1 min incubation at room temperature, 1 mL of dichromate (5%) prepared in acetic acid at 1% was added. The mixture was homogenized and incubated at 100°C for 10 min then cooled in ice bath and the absorbance was recorded at 570 nm using Schimadzu 1501 spectrophotometer Japan. One unit of activity is equal to 1 mmol L^{-1} of H_2O_2 degraded per minute and is expressed as units per milligram of protein.

POD (Peroxidase EC 1.11.1.7) assay: Peroxidase activity was determined in tissues and serum using the Habbu *et al.*[20] method. To 0.5 mL tested sample were added 1 mL of 10 mM potassium iodide solution and 1 mL of 40 mM sodium acetate. The absorbance of potassium iodide was read at 353 nM which indicates the amount of peroxidase. Then 20 µL of H_2O_2

(15 mM) was added and the change in the absorbance in 5 min was recorded. Units of peroxidase activity were expressed as the amount of enzyme required to change the optical density by 1 unit per min. The specific activity was expressed in terms of units per mg of proteins.

Non enzymatic parameters

Malondialdehyde (MDA) assay: The extent of peroxidation in tissues and serum was assessed by measuring the level of malondialdehyde (MDA) according to the method of Fodouop *et al.*[19] with some modifications and peroxidation in the tissues was calculated based on the molar extinction coefficient of malondialdehyde (MDA) (153 mM^{-1} cm^{-1}) and expressed in terms of micromoles of MDA/g of tissue.

Nitric oxide (NO) assay: To 340 µL of the experimental sample, 340 µL of freshly prepared 1% sulfanilamide in 5% orthophosphoric acid were added after 5 min of incubation in the dark at room temperature, 340 µL of the NED (N-(1-Naphthyl) ethylenediamine) solution (0.1% NED in water) were also added. The resulting solution was well mixed and then incubated at room temperature for 5 min protected from light. The absorbency of the colored azo compound formed was measured at 520 nm within 30 min. A standard curve was plotted using nitrite ($NaNO_2$) (100, 50, 25, 12.5, 6.25, 3.13 and 1.56 µM). The results were expressed as Micromolar of Nitrite Equivalents (µMNE) per gram (g) of tissue or per milliliter of blood.

Reduced GSH activity: The GSH was determined in tissue by the Oyedemi *et al.*[21] method with some modifications. A total of 0.8 mL of 0.3 mol L^{-1} dihydrate sodium phosphate solution was added to 0.2 mL of homogenate. It was centrifuged at 5000×g for 5 min and 0.5 mL of 0.4 mg mL^{-1} dithiobis nitrobenzoate (prepared in 1% sodium citrate) was added to the supernatant. The optical density was recorded at 412 nm. The total GSH was calculated based on the molar extinction coefficient of GSH (1.36×105 mM^{-1} cm^{-1}) and expressed in terms of micromoles of GSH/g of tissue.

Ethical consideration: Experimental protocol used in this study strictly conformed with internationally accepted standard ethical guidelines for laboratory animal use and care as described in the European community guideline, EEC Directives 86/609/EEC, of the 24th November, 1986[22].

Statistical analysis: Results were expressed as Mean±standard error of mean (S.E.M.). Within group, comparisons were performed using ANOVA one way test. Significant difference ($p \leq 0.05$) between control and experimental groups was assessed using Waller Duncan test.

RESULTS

***In vivo* anti-coccidial evaluation:** Table 1 revealed that *Eimeria*-infected Rabbits treated with methanolic extract in different groups showed a significant reduction in oocyst count between treatment groups. The oocyst counts in the treated group with distilled water increased continuously from the initial count on Day 0 as opposed to the treated groups. The highest oocyst count reduction rate was 95% in the group which received the standard anti-coccidial drug. Among the groups that received the plant extract, the highest oocyst reduction rate was 69.41% at a dose of 500 mg kg^{-1} and

Table 1: Mean oocyst counts per gram (Means±standard deviation) and faecal oocyst count reduction(%) in rabbits treated with methanolic extracts of *P. macrophylla* as a function of doses and days

	Oocyst counts at different days of treatment			
Treatments	Day 0 (Initial)	Day 1	Day 4	Day 7 (Final)
500	7084.43±743.36^{aD}	6977.76±601.23^{aC}	5600±676.6^{aB}	2166.66±166.66^{abA}
	(0%)	(1.51%)	(20.95%)	(69.41%)
250	7466.66±1353.2^{aB}	7266.66±1466.66^{aB}	5511.1±1202.46^{aAB}	3066.66±584.03^{bA}
	(0%)	(2.68%)	(26.19%)	(58.92%)
125	7088.9±2696.5^{aB}	6688.9±1702.73^{aB}	5088.9±1108.23^{aB}	3188.9±816.73^{bA}
	(0%)	(5.64%)	(28.21%)	(55.2%)
ITD	7577.766±2505.4^{aA}	6744.43±2450.46^{aA}	10222.23±3166.73^{cAB}	12377.76±2329.83^{cB}
	(0%)	(10.99%)	(-34.89%)	(-63.34%)
ITA (5 mg kg^{-1})	7084.43±743.36^{aC}	6977.76±601.23^{aC}	5600±676.6^{aB}	2166.66±166.66^{abA}
	(0%)	(14.5%)	(74.72%)	(95.9%)
NNT	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	(0%)	(0%)	(0%)	(0%)

ITD: Infected and treated with DMSO, ITA: Infected and treated with Amprocox at 5 mg kg^{-1} and NNT: Non infected-non treated. Values are Mean±SEM. a,b,c: For the same column, values carrying the same superscript letter are not significantly different at p>0.05 (Student-Newman-Keuls test). A,B,C: For the same row and same concentrations values carrying the same superscript letter are not significantly different at p>0.05 (Student-Newman-Keuls test)

reduced in a dose-dependent manner from 58.92% (250 mg kg^{-1}) and then 55.01% (125 mg kg^{-1}).

Effects of the methanolic extract on growth parameters of rabbit: The growth parameters of *Eimeria*-infected rabbits treated with methanolic *P. macrophylla* extract are presented in Table 2. From this Table 2, it appeared that there were no significant difference in the mean total feed consumed between the different groups receiving the metabolic *P. macrophylla* extract at the dose of 500 and 250 mg kg^{-1} when compared to the group receiving Amprocox ($p>0.05$). Among extract treated groups, the maximum weight gain was shown by the group treated with 500 g followed by the groups treated with 250 and 125 mg kg^{-1} of body weight. There was no significant ($p>0.05$) difference between the weight gains of groups treated with *P. macrophylla* methanolic extract and that of amprocox. The FCR values of the treated groups were numerically lower compared with the infected non-treated groups receiving DMSO. Among the treated groups, the lowest FCR was observed in the group treated with 250 mg of methanolic extract per body weight followed by the groups treated with 125 and 500 mg kg^{-1}.

Table 2: Mean total feed consumed, total weight gain and feed conversion ratio (Mean±SD) of rabbits treated with methanolic *P. macrophylla* as a function of doses

	Pentaclethra macrophylla		
Treatments	MTFC	TWG	FCR
500	384.0±2.6^{c}	129.12±4.25^{ab}	2.97^{a}
250	350.8±5.9^{b}	126.96±1.15^{ab}	2.76^{a}
125	324.0±2.9^{a}	112.00±2.13^{a}	2.89^{a}
ITD	384.0±2.6^{c}	84.16±2.37^{a}	4.56^{b}
ITA (5 mg kg^{-1})	369.2±0.6^{bc}	133.68±3.11^{ab}	2.76^{a}
NNT	414.8±1.2^{d}	149.28±0.33^{b}	2.77^{a}

ITD: Infected and treated with DMSO, ITA: Infected and treated with Amprocox at 5 mg kg^{-1}, NNT: Non infected-non treated, MTFC: Mean total feed consumed, TWG: Total weight gain and FCR: Feed conversion ratio. Values are Mean±SD. For the same column, values carrying the same superscript letter are not significantly different at $p>0.05$ (Student-Newman-Keuls test)

Effects of the methanolic extract on mortality rate: Table 3 presented the effect of treatment on mortality (%) in Rabbits. It follows from the analysis of this table that infection increase the mortality rate (negative control). The administration of the extract to the infected rabbits reduced mortality in a dose dependent manner. Among treated groups, the mortality was numerically lower in Amprocox treated group compared with the other treated groups. Among the extracts, treated groups maximum mortality was observed in 125 mg kg^{-1}.

Effect of treatment on transaminases (ALT and AST),serum creatinine, blood urea nitrogen, bilirubin and total proteins: The recorded data in Table 4 revealed that, administration of sporulated oocysts of *E. intestinalis* to normal rabbits exhibited a significant increase in serum ALT and AST activities after 7 days of infection when compared to the normal control group. There was a significant difference ($p<0.05$) in serum AST activities, when compared with the normal control. Treatment with *P. macrophylla*at the dose of 500 mg kg^{-1} showed a hyper-creatinine level compared to the controls. It appears that infection resulted in an increase of total and direct bilirubin as compared to neutral control. Administration of different doses of *P. macrophylla* led to a decrease in total and direct bilirubin compared to the negative control. It appears that there was a significant decrease ($p<0.05$) in total protein levels in the infected non-treated group when compared to the normal control, except the dose of 125 mg kg^{-1} of *P. macrophylla* which had no significant difference when compared with the negative control.

Effects of the methanolic extract on haematological parameters: The haematological parameters for *Eimeria*-infected rabbits treated with methanolic extract of *P. macrophylla* were presented in Table 5. Analysis of blood parameters after treatment shows that, apart from the red

Table 3: Effect of treatment on mortality (%) in Rabbits experimentally inoculated with sporulated oocysts of *Eimeria intestinalis*

		Mortality days during treatment								
Plant	Groups and doses	1	2	3	4	5	6	7	Total mortality	Mortality (%)
Pentaclethra macrophylla	125 mg	0	0	1	2	0	0	0	3	37.5^{a}
	250 mg	0	0	1	1	0	0	0	2	25.0^{a}
	500 mg	0	0	0	1	0	0	0	1	12.5^{a}
Positive control	ITA	0	0	0	0	0	0	0	0	0.0^{a}
Negative control	ITD	0	0	1	1	1	2	0	5	62.5^{a}
Normal control	NNT	0	0	0	0	0	0	0	0	0.0^{a}
	P									0.423

ITD: Infected and treated with DMSO, ITA: Infected and treated with Amprocox at 5 mg kg^{-1} and NNT: Non infected-non treated. Number of animal per group = 8, For the same column, values carrying the same superscript letter are not significantly different

Table 4: Effect of treatment on transaminase (ALT and AST), serum creatinine, blood urea nitrogen, bilirubin and total proteins parameters as a function of treatment

Plants	Groups and doses	AST (UI/L)	ALAT (UI/L)	Creatinine (mg dL^{-1})	BUN (mg dL^{-1})	Total bilirubin (mg dL^{-1})	Direct bilirubin (mg dL^{-1})	Total protein (mg mL^{-1})
P. macrophylla	125 mg	17.72±1.21^{b}	3.27±0.41^{ab}	5.02±0.13^{cd}	55.41±0.13^{c}	1.27±0.02^{c}	0.63±0.01^{c}	5.82±0.54^{a}
	250 mg	21.54±1.27^{bc}	3.05±0.20^{ab}	4.73±0.21^{c}	43.55±0.12^{b}	0.13±0.00^{a}	0.01±0.01^{a}	8.36±0.66^{b}
	500 mg	17.37±0.21^{b}	4.43±0.21^{b}	6.23±0.21^{e}	37.31±0.12^{a}	0.29±0.14^{ab}	0.19±0.02^{ab}	11.00±1.22^{c}
Positive control	ITA	10.08±1.31^{a}	3.27±0.31^{ab}	1.50±0.25^{a}	64.40±0.05^{d}	0.16±0.05^{a}	0.10±0.00^{ab}	8.80±0.31^{b}
Negative control	ITD	31.22±1.11^{c}	5.04±0.42^{b}	5.88±0.29^{d}	43.22±0.39^{b}	3.12±0.00^{d}	0.96±0.04^{d}	4.45±0.12^{a}
Normal control	NNT	10.58±1.04^{a}	2.06±006^{a}	1.03±0.13^{a}	36.48±0.14^{a}	0.15±0.09^{a}	0.06±0.07^{a}	15.24±0.70^{d}

ITD: Infected and treated with DMSO, ITA: Infected and treated with Amprocox at 5mg kg^{-1} and NNT: Non infected- non treated, BUN: Blood urea nitrogen, For the same column, values carrying the same superscript letter are not significantly different at p>0.05

Table 5: Effect of methanolic extraction red blood cells and white blood cells as a function of treatment

	P. macrophylla					
Treatments	RBC ($X10^{6}$/µL)	HGB (g dL^{-1})	HCT (%)	MCV	MCH (pg)	MCHC (g dL^{-1})
125	6.28±0.09^{ab}	12.30±0.43^{ab}	44.90±3.30^{c}	63.06±2.30^{ab}	18.43±0.76^{ab}	29.30±0.65^{b}
250	6.57±0.25^{ab}	12.83±0.49^{ab}	43.20±4.19^{c}	67.76±1.84^{ab}	20.16±0.32^{b}	29.96±0.76^{b}
500	6.35±0.24^{ab}	13.30±2.17^{b}	38.23±1.07^{b}	68.73±4.61^{ab}	21.00±1.38^{b}	30.56±0.11^{b}
ITD	5.74±0.10^{a}	11.83±1.25^{a}	45.60±3.63^{c}	75.86±4.41^{b}	17.40±0.87^{a}	20.33±0.83^{a}
ITA	6.66±0.19^{b}	12.83±0.55^{ab}	40.36±2.37^{c}	73.63±1.73^{ab}	20.83±1.06^{b}	29.80±1.58^{b}
NNT	7.12±0.26^{c}	13.16±0.83^{b}	32.5±1.22^{a}	64.80±1.70^{a}	19.73±1.42^{ab}	30.50±1.90^{b}
	P. macrophylla					
Treatments	WBC ($X10^{3}$/µL)	LYM (%)	Monocytes (%)	Granulocytes (%)	Platelets (X 10^{3}/µL)	MPV (fl)
125	6.06±2.83^{ab}	93.16±2.95^{b}	3.16±0.28^{c}	7.80±1.10^{c}	255.66±8.96^{a}	7.00±0.10^{a}
250	5.73±1.06^{ab}	90.10±1.21^{ab}	1.76±0.11^{ab}	3.40±0.17^{a}	245.66±15.04^{a}	7.36±0.28^{a}
500	6.70±1.21^{ab}	94.30±0.51^{b}	2.53±0.28^{abc}	3.73±0.23^{a}	464.00±29.44^{a}	8.26±0.23^{bc}
ITD	7.10±0.60^{b}	95.36±0.70^{b}	3.63±0.15^{c}	6.03±0.20^{bc}	171.00±40.44^{a}	8.66±0.37^{c}
ITA	5.43±0.32^{a}	90.63±2.51^{ab}	2.53±0.32^{abc}	4.53±0.35^{a}	235.00±34.58^{a}	7.33±0.45^{c}
NNT	4.76±0.68^{a}	86.23±5.09^{a}	1.63±0.28^{a}	2.86±0.23^{a}	236.33±18.50^{a}	7.40±0.40^{a}

ITD: Infected and treated with DMSO, ITA: Infected and treated with Amprocox at 5 mg kg^{-1} and NNT: Non infected-non treated, HCT: Hematocrit, HGB: Hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, MCV: Mean corpuscular volume. For the same column, values carrying the same superscript letter are not significantly different at p>0.05

Table 6: Effect of treatment on superoxide dismutase, catalase and total peroxidase in the serum and tissues (intestine) as a function of extract doses

		Superoxide dismutase		Catalase		Total peroxidase	
Plant	Groups and doses	Serum (µmole mL^{-1})	Intestine (µmole g^{-1})	Serum (µmol/min/mL)	Intestine (µmol/min/g)	Serum (µmol/min/mL*10^{-2})	Intestine (µmol/min/g)
Pentaclethra macrophylla	125 mg	0.999±0.00^{d}	3.129±1.71^{a}	0.260±0.005^{b}	14.743±1.541^{b}	2.527±1.303^{a}	0.578±0.490^{ab}
	250 mg	0.922±0.00^{c}	2.872±0.89^{a}	0.261±0.047^{b}	20.419±1.229^{c}	2.830±0.221^{a}	0.573±0.505^{ab}
	500 mg	0.629±0.10^{a}	1.957±0.23^{a}	0.326±0.035^{c}	20.335±1.050^{c}	3.238±1.009^{a}	0.784±0.069^{b}
Positive control	ITA	0.999±0.00^{d}	1.913±0.39^{a}	0.317±0.011^{c}	18.093±1.732^{b}	3.250±1.425^{a}	0.729±0.553^{b}
Negative control	ITD	1.190±0.01^{e}	2.510±0.87^{a}	0.213±0.013^{a}	10.028±1.57^{a}	2.076±1.151^{a}	0.366±0.114^{a}
Normal control	NNT	0.559±0.00^{a}	2.848±0.81^{a}	0.375±0.054^{d}	21.876±1.507^{bc}	3.448±1.881^{ab}	0.957±0.095^{c}

ITD: Infected and treated with distilled water, ITA: Infected and treated with Amprocox at 5 mg kg^{-1}, NNT: Non infected-non treated for the same column, values carrying the same superscript letter are not significantly different at p>0.05

blood cells, hemoglobin, mean corpuscular haemoglobin concentration (MCHC) and the mean corpuscular haemoglobin (MCH) which increased in a dose-dependent manner, in the extract treated groups and white blood cells, lymphocytes and granulocyte which increase in the infected non treated group the rest of the hematological parameters did not show any significant treatment related variation.

In vivo antioxidant activities of extracts

Enzymatic antioxidant parameters

Effect of treatment on superoxide dismutase, catalase and total peroxidase activity: From Table 6, it appeared that infection resulted in an increase in tissues and serum SOD activity compared to the neutral control. Administration of different extracts at different doses

Table 7: Effect of treatment on malondialdehyde, nitric oxide and reduced glutathione as a function of extract doses

		Malondialdehyde		Nitric oxide		Reduced glutathione	
Plant	Groups and doses	Serum ($\mu M\ mL^{-1}$)	Intestine ($\mu M\ g^{-1}$)	Serum ($\mu mole\ mL^{-1}$)	Intestine ($\mu mole\ g^{-1}$)	Serum ($\mu mole\ mL^{-1}$)	Intestine ($\mu mole\ g^{-1}$)
Pentaclethra macrophylla	125 mg	0.009 ± 0.000^{f}	0.030 ± 0.001^{d}	5.321 ± 0.120^{b}	0.388 ± 0.032^{a}	0.976 ± 0.66^{a}	3.976 ± 0.67^{b}
	250 mg	0.008 ± 0.000^{e}	0.022 ± 0.003^{c}	5.769 ± 0.199^{b}	0.394 ± 0.020^{a}	0.681 ± 0.13^{a}	1.894 ± 0.38^{a}
	500 mg	0.006 ± 0.001^{cd}	0.013 ± 0.002^{b}	4.928 ± 0.253^{ab}	0.366 ± 0.091^{a}	0.684 ± 0.13^{a}	1.397 ± 0.54^{a}
Positive control	ITA	0.004 ± 0.000^{b}	0.013 ± 0.004^{b}	4.601 ± 1.412^{ab}	0.342 ± 0.097^{a}	0.591 ± 0.05^{a}	1.476 ± 0.61^{a}
Negative control	ITD	0.065 ± 0.005^{g}	0.160 ± 0.107^{e}	8.743 ± 0.987^{c}	0.498 ± 0.055^{b}	1.087 ± 0.57^{a}	7.049 ± 1.32^{c}
Normal control	NNT	0.009 ± 0.000^{f}	0.030 ± 0.001^{d}	4.003 ± 0.575^{a}	0.367 ± 0.027^{a}	0.599 ± 0.03^{a}	1.913 ± 0.39^{a}

ITD: Infected and treated with distilled water, ITA: Infected and treated with Amprocox at 5 $mg\ kg^{-1}$, NNT: Non infected-non treated for the same column, values carrying the same superscript letter are not significantly different at $p>0.05$

resulted in a decrease in the rate of serum SOD activity compared to the negative control.

Infection resulted in a significant ($p<0.05$) decrease in the catalase activity in all the groups compared to normal control. However, treatment with the various doses of extract resulted in significantly higher ($p<0.05$) tissues and serum catalase level when compared to the negative control rabbits. The result showed a decreased POD level, in the tissues of the negative control animals compared to the normal control groups. However, the treatment with the various doses of extract increased the tissues and serum peroxidase level when compared with the negative control rabbits.

Non enzymatic antioxidant parameters

Effect of treatment on malondialdehyde (MDA), nitric oxide (NO) and reduced glutathione (GSH): Table 7 showed the effect of treatment on serum and tissues concentration of malondialdehyde. It followed from the analysis of Table 7 that significantly increased levels of MDA ($p<0.05$) were observed in tissues and serum negative control compared to the normal control and the animals receiving different doses. Infection resulted in an increase in the tissues and serum NO compared to normal control. This increase persisted in the untreated group and decreased with the administration of different doses of *P. macrophylla* extract. It follows from the analysis of this Table that infection resulted in an increase in tissues and serum GSH. Even though not significant in the serum, the administration of different extracts at different doses resulted in a dose dependent decrease in the rate of tissues and serum GSH compared to the negative control.

DISCUSSION

In the present study, higher doses of the methanolic extract of *P. Macrophylla* administered have shown good anticoccidial activity in rabbits challenged with *Eimeriaintestinalis* by means of improved weight gains, better feed conversion ratio and lower oocyst count. There was a significant reduction in faecal oocyst count with an increase in *P. macrophylla* doses. The reduction in oocyst count probably indicates that *P. macrophylla* extracts impair the development of parasites in the host before the relatively inert oocysts are formed and finally released. However, the lowest oocysts per gram was recorded in amprocox treated groups in the experiments indicating the highest prophylactic efficacy among all groups. Similar results were reported by Cedric *et al.*[13] on *Eimeria intestinalis* in rabbits.

The highest feed conversion ratio reported in infected rabbits resulted in a significant reduction in the body weight. The loss of body weight may be due to excessive loss of body water i.e., diarrhea, which contribute substantially to body weight. The study revealed that groups of rabbits not infected with coccidial oocysts consume more feed, while infected groups showed lower feed intake due to coccidial stress. Seddiek and Metwally[23] and Cedric *et al.*[13] also reported a significant reduction in body weight in rabbits infected with oocysts of *E. stiedae* and *E. intestinalis*. The effect of infection on growth performance may be related to the degree of infection. Under conditions of more severe infection with *Eimeria*, weight gain is generally reduced[24].

No mortality was observed in un-infected un-medicated control group because no infection was given to the rabbits of this group. While in groups medicated with *P. macrophylla* extract mortalities were present and the highest mortality (62.5%) was recorded in the infected non-treated group. In the extracts medicated groups mortality rate was inversely proportional to the dose of the extracts this is an indication of the therapeutic activity of the extracts these results are in line with the results described by Seddiek and Metwally[23].

This research showed that infection of *E. intestinalis* oocysts in rabbits infected with 1×10^{3} oocysts caused a decrease of erythrocytes and haemoglobin concentrations as well as an increase of MCV, leukocytes and lymphocytes compared to the neutral group. This data showed that rabbits

infected with *E. intestinalis* with a dose of 1×10^3 oocysts suffered from anemia. Anemia that occurred in rabbits in this study was probably due to damage of the intestinal mucosal epithelium and blood vessels by *E. intestinalis*. A decrease in the number of erythrocytes and hemoglobin was reported in rabbits suffering from hepatic coccidiosis[25]. Leukocytosis (increase in the number of white blood cells) is part of a complex clinical intestinal coccidiosis feature, where most leukocytes will be transported to areas of inflammation to defend against *E. intestinalis* infection. Leukocytosis was also reported in chickens, dogs and sheep, during acute phase of *Eimeria* sp. Infection[26]. Rabbits are very vulnerable to stress, and leukocytosis can be seen during stress due to illness[27]. Lymphocytes play an important role in the immunologic response. Functional tissue associated with immunity against coccidiosis is the gut associated lymphoid tissue (GALT) located in the intestine, along the mucosal layer and lamina propria. This lymphocyte forms an obstacle against the infection and takes part in the formation of antibody during development of immunity against coccidiosis[28]. The large number of lymphocytes that migrate actively to the mucosa of intestine confirms the presence of physiological response due to stimulation of sporozoites of *E. intestinalis* that damages the intestine. Blood platelets are implicated in blood clotting. Low platelet concentration suggested that the process of clot-formation (blood clotting) will be prolonged resulting in excessive loss of blood in the case of coccidiosis. The increase number of platelets in treated groups is probably due to the fact that *P. macrophylla* have immune stimulatory properties and it is this property that it uses to raise platelets against coccidial infections[28]. Since platelets are raised to stop haemorrhage which is as a result of the parasitic infection, it is therefore suggested that this is the reason for the high number of platelets in the treated groups.

From the results of the safety study, the reduction in AST and ALT activities compared to control shows that extract of *P. macrophylla* are not hepatotoxic. Infection with *E. intestinalis* parasites in rabbits showed highly significant increase in serum ALT and serum AST level as compared with control group. Similarly, Al-Mathal[5] reported that, infected rabbits with *Eimeria stiedae* showed a significant increase in the serum ALT, AST. Increase in serum AST and ALT activities may be due to hepatocellular damage[29].

Infection with coccidia parasites in rabbits exhibited a significant decrease in serum total protein as compared with control group. These results corroborate with the findings of Seddiek and Metwally[23]. Mondal *et al.*[30] suggested that, a fall in total plasma protein (hypo-proteinemia) in coccidia infected birds might be due to acute stress that leads to cortisol secretion and catabolism of protein.

Administration of *P. macrophylla* extract to apparently healthy rabbit's significantly reduced ($p<0.05$) direct bilirubin and total bilirubin across the groups treated with the extracts, compared to the negative control. Increased bilirubin in infected and untreated animals is a body response to fight against free radicals whose formation was induced by the infection. Indeed several studies have shown that free and bound bilirubin are potent radical scavengers and also protect human cells from lipid peroxidation[31]. These results corroborate those of Yamaguchi *et al.*[32], which suggested that bilirubin serves as a physiological antioxidant in ischemia-infusion *In vivo.* Serum creatinine is a specific indicator for renal function. The decrease in serum creatinine and urea levels across the dose levels when compared to the control shows that the extracts are not nephrotoxic.

The increased level of NO in the tissues of negative control animals suggests that macrophages have excessively produced that compound (NO) to destroy *E. intestinalis.* There was an imbalance between prooxidants and antioxidants. Higher NO and MDA levels in infected rabbits are probably due to oxidative stress occurring after coccidial infection. Similar results on higher NO and MDA levels in parasitic diseases have been reported by others including[13,33]. This result is similar to the findings of Cedric *et al.*[13] who showed that *Psidium guajava* treatment lowered MDA and NO. The increase of malondialdehyde (MDA) level in organs and serum induced by infection suggests increased membrane peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals.

Treated groups with 250 and 500 mg kg^{-1} *P. macrophylla* extract generally showed significant increase in CAT activity, and this could be responsible for the cure effect of the extract. In fact, administration of the methanolic extract to treated rabbits enhanced catalase and peroxidase profiles, dose-dependently, by acting as a strong free radical quencher and protecting the tissues. Therefore, peroxidase and catalase are essential for the endogenous antioxidative defense system to scavenge reactive oxygen species and maintain the cellular redox balance. Catalase (CAT) and peroxidase (POD) are enzymatic antioxidants widely distributed in all animal tissues and the highest activity is found in the red blood cells. The CAT and POD decompose hydrogen peroxide and protect the tissues from highly reactive hydroxyl radicals[34]. Therefore, reduction in the activity of CAT and POD in negative control animals may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide. The decrease in GSH levels in animals treated with different

extracts might be due to the free radical's neutralizing activities of this extract. They could have reactivated the hepatic GSH reductase which is reflected by decreasing the level of lipid peroxidation. Reduced GSH is an enzymatic biological antioxidant mostly present in the liver, which protects cellular proteins against reactive oxygen species generated from exposure to pro oxidant[35]. Decreased level of GSH is associated with increased lipid peroxidation which is also confirmed in this study. These observations corroborated the report of Cedric *et al.*[13].

The observed decrease in SOD activity following *E. intestinalis* infection and treatment with different doses of plant extract might be due to the oxidation of CAT and GSH-Px enzymes. Pawel *et al.*[33] showed a decrease in SOD and CAT activities in rabbits infected with *E. stiedae* compared to control rabbits. Farombi *et al.*[34] suggested that superoxide radicals by themselves, or after their transformation to H_2O_2, caused oxidation of CAT and GSH-Px enzymes and thus decrease SOD activity.

CONCLUSION

It can be concluded that, our findings, therefore corroborate the use of *P. macrophylla* as an anticoccidial agent in Cameroonian folk medicine. However, further studies to determine the toxicity are needed to establish an anticoccidial prototype.

SIGNIFICANCE STATEMENT

This study discovered the anticoccidial and antioxidant efficacy of *Pentaclethra macrophylla* methanolic extract. These results also showed that methanolic extract, possessed strong antioxidant and anticoccidial activities. Among the groups that received the plant extract, the highest oocyst reduction rate was 69.41% at a dose of 500 mg kg^{-1} and reduced in a dose-dependent manner from 58.92% (250 mg kg^{-1}) and then 55.01% (125 mg kg^{-1}). This study will help the researcher to uncover critical areas of coccidiosis and oxidative stress that many researchers were not able to explore. Thus a new theory on the usage of *Pentaclethra macrophylla* against coccidiosis by agro pastoral farmers in Cameroon may be arrived at.

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