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Leaf Extract Against *Encephalitozoon cuniculi* Infection in New Zealand White Rabbits: Impacts on Growth Performance, Immune Response, Caecal Microflora and Hepatic Histology



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Abstract

Microsporidiosis caused by *Encephalitozoon cuniculi* poses a severe threat to rabbit health, leading to growth depression, intestinal dysbiosis, systemic immunosuppression, and hepatic damage. This study evaluated the protective and therapeutic effects of *Papaver somniferum* leaf extract against *E. cuniculi* challenge in rabbits. Fifty male New Zealand White rabbits were randomly assigned to five treatment groups (n=10, 5 replicates of 2 animals) in a Completely Randomized Design (CRD) over a 74-day trial (14-day quarantine + 60-day experimental period). Treatments comprised: T1 (unchallenged control, basal diet), T2 and T3 (basal diet + intraperitoneal injection of 0.2 mL and 0.4 mL *E. cuniculi* spore suspension on Day 15, respectively), and T4 and T5 basal diet + 0.2 mL and 0.4 mL *E. cuniculi* injection + daily oral drench of 2 mL and 4 mL *P. somniferum* extract respectively. Quantitative phytochemical profiling revealed that *P. somniferum* extract was rich in phenols (952.00 mg/g), flavonoids (520.00 mg/g), terpenoids (460.00 mg/g), tannins (200.00 mg/g), and alkaloids (86.30 mg/g). Pathogen injection in untreated groups (T2 and T3) significantly (p<0.05) depressed daily feed intake and total weight gain, increased Feed Conversion Ratio, elevated mortality (2.00-2.50 %), and decreased serum immunoglobulins (IgA, IgG, IgM) and hematological parameters. Furthermore, T2 and T3 exhibited increased caecal *E. cuniculi* loads, suppressed *Lactobacillus* populations, hyper-elevated liver enzymes (ALT, AST, ALP), and severe hepatic cell necrosis. Conversely, daily administration of *P. somniferum* extract in T4 and T5 completely neutralized parasitic damage, achieving significantly higher weight gain, optimized FCR and no mortality, near-total elimination of caecal *E. cuniculi*, proliferation of beneficial *Lactobacillus* spp and improved serum immunoglobulins, normalized liver enzymes, and normal hepatic histology. *Papaver somniferum* leaf extract exhibits potent antiparasitic, immunomodulatory, and hepatoprotective properties, making it an effective natural therapeutic agent against *E. cuniculi* infection in rabbits.

Keywords: *Papaver somniferum*; Rabbits; Phytochemical; Growth; Extract; Haematology; Serum

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Introduction

Rabbit production (*Oryctolagus cuniculus*) plays a vital role in global livestock agriculture, serving as a high-quality source of protein, fur as well as laboratory animal models for research [1]. However, the intensification of rabbit farming is frequently undermined by infectious diseases that compromise animal welfare, reduce growth performance, and cause serious economic losses [2]. Among the pathogenic threats impacting is Cuniculture, microsporidiosis which is primarily caused by the obligate intracellular pathogen *Encephalitozoon cuniculi* [2]. It represents one of the most widespread, debilitating, and difficult to-manage parasitic infections in many countries [3].

Encephalitozoon cuniculi is a spore-forming microsporidian fungus with a broad host range, though rabbits serve as its primary definitive host [4, 5]. The pathogen infects host cells through a unique, highly specialized mechanism involving the extrusion of a coiled tube, which penetrates

the host cell membrane to inject sporoplasm directly into the cytoplasm [6]. Once inside, *E. cuniculi* proliferates rapidly, causing impairment and injury to the liver, kidney and other vital organs in the body [7, 8]. The disease also targets the central nervous system and gastrointestinal tract by creating dysbiosis [9].

Conventional control of microsporidiosis relies heavily on synthetic antimicrobials and benzimidazole anthelmintics (such as albendazole, fenbendazole amongst others) [2, 6]. While these pharmaceuticals can reduce spore shedding, their prolonged usage is increasingly limited by high treatment costs, risk of pharmaceutical residues in meat products and their potential drug resistance [10]. Consequently, there is an urgent need to identify natural, plant-derived phytogenic alternatives capable of suppressing *E. cuniculi* proliferation while simultaneously enhancing growth performance, gut health, immune responsiveness, and tissue protection [11]. Plant extracts offer a promising frontier in veterinary parasitology due to their complex mixtures of bioactive secondary metabolites that act synergistically across multiple physiological targets [12].

Papaver somniferum belongs to the Papaveraceae plant family. *Papaver somniferum* is known to have originated from the Southern Europe and North Africa, although it can also be found in most parts of the world including Asia, Central and South America and Australia [13]. The plant parts are important for the traditional treatment of a variety of infections, whilst the leaves are used as analgesics for the treatment of headache, toothache and general body pain [14]. Both the seeds and the leaves are combined for the treatment of asthma, coughs, rheumatism. Skin infections, diarrhoea, fevers and intestinal infections are all treated using the root of the plant [15]. The stems are used for the treatment of toothache, stomachache, dysentery and bronchitis. In Ghana for example, the leaves, flowers and roots are used for the treatment and management of a range of infections, and of disease conditions like gastro intestinal disorder, ulcers, malaria, dermatitis, bronchitis, tooth decay, malaria, stomach cramps and dysentery [16].

The plant is widely recognized in traditional medicine for its rich pharmacological profile, contains potent secondary metabolites with reported antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties [14, 17]. Furthermore, it was shown that a methanolic extract of *P. somniferum* inhibited the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Klebsiella pneumoniae* [18, 19].

Previous studies have shown that supplementation of phytogenics in the diet of rabbits have been able to avert villous atrophy, mucosal ulceration, decreased daily feed intake, depressed weight gain, poor feed conversion efficiency, disruption caecal microbial ecology, hepatocyte degeneration when animals were challenged with different pathogens [20-24].

Despite the known medicinal value of *Papaver somniferum*, its therapeutic potential in mitigating microsporidian infections in livestock remains under-investigated. Therefore, this study was designed to evaluate the therapeutic and prophylactic efficacy of *Papaver somniferum* leaf extract in New Zealand White rabbits experimentally challenged with *Encephalitozoon cuniculi*.

Materials and Methods

Experimental site and ethical consideration

The study was conducted under controlled laboratory conditions in the Animal House Facility at Gandhi Agricultural Research College, Rajasthan, India, located between latitude 26°55' N, Longitude 75°49' E; altitude of 390 m above sea level). The regional climate during this period is semi-arid, characterized by mean daily ambient temperatures ranging between 28.5°C and 41.2°C, a relative humidity of 32% to 58%, and an average annual rainfall of approximately 520 mm. All handling procedures and experimental protocols strictly adhered to standard guidelines for experimental animal welfare and were approved by the Institutional Animal Ethics Committee (AAF/GNDC/20008A).

Collection and extraction of *Papaver somniferum* leaves

Fresh, healthy leaves of *Papaver somniferum* were collected from areas within Gandhi College of Agriculture, Rajasaathan and sent to the department of Biological Sciences in the same institution for proper identification and assigned reference number ADF/202011/08F. Leaves were thoroughly rinsed with distilled water to remove dirt and other contaminants, and shade-dried at room temperature for 14 days until a constant weight was achieved. The dried leaves were pulverized into fine powder using an electric multi-purpose blender. Extraction was carried out via maceration technique. Briefly, 500 g of powdered leaf material was soaked in 80% ethanol for 72 hours with occasional agitation. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated at 40°C under reduced pressure using a rotary evaporator (Buml, Model AA088, Korea). The resulting crude extract was stored in airtight glass containers at 4°C until use. Quantitative phytochemical screening of *Papaver somniferum* extract was carried out according to the standard procedures recently published by Singh *et al.,* [25]. Each compound was quantified at different optical densities.

Pathogen collection, culturing, and inoculation protocol

The pathogen *Encephalitozoon cuniculi* was collected from the Sumitra Veterinary Research Institute in Gujarat, India. Microsporidian spores were maintained and propagated in Rab-9 (rabbit skin fibroblast) cell lines. Cells were grown in tissue culture flasks containing Eagle's Minimum Essential Medium (EMEM) supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin, incubated at 37°C in a humidified atmosphere with 5% CO_2. Spores released into the cell culture supernatant were collected continuously. Supernatants were centrifuged at 1,500×g for 15 minutes at a temperature of 4°C. The resulting spore pellet was washed three times in sterile Phosphate-Buffered Saline (PBS, *pH* 7.2) to remove culture medium. Spore concentration was quantified using a bright-line hemocytometer under light microscopy and adjusted to a standard concentration of 1×107 spores/mL using sterile Phosphate-Buffered Saline.

Experimental animals and design

Fifty (50) healthy, pathogen-free male New Zealand White bucks (aged 8-10 weeks, average initial weight of 1150 g) were purchased from a reputable breeding farm in Rajasthan in the month of August, 2026 using a plastic crate to an already disinfected cages, watering and feeding troughs placed in an open sided pen. On arrival, they were given anti-stress (100 g Glucose + 100 g Aminovit wsp® to 25 liters of water) for 3 days, fed standard growers mash (basal) compounded according to their nutritional specifications (NRC, 1977) and dewormed with Oramec super® to prevent ecto and endo-parasites during the 14 days adjustment period. On day 15, (immediately following the 14-day quarantine) rabbit weights were stratified and randomly assigned into five treatment groups using a Completely

Randomized Design (CRD). Each treatment consisted of 10 rabbits divided into 5 replicates, with 2 rabbits per replicate cage and animals in treatment groups T2, T3, T4, and T5 were injected Intraperitoneally (I.P.) using sterile 23-gauge needles under aseptic conditions. Feeders and drinkers were washed daily, rabbits were fed trice daily (7:30 H, 12:00 H and 16:00 H). Water was offered at all times fluorescent lamp was placed in the pen to increase the lighting period to 16 hour per day in order to increase feed intake. Other hygiene and biosecurity measures were also taken throughout the study period.

Treatment layout

T1 (Unchallenged Control): Basal diet alone + sham injection (0.3 mL sterile PBS).

T2 (Low Pathogen Challenge): Basal diet + 0.2 mL *E. cuniculi* injection (I.P.).

T3 (High Pathogen Challenge): Basal diet + 0.4 mL *E. cuniculi* injection (I.P.).

T4 (Low Challenge + Low Extract): Basal diet + 0.2 mL *E. cuniculi* injection + daily oral drench of 2 mL *P. somniferum* extract.

T5 (High Challenge + High Extract): Basal diet + 0.4 mL *E. cuniculi* injection + daily oral drench of 4 mL *P. somniferum* extract.

Extract drenching commenced on Day 15 immediately following pathogen injection and continued daily for 60 consecutive days until Day 74.

Data Collection and Laboratory Analysis

Feed consumption, body weight gain, feed conversion ratio and mortality: The amount of feed consumed per rabbit was determined as the difference between the feed offered and refused. Animals were weighed at the begining and end of the trial. Body weight gain was calculated as the difference between the final and initial body weight. Daily feed consumption was computed by dividing total feed consumption by the duration of experiment (in days). Feed Conversion Ratio (FCR) was calculated as total feed consumed (g) divided by total weight gained (g). Mortality was monitored daily.

Blood sample collection and analysis: On Day 74, two sets of blood samples were collected from each animal through marginal ear vein puncture using syringes into labeled sample bottles. Blood samples were collected very early in the morning to minimize stress. One set of the blood samples (3 mL) was collected into ethylenediaminetetraacetic acid bottles for the determination of hematological parameters and placed in an ice pack cooler. The other set of blood samples (3 mL) was collected into plain tubes without anticoagulant, allowed to coagulate at room temperature and centrifuged for 5 min at 3000 rpm. Haematological analysis was carried out using Mindray BC-2800 Vet Auto Hematology Analyzer (Mindray Bio-Medical Electronics Co., Shenzhen, China), which set at absorption 540 nm, CV<1.5, Sensitivity: down to 0.1g/dL. Blood components were determined using electrical impedence procedure. Serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) levels were measured using Nerre automated clinical biochemistry analyzer (Model 3200X, Netherlands).

Caecal microbiology: On Day 74, caecal digesta samples were collected under sterile conditions from five randomly selected rabbits per treatment. Samples were collected in a labeled test tubes with peptone solution. Quantification *Lactobacillus* species (log10 CFU/g) was determined using selective agar media while *E. cuniculi* spore counts was determined using qPcr.

Serum immunoglobulins determination: Blood samples were collected via marginal ear vein puncture on Day 74. Serum concentrations of IgA, IgG, and IgM were quantified using immunosorbent assay (ELISA) kits Model SC2210V, China.

Hepatic histopathology: Liver tissues were harvested from five randomly selected rabbits per treatment (from animals used for caecal microbiology), fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 μm and stained with Hematoxylin and Eosin for microscopic evaluation.

Proximate composition of experimental diet: Proximate composition of experimental diet was determined using Aligent Cary NIR spectrophotometer (Model 6300C, USA) adjusted at a spectral range between 800-3000 nm and resolution (4 cm-8cm). Feds Atomic Absorption Spectrophotometer (Model 230 VX, China) was used for the determination of dietary calcium and phosphorus level. Each kit follows strictly was operated according to the manufacturers' protocol to ensure precision in outcome.

Data analysis

Data were subjected to Analysis Of Variance (ANOVA) for a complete randomized design, with a model that included the diet as treatment effects, using Statistical Package for the Social Sciences (SPSS 2009). When the ANOVA was significant, means were sepa rated using Duncan's multiple range test at the level of $P \leq 0.05$.

Experimental Results

Table 1 presents the phytochemical constituents of *P. somniferum* extract. The compounds contained phenols at 952.0 mg/g, flavonoids (520.0 mg/g), terpenoids (460.0 mg/g), tannins (201.0 mg/g), alkaloids (86.30 mg/g), saponins (65.22 mg/g) and steroids (35.09 mg/g). The extracts showed this rank order: phenol > flavonoids > terpenoids > tannins > alkaloids > saponins > steroids.

Table 2 shows the nutrient and chemical composition of basal diet. The feed contained crude protein (16.02 %), crude fibre (13.16 %), ether extract (3.88 %), calcium (1.35 %), phosphorus (0.72 %) and energy (2604.7 Kcal/kg).

Table 3 presents the growth performance of New Zealand White rabbits challenged with *Encephalitozoon cuniculi*. Final body weight, total weight gain and daily feed intake were similar (p>0.05) among rabbits which recieved T4 and T5 but signifcantly higher (p<0.05) than other groups. Feed conversion ratio and motality ranged from 4.93-8.36 and 0.00-2.50 % respectively. Values obtained for mortality and feed conversion ratio were more (p<0.05) in T2 and T3 compared to the other treatments.

Table 1: Phytochemical constituents of *P. somniferum* extract.

Phytochemical Constituent	Qualitative content (mg/g)
Phenols	952.0
Flavonoids	520.0
Terpenoids	460.0
Tannins	201.0
Alkaloids	86.30
Saponins	65.22
Steroids	35.09

Table 2: Gross composition of basal diet used for the experiment.

Feedstuff	% Inclusion
Maize	44.30
Wheat offal	15.00
Soybean meal	22.00
Palm Kernel Cake	15.00
Dicalcium phosphase	2.50
Lysine	0.25
Methionine	0.25
Premix	0.25
Salt	0.30
Toxin binder	0.15
Total	100.0
Determined analysis (%)	
Crude protein	16.02
Crude fibre	13.16
Ether extract	3.88
Calcium	1.35
Phosphorus	0.72
Energy (Kcal/kg)	2604.7

2.5 kg Premix contains the follwing nutrients: Vitamin A, 10,000.000 I.U., Vitamin D3: 3,000.00 I.U, vitamin E, 40,000., vitamin K, 2000.00., vitamin B1, 2000., vitamin B2, 5000., vitamin B12 3000., Niacin 15 mg., Panthothenic acid, 40,000 mg., Folic acid, 1,000 mg., Biotin 100 mg., Choline, 30,000 mg., Manganese, 70,000 mg., Zinc, 40,000 mg., Iron, 40,000 mg., Copper, 5000 mg., Iodine 1,200 mg., Selenium, 200 mg., Cobalt, 250 mg and Antioxidant 125 mg.

In Table 4, caecal microbiota of New Zealand White rabbits challenged with *Encephalitozoon cuniculi*. *E. cuniculi* and *Lactobacillus* spp count varied from 0.20-7.12 ($\times 10^6$ spores/g) and 2.45-9.20 (log10 CFU/g). *E. cuniculi* population was higher in T2 and T3 than the other groups. Conversely, *Lactobacillus* spp count was lower in T2 and T3 compared to the other treatments ($p<0.05$).

In Table 5, serum immunoglobulins of New Zealand White rabbits challenged with *Encephalitozoon cuniculi*. Immunoglobulin A, G and M concentrations were significantly affected ($p<0.05$) by the treatment and their respective values ranged from 145.20-225.80 mg/dL, 310.0-930.4 mg/dL and 48.30-172.0 mg/dL. Values obtained were more in T4 and T5, intermediate in T1 and lower in T2 and T3 ($p<0.05$).

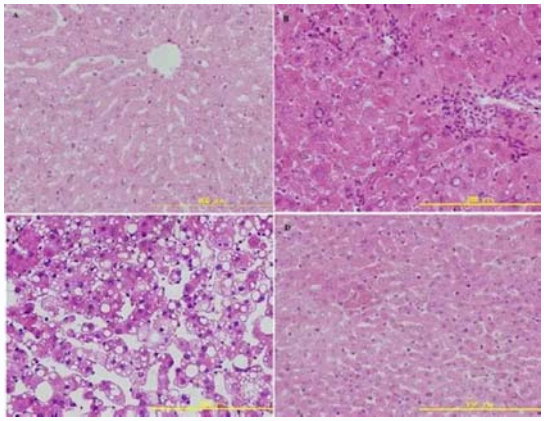


Plate 1: (Unchallenged Control): Basal diet alone + sham injection (0.3 mL sterile PBS).

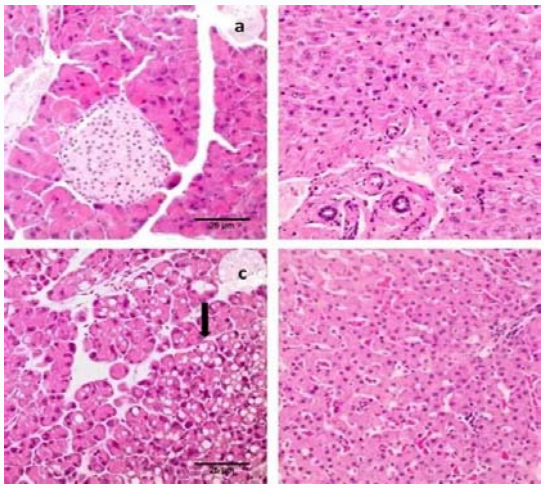


Plate 2: (Low Pathogen Challenge): Basal diet + 0.2 mL *E. cuniculi* injection (I.P.).

Table 6 displays some haematology and Serum Parameters of New Zealand White rabbits challenged with *Encephalitozoon cuniculi*. Pack cell volume (23.10-42.50%), hemoglobin (7.20-14.20 g/dL), total protein (3.80-7.60 g/dL), Serum Alanine Aminotransferase (34.80-124.6 U/L), Aspartate Aminotransferase (39.20-148.9 U/L), and Alkaline Phosphatase (58.90-195.4 U/L) were significantly affected by the treatment (Figure 1).

Table 3: Growth performance of New Zealand white rabbits challenged with *Encephalitozoon cuniculi.*

Parameter	T1 (Control)	T2 (0.2 mL EC)	T3 (0.4 mL EC)	T4 (0.2 mL EC + 2 mL PS)	T5 (0.4 mL EC + 4 mL PS)	SEM	*p*-value
Initial Body Weight (g)	1150.20	1152.00	1148.50	1151.10	1150.80	12.45	<0.001
Final Body Weight (g)	2450.00b	1820.00d	1680.00e	2680.00a	2710.00a	42.15	<0.001
Total Weight Gain (g)	1299.80b	668.00d	531.50e	1528.90a	1559.20a	31.80	<0.001
Daily Feed Intake (g/day)	110.50b	82.30d	74.10e	124.60a	128.20a	3.20	<0.001
Feed Conversion Ratio (FCR)	5.10b	7.40a	8.36a	4.89c	4.93c	0.21	<0.001
Mortality (%)	0.00b	2.00a	2.50a	0.00b	0.00b	0.11	0.013

Means within a row bearing different superscript letters (a,b,c,d,e) differ significantly ($p<0.05$); SEM: Standard Error of Mean; T1 (Unchallenged Control): Basal diet alone + sham injection (0.3 mL sterile PBS).
T2 (Low Pathogen Challenge): Basal diet + 0.2 mL *E. cuniculi* injection (I.P.).
T3 (High Pathogen Challenge): Basal diet + 0.4 mL *E. cuniculi* injection (I.P.).
T4 (Low Challenge + Low Extract): Basal diet + 0.2 mL *E. cuniculi* injection + daily oral drench of 2 mL *P. somniferum* extract.
T5 (High Challenge + High Extract): Basal diet + 0.4 mL *E. cuniculi* injection + daily oral drench of 4 mL *P. somniferum* extract.

Table 4: Caecal microbiota of New Zealand white rabbits challenged with *Encephalitozoon cuniculi.*

Parameter	T1 (Control)	T2 (0.2 mL EC)	T3 (0.4 mL EC)	T4 (0.2 mL EC + 2 mL PS)	T5 (0.4 mL EC + 4 mL PS)	SEM	*p*-value
Microbiology							
E. cuniculi (×10^6 spores/g)	1.20c	5.85b	7.12a	0.42d	0.21d	0.16	<0.001
Lactobacillus spp (log10 CFU/g)	6.40b	3.10c	2.45c	8.90a	9.20a	0.28	<0.001

Means within a row bearing different superscript letters (a,b,c,d) differ significantly (p<0.05); SEM: Standard Error of Mean; T1 (Unchallenged Control): Basal diet alone + sham injection (0.3 mL sterile PBS).
T2 (Low Pathogen Challenge): Basal diet + 0.2 mL *E. cuniculi* injection (I.P.).
T3 (High Pathogen Challenge): Basal diet + 0.4 mL *E. cuniculi* injection (I.P.).
T4 (Low Challenge + Low Extract): Basal diet + 0.2 mL *E. cuniculi* injection + daily oral drench of 2 mL *P. somniferum* extract.
T5 (High Challenge + High Extract): Basal diet + 0.4 mL *E. cuniculi* injection + daily oral drench of 4 mL *P. somniferum* extract.

Table 5: Serum immunoglobulins of New Zealand white rabbits challenged with *Encephalitozoon cuniculi.*

Parameter(mg/dL)	T1 (Control)	T2 (0.2 mL EC)	T3 (0.4 mL EC)	T4 (0.2 mL EC + 2 mL PS)	T5 (0.4 mL EC + 4 mL PS)	SEM	*p*-value
IgA	145.20b	82.10c	71.40c	210.50a	225.80a	7.90	<0.001
IgG	620.00b	340.50c	310.00c	890.20a	930.40a	22.40	<0.001
IgM	110.40b	55.20c	48.30c	165.10a	172.00a	5.80	<0.001

Means within a row bearing different superscript letters (a,b,c) differ significantly (p<0.05); SEM: Standard Error of Mean; T1 (Unchallenged Control): Basal diet alone + sham injection (0.3 mL sterile PBS).
T2 (Low Pathogen Challenge): Basal diet + 0.2 mL *E. cuniculi* injection (I.P.).
T3 (High Pathogen Challenge): Basal diet + 0.4 mL *E. cuniculi* injection (I.P.).
T4 (Low Challenge + Low Extract): Basal diet + 0.2 mL *E. cuniculi* injection + daily oral drench of 2 mL *P. somniferum* extract.
T5 (High Challenge + High Extract): Basal diet + 0.4 mL *E. cuniculi* injection + daily oral drench of 4 mL *P. somniferum* extract.

Table 6: Haematology and serum parameters of New Zealand white rabbits challenged with *Encephalitozoon cuniculi.*

Parameter	T1 (Control)	T2 (0.2 mL EC)	T3 (0.4 mL EC)	T4 (0.2 mL EC + 2 mL PS)	T5 (0.4 mL EC + 4 mL PS)	SEM	*p*-value
Packed Cell Volume (%)	38.50b	26.20c	23.10c	41.20a	42.50a	1.15	<0.001
Hemoglobin (g/dL)	12.80b	8.40c	7.20c	13.90a	14.20a	0.45	<0.001
Total Protein (g/dL)	6.50b	4.20c	3.80c	7.40a	7.60a	0.22	<0.001
Liver Enzymes (U/L)							
ALT	38.50b	98.40a	124.60a	36.10b	34.80b	3.85	<0.001
AST	42.10b	115.20a	148.90a	40.50b	39.20b	4.90	<0.001
ALP	65.30b	162.00a	195.40a	61.20b	58.90b	7.10	<0.001

Means within a row bearing different superscript letters (a,b,c) differ significantly (p<0.05); SEM: Standard Error of Mean; Serum Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP); T1 (Unchallenged Control): Basal diet alone + sham injection (0.3 mL sterile PBS);
T2 (Low Pathogen Challenge): Basal diet + 0.2 mL *E. cuniculi* injection (I.P.).
T3 (High Pathogen Challenge): Basal diet + 0.4 mL *E. cuniculi* injection (I.P.).
T4 (Low Challenge + Low Extract): Basal diet + 0.2 mL *E. cuniculi* injection + daily oral drench of 2 mL *P. somniferum* extract.
T5 (High Challenge + High Extract): Basal diet + 0.4 mL *E. cuniculi* injection + daily oral drench of 4 mL *P. somniferum* extract.

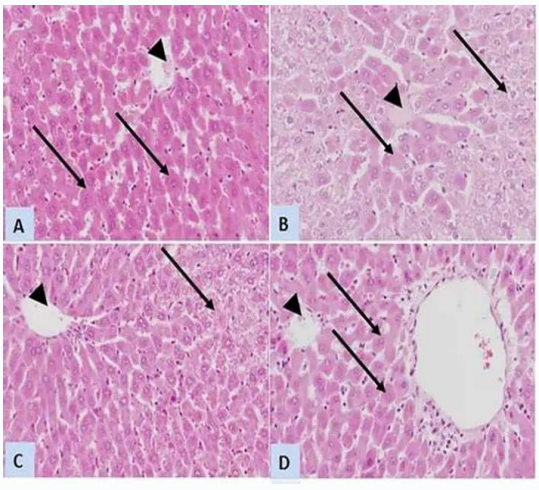


Plate 3: (High Pathogen Challenge): Basal diet + 0.4 mL *E. cuniculi* injection (I.P.).

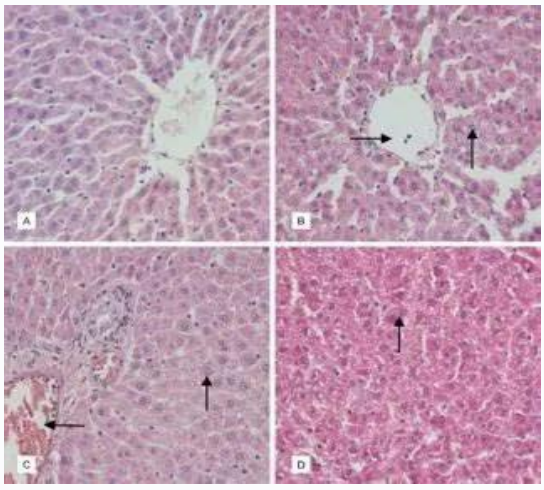


Plate 4: (Low Challenge + Low Extract): Basal diet + 0.2 mL *E. cuniculi* injection + daily oral drench of 2 mL *P. somniferum* extract.

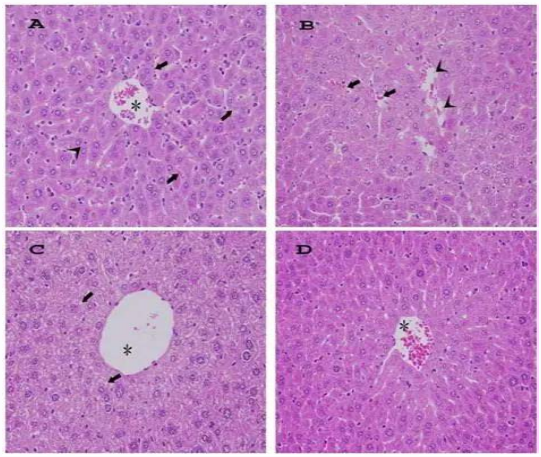


Plate 5: (High Challenge + High Extract): Basal diet + 0.4 mL *E. cuniculi* injection + daily oral drench of 4 mL *P. somniferum* extract.

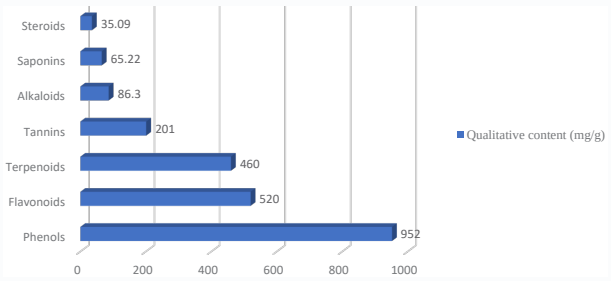


Figure 1: Graphical representation of phytocomponents in *P. somniferum* extract.

Discussion

The administration of *E. cuniculi* without herbal intervention (T2 and T3) severe influenced rabbit growth and physiological stability. *Encephalitozoon cuniculi* is an obligate intracellular microsporidian parasite that targets target tissues including the renal tubular epithelium, vascular endothelium, and hepatic parenchyma [26]. The significant drop in daily feed intake and total weight gain in T2 (668.0 g) and T3 (531.5 g) compared to T1 (1299.8 g) reflects chronic parasitic stress, systemic inflammation, and metabolic exhaustion [27]. Parasite invasion damages intestinal mucosal structures, impairing digestion and nutrient uptake, leading to elevated feed conversion ratio values (7.40 and 8.36). Mortality recorded in T2 (2.00%) and T3 (2.50%) directly stems from systemic organ failure and acute tissue necrosis caused by unchecked parasitic proliferation. In contrast, oral administration of *P. somniferum* leaf extract in T4 and T5 fully reversed these deleterious effects, achieving superior final body weights (2680.0 g) and (2710.0 g) and lower FCRs (4.89 and 4.93) compared to all other groups, including the uninfected control (T1). This performance boost is attributable to the synergistic action of the extract's bioactive constituents: flavonoids (520 mg/g) and phenols (952 mg/g) act as potent antioxidants and digestive stimulants [28, 29], boosting brush-border enzymatic activity and nutrient absorption [30, 31]. tannins (200 mg/g) at low-to-moderate concentrations form protective protein complexes across the gut mucosa, enhancing intestinal barrier function and reducing systemic toxin absorption [32, 33] and saponins (65.22 mg/g) enhance cell membrane permeability for nutrient transport while demonstrating antimicrobial action against intestinal pathogens [34]. The result obtained in this study is in consonance with the Metwally *et al.,* [35] when Litchi chinensis peel extract was supplemented in the diet of rabbits. Cho *et al.,* [36]; Dragan *et al.,* [37] also recorded a higher feed intake in birds fed phytogenics after oral challenge with Clostridium perfringens and Eimeria tenella respectively. Onyiche *et al.,* [38] also recorded no moratlity in animals fed diet supplemented with *Azadirachta indica* aqueous leaf extracts after infected with Eimeria oocysts.

The caecal microbial profile demonstrated a clear inverse relationship between *E. cuniculi* load and *lactobacillus* species. In T2 and T3, *E. cuniculi* populations expanded markedly (5.85 log10 CFU/g and 7.12 log10 CFU/g), causing severe dysbiosis that suppressed beneficial *Lactobacillus* counts down to 3.10 log 10 CFU/g and 2.45 log10 CFU/g. In groups receiving *P. somniferum* extract (T4 and T5), *E. cuniculi* populations were drastically suppressed pathogenic levels (0.21-0.42 log10 CFU/g), while *Lactobacillus* proliferated up to 8.90 to 9.20 log10 CFU/g. This outcome suggests that phytocompounds in the extract penetrate the microsporidian spore wall, destabilizing cellular membranes and interfering with spore polar tube extrusion mechanisms, thereby arresting intracellular replication [39]. Proliferating *Lactobacillus* species produces Short-Chain Fatty Acids (SCFAs) and bacteriocins, lowering caecal *pH* and creating an inhospitable environment for pathogenic microsporidia *via* competitive exclusion [40]. The outcome of this experiment corresponds with the result of Giannenas *et al.,* [41]; Awaad *et al.,* [42] when peppermint and eucalyptus essential oils was supplemented in birds after experimental infection with vVND.

Microsporidian infections typically evade host immune system by suppressing humoral immune activation [43]. This was evidenced in T2 and T3 by marked reductions in immunoglobulins IgA, IgG and IgM as well as depressed hematological parameters (PCV, Hb, and serum total protein). Parasite-induced inflammation damages vascular systems and hematopoietic tissue, causing anemia and hypoproteinemia [44, 45]. Daily drenching with *P. somniferum* extracts in T4 and T5 significantly improved immunoglobulin counts and restored hematological values above control levels (T1). Steroids and Saponins function as natural immunoadjuvants, stimulating B-lymphocyte differentiation and macrophage phagocytic activity [46, 47]. Mucosal IgA binds *E. cuniculi* spore surface antigens, preventing epithelial adhesion, while IgG and IgM facilitate systemic colonization and complement-mediated destruction of circulating spores [48]. The result of this trial is in agreement with the report of El-Ghareeb *et al.,* [49].

Serum ALT, AST, and ALP serve as clinical biomarkers for hepatocellular damage or liver injury [46]. In T2 and T3, these enzymes were significantly elevated indicating acute liver injury. Histopathological examination of T2 and T3 liver tissues revealed extensive cellular lesions, hepatocyte necrosis, vacuolar degeneration, hepatic cord disruption, and diffuse lymphocytic infiltration triggered by intracellular spore multiplication [50, 51]. In T4 and T5, serum liver enzymes remained within normal basal ranges (34.80 to 36.10 U/L), matching the healthy control (T1). Corresponding hepatic histology in T4 and T5 displayed intact hepatic architecture with regular lobular structures, well-preserved hepatocytes, normal sinusoids, and no necrotic foci. The striking hepato-protective effect is driven by the extreme concentration of phytocomponents in *P. somniferum*. These compounds neutralize reactive oxygen species,

inhibit lipid peroxidation of hepatocyte cell membranes, and preserve hepatic structural integrity during pathogenic challenge.

Conclusion

This study demonstrates the potent therapeutic efficacy of *Papaver somniferum* leaf extract against *Encephalitozoon cuniculi* infection in New Zealand White rabbits. Experimental challenge with *E. cuniculi* in untreated animals caused depressed weight gain and feed intake, poor feed conversion efficiency, caecal dysbiosis (*E. cuniculi* proliferation and *Lactobacillus* depletion), systemic humoral immunosuppression, elevated liver enzymes (ALT, AST, ALP), acute hepatocyte necrosis, and mortality. Daily oral administration of *P. somniferum* leaf extract at 2 mL and 4 mL doses completely neutralized pathogen-induced damage, eradicate caecal *E. cuniculi* populations through direct antiparasitic action and membrane disruption. Promote beneficial caecal *Lactobacillus* proliferation, restoring gut microbial homeostasis. Stimulate humoral immunity, marked by elevated serum IgA, IgG, and IgM concentrations. Prevent hepatocellular leakage and preserve normal hepatic microarchitecture and enhance overall growth performance and feed conversion efficiency beyond non-infected controls with zero mortality.

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Author Contribution

Alagbe Olujimi John, designed the work, laboratory analysis and correction of manuscripts, Kadiri Mercy Cincinsoko did the statistical analysis, data collection and correction of manuscript.

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Conflict of Interest

The authors declare that there are no conflicts of interests.

Ethical Approval

The Animal ethical guidelines are followed in the study for species observation & identification (Ethical approval code: AAF/GNDC/20008A).

Informed Consent

Not applicable.

Data Materials Availability

All data associated with this study will be available based on the reasonable request to corresponding author.

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