

Investigating the Effects of Dietary Supplementation of *Eucalyptus camaldulensis* Essential Oil on Hemato-biochemical Indices, Immune Response, and Oxidative Stress of Weaned Rabbits

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Abstract

Aims: The aim of this experiment was to investigate the effects of dietary supplementation of *Eucalyptus camaldulensis* essential oil on haemato-biochemical indices, immune response and oxidative stress of weaned rabbits. **Methods:** Sixty healthy weanling crossbred male rabbits (4 weeks old) with an initial body weight (BW) of 460.1 ± 0.25 g were grouped based on their BW into four treatments containing treatment 1 (T1) of a base diet without *E. camaldulensis* oil (ECO), T2, T3, and T4 were fed basal diets with 200 mg, 400 mg, and 600 mg/kg, respectively, in a completely randomized design. Feed and clean water was supplied ad libitum throughout the experiment which lasted for 56 days. **Results:** All haematological parameters (pack cell volume, red blood cell, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentrations, white blood cells and its differentials) were higher ($P < 0.05$) in T2, T3 and T4 relative to T1. Total protein, albumin, globulin, albumin/globulin ratio, cholesterol, aspartate transaminase, alanine transaminase and alanine phosphatase values were greater in ECO supplemented diets whereas urea nitrogen, creatinine, calcium, phosphorus, potassium, sodium and bicarbonate were influenced by the treatments. **Conclusion:** However, all values were within the optimal range for healthy rabbits. Immunoglobulins, melanodialdehyde, superoxide dismutase, glutathione peroxidase, catalase and total antioxidant capacity were higher ($P < 0.05$) in T2, T3 and T4 than in other diet. Result revealed that ECO can be supplemented up to 600 mg/kg without negatively affecting the performance and health status of rabbits.

Keywords: *Eucalyptus camaldulensis* oil, immune response, oxidative stress, phytochemical, rabbits

INTRODUCTION

A variety of plants, including herbs, spices, and essential oils and oleoresins generated from plants, are used as phytochemical feed additives. Numerous biological functions are expressed by essential oils, including antibacterial, anti-inflammatory, immunostimulatory, hepatoprotective, and antifungal ones.^[1] Numerous studies were done to investigate options after antimicrobial growth promoters were outlawed in the European Union and other nations in 2009.^[2] The use of antibiotics in chickens must be reduced to lower the danger of antibiotic resistance globally.^[3]

In particular plant components, such as leaves (*Eucalyptus*), bark (cinnamon), seeds (*Polyalthia longifolia*, anise, and pepper), bulbs (garlic), roots (ginger), or fruit peels (lemon and orange), essential oils tend to collect. A number of active

components in *Eucalyptus camaldulensis* oil (ECO) allow this phytochemical to have anti-inflammatory, antioxidant, antiprotozoal, and immunomodulatory qualities in addition to its antibacterial action.^[4,5] *E. camaldulensis* leaves and oils have been utilized to treat malaria, gastrointestinal illnesses, diarrhea, cough, and dysentery.^[6] Natural phytochemical feed additives such as *E. camaldulensis* oil have been thoroughly researched, and the advantages of chicken feed containing

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essential oils are well established, all of which aim to improve the performance of birds.^[7]

Numerous essential oils have been studied in rabbits, including those from thyme, rosemary, lemon grass, ginger, garlic, and turmeric.^[8] According to several scientific investigations, certain essential oils can cause cell toxicity when used at greater doses.^[9,10] According to research,^[11,12] *E. camaldulensis* oil may stimulate bile secretion in the intestinal tract, promote enzyme production, speed up digestion, and guard against the entry of pathogenic microorganisms into animals' bodies. The differences in efficacy of essential oils are explained by the chemical makeup of essential oils, which underlay some variance caused by their constituents and other influencing elements including climate, harvest, stage, and storage conditions. Despite being necessary, it has been demonstrated to have antibacterial characteristics and a natural antibiotic alternative. On the other hand, there are conflicting data regarding their effectiveness and the right dosage in animal feeds. Information on the impact of supplemental feeding of *E. camaldulensis* oil on the physiological parameters of rabbits is scarce.

Therefore, this experiment aimed to investigate the effects of dietary supplementation of *E. camaldulensis* essential oil on hemato-biochemical indices, immune response, and oxidative stress of weaned rabbits.

MATERIALS AND METHODS

Experimental site, ethical consent, and extraction of *Eucalyptus camaldulensis* oil

The study was carried out at the Livestock Unit of Sumitra Institute located between 23° 13' N and 72° 41' E. The study was carried out according to the rules and specifications of protocols approved by the Research Ethics Committee of Sumitra Research Institute, Gujarat, India.

Fresh *E. camaldulensis* leaves were harvested within the Research Institute and taken to the Department of Biological Sciences where a detailed identification and authentication was carried out. To eliminate dirt and other particles, leaves were washed. The samples were then divided into smaller pieces to minimize their surface area before being transported to the laboratory and kept in a sealed, tagged container to allow water to drain from the plant. Steam distillation technique is the extraction method used. The equipment needed for the laboratory procedure includes a steam generator, round-bottom flask, beaker, condenser, safety tube, separatory funnel thermometer, and a distillation flask connected to the steam generator by a delivery tube. Two hundred and fifty milliliters of distilled water was added after 80 g of thinly cut *E. camaldulensis* leaf was put into a 500-mL round-bottom flask. Setting up the distillation flask, condenser, and receiver, the mixture in the round-bottom flask was heated to 70°C for 15 min. The steam that was produced passed through the condenser, and when it was cold, oil was collected in the receiver (beaker). For the purpose of obtaining pure *E. camaldulensis* essential oil (ECO), distillate is put through a separatory funnel.

Animals and their care

A respected breeding farm in Gujarat, India, sold 60 weaned male crossbreed rabbits with an initial body weight (BW) of 460.1 ± 0.25 g that were approximately 4 weeks old and in clinically good health. Two weeks before the start of the experiment, galvanized cages were thoroughly disinfected using Morigad®. Nipple drinkers and automatic feeders were also included. The rabbits were thoroughly checked once more and placed in semi-housed open pens with individual cages that measured 35 cm × 71 cm × 50 cm (length × width × height). According to the nutritional requirements for rabbits (Nutrient Requirements of Rabbits (NRC), 1979), rabbits were confined for 14 days and fed a basal diet that was appropriate in all nutrients [Table 1]. During their period of acclimation, they also received preventative treatment against parasites, including sulfadimidine sodium BP solution to prevent coccidiosis and Ivermectin® injection (subcutaneously administered at 0.1 mL/kg). In a perfectly randomized design, the BW of the rabbits was used for splitting them into four treatment groups of 15 rabbits each.

Experimental arrangement

Treatment 1 (T1) utilizes a base diet without *E. camaldulensis* essential oil (ECO); Treatment 2 (T2) uses a base diet with

Table 1: Experimental diets' chemical composition

Feedstuffs	Quantity (kg)
Maize	32.00
Wheat ofal	15.00
Palm kernel meal	20.10
Soya meal	25.00
Bone meal	4.00
Limestone	2.00
Lysine	0.20
Methionine	0.10
Minimum/vitamin premix*	0.25
Salt	0.35
Total	100.0
Calculated analysis (% dry matter)	
Crude protein	14.81
Crude fiber	13.30
Ether extract	1.91
Calcium	1.91
Phosphorus	0.72
Energy (kcal/kg)	2410.7
Determined analysis (% dry matter)	
Crude protein	15.73
Crude fiber	12.11
Ether extract	2.00
Calcium	1.93
Phosphorus	0.88
Energy (kcal/kg)	2513.6

*Minimum/vitamin premix supplied per kg diet – Vitamin A, 6800 IU; Vitamin E, 16.0 mg; Vitamin D, 2000 IU; Vitamin K, 5.00 mg; Vitamin B2, 5.0 mg; niacin, 65 mg; Vitamin B12, 20 mg; choline chloride, 70 mg; manganese, 3.0 mg; zinc, 35.1 mg; copper, 2.0 g; folic acid, 2.5 mg; iron, 7.1 g; pantothenic acid, 18 mg; biotin, 35.5 g; antioxidant, 60 mg

200 mg/kg ECO; Treatment T3 employs a base diet with 400 mg/kg ECO; and Treatment T4 includes a base diet with 600 mg/kg ECO.

Blood samples collection

On the last day of the experiment, blood samples were collected from ten randomly selected rabbits per treatment for hematological and serum biochemical indices. Blood for hematological studies (2 mL) was collected from the marginal ear into a sample bottle with anticoagulants, and another 2 mL was collected into bottles free from anticoagulant for serum biochemical analysis. All samples were placed in ice packs and transferred to the laboratory for further analysis. H360 fully automated three-part hematology analyzer (model HEM0028, Czech Republic) was used for hematological studies; this kit was employed because it is easy, efficient, and reliable. It has the following technical specifications: manual closed and open tube volumes at 100 μ L each, workstation (Intel Pentium Dual Core 2.00 GHz 200 W desktop/tower), (3Gb/s 7200 RPM 16 MB Cache hard drive; 2 GB CD-RW memory module) and (11 inch TORCH screen with LCD monitor).

Serum biochemical analysis was carried out using Pictus 700 automatic analyzer (model F1209-06A, Hungary) with the following technical specifications: photometric module (eight interference filters: 340, 405, 505, 546, 578, 630, 710, and 872 nm), measuring module (25 μ L flow cell volume), 15 mm² cuvette, minimum aspiration volume: 200 μ L, and analysis mode.

Immune response analysis

Blood samples were collected from ten randomly selected rabbits (the same rabbits used for blood analysis) for immunological analysis. Blood samples were collected into a sterile labeled plain bottle early in the morning and placed in an ice pack before they are transferred to the laboratory for further studies. Analysis was carried out using Accu-Tell[®] Elisa Reader (Model: ABER-2, Belgium) with the following technical specifications: work environment temperature (0°C–40°C), humidity (85%), standard wavelength (405, 450, 492, and 630 nm and

substitute filters from 400 to 700 nm), abs range (0–3.500 A), measurement range (0–2.000 A), accuracy (0–2.0 A: \pm 1.0% or \pm 0.007 A), precision (0–2.0 A: \pm 0.5% or \pm 0.005 A), power (a.c. 110 V), reading speed (continuous mode <5s), and fuses (T315AL250V).

Oxidative stress indices

Total antioxidant capacity and activities of superoxide dismutase, catalase, glutathione peroxidase, and malondialdehyde were measured using Getein 1180 immunofluorescence quantitative analyzer (Model 88-OL90D, Belgium). Calibration, buffering, and other operations were strictly according to the manufacturer's recommendations.

Analysis of experimental diet

Analysis of experimental diet was carried out using PerkinElmer near infrared (Model DA 7250, England) which analyzes sample in 60 s. The machine has the following technical specifications: operating temperature range (5°C to 40°C), wavelength range (900–1700 rpm), and wavelength accuracy (<0.05 nm).

Statistical analysis

All data obtained were subjected to statistical analysis of variance using Statistical Analytical System (SAS, (2003) SAS Release 9.1 for windows, SAS Institute Inc.Cary, NC, USA). Treatment means were compared using Duncan's multiple-range test of the same software.

The statistical model used is shown below:

$$Y_i = \mu + T_{ij} + e_{ij}$$

Where Y_i = the effect of the j th observation in the i th treatment, μ = general mean of the population, and T_i = the effect of the i th treatment where $i = 4$ e_{ij} = random error associated with the j th observation in the i th treatment.

Hematological parameters of weaned rabbits fed diet supplemented with *Eucalyptus camaldulensis* oil

The hematological parameters of weaned rabbits fed diet supplemented with *E. camaldulensis* oil (ECO) are displayed in Table 2. The dietary treatments influenced ($P < 0.05$) all

Table 2: Hematological parameters of weaned rabbits fed diet supplemented with *Eucalyptus camaldulensis* oil

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	SEM	Range
Pack cell volume (%)	34.18 ^b	40.70 ^a	40.92 ^a	41.00 ^a	0.82	31–51
RBC ($\times 10^6/L$)	9.12 ^b	12.33 ^a	12.85 ^a	12.90 ^a	0.03	8–18
Hemoglobin (g/L)	100.6 ^b	127.6 ^a	129.5 ^a	131.6 ^a	3.18	100–180
MCH (pg)	19.35 ^b	27.71 ^a	28.83 ^a	29.96 ^a	0.39	16–30
MCV (fl)	49.50 ^b	59.40 ^a	60.85 ^a	61.23 ^a	0.97	30–60
MCHC (%)	35.01 ^b	41.57 ^a	43.08 ^a	44.26 ^a	0.80	29–50
WBC ($\times 10^9/L$)	8.42 ^b	11.55 ^a	12.50 ^a	12.58 ^a	0.02	5–21
Lymphocytes (%)	51.48 ^b	67.62 ^a	67.95 ^a	70.10 ^a	0.91	20–85
Neutrophils (%)	23.70 ^b	32.18 ^a	33.04 ^a	33.86 ^a	0.65	21–50
Monocytes (%)	1.77 ^b	2.03 ^a	2.19 ^a	2.85 ^a	0.01	1–5
Eosinophils (%)	1.45 ^b	2.99 ^a	3.02 ^a	3.08 ^a	0.01	1–4

^{a,b}Means in a row without a similar superscript differ ($P < 0.05$). SEM: Standard error of the mean, RBC: Red blood cell, MCH: Mean corpuscular hemoglobin, MCV: Mean corpuscular volume, MCHC: MCH concentration, WBC: White blood cell

the hematological parameters analyzed. However, the values were within the established ranges for healthy rabbits (Merck Veterinary Manual, 2011) recommending the absence of health challenges among rabbits. The pack cell volume, hemoglobin, red blood cells, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentrations were greater ($P < 0.05$) in T2, T3, and T4 than in T1. Higher pack cell volume, red blood cell, and hemoglobin values could lead to an increased cell ability to transport oxygen which translates to efficient distribution of nutrients in the body of animals.^[13] This observation agreed with the findings of Alagbe where increase in pack cell volume, hemoglobin, and red blood cell was reported in weaner pigs supplemented with *Eucalyptus* oil.^[14] Mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentrations were within the normal ranges by Alessandro, suggesting that there was acute inflammation in the cells of rabbits.^[15] White blood cells, lymphocytes, monocytes, neutrophils, and eosinophils were highest ($P < 0.05$) in T2, T3, and T4 relative to T1 indicating that the animal's immune system is strengthened.^[16,17] White blood cell is vital in body defense and immunity, and it also detects foreign organisms and destroys them.^[18] Rising white blood cell in the bloodstream usually indicates a physiological problem/infection. Neutrophils are indices for stress, and they also protect the body against bacterial infection such as acute inflammation.^[19] Elevation in the number of basophils occurs during acute hypersensitive reaction or allergy.^[20] Eosinophils play a special role in the elimination of parasites such as worms and allergic infections.^[21] Monocytes and lymphocytes are indices for immune response.^[22]

Serum biochemical indices of weaned rabbits fed diet supplemented with *Eucalyptus camaldulensis* oil

Table 3 shows the dietary treatment influenced the serum biochemical indices with the exception of creatinine, urea nitrogen, calcium, potassium, sodium, bicarbonate, and phosphorus ions. Total protein, albumin, and globulin values were maximum ($P < 0.05$) in T2, T3, and T4 relative to T1. However, all values were within the optimum range for healthy rabbits. The increase in globulin levels among rabbits fed ECO suggests efficient transportation of essential metals through the bloodstream to all parts of the rabbit's body. Animals with severe infections frequently exhibit high globulin levels as a result of unusually elevated antibody production. Increased total protein and albumin/globulin ratio in T2, T3, and T4 suggest that rabbits' ability to use protein is uninhibited. Cholesterol values were higher ($P < 0.05$) in T1 than in other diets; this reveals that the ECO has hypocholesterolemic potential which could promote food safety and prevent the risk of heart diseases.^[23,24] Urea nitrogen and creatinine levels were within the normal range for healthy rabbits reported by Özkan *et al.*, 2012; this suggests the absence of kidney or liver damage and other metabolic disorders.^[25,26] Aspartate transaminase, alanine transaminase, and alanine phosphatase are indices for liver damage; these values were within the optimal ranges

for healthy rabbits indicating the absence of chronic liver inflammation, bile ducts, cholestasis as well as muscle damage in rabbits.^[27-29] Calcium, potassium, sodium, phosphorus, and bicarbonate levels were not influenced ($P > 0.05$) by the treatments. However, all values were within the optimum ranges for rabbits reported by Haghjooyjavanmard *et al.*, suggesting that the experimental diets were able to regulate chemical reaction, maintain fluid balance and blood volume as well as energy processing in deoxyribonucleic acid and adenosine triphosphate.^[30-32] Harcourt-Brown reported that a normal serum bicarbonate level is a definite sign that kidney infections are absent.^[33]

Immune response of weaned rabbits fed diet supplemented with *Eucalyptus camaldulensis* oil

Table 4 reveals the immune response of weaned rabbits fed diet supplemented with *E. camaldulensis* oil (ECO). Immunoglobulin A, immunoglobulin G, and immunoglobulin M values were altered ($P < 0.05$) by supplementing ECO in the diets of rabbits. Immunoglobulins (A, G, and M) were higher ($P < 0.05$) in T2, T3, and T4 relative to T1, suggesting that ECO contains bioactive compounds, especially eucalyptol and α -pinene which have immunostimulatory properties.^[34] Oluwafemi *et al.* (2021) also explained that phytochemicals or bioactive compounds have several pharmacological activities including hepatoprotective, antimicrobial, antioxidants, antiviral, antihelminthic, and antifungal among others.^[35] Phenols are antioxidants or immune boosters which prevent the body against the invasion of pathogenic organisms.^[36] The results obtained in this study corroborated with the findings by Oloruntola *et al.* (2016) where increased immunoglobulin was reported in rabbits fed diet supplemented with *Alchornea cordifolia* leaf. Similar results were recorded when phytogenics (*Gliricidia* leaf) were supplemented in the diets of weaned rabbits.^[37,38]

Oxidative stress indices of weaned rabbits fed diet supplemented with *Eucalyptus camaldulensis* oil

The oxidative stress indices of weaned rabbits fed diet supplemented with *E. camaldulensis* oil (ECO) are presented in Table 5. Total antioxidant capacity values varied from 3.00 to 4.74 (nmol/L), superoxide dismutase (28.61–42.15 nmol/L), glutathione peroxidase (21.88–36.04 nmol/L), catalase (11.50–21.01 nmol/L), and malondialdehyde (58.07–71.87 nmol/L). Total antioxidant capacity, superoxide dismutase, glutathione peroxidase, catalase, and malondialdehyde values were greater ($P < 0.05$) in T2, T3, and T4 relative to T1. This result suggests that ECO has antioxidant properties (α -pinene and eucalyptol), which protects against toxic effects of free radicals.^[39] According to Alagbe *et al.*, the protective mechanisms of antioxidants can occur at different levels, preventing the initiation of chain reactions by removing free radicals, interrupting chain sequence of scavenging free radicals generated in the chain reactions, and removing the peroxidases, thereby preventing further generation of reactive oxygen species.^[40,41] The results

Table 3: Serum biochemical indices of weaned rabbits fed diet supplemented with *Eucalyptus camaldulensis* oil

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	SEM	Reference value
Total protein (g/L)	3.78 ^b	4.93 ^a	4.95 ^a	5.02 ^a	0.02	5.4–7.5
Albumin (g/L)	1.84 ^b	2.50 ^a	2.51 ^a	2.52 ^a	0.01	2.7–5.0
Globulin (g/L)	1.94 ^b	2.43 ^a	2.44 ^a	2.50 ^a	0.01	1.5–2.7
Albumin/globulin ratio	0.95 ^b	1.02 ^a	1.03 ^a	1.01 ^a	0.03	0.5–1.8
Cholesterol (mmol/L)	76.81 ^a	54.50 ^b	53.28 ^b	50.91 ^b	1.02	11–70
Urea nitrogen (mmol/L)	3.21	3.27	3.08	3.11	0.01	1.0–4.0
Creatinine (mmol/L)	2.09	2.11	2.08	2.21	0.01	0.91–3.0
AST (U/L)	115.7 ^a	100.6 ^b	98.12 ^b	98.00 ^b	1.23	35–130
Alanine transaminase (U/L)	72.56 ^a	60.80 ^b	60.11 ^b	60.02 ^b	1.01	45–80
Alanine phosphatase (U/L)	54.12 ^a	48.04 ^b	44.16 ^b	44.05 ^b	0.09	12–96
Calcium (mmol/L)	2.50	2.68	2.80	2.88	0.01	1.0–3.0
Potassium (mmol/L)	1.51	1.55	1.61	1.66	0.02	1.0–4.0
Sodium (mmol/L)	130.6	134.7	138.5	139.1	2.93	100–141
Bicarbonate (mmol/L)	27.4	28.5	29.1	29.6	0.09	15–40
Phosphorus (mmol/L)	1.40	1.49	1.51	1.55	0.01	1.0–3.5

^{a,b}Means in a row without a similar superscript differ ($P<0.05$). AST: Aspartate transaminase, SEM: Standard error of the mean

Table 4: Immune response of weaned rabbits fed diet supplemented with *Eucalyptus camaldulensis* oil

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	SEM
Immunoglobulin A (µg/mL)	1.77 ^b	2.97 ^a	3.05 ^a	3.11 ^a	0.02
Immunoglobulin G (µg/mL)	3.00 ^b	4.07 ^a	4.14 ^a	4.58 ^a	0.13
Immunoglobulin M (µg/mL)	0.92 ^b	1.26 ^a	1.55 ^a	1.57 ^a	0.01

^{a,b}Means in a row without a similar superscript differ ($P<0.05$). SEM: Standard error of the mean

Table 5: Oxidative stress indices of weaned rabbits fed diet supplemented with *Eucalyptus camaldulensis* oil

Parameters	Treatment 1	Treatment 2	Treatment 3	Treatment 4	SEM
Total antioxidant capacity (nmol/mL)	3.00 ^b	4.52 ^a	4.68 ^a	4.74 ^a	0.02
Superoxide dismutase (nmol/mL)	28.61 ^b	38.90 ^a	40.11 ^a	42.15 ^a	0.17
Glutathione peroxidase (nmol/mL)	21.88 ^b	30.14 ^a	35.49 ^a	36.04 ^a	0.05
Catalase (nmol/mL)	11.50 ^b	19.60 ^a	20.56 ^a	21.01 ^a	0.02
Malondialdehyde (nmol/mL)	71.87 ^a	59.93 ^b	59.34 ^b	58.07 ^b	0.08

^{a,b}Means in a row without a similar superscript differ ($P<0.05$). SEM: Standard error of the mean

obtained in this study agreed with the findings by Awad *et al.* where the activities of free radicals were scavenged in animals fed diet supplemented with probiotics.^[42-44] Similar results were reported by previous study who recorded a positive response when natural plant extract was supplemented in the diet of rabbits.^[45-48]

CONCLUSION

ECO expresses a wide range of biological activities including antimicrobial, antiviral, immunostimulatory, hepatoprotective, and antioxidants among others. ECO is safe at 600 mg/kg because it does not pose any detrimental effect on hematological and serum biochemical parameters as well as the immune response of rabbits. Its ability to protect against toxic effects of free radicals will prevent diseases/mortality and promote food safety.

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Conflicts of interest

There are no conflicts of interest.

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