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Effect of Dietary Supplementation of Lantana Camara Oil on the Hamato-Biochemical and Immune Response of Sirohi Bucks

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A B S T R A C T

The aim of this study was to evaluate effect of supplementation of Lantana camara oil on haematological, serum and immune response of Sirohi male bucks. 40 healthy Sirohi bucks with 11.22 ± 0.21 kg initial body weight (8 months of age) were grouped into four treatments of ten bucks each. Goats in treatment 1(T1) was fed experimental diet without Lantana oil while those in T2, T3 and T4 received experimental diet supplemented with oil at 1 mL, 2mL and 3mL per kg diet. The experimental duration was 12 weeks, animals had free access to water and a completely randomized design was used. The major bioactive compound observed in Lantana camara oil include, β-Caryophyllene (30.61 %), Germacrene (16.88 %), Valencene (15.40 %), β-Linalol (10.50 %), β-Guaiene (7.11 %) and α-Pinene (6.90 %). In T3 and T4, haematocrit, total erythrocytes, haemoglobin, leucocytes, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration, lymphocytes and monocytes were higher, it was intermediate in T2 and lower in T1 except for basophils and eosinophils count which were not affected ($p>0.05$). Total protein, albumin, globulin, glucose and cholesterol were influenced by the treatment ($p<0.05$) whereas, urea, creatinine, alkaline phosphatase, alanine aminotransferase and aspartate amino transferase were not significantly different. Immunoglobulin A, G and M value was lower in T1 than in T2, T3 and T4 ($p<0.05$). In conclusion, all values recorded in this experiment fell within the reference range for clinically healthy bucks, indicating that supplementation of Lantana camara oil to the diet gas no adverse impact on their health status

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INTRODUCTION

The ban on the use of antibiotics as growth promoters in most countries of the world especially in the diet of ruminants has been maintained due to antimicrobial resistance and deposit of toxic residues in animal products which could be pose a serious threat to human health (Alagbe, 2024). This scenario has led animal scientist to search for safer alternatives to antibiotics. Among the potential alternatives is the use of plant extracts such as essential oils since they are less toxic, eco-friendly, effective and has no withdrawal period (Agubosi et al., 2022; Musa et al., 2020). According to (Ojediran et al., 2024a) medicinal plants produce essential oil which contains several phyto-constituents such as, tannins, flavonoids, terpenoids, saponins, phenols, alkaloids, triterpenoids amongst others which have numerous pharmacological functions including, anti-inflammatory, anti-cancer, anti-diabetic, antifungal, anti-helminthic, antifungal, immuno-modulatory, hepat-protective amongst others (Ojediran et al., 2024b; Shittu et al., 2023). These phyto-constituents or bioactive compounds can be influenced by storage, age of plant, specie, geographical location, method of processing and harvesting (Adewale et al., 2021). For more sustainable livestock production and safeguard animal protein, the utilization of essential oils from medicinal plants is rapidly gaining interest (John, 2024b; Daniel et al., 2023). Among the potential plants with therapeutic properties is Lantana camara.

Lamara camara is a shrub belonging to the family Verbenuceae, it grows mostly in tropical, sub-tropical and temperate regions of the world (Sathish et al., 2011; Khan et al., 2016). The leaves have high medicinal value and it also contains some essential minerals like, potassium, manganese, zinc and cobalt (Sousa et al., 2013; Tesch et al., 2011). Aqueous extract from the leaves of Lantana camara have been utilized for the treatment of toothache, skin infections, tumors, gastro-intestinal problems, rheumatism, fever, cancer, respiratory problems and sexually transmitted infections (Goswami-Giri and Oza, 2014; Bashir et al., 2019). The leaf is loaded with triterpenoids, glycosides, flavonoids, alkaloids, saponins and phenols which have high efficacy to eliminate pathogenic organisms in the gastro-intestinal tract (Bhuvaneswari and Giri, 2018). According to (Elansary et al., 2012; Zoubiri and Baaliouamer, 2012), essential oils from Lantana camara majorly contains bicyclogermacrene at 19.40 %, isocarophyllene (16.70 %), valencene (12.90 %) and germacrene (12.30 %). These compounds have numerous pharmacological functions including, anti-amoebic, anti-tumor, anti-cancer, anti-diarrhoea, anti-inflammatory, antifungal, antiviral, antidiabetic, gastro-protective, antioxidant, antimicrobial, hypolipidemic amongst others (Yaradua and Shah, 2020; Verma et al., 2013).

Though there is dearth of information on the dietary supplementation of Lantana camara essential oil in the diet of ruminants. However, previous studies have shown that supplementation of essential oils in the diet of ruminants can help to improve feed efficiency (Morsy et al., 2021; Maekawa et al., 2002), growth performance (Weng et al., 2023; Soltan et al., 2021), rumen modification and prevent greenhouse emission (Amr et al., 2025; Ihejirika et al., 2024). However, there conflicting reports about their appropriate dose in the diet of ruminants. Supplementation of essential oils at a higher could be toxic and detrimental to the health of animals. Therefore, it is necessary to establish a safe optimum level for animals in order to promote livestock production and food safety.

This study was designed examine the dietary supplementation of Lantana camara essential oil on the haemato-biochemical and immune response of Sirohi bucks.

LITERATURE REVIEW

Study Area

The study was undertaken at the Ruminant Unit of Gandhi College of Agriculture, Rajasthan, India after approval by the ethics committee at the Department of Animal Production of the same institution. The college lies between latitude 5o 18'N and 36o 12'E and longitude 4o 00'N and 6o 012'E. It has an annual rainfall ranging from 1200 – 1800 mm and with temperature of 44°C.

Collection of Lantana Camara Leaves and Oil Extraction

Fresh and healthy leaves of Lantana camara were collected from Gujarat in the month of October 2024. Botanical authentication and identification was carried out at the department of Taxonomy, Gandhi College of Agriculture, Rajasthan by Liu Xing with reference number (HPF/05T/2024). Leaves were sorted, air dried for 9 days before they were pulverized into powder using electric blender. The pulverized powder was transferred into a labelled zip lock and placed under room temperature. Oil extraction was carried out using hydro-distillation technique as described by John (2024). Briefly, 200 g of pulverized Lantana camara leaf powder was measured into a round bottom flask, placed on a heating mantle and heated at 65°C for 20 minutes the steam produced passes through the glass condenser mounted on the top of the round bottom flask. After cooling it is collected into a beaker and transferred into a separator to separate the oil from other liquid. The collected oil is kept in the refrigerator at a temperature of 4°C before it was taken to the biochemistry department of Sumitra Research Institute, Gujarat for further evaluation.

Evaluation of Bioactive Compounds in Lantana Camara Oil

Bioactive compounds in Lantana camara oil was done using Quadrupole gas chromatography and mass spectrometry (GC-MS DW-EXPEC-3700, Netherlands). 10 mL of Lantana camara oil was passed into the sample carrier of the machine and the gas chromatography unit is set at a peak area repeatability of < 0.5 % and retention time repeatability < 0.008 %. Column oven is maintained at a temperature setting accuracy of ≤ 0.1°C supported carrier and make up gas of nitrogen, helium with two injectors ports, split ratio up to 12500:1 to avoid column overload, flow control range 0-500 mL/min (N_2), 0-1000 mL (H_2 and He). While the mass spectrometry was adjusted to an ionization energy (0-240 eV), ion source temperature (50 to 350 °C) and transfer line temperatures of 50 to 400 °C. Identification of bioactive components in the essential oil was based on comparison with their retention time and mass spectra by matching with those of the National Institute of Standard and Technology (NIST, 2011) database.

Experimental Goats, Diet, Housing and Management

40 Sirohi male bucks of about 8 months of age with an initial body weight of 11.22 ± 0.21 kg was purchased from Sumitra Research Institute, Gujarat and used for the experiment. Open sided pens where the pens were kept was disinfected thoroughly before the arrival of the animals. On arrival of the goats, they were quarantined for 2 weeks and treated against parasites (endo and ecto-parasites) using Ivermectin Plus® and also vaccinated against PPR (Peste Des Petits). Animals were fed basal diet which meets their nutritional requirement according to NRC (1994) standard as presented in Table 1. After the period of acclimatization, goats were stratified based on their weights and grouped into 4 treatments. Each goat served as a replicate and were individually housed in a wooden cage of 1.8 m2 each. Clean fresh water was provided unrestrictedly and the animals were fed 5 % of their body weight thrice daily at 07:00 H, 12:00 H and 17:00 H. Goats in treatment 1 received basal diet without Lantana camara oil while those in treatment 2, 3 and 4 were given basal diet with Lantana camara oil at 1 mL, 2 mL and 3 mL per kg diet respectively. Feed intake was estimated by subtracting the feed left over from the feed served.

Chemical Analysis of Experimental Diet

Crude protein and ether extract content of basal diet was done using Gild automated near –infra red feed analyzer (model HJ/09CV-32, China). Machine was adjusted according to the technical specifications of the machine to ensure precision in results. Ash content was determined with Leans Atomic Absorption Spectrophotometer (Model POD-06A-3Y, Netherlands). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were determined according to scientific procedures outlined by AOAC (2010). Organic matter, cellulose, hemicellulose, lignin and non-structural carbohydrates were estimated using the formula below:

Organic matter = 100 – ash content

Non-structural carbohydrate = 100 – (Crude protein + ether extract + ash + NDF)

Hemicellulose = Neutral detergent fibre – acid detergent fibre

Cellulose = ADF – lignin

Lignin = ADF – cellulose

Blood collection and evaluation

At the end of the trial, 5 mL of blood was collected from the jugular vein of each goat in the early hours of the morning before feeding into 2 labeled sample bottles (one for haematology and the other for serum). Sample bottles for haematology had anticoagulant (EDTA) while those for serum analysis were EDTA free. Collected samples were placed in an ice pack and sent to the biochemistry laboratory at Sumitra Research Institute, Gujarat India. Haematological parameters were analyzed using MS-H630 auto-haematology analyzer (China). Kit was adjusted to a reagent volume of 10 – 35 μL and sample volume of 1.5 μL to ensure precision in the results. Fully automated random access chemical chemistry analyzer (model VB-09H, China) was used to analyze serum parameters. It was placed at a reaction temperature of 37°C, reagent volume (15 to 300 μL) and sample volume of 2 – 70 μL. LIS software was used for result processing via the monitor.

Samples for serum parameters were also used to determine immune parameters (IgA, IgG and IgM) using Mindray clinical chemistry analyzer (BS-480 model, USA) with automatic sample dilution, pre-dilution and post-dilution ratio up to 1:150. Other components in the machine were adjusted to a reagent volume of 10 – 400 µL and sample volume (1.5-5.0 µL).

METHODOLOGY

Data Analysis

In Statistical Analysis for Social Sciences (SPSS), data were subjected to analysis of variance. To test the significant differences Duncan multiple range test was employed where $p<0.05$ level was declared significant. The following model was used:

$$Hey = \mu + Se + Dey$$

Where Hey = response variable to the parameters under trial; µ = general mean; Se = fixed effect of dietary treatment on the parameters observed; Dey = random error.

RESULTS AND DISCUSSION

Outcome on the bioactive compounds in Lantana camara oil suggests the major compounds includes, β-Caryophyllene (30.61 %), Germacrene (16.88 %), Valencene (15.40 %), β-Linalol (10.50 %), β-Guaiene (7.11 %) and α-Pinene (6.90 %). The presence of these compounds confirms the earlier reports of (Erianio et al., 2012). (John, 2024a; John, 2024b; Adewale et al., 2021), reports that bioactive compounds in essential oils can be influenced by method of preparation, specie, period and method of harvesting, age, geographical location amongst others. However, the presence of these major compounds shows that it can perform several pharmacological roles in goats such as, neuroprotective, cytotoxic, analgesics, gastro-protective, immune-modulatory, anti-cancer, anxiolytic, mutagenic, hepato-protective, hypolipidemic, anti-inflammatory, antioxidant, antifungal, antiviral, anti-diarrhea, appetite stimulator amongst others. Bioactive compounds are the power house of medicinal plants and have been reported to be environmental friendly, effective, non-toxic and has no withdrawal period thus alleviating the fear of antimicrobial resistance (Musa et al., 2020; Alagbe, 2024).

Results on haematological parameters of Sirohi male goats fed diets supplemented with Lantana camara oil (Table 3) shows that total erythrocyte, haemoglobin, hematocrit counts decreased in T1, although it was greater in T3 and T4 and intermediate in T2 ($p<0.05$). Similarly, in T3 and T4 mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrations were higher, intermediate in T2 and in T1 it was lower ($p<0.05$). (Madan et al., 2016) reported that total erythrocytes, haematocrit and haemoglobin values were within 8.00 -15.00 (×106/µL), 28.0 – 36.0 % and 8.00 – 13.00 g/dL. The result demonstrates oxygen sufficiency in the body of animals. Low red blood cell may be the sign of bone marrow disorder, folate deficiency and gastro-intestinal infection (Omokore and Alagbe, 2019; John, 2024c).

Haemoglobin and hematocrit are important in accessing conditions like, anaemia and renal disorder. Significant difference of the treatment was observed in mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration levels ($p<0.05$). The result obtained suggests the absence iron deficiency anaemia in goats (Arfuso et al., 2016; Egbe-Nwiyi et al., 2015). However, the results obtained aligns with reports of (Egbe-Nwiyi et al., 2015, Zumbo et al., 2011), who recorded a range of 16.0 – 40.0 fl, 25.00 – 45.00 pg and 40.00 – 76.00 g/dL for mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration. Total leucocyte, lymphocytes, monocytes count varied from [(8.00 – 15.00 (×103/μL)], 60.00 – 79.00 %, 1.00 – 4.00 % were affected by the treatment ($p<0.05$) except for basophils (0.80 – 2.00 %) and eosinophils (0.01 – 1.00 %) ($p>0.05$). Values obtained were within the established range for goats according to (John, 2024a). Leucocytes, lymphocytes and monocytes actively participant in the defense against diseases and infections in the body (John, 2024d). Results obtained suggests that goats were healthy throughout the period of the experiment. Eosinophils and basophils level are mostly triggered during parasitic infections and allergic state (John, 2024e; Daniel et al., 2023).

Although albumin, globulin and total protein values were higher in T2, T3 and T4 compared to those in T1 ($p<0.05$) however, values were within the established range mentioned by (Sharma and Kataria, 2012). According to (Piccione et al., 2012), albumin, globulin and total protein values were within 2.40 – 4.00 g/dL, 2.50 – 4.50 g/dL and 5.00 – 7.50 g/dL. The result obtained demonstrates sufficiency in dietary protein across the treatment (Arfuso et al., 2016; Alagbe, 2025). Glucose level was lower in T1 (51.72 mg/dL) than in T2 (60.80 mg/dL), T3 (62.10 mg/dL) and T4 (63.10 mg/dL) ($p<0.05$) whereas cholesterol level was greater in T1 (109.3 mg/dL) than T2 (89.03 mg/dL), T3 (85.19 mg/dL) and T4 (85.00 mg/dL) ($p<0.05$). Cholesterol and glucose level was within 102.8 – 250 mg/dL and 45.00 – 75.00 mg/dL reported by (). Result obtained shows that dietary supplementation of Lantana camara oil can reduce the risk of heart infection in goats due to its hypo-lipidemic nature (Agubosi et al., 2022a; Oluwafemi et al., 2021a, b). Creatinine, urea and bilirubin values were not affected ($p>0.05$) by the treatments. However, values were within the range [(creatinine: 1.20 – 3.00 mg/dL); urea (2.00 – 6.70 mg/dL); 0.01 – 0.05 mg/dL)] noted by (Latimer, 2011). Elevation in serum bilirubin level is caused by hepatic disorder (Singh et al., 2022; Daniel et al., 2023). Stability in the values of creatinine and urea suggests the absence of renal disorder (Shittu et al., 2023). Alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate amino transferase (AST) values recorded in this study were within 90.00 – 240.0 (U/L); 14.00 – 25.00 (U/L) and 35.00 – 70.00 (U/L) stated by (Njidda et al., 2013). Similarly, (Shahab et al., 2017) noted that ALP and AST values in different goat breeds in Sultanate of Oman ranged from 87.00 – 240.0 (U/L) and 35.00 – 60.00 (U/L). This result suggests the non-toxicity effect of Lantana camara oil when supplemented up to 3 mLs in the diet of goats. Conditions which cause large amount of serum enzymes in the blood (ALP, AST and ALT) could include, deficiency of vitamins as well as damaged liver cells in the body of animals.

Immunoglobulin parameters of Sirohi male goats fed diets supplemented with Lantana camara oil (Table 5). Treatment had significant influence on IgA, IgG and IgA levels. The values obtained in this study decreased in T1, although it was higher in T2, T3 and T4 ($p<0.05$). This result demonstrates that dietary supplementation of Lantana camara oil could trigger the production of more antibodies securing the body against the attack of disease (Alagbe et al, 2025). The values obtained were similar to those stated by (Mishra et al., 2013).

Table 1. Ingredient and Chemical Composition of Basal Diet (% Dry Matter)

Components	Quantity
Maize	30.00
Corn barn	25.00
Cowpea husk	22.00
Groundnut cake	20.00
Oyster shell	2.50
Growers premix	1.00
Salt	0.50
Total	100.00
Chemical composition	
Organic matter	92.10
Crude protein	16.07
Ether extract	3.06
Ash	7.90
Non-structural carbohydrate	35.36
Acid detergent fibre	22.72
Acid detergent lignin	3.11
Neutral detergent fibre	37.66
Cellulose	19.61
Hemicellulose	14.94
Energy (Kcal/kg)	2556.7

Table 2. Bioactive Compounds of Lantana Camara Oil

Compounds	Retention time	Composition (%)
Methyl-cyclohexane	405.7	0.62
4-Methyl-2-pentanone	429.5	0.88
Valencene	440.8	15.40
Ethyl benzene	501.2	0.91
Germacrene	533.0	16.88
α-Pinene	546.7	6.90
Octane	560.3	0.01
Toluene	572.5	0.07
3-Octanol	588.6	1.08
Linalool	620.9	0.59
Oleic acid	660.3	0.04

β-Guaiene	671.7	7.11
Palmitic acid	680.9	0.26
n-Hexadecanoic acid	716.1	0.10
Pentadecanoic acid	742.9	0.55
Humulane-1,6-dien-3-ol	803.4	0.03
β-Linalol	852.9	10.50
α-Cadinol	875.4	0.93
D-Limonene	894.5	0.52
2,4,6-Trimethyldecane	903.6	0.05
β-Caryophyllene	911.4	30.61
α-Muurolene	947.0	0.02
α-Terpinolene	964.2	0.08
Trans-2-Nonenal	978.3	0.04
β-Cymene	981.5	1.09
Benzene isothiocyanate	990.1	0.11
β-Selinenol	992.3	0.66
Isopropyltetradecanoate	998.6	0.81

Table 3. Haematological Parameters of Sirohi Male Goats Fed Diets Supplemented with Lantana Camara Oil

Parameters	T_1	T_2	T_3	T_4	SEM
Total erythrocytes count ($\times 10^6/\mu L$)	10.07^b	12.90^b	14.18^a	14.25^a	2.09
Haemoglobin (g/dL)	8.12^b	10.10^b	11.13^a	11.18^a	1.88
Hematocrit (%)	28.85^b	30.40^b	32.06^a	32.41^a	3.37
Mean corpuscular volume (fl)	18.59^b	22.97^b	27.11^a	27.18^a	2.92
Mean corpuscular haemoglobin (pg)	29.06^b	30.81^b	34.05^a	34.72^a	3.01
Mean corpuscular haemoglobin concentration (g/dL)	40.80^b	51.72^b	53.07^a	53.21^a	7.28
Total leucocyte count ($\times 10^3/\mu L$)	9.71^b	11.13^a	11.21^a	11.29^a	0.19
Lymphocytes (%)	52.66^b	70.12^a	73.88^a	74.05^a	5.02
Monocytes (%)	1.83^b	2.51^a	2.64^a	2.72^a	0.09
Eosinophils (%)	1.51	1.60	1.66	1.68	0.04
Basophils (%)	0.32	0.29	0.30	0.33	0.01

Means with different superscripts along the row were significantly ($p<0.05$) influenced; SEM: standard error of mean; T1: 0 % Lantana camara oil supplementation; T2, T3 and T4: 1mL, 2 mL and 3 mL dietary supplementation of Lantana camara oil per kg diet.

Table 4. Serum Biochemical Parameters of Sirohi Male Goats Fed Diets Supplemented with Lantana Camara Oil

Parameters	T_1	T_2	T_3	T_4	SEM
Albumin (g/dL)	2.90^b	3.30^a	3.30^a	3.32^a	0.02
Globulin (g/dL)	3.08^b	3.39^a	3.50^a	3.50^a	0.03
Total protein (g/dL)	5.98^b	6.69^a	6.80^a	6.82^a	0.07

Glucose (mg/dL)	51.72[b]	60.80[a]	62.10[a]	63.01[a]	3.47
Creatinine (mg/dL)	1.34	1.37	1.30	1.33	0.05
Urea (mg/dL)	5.90	5.06	5.01	5.00	0.12
Cholesterol (mg/dL)	109.3[a]	89.03[b]	85.19[b]	85.00[b]	2.06
Total bilirubin (mg/dL)	0.02	0.01	0.01	0.01	0.001
ALP (U/L)	110.3	115.8	118.6	118.9	5.72
AST (U/L)	40.83	42.10	43.56	43.95	1.17
ALT (U/L)	14.80	15.01	15.66	15.80	0.19

Means with different superscripts along the row were significantly ($p<0.05$) influenced; SEM: standard error of mean; T1: 0 % Lantana camara oil supplementation; T2, T3 and T4: 1mL, 2 mL and 3 mL dietary supplementation of Lantana camara oil per kg diet.

Table 5. Immunoglobulin Parameters of Sirohi Male Goats Fed Diets Supplemented with Lantana Camara Oil

Parameters (mg/dL)	T_1	T_2	T_3	T_4	SEM
Immunoglobulin A (IgA)	1.12[b]	1.96[a]	1.98[a]	2.00[a]	0.06
Immunoglobulin G (IgG)	0.17[b]	0.33[a]	0.35[a]	0.39[a]	0.03
Immunoglobulin M (IgM)	0.86[b]	1.02[a]	1.13[a]	1.18[a]	0.05

Means with different superscripts along the row were significantly ($p<0.05$) influenced; SEM: standard error of mean; T1: 0 % Lantana camara oil supplementation; T2, T3 and T4: 1mL, 2 mL and 3 mL dietary supplementation of Lantana camara oil per kg diet.

CONCLUSIONS AND RECOMMENDATIONS

Based on the obtained results, it can be concluded that the effect of dietary supplementation of Lantana camara oil in Sirohi male goats had statistical significant effect on blood parameters (haematology and serum) as well as their immune system. Though higher values were recorded among goats fed 1mL, 2mL and 3mL compared to 0 mL (control). However, all values obtained were within the reference range for clinically healthy goats.

FURTHER STUDY

This research still has limitations, so further research is needed related to the topic of Effect of Dietary Supplementation of Lantana Camara Oil on the Hamato-Biochemical and Immune Response of Sirohi Bucks in order to perfect this research and increase insight for readers.

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