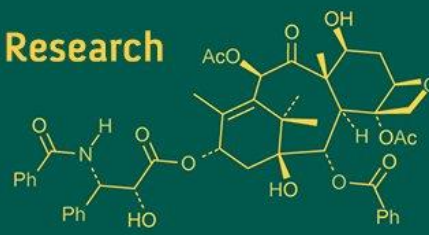
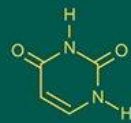
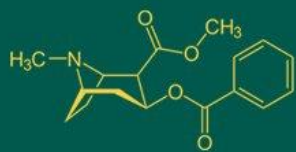


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Comparative therapeutic evaluation of an ethnoveterinary herbal spray and conventional topical antifungal therapy for bovine dermatophytosis

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Abstract

Dermatophytosis is a highly contagious superficial fungal infection of keratinized tissues that adversely affects the health, productivity, and economic value of dairy animals while posing a significant zoonotic risk. Although conventional topical antifungal agents are effective, their prolonged use, higher cost, and potential adverse effects have encouraged the exploration of ethnoveterinary alternatives. The present study was undertaken to compare the therapeutic efficacy of an ethnoveterinary herbal spray with a conventional topical antifungal formulation in naturally occurring bovine dermatophytosis. A total of 137 cattle and buffaloes from organized and unorganized dairy farms in and around Mhow, Madhya Pradesh, India, were clinically screened for dermatophytosis. Diagnosis was confirmed by direct microscopic examination of 20% potassium hydroxide (KOH)-treated skin scrapings and fungal culture on Sabouraud Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM). Twenty-nine animals (21.17%) were confirmed positive, of which sixteen culture-positive animals were randomly allocated into two treatment groups (n = 8 each). Group I received a topical formulation containing clobetasol propionate, ofloxacin, miconazole nitrate, and zinc sulphate, whereas Group II was treated with an ethnoveterinary herbal spray twice daily for ten days. Clinical response, lesion severity, haematological and biochemical parameters, and mycological recovery were evaluated on Days 0, 14, 28, and 45 post-treatment. Dermatophytosis was characterized by alopecia, crust formation, erythema, and pruritus, predominantly affecting the neck, hump, and dorsal trunk. Significant ($P < 0.05$) improvements in haemoglobin concentration, packed cell volume, total erythrocyte count, neutrophil, and lymphocyte percentages were observed in both groups during recovery, whereas physiological parameters, total leukocyte count, serum total protein, creatinine, and aspartate aminotransferase remained within normal limits. Mycological cure was achieved in 87.5% of animals receiving conventional therapy and 75.0% of animals treated with the herbal spray. No relapse or treatment-related adverse effects were observed during the 45-day follow-up period. The findings indicate that the ethnoveterinary herbal spray is a safe, economical, and farmer-friendly alternative for the management of bovine dermatophytosis, although conventional topical antifungal therapy demonstrated a comparatively higher cure rate.

Keywords: Bovine dermatophytosis, ethnoveterinary herbal spray, antifungal therapy, *Trichophyton* spp., mycological cure

Introduction

Dermatophytosis (ringworm) is a highly contagious superficial fungal disease affecting the keratinized tissues of animals, including the skin, hair, horns, and hooves. It is caused by keratinophilic fungi belonging primarily to the genera *Trichophyton*, *Microsporum*, and *Epidermophyton*, with *Trichophyton verrucosum* recognized as the principal etiological agent in cattle worldwide (Fratti *et al.*, 2023). Dermatophytes invade keratinized tissues by producing keratinolytic enzymes, resulting in alopecia, scaling, crust formation, and characteristic circular lesions. Although the infection is generally confined to superficial tissues, it causes considerable discomfort, reduces animal productivity, and represents an important zoonotic hazard for veterinarians, dairy workers, and animal handlers (Moriello *et al.*, 2017)^[4, 7].

Bovine dermatophytosis has been reported globally with prevalence varying according to geographical location, climatic conditions, management practices, animal age, nutritional

status, and hygiene. Young calves are particularly susceptible because of their immature immune system, while overcrowding, poor ventilation, high humidity, nutritional deficiencies, and environmental contamination facilitate transmission and persistence of infection (Robi *et al.*, 2023). Dermatophyte spores can survive for prolonged periods in the environment, making contaminated housing, bedding, grooming equipment, and fences important sources of infection^[11].

Although dermatophytosis is rarely fatal, it has substantial economic significance due to decreased milk production, reduced weight gain, deterioration of hide quality, increased treatment costs, and temporary restrictions on animal movement. The disease also compromises animal welfare through persistent pruritus and skin irritation while increasing the risk of zoonotic transmission, particularly in dairy production systems (Fratti *et al.*, 2023)^[4]. Diagnosis of dermatophytosis is based on clinical examination supported by laboratory confirmation. Direct microscopic examination of potassium hydroxide (KOH)-treated skin scrapings provides rapid presumptive diagnosis, whereas fungal isolation on Sabouraud Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM) remains the conventional gold standard for confirmation. Molecular diagnostic techniques such as polymerase chain reaction (PCR) offer greater sensitivity and specificity but are still unavailable in many field veterinary laboratories because of their cost and technical requirements (Lagowski *et al.*, 2021)^[6].

Topical antifungal agents, including miconazole, clotrimazole, ketoconazole, enilconazole, and other azole compounds, remain the cornerstone of bovine dermatophytosis treatment. However, prolonged treatment duration, repeated application, high cost, occasional adverse reactions, and concerns regarding antifungal resistance limit their widespread use, particularly under field conditions in developing countries (Shaheen, 2005). Consequently, there has been increasing interest in ethnoveterinary medicine as a sustainable alternative for managing infectious diseases in livestock^[12].

Ethnoveterinary medicine encompasses traditional knowledge and the use of medicinal plants for the prevention and treatment of animal diseases. Herbal formulations contain diverse bioactive constituents, including flavonoids, tannins, phenolic compounds, terpenoids, alkaloids, and essential oils, which possess antifungal, antioxidant, anti-inflammatory, immunomodulatory, and wound-healing activities. These phytochemicals inhibit fungal growth through multiple mechanisms, including disruption of fungal cell membranes, inhibition of ergosterol biosynthesis, suppression of spore germination, and promotion of epithelial regeneration (Nazzaro *et al.*, 2017). Despite their widespread traditional use, well-designed comparative clinical studies validating their efficacy in naturally occurring bovine dermatophytosis remain limited^[9].

Therefore, the present study was undertaken to compare the therapeutic efficacy of an ethnoveterinary herbal spray with a conventional topical antifungal preparation in naturally occurring bovine dermatophytosis. Clinical recovery, hematological and biochemical responses, and mycological cure were evaluated to determine the safety and effectiveness of the herbal formulation as a practical and economical alternative for the management of bovine dermatophytosis under field conditions.

Materials and methods

Study location: The present study was conducted at the Department of Veterinary Medicine, Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Mhow, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India. The investigation was carried out between February and May 2024 following approval from the Institutional Research Committee. Animals were enrolled after obtaining informed consent from their owners.

Study animals: A total of 137 bovines (cattle and buffaloes) of different breeds, age groups, and either sex presented to the Veterinary Clinical Complex and animals maintained under organized and unorganized dairy farms in and around Mhow were clinically screened for dermatological disorders. Animals exhibiting characteristic skin lesions suggestive of dermatophytosis were subjected to detailed clinical and mycological investigations.

Inclusion criteria: Animals were included in the study when they fulfilled the following criteria:

- Presence of circular or irregular alopecic lesions.
- Dry, thickened crusts and scales.
- Localized erythema and pruritus.
- Hyperpigmentation around lesions.
- Positive KOH examination and fungal culture.

Animals receiving systemic antifungal drugs within the previous four weeks or having generalized debilitating diseases were excluded from therapeutic evaluation.

Clinical examination: A detailed clinical examination was performed in each animal. Particular attention was paid to:

- Distribution of lesions
- Number and size of lesions
- Presence of crusts and scales
- Degree of alopecia
- Pruritus
- Secondary bacterial infection

General physiological parameters including rectal temperature (°F), pulse rate (beats/minute), and respiration rate (breaths/minute) were also recorded before initiation of treatment and during subsequent follow-up examinations.

Lesion scoring: Severity of dermatophytosis was evaluated using a modified clinical scoring system described by Westhoff *et al.* (2010)^[15].

Table 1: Scoring system for the judgement of lesion's severity of dermatophytosis

Score	Clinical findings
1	Normal skin
2	Mild alopecia with erythema
3	Alopecia with mild crust formation
4	Extensive alopecia with thick crusts and scales
5	Severe inflammatory lesions with plaques, papules and pustules

Clinical improvement was assessed by reduction in lesion score during follow-up examinations on Days 14, 28 and 45.

Collection of skin scrapings: After cleaning the lesion with 70% alcohol and allowing it to dry, skin scrapings were collected aseptically from the active margins of lesions using sterile blunt scalpels. The advancing edge of the lesion was selected because it contains actively growing fungal hyphae. Each sample was divided into two portions:

- Direct microscopic examination
- Fungal culture

Post-treatment skin scrapings were collected from the same lesion sites on Days 14 and 28 for evaluation of mycological cure.

Direct microscopic examination

Approximately 20-30 mg of skin scraping was placed on a clean grease-free glass slide. Two to three drops of 20% potassium hydroxide (KOH) solution were added. Slides were gently heated over a spirit lamp for approximately five minutes to facilitate digestion of keratin. After cooling, preparations were examined under low (10×) and high (40×) power objectives of a compound microscope. Samples showing branching septate hyphae and/or arthroconidia were considered positive for dermatophytosis.

Fungal culture and identification

For confirmation of dermatophytosis, skin scrapings were inoculated onto sabouraud dextrose agar (SDA) and dermatophyte test medium (DTM). Culture media were supplemented with chloramphenicol and cycloheximide to suppress bacterial and saprophytic fungal contamination. The inoculated SDA plates were incubated at 37°C, whereas DTM cultures were maintained at 25°C. Cultures were observed daily for fungal growth for up to four weeks. Dermatophytes were identified based on:

Colony morphology

- Surface texture
- Growth rate
- Pigmentation
- Reverse colony colour

Microscopic morphology

Lactophenol cotton blue mounts were prepared from representative colonies. Identification was performed according to the criteria described by Quinn *et al.* (2011)^[10].

Haematological examination

Five millilitres of jugular blood were collected aseptically from each animal before treatment (Day 0) and on Days 14 and 28 after treatment. Approximately 2 mL blood was transferred into EDTA vacutainers for haematological analysis using an automated veterinary haematology analyser. The following parameters were estimated:

- Haemoglobin (Hb)
- Packed Cell Volume (PCV)
- Total Erythrocyte Count (TEC)
- Total Leukocyte Count (TLC)
- Differential Leukocyte Count: Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils

Biochemical examination

The remaining blood samples were allowed to clot at room temperature. Serum was separated by centrifugation at 2500

rpm for 10 minutes and stored at -20°C until analysis. Commercial diagnostic kits were used for estimation of:

- Total Protein
- Serum Creatinine
- Aspartate Aminotransferase (AST)

Biochemical analyses were performed using a semi-automatic biochemical analyser according to the manufacturer's instructions.

Therapeutic trial: On the basis of the results of direct microscopic examination and culture the infected animals were subjected to therapeutic regimen. The infected animals were divided into 2 groups, each comprising of 8 animals for this study.

Table 2: Specific therapies for various groups of bovines under study

Group 1	Group 2
Clobetasol propionate+ ofloxacin+ Miconazole nitrate+ Zinc sulphate topical ointment topical twice daily for 10 days	Ethnovet herbal formulation (Spray) topical twice daily for 10 days

The assessment was made on 14th, 28th, and 45th day post treatment. Animals were treated with an ethnoveterinary herbal antifungal spray supplied by EW Nutrition India Pvt. Ltd.

The spray was applied twice daily directly over the affected lesions after cleaning for ten consecutive days. No additional antifungal medication was administered during the study period.

- **Follow-up:** Recovered animals were re-examined on Day 45 to detect relapse or recurrence.
- **Statistical analysis:** The statistical analysis of data was carried out by using two-way ANOVA and interpretation based on statistical findings (Snedecor and Cochran, 1994)^[13].
- **Ethical approval:** The study was conducted following institutional guidelines for the ethical use of animals in research. All procedures were performed with the informed consent of the animal owners. No invasive procedures beyond routine clinical sampling were undertaken.

Results

Prevalence of dermatophytosis

Out of 137 bovines (cattle and buffaloes) examined in organized and unorganized dairy farms in and around Mhow, 29 animals were confirmed positive for dermatophytosis, resulting in an overall prevalence of 21.17%. Diagnosis was established based on characteristic clinical lesions, positive 20% KOH examination, and fungal culture on Sabouraud Dextrose Agar (SDA) and Dermatophyte Test Medium (DTM).

The observed prevalence suggests that dermatophytosis is a common dermatological disorder among bovines in the study region and warrants routine surveillance and timely therapeutic intervention.

Table 3: Prevalence of dermatophytosis in and around Mhow region

No. of Animals		Prevalence (%)
Total	Positive	
137	29	21.1%

Clinical manifestations

Affected animals exhibited classical dermatophytic lesions characterized by well-circumscribed circular areas of alopecia associated with grayish-white crusts, scaling, erythema, and varying degrees of pruritus. Lesions were predominantly distributed over the neck, hump, and dorsal trunk, followed by the abdomen and limbs. Lesions on the tail and distal extremities were comparatively less frequent (Figure 01).

Most animals presented with lesion severity scores of 3 or 4, indicating moderate to severe dermatophytosis at the time of enrolment. Progressive regression of crusts, reduction in inflammation, disappearance of pruritus, and regrowth of hair were observed in both treatment groups during the observation period.

Mycological findings

Direct microscopic examination of skin scrapings treated with 20% potassium hydroxide revealed septate fungal hyphae and arthroconidia in all clinically affected animals.

Fungal culture produced characteristic dermatophyte colonies on SDA and DTM. Lactophenol cotton blue preparations demonstrated hyaline septate hyphae with abundant arthroconidia arranged around infected hairs, suggestive of *Trichophyton* species (Figure 02).

Culture examination after treatment demonstrated progressive elimination of fungal growth in both treatment groups.

Physiological parameters

The mean values of rectal temperature, pulse rate, and respiration rate in both treatment groups are presented in Table 04. Throughout the study period, all physiological parameters remained within the normal physiological range for bovines. Statistical analysis revealed no significant ($P>0.05$) differences either between treatment groups or across the observation period, indicating that neither dermatophytosis nor the administered therapies produced any appreciable systemic physiological alterations.

Table 4: Mean ($\pm$ SE) values of physiological parameters in bovines with dermatophytosis before and after treatment

Observation day	Rectal temperature ($^{\circ}$ F)		Pulse rate (beats/min)		Respiration rate (breaths/min)	
	Group I	Group II	Group I	Group II	Group I	Group II
Day 0	101.46 $\pm$ 0.38	101.52 $\pm$ 0.62	60.86 $\pm$ 0.57	61.39 $\pm$ 0.19	31.64 $\pm$ 0.81	30.94 $\pm$ 0.43
Day 14	101.58 $\pm$ 0.25	101.61 $\pm$ 0.44	62.25 $\pm$ 0.34	61.93 $\pm$ 0.31	31.27 $\pm$ 0.57	31.53 $\pm$ 0.59
Day 28	101.24 $\pm$ 0.41	101.33 $\pm$ 0.27	62.64 $\pm$ 0.71	62.81 $\pm$ 0.47	30.89 $\pm$ 0.63	31.06 $\pm$ 0.82

Scoring system for the judgement of lesion's severity of dermatophytosis: The hump, neck and back were the areas with the most prevalent lesions, followed by the legs and abdomen. The frequency of tail and hoof lesions was lesser. In both treatment groups, the distribution of the lesions was similar with lesion severity being more frequently assessed at day 0 with a 3 or 4 than with a 5. Lesions with severity scores of 1-2 were considered moderate, whereas those with scores of 3-5 were considered more severe (Table 01).

Haematological alterations

Haemoglobin: Mean haemoglobin concentration increased significantly ($P<0.05$) throughout the treatment period in both groups. Group I increased from 10.13 $\pm$ 0.33 g/dL before treatment to 12.64 $\pm$ 0.31 g/dL on Day 28, whereas Group II increased from 10.97 $\pm$ 0.20 g/dL to 12.53 $\pm$ 0.15 g/dL (Table 05).

Packed Cell Volume

Packed cell volume showed a significant ($P<0.05$) increase during recovery. Group I improved from 33.24 $\pm$ 1.04% to 38.11 $\pm$ 0.84%, while Group II increased from 33.87 $\pm$ 0.62% to 38.89 $\pm$ 0.36% (Table 05).

Total Erythrocyte Count

TEC also increased significantly ($P<0.05$). Values increased from 6.57 $\pm$ 0.26 to 9.31 $\pm$ 0.17 million/ μ L in Group I and 6.11 $\pm$ 0.20 to 9.39 $\pm$ 0.17 million/ μ L in Group II (Table 05).

Total Leucocyte Count

No significant variation was observed in TLC throughout treatment. The values remained close to 10 $\times 10^3/\mu$ L, indicating absence of systemic inflammatory response (Table 05).

Table 5: Mean ($\pm$ SE) values of haematological parameters in bovines with dermatophytosis before and after treatment

Days of observation	Haemoglobin (g/dL)		Packed Cell Volume (%)		Total Erythrocyte Count ($\times 10^6/\mu$ L)		Total Leukocyte Count ($\times 10^3/\mu$ L)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
Day 0	10.13 ^a $\pm$ 0.33	10.97 ^a $\pm$ 0.20	33.24 ^a $\pm$ 1.04	33.87 ^a $\pm$ 0.62	6.57 ^a $\pm$ 0.26	6.11 ^a $\pm$ 0.20	10.47 $\pm$ 0.28	10.51 $\pm$ 0.27
Day 14	11.89 ^b $\pm$ 0.32	11.72 ^b $\pm$ 0.19	36.83 ^b $\pm$ 0.96	36.36 ^b $\pm$ 0.57	7.80 ^b $\pm$ 0.22	8.34 ^b $\pm$ 0.19	10.13 $\pm$ 0.26	10.28 $\pm$ 0.30
Day 28	12.64 ^c $\pm$ 0.31	12.53 ^c $\pm$ 0.15	38.11 ^c $\pm$ 0.84	38.89 ^c $\pm$ 0.36	9.31 ^c $\pm$ 0.17	9.39 ^c $\pm$ 0.17	10.32 $\pm$ 0.17	10.19 $\pm$ 0.21

Means bearing different superscripts (a, b, c) within the same treatment group differ significantly ($P<0.05$). Total leukocyte count did not differ significantly ($P>0.05$) during the study period.

Differential Leukocyte Count

The mean ($\pm$ SE) values of differential leukocyte count are presented in Table 06. Neutrophil percentage decreased

significantly ($P<0.05$) during the treatment period in both groups, indicating resolution of the inflammatory response. Conversely, lymphocyte percentage increased significantly ($P<0.05$) from Day 0 to Day 28 in both treatment groups, reflecting progressive recovery. Monocyte, eosinophil, and basophil percentages remained within the normal physiological range and did not show significant ($P>0.05$) variation throughout the study.

Table 6: Mean ($\pm$ SE) values of differential leucocyte count (%) in bovines with dermatophytosis before and after treatment

Days of Observation	Neutrophils (%)		Lymphocytes (%)		Monocytes (%)		Eosinophils (%)		Basophils (%)	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
Day 0	37.12 ^a $\pm$ 0.85	37.87 ^a $\pm$ 0.97	59.17 ^a $\pm$ 0.51	57.62 ^a $\pm$ 0.86	2.12 $\pm$ 0.47	2.87 $\pm$ 0.54	1.25 $\pm$ 0.25	1.12 $\pm$ 0.29	0.34 $\pm$ 0.18	0.52 $\pm$ 0.18
Day 14	34.25 ^{bc} $\pm$ 1.03	35.37 ^b $\pm$ 0.98	61.83 ^b $\pm$ 0.87	60.25 ^b $\pm$ 0.92	2.12 $\pm$ 0.29	2.75 $\pm$ 0.31	1.00 $\pm$ 0.26	1.25 $\pm$ 0.25	0.80 $\pm$ 0.18	0.38 $\pm$ 0.18
Day 28	32.50 ^{bc} $\pm$ 0.82	32.12 ^c $\pm$ 0.74	63.50 ^c $\pm$ 0.94	63.00 ^c $\pm$ 0.62	2.50 $\pm$ 0.18	2.25 $\pm$ 0.45	1.12 $\pm$ 0.22	1.25 $\pm$ 0.25	0.38 $\pm$ 0.18	0.38 $\pm$ 0.18

Means bearing different superscripts (a, b, c) within the same treatment group differ significantly ($P < 0.05$). Monocyte, eosinophil, and basophil percentages did not differ significantly ($P > 0.05$) during the study period.

Serum biochemical findings

The mean ($\pm$ SE) values of serum biochemical parameters are presented in Table 07. Serum total protein, creatinine, and

aspartate aminotransferase (AST) activities remained within the normal physiological range throughout the study period in both treatment groups. No significant ($P > 0.05$) differences were observed either within or between groups, indicating that both the conventional topical antifungal formulation and the ethnoveterinary herbal spray were well tolerated and did not produce any detectable adverse effects on hepatic or renal function.

Table 7: Mean ($\pm$ SE) values of serum biochemical parameters in bovines with dermatophytosis before and after treatment

Days of Observation	Total Protein (g/dL)		Serum Creatinine (mg/dL)		AST (IU/L)	
	Group I	Group II	Group I	Group II	Group I	Group II
Day 0	6.50 $\pm$ 0.08	6.74 $\pm$ 0.07	0.89 $\pm$ 0.03	0.93 $\pm$ 0.03	79.26 $\pm$ 0.82	78.24 $\pm$ 0.53
Day 14	6.59 $\pm$ 0.06	6.69 $\pm$ 0.04	0.86 $\pm$ 0.32	0.95 $\pm$ 0.02	78.74 $\pm$ 0.55	78.24 $\pm$ 0.47
Day 28	6.67 $\pm$ 0.05	6.66 $\pm$ 0.05	0.80 $\pm$ 0.31	0.95 $\pm$ 0.02	77.98 $\pm$ 0.55	77.99 $\pm$ 0.56

Therapeutic efficacy

Fungal culture performed on 14th and 28th day post treatment demonstrated negative results in seven animals in Group I with one animal being asymptomatic carrier. Group II revealed recovery rate of 75 per cent by 10 days of treatment. Two animals did not recover even by 10 days of treatment probably due to irregular treatment. Follow up of all recovered animals by 45 days showed no relapse in animals after discontinuation of treatment. Progressive reduction in itching and regrowth of hair during treatment suggested antifungal activity of ethnoveterinary herbal preparation (Table 08).

Follow-up evaluation performed 45 days after treatment revealed no recurrence among recovered animals.

Table 8: Comparative efficacy of antifungal drugs

Groups	No. of animals		Efficacy (%)
	Total	Cured	
I	8	7	87.5
II	8	6	75

Discussion

Dermatophytosis remains one of the most important contagious fungal diseases affecting cattle worldwide because of its economic impact and zoonotic significance. The overall prevalence of 21.17% observed in the present study indicates that bovine dermatophytosis continues to be an important dermatological problem in central India. Similar prevalence has been reported in cattle from different geographical regions, although considerable variation exists depending on climatic conditions, management practices, animal density, and environmental hygiene (Dalis *et al.*, 2019; Fratti *et al.*, 2023). Dermatophyte spores survive for prolonged periods in the environment, particularly under humid conditions, thereby facilitating transmission within herds (Robi *et al.*, 2023)^[3, 4, 11].

The predominance of lesions over the neck, hump, and dorsal trunk agrees with previous observations of Swai and Sanka

(2012)^[14] and more recent findings by Lagowski *et al.* (2021). These anatomical regions are frequently subjected to friction, minor trauma, rain exposure, and direct animal-to-animal contact, which favour fungal inoculation and colonization. The characteristic circular alopecic lesions with crust formation observed in the present study reflect the centrifugal growth pattern of dermatophytes accompanied by central healing resulting from host immune responses (Moriello *et al.*, 2017)^[6, 7].

Direct microscopic examination using 20% potassium hydroxide provided rapid presumptive diagnosis, whereas fungal culture on Sabouraud Dextrose Agar and Dermatophyte Test Medium confirmed infection and facilitated morphological identification of dermatophytes. Hyaline septate hyphae and ectothrix invasion observed in the present investigation were characteristic of *Trichophyton* species, the predominant dermatophytes affecting cattle worldwide (Lagowski *et al.*, 2021; Fratti *et al.*, 2023)^[4].

No significant alterations were observed in rectal temperature, pulse rate, or respiration rate during the study period, indicating that dermatophytosis remains primarily a localized superficial infection without marked systemic involvement. Similar observations have been reported in naturally occurring bovine dermatophytosis by Bhikane *et al.* (2015) and Robi *et al.* (2023)^[2, 6, 11].

Significant improvement in haemoglobin concentration, packed cell volume, and total erythrocyte count following treatment probably reflects restoration of normal physiological status after elimination of chronic infection and improvement in feed intake. Comparable hematological improvements have been reported following successful treatment of bovine dermatophytosis (Gupta *et al.*, 2014) and are consistent with recovery from localized inflammatory stress (Moriello *et al.*, 2017)^[5, 7]. The absence of significant changes in total leukocyte count further suggests that uncomplicated dermatophytosis rarely induces marked systemic inflammatory responses.

The decline in neutrophil percentage accompanied by a significant increase in lymphocyte percentage indicates progressive resolution of inflammation and restoration of adaptive immune responses following antifungal therapy. Similar leukocyte trends have been described by Bhikane *et al.* (2015) and are consistent with the immunopathogenesis of dermatophyte infections discussed by Moriello *et al.* (2017). Monocyte, eosinophil, and basophil counts remained within normal physiological limits throughout the study [2, 7].

Serum biochemical parameters remained stable throughout the observation period. The absence of significant alterations in serum total protein, creatinine, and AST indicates that neither the conventional topical antifungal preparation nor the ethnoveterinary herbal spray produced detectable hepatic or renal adverse effects. These findings corroborate previous reports demonstrating the safety of topical antifungal therapy in bovine dermatophytosis (Gupta *et al.*, 2014) [5].

Both treatment protocols resulted in progressive clinical improvement accompanied by mycological cure in the majority of animals. Conventional topical antifungal therapy achieved a cure rate of 87.5%, whereas the ethnoveterinary herbal spray produced 75.0% recovery. Although the conventional formulation demonstrated comparatively higher efficacy, the herbal preparation also produced substantial clinical improvement without evidence of toxicity or relapse during the follow-up period. The slightly lower efficacy observed in the herbal treatment group was likely influenced by irregular application in two animals, emphasizing the importance of owner compliance during topical therapy. Similar beneficial effects of polyherbal formulations in bovine dermatomycosis have been reported by Bhikane *et al.* (2015), while increasing interest in ethnoveterinary therapies has also been highlighted by McGaw and Abdalla (2020) [2, 8].

The encouraging therapeutic response observed with the ethnoveterinary herbal spray may be attributed to the synergistic activity of plant-derived phytochemicals possessing antifungal, anti-inflammatory, antioxidant, and wound-healing properties. Bioactive compounds including flavonoids, phenolic compounds, terpenoids, tannins, alkaloids, and essential oils inhibit fungal growth by disrupting cell membrane integrity, interfering with ergosterol biosynthesis, suppressing fungal enzyme activity, and reducing oxidative stress while simultaneously promoting epithelial regeneration (Nazzaro *et al.*, 2017; Robi *et al.*, 2023). Similar antifungal activities of medicinal plant extracts against dermatophytes have also been demonstrated by Begum and Kumar (2021) [1, 9, 11].

An additional practical advantage of the herbal spray is its suitability for field application. Unlike ointments that require manual rubbing, spray formulations allow rapid and uniform application over extensive lesions with minimal animal restraint, thereby reducing labour requirements and improving farmer compliance. Furthermore, the absence of treatment-related adverse effects indicates that the formulation is safe for repeated topical application in dairy cattle.

The present investigation has certain limitations. The therapeutic trial involved only sixteen animals, thereby limiting statistical power for direct comparison between treatments. In addition, fungal identification relied on conventional morphological methods rather than molecular confirmation using PCR or sequencing. Future multicentric studies involving larger sample sizes, molecular

characterization of dermatophytes, quantitative lesion scoring, and longer follow-up periods are warranted to further validate the clinical efficacy of ethnoveterinary antifungal preparations. Overall, the findings demonstrate that the ethnoveterinary herbal spray possesses appreciable clinical and mycological efficacy against bovine dermatophytosis and may serve as a safe, economical, and farmer-friendly alternative where access to conventional antifungal therapy is limited. Nevertheless, under the conditions of the present study, the conventional topical antifungal preparation achieved a comparatively higher cure rate.

Conclusion

The present study demonstrated that both the conventional topical antifungal preparation and the ethnoveterinary herbal spray were effective in the treatment of naturally occurring bovine dermatophytosis. The conventional antifungal therapy achieved a higher clinical and mycological cure rate (87.5%) compared with the ethnoveterinary herbal spray (75.0%). Nevertheless, the herbal spray produced substantial clinical improvement, promoted healing of skin lesions, and resulted in mycological recovery in the majority of treated animals without any observable adverse effects or relapse during the follow-up period. Hematological and biochemical parameters remained within normal physiological limits throughout the study, indicating that both treatment protocols were safe for topical use.

The aerosol formulation of the ethnoveterinary herbal spray offered practical advantages under field conditions by allowing easy, rapid, and uniform application over affected areas with minimal handling of animals. Its ease of application reduced labour requirements and minimized the need for repeated hospital visits, making it particularly suitable for use by livestock owners under rural farming conditions. In addition, the herbal preparation represents a cost-effective, eco-friendly, and farmer-friendly alternative to conventional antifungal therapy, especially in situations where access to commercial antifungal drugs is limited or prolonged treatment is economically challenging.

Although the conventional topical antifungal preparation demonstrated comparatively superior therapeutic efficacy, the encouraging response obtained with the ethnoveterinary herbal spray highlights its potential as an alternative treatment option for bovine dermatophytosis. Further multicentric studies involving larger numbers of animals, molecular confirmation of dermatophyte species, and longer follow-up periods are recommended to validate its efficacy and facilitate its wider adoption in veterinary practice.

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