Original Research Article

Therapeutic Efficacy of Eucalyptus Oil as Teat Dip in Subclinical Mastitis in Goats

Abstract

Mastitis, an inflammation of the parenchyma of mammary gland, results in alterations to the chemical and physical composition of milk due to a variety of infectious and non-infectious causes.The present work was conducted in the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur,Madhya Pradesh (M.P.).A total of 256 lactating goats irrespective of age, breed, parity and stage of lactation belonging to both organized and unorganized sectors at Jabalpur were screened from “February 2024 to July 2024”. On the basis of modified California mastitis test (MCMT) score,out of 256 lactating goats, 98 goats were found positive for subclinical mastitis with overall occurrence as 38.28% on animal basis and 33.33% on udder halves basis. Therapeutic study on24 milk samples positive for subclinical mastitis by MCMT was conducted and categorized into four treatment groups (G1, G2, G3 and G4), each group comprised of 6 goats. Six apparently healthy goats were selected as healthy control group (G5). Two different teat dips viz. Eucalyptus oil 98% + DMSO 2% BID for 14 days in groups G1 and G3 and 5% Povidone iodine BID for 14 days in groups G2 and G4 were used as teat dips, respectively. Moreover, goats of groups G3 and G4 were commonly treated with parenteral antibiotic amoxicillin sulbactam @10 mg/kg b.wt. I/M OD for 3 days.Significantly higher values of somatic cell count, standard plate count, pH, freezing point and significantly lower value of lactose were observed in subclinical mastitis affected goats. The mean density and mean water percentage values varied within normal physiological range. On the basis of restoration of milk parameters and standard plate count, the most effective therapy was elicited by Eucalyptus oil 98%+ DMSO 2% as teat dip in combination with antibiotic amoxicillin sulbactam (G3).

Keywords: Subclinical mastitis,modified California mastitis test, somatic cell count, Goats, Standard plate count, Eucalyptus oil.

1. INTRODUCTION

Mastitis is an inflammation of mammary gland, is one of the most important and economically devastating disease affecting the dairy animals throughout the world [1]. The economic losses are related to lower milk production, low-quality milk, early culling, veterinary care costs, lower milk and milk product exports, and higher management expenses.Mastitis affects both quantity and quality of milk and is characterized by physical, chemical, microbiological, and pathological changes in the udder and milk [2]. Subclinical mastitis is common in goats with mammary tissue showing no obvious anomalies, and the milk appears normal. It typically occurs before the clinical form and serves as a reservoir of microorganisms that can infect healthy animals [3].

Antibiotic therapy is implemented for treatment of both clinical and subclinical mastitis cases. Antimicrobial Resistance (AMR), a major concern, is being caused by a large number of bacterial strains that are resistant to the antibiotics that are employed. In order to treat, prevent and control both clinical and subclinical mastitis, the current focus is shifting towards the use of ethnoveterinary treatments such herbs and herbal products. Essential oils and plant derived antimicrobials are safe with antibacterial and antioxidant properties and unlike antibiotics has no resistance on prolonged exposure to bacteria [4]. In general, both medications and herbal remedies can be used to treat mild to severe cases of bacterial infections [5]. One such simple, useful and affordable method for lowering the load and entrance of pathogens on teats is teat dipping. It promotes the healing of skin lesions and maintains the health of the teat skin, which helps in mastitis control [6].

The popularity of natural and herbal medications is growing fastidiously, easy availability and reduced cost have led to many polyherbal teat disinfectants formulations. *Eucalyptus globulus* also known as blue gum was brought in 1843 to India as a fuel tree. According to reports, the plant has strong

antibacterial, astringent, deodorant, diaphoretic, expectorant, inhalant, insect repellent, rubefacient and suppurative qualities that can be easily used [7].

Hence, this study was conducted to evaluate the therapeutic efficacy of Eucalyptus oil that could be applied as a post-milking teat dip for the control of subclinical mastitis in goats at Jabalpur (M.P.).

2. MATERIALS AND METHODS

2.1 Location and place of work

The study was conducted in the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University (N.D.V.S.U), Jabalpur (M.P.). for a period of six months i.e. from February 2024 to July 2024.

2.2 Experimental design

For this study, a total of 256 goats were screened from February 2024 to July 2024. After confirmation of subclinical mastitis, 24 goats having MCMT score of+2 and+3 were subjected to estimation of somatic cell count (SCC), different milk parameters such as milk PH, lactose and density, total number of viable bacteria in milk and different therapeutic regimen. Additionally, 6 apparently healthy goats(mastitis free) were taken as healthy control group.

2.3 Modified California Mastitis Test (MCMT)

MCMT was carried out for the screening of goats for subclinical mastitis. Equal quantity of both milk and MCMT reagent (i.e. 5ml) was added in a MCMT plastic paddle, giving it a gentle swirling motion with minimum agitation. The reaction was graded by intensity of gel formation and colour changes (Table 01).

Table 01: MCMT score of milk

MCMT score	Reaction
Negative	No reaction
Trace	Slight slime
1	Distinct slime but without gel
2	Immediate gel formation; moves as a mass during swirling
3	Gel develops a convex surface and adheres to the bottom of the cup

2.4 Somatic cell count (SCC)

The somatic cell count was performed in milk samples on day 0 (pretreatment) and on day 15 (post-treatment) in all the goats of different treatment group. The SCC was performed with the help of automatic somatic cell counter cum lactoscan combo machine.

2.5 Estimation of milk parameters

Following milk parameters were estimated using automatic somatic cell counter cum lactoscan combo on day ‘0’ pre- treatment and on day ‘15’ post treatment in 15 ml of milk.

2.5.1 Milk parameters

- pH
- Lactose (%)
- Density (g/L)

2.6 Standard Plate Count to examine bacteriological quality of milk

The Standard Plate Count (SPC) is a method for estimating the total number of viable bacteria. For this, milk samples were collected aseptically on day ‘0’ pre-treatment and on day ‘15’ of post-

treatment in sterile collecting tubes. These tubes were properly labelled and kept in an ice box maintained at 4-5 ℃. The raw milk samples were analyzed within 4 hours of their collection. The SPC was performed according to the standard method described [8].

For this 1 ml of milk was diluted in 9 ml of normal saline solution (1:10 dilution). Further, serial dilutions were made by taking 1ml of diluted sample in 9ml of normal saline. Then 100 µl of diluted sample was spread with the help of disposable plastic spreader on the Plate Count Agar plates in duplicate and then incubated at 37°C for 24-48 hours. After incubation, plates inoculated with milk sample dilution yielding between 30-300 colonies were counted and count was made as "Colony Forming Unit" (cfu/ml).

2.6.1 Calculation

CFU/ml= (no. of colonies x dilution factor) / volume of culture plate

2.7 Procedure for preparation of teat dips

The herbal teat dip was prepared by mixing of Eucalyptus oil procured from Indian pharmacopeia and Dimethyl Sulfoxide procured from Bio Balance through agitation in the ratio of 98:2, as DMSO is a polar solvent and has ability to dissolve both polar and non-polar compounds[9].

2.8 Therapeutic regimen

A total of 24 goats confirmed for subclinical mastitis were selected randomly and divided into four treatment groups viz. G1, G2, G3 and G4. Each treatment group was comprised of six goats. Moreover, six apparently healthy goats were kept as healthy control group (G5) (Table 02).

Table 02: Therapeutic regimen of subclinical mastitis in goats

Groups	No. of animals	Drugs and dosage
G1	06	Eucalyptus oil 98% and DMSO 2% x BID for 14 days as teat dip
G2	06	5% Povidone iodine x BID for 14 days as teat dip
G3	06	Inj. Amoxicillin + sulbactam @ 10 mg/kg b.wt. I/M x OD x 3 days + Eucalyptus oil 98% and DMSO 2% x BID for 14 days as teat dip
G4	06	Inj. Amoxicillin + sulbactam @ 10 mg/kg b.wt. I/M x OD x 3 days + 5% Povidone iodine x BID for 14 days as teat dip
G5	06	Apparently healthy control group

2.9 STATISTICAL ANALYSIS

The alterations in different treatment groups at different intervals were analyzed using one-way ANOVA in R software using dplyr package and means were compared using post Hoc- Duncan's multiple range tests as per the standard procedure [10].

3. RESULTS AND DISCUSSION

3.1 Milk parameters

3.1.1 Somatic cell count (SCC)

Somatic cell count (SCC) in all the 24 lactating goats on day 0 (pre-treatment) and day 15 (post treatment) were recorded under therapeutic trial and compared with the SCC of healthy control goats.

Results showed that mean SCC on day 0 before treatment in goats of groups G1, G2, G3 and G4 were 15.72±0.52 x 10^5 cells/ml, 16.43±0.63 x 10^5 cells/ml, 16.33±0.43 x 10^5 cells/ml and 16.05±0.68 x 10^5 cells/ml, respectively. After treatment the mean SCC on day 15 in goats of groups G1, G2, G3 and G4 were 8.15±0.62 x 10^5 cells/ml, 9.69±0.48 x 10^5 cells/ml, 5.33±0.12 x 10^5 cells/ml and 6.09±0.39 x 10^5

cells/ml, respectively. The mean SCC in healthy control group (G5) on day 0 and day 15 was 4.56±0.14 x 10^5 cells/ml and 4.48±0.11 x 10^5 cells/ml, respectively.

Significant increase in mean SCC was observed in goats of all treatment groups at day 0 (pretreatment) as compared to healthy control group. A significant reduction in the SCC of goats was recorded in all treatment groups on day 15 (post treatment). However, maximum reduction was seen in goats of group G3 (Table 03; Figure 01).

Table 03: Mean SCC (10^5 cells/ml) in goats in different treatment groups at different intervals

Groups (n=6)	SCC (Mean±SE)	
	Day 0	Day 15
G1	15.72^{aA}±0.52	8.15^{aB}±0.62
G2	16.43^{aA}±0.63	9.69^{aB}±0.48
G3	16.33^{aA}±0.43	5.33^{bB}±0.12
G4	16.05^{aA}±0.68	6.09^{bB}±0.39
G5	4.56^{bB}±0.14	4.48^{bB}±0.11

Mean values with different superscript between group (lowercase) and between days (uppercase) differ significantly ($p<0.05$)

The result of present study is in accordance with findings of Contreras *et al*. [11], Al-Ramahi *et al*. [12], Persson and Olofsson [3] and Singh *et al*. [13] who have reported an increase in SCC in milk of goats affected with subclinical mastitis. Somatic cells also known as milk-secreting epithelial cells sheds from the white blood cells and lining of the mammary gland that enters the gland in reaction to any infection or injury [14]. Since, there is a direct relationship between inflammatory cells and intramammary infection, milk SCC is increased during intramammary infection and have been used as a reliable indicator for the detection of mastitis and to monitor udder health.

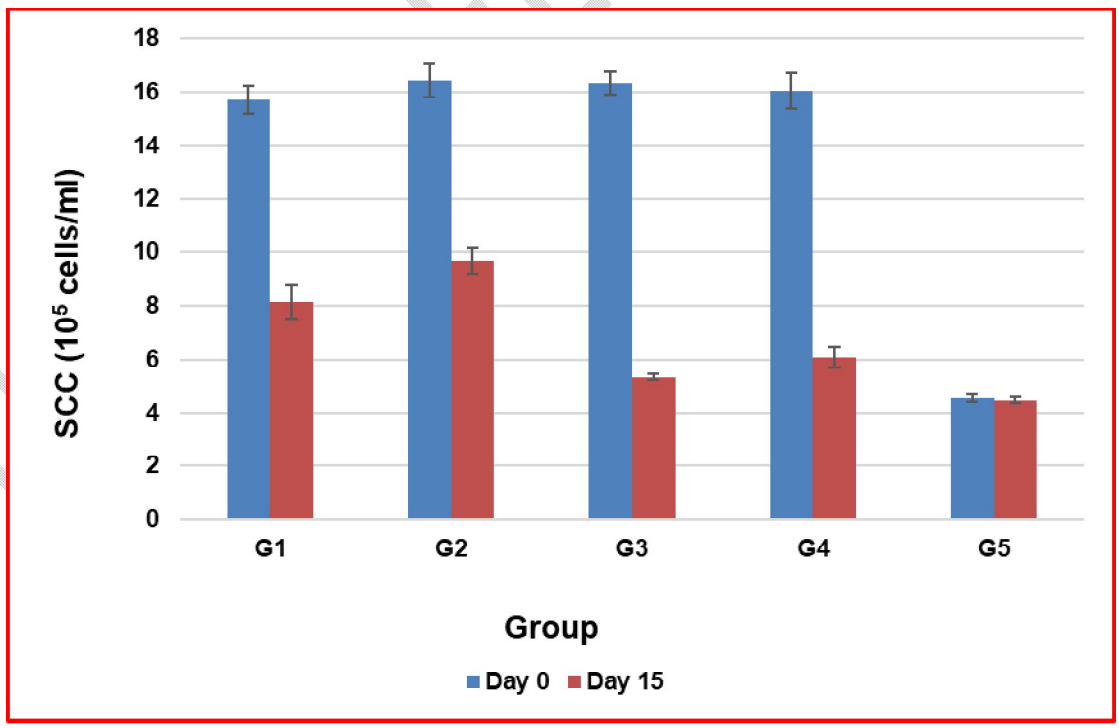

Figure 01: Mean SCC (10^5 cells/ml) in goats in different treatment groups at different intervals

3.1.2 Milk pH

Milk pH in all the 24 lactating goats on day 0 (pretreatment) and day 15 (post treatment) were recorded under therapeutic trial and compared with the healthy control goats.

Results showed that mean pH on day 0 before treatment in goats of groups G1, G2, G3 and G4 were 7.32±0.05, 7.37±0.04, 7.28±0.06 and 7.29±0.06, respectively. On day 15 (post treatment) the mean pH in goats of groups G1, G2, G3 and G4 were 6.96±0.03, 7.09±0.03, 6.77±0.03 and 6.87±0.02, respectively. The mean pH in healthy control group (G5) on day 0 and day 15 was 6.60±0.03 and 6.63±0.03, respectively.

Milk pH on day 0 (pretreatment) showed a significant increase in goats of all treatment groups in comparison to healthy control. A significant improvement(decrease) in the milk PH of goats was reported on day 15 (post treatment) in all treatment groups. However, maximum improvement was seen in goats of group G3. (Table 04; Figure 02).

Table 04:Mean milk PH in goats in different treatment groups at different intervals

Groups (n=6)	PH (Mean±SE)	
	Day 0	Day 15
G1	7.32^{aA}±0.05	6.96^{abB}±0.03
G2	7.37^{aA}±0.04	7.09^{aB}±0.03
G3	7.28^{aA}±0.06	6.77^{cB}±0.03
G4	7.29^{aA}±0.06	6.87^{bB}±0.02
G5	6.60^{bA}±0.03	6.63^{dA}±0.03

Mean values with different superscripts between group (lowercase) and between days (uppercase) differ significantly ($p<0.05$)

The results of present study is in agreement with the findings of Islam *et al*. [15], Gajarlawar*et al*. [16], Hassan [17] and Kumhar *et al*. [18] who also recorded higher PH of milk in goats affected with subclinical mastitis. The probable reason for increase in milk PH in subclinical mastitis may be because of the gland tissue's enhanced permeability to blood components, which results in increased level in milk. This may be due to escalated movement of bicarbonate ions into milk making it more alkaline [19]. Therefore, milk PH can be a useful tool for evaluating the health of the udder and variations in milk PH can reveal the severity of an intramammary infection.

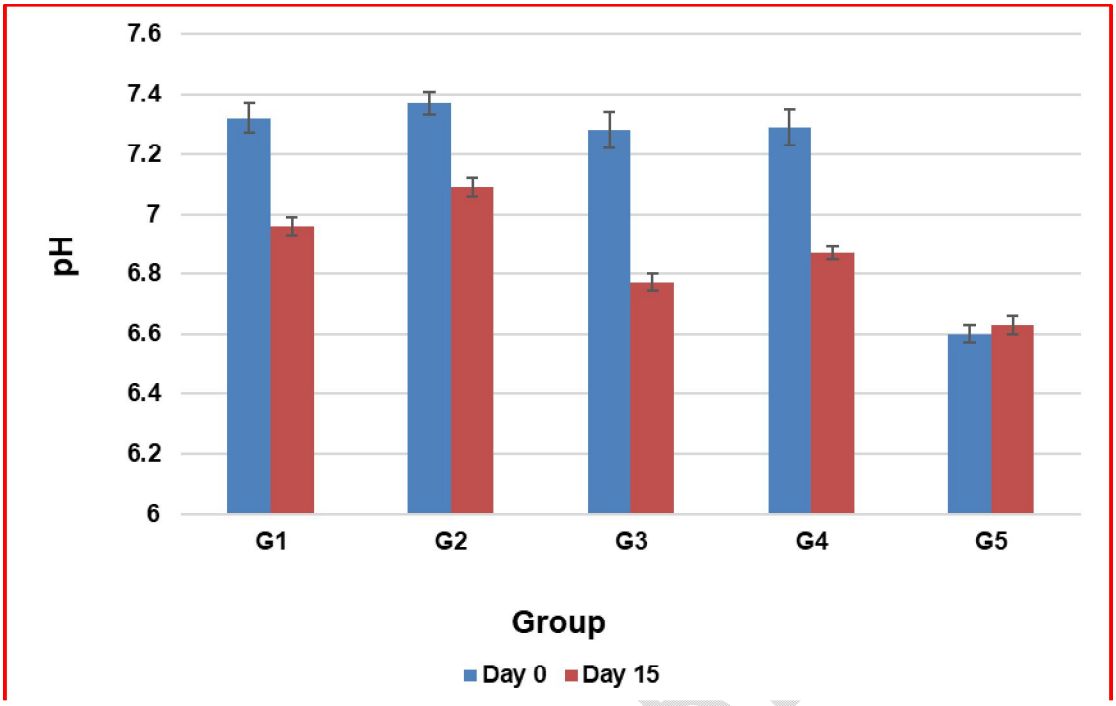

Figure 02:Mean milk PH in goats in different treatment groups at different intervals

3.1.3 Milk lactose

Milk lactose in all the 24 lactating goats on day 0 (pre-treatment) and day 15 (post treatment) were recorded under therapeutic trial and compared with the healthy control goats.

Results revealed that mean lactose on day 0 before treatment in goats of groups G1, G2, G3 and G4 were 4.17±0.06, 4.16±0.04, 4.21±0.05 and 4.20±0.04, respectively. On day 15 (post treatment) the mean lactose in goats of groups G1, G2, G3 and G4 were 4.57±0.04, 4.50±0.01, 4.76±0.02 and 4.69±0.01, respectively. The mean lactose in healthy control group (G5) on day 0 and day 15 was 4.82±0.01 and 4.83±0.01, respectively.

Milk lactose on day 0 (pretreatment) showed a significant decrease in goats of all treatment groups in comparison to healthy control. A significant improvement in the milk lactose of goats was reported on day 15 (post treatment) in all treatment groups (Table05; Figure 03).

Table 05:Mean milk lactose (%) in goats in different treatment groups at different intervals

Groups (n=6)	Lactose (Mean±SE)	
	Day 0	Day 15
G1	4.17^{bB}±0.06	4.57^{cA}±0.04
G2	4.16^{bB}±0.04	4.50^{cA}±0.01
G3	4.21^{bB}±0.05	4.76^{abA}±0.02
G4	4.20^{bB}±0.04	4.69^{bA}±0.01
G5	4.82^{aA}±0.01	4.83^{aA}±0.01

Mean values with different superscripts between group (lowercase) and between days (uppercase) differ significantly ($p<0.05$)

The result of present study correlates with the findings of Leitner *et al*. [20], Merin *et al*. [21] who reported decreased lactose per cent in milk of goats during SCM. Bagri *et al*. [22] observed similar

findings in lactating cows and reported decrease in lactose per cent in milk of cows suffering from SCM. The most osmotically active ingredient in milk is lactose, a sugar found in milk. Lactose cannot pass through the healthy mammary gland. The udder's gland cells use glucose and galactose to create lactose. These processes are somewhat inhibited during infections, which lowers the amount of lactose in the milk. Thus, lactose may be one of the good indicators to detect mastitis and various intramammary infections [23].

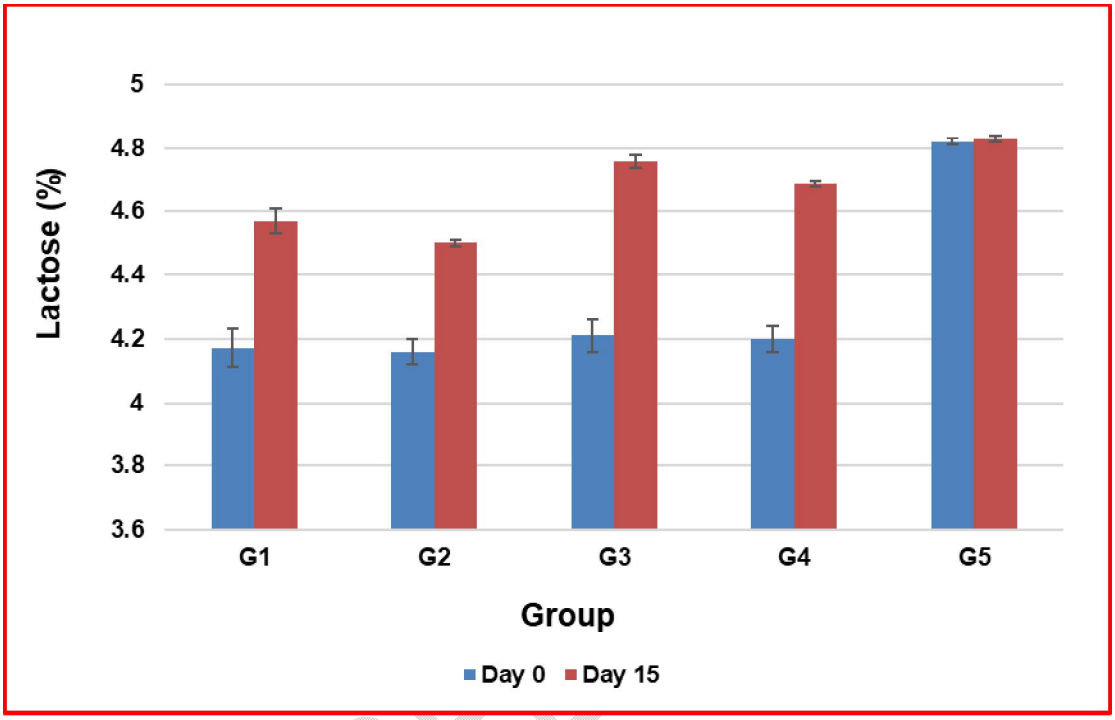

Figure 03:Mean milk lactose (%) in goats in different treatment groups at differentintervals

3.1.4 Milk density

Milk density in all the 24 lactating goats on day 0 (pretreatment) and day 15 (post treatment) were recorded under therapeutic trial and compared with the healthy control goats.

Results showed that mean density on day 0 before treatment in goats of groups G1, G2, G3 and G4 were 30.33±0.23, 30.41±0.19, 30.46±0.19 and 30.49±0.16, respectively. On day 15 (post treatment) the mean density in goats of groups G1, G2, G3 and G4 were 30.26±0.17, 30.48±0.22, 30.62±0.15 and 30.52±0.16, respectively. The mean density in healthy control group (G5) on day 0 and day 15 was 31.30±0.25 and 30.34±0.12, respectively.

No significant difference in mean milk density was observed in goats of different treatment groups on day 0 (pretreatment) and day 15 (post treatment). However, a significant difference was recorded in goats of all treatment groups on day 0 (pretreatment) when compared with healthy control group but it was within normal physiological range (Table 06; Figure 04).

Table 06:Mean milk density (g/L) in goats in different treatment groups at different intervals

Groups	Density (Mean±SE)	
(n=6)	Day 0	Day 15
G1	30.33^{b}±0.23	30.26^{a}±0.17
G2	30.41^{b}±0.19	30.48^{a}±0.22
G3	30.46^{ab}±0.19	30.62^{a}±0.15
G4	30.49^{ab}±0.16	30.52^{a}±0.16

G5	31.30[a]±0.25	30.34[a]±0.12

Mean values with different superscripts between group (lowercase) differ significantly ($p<0.05$)

The result of present study revealed mean density of milk in goats in normal range during subclinical mastitis. However, Ali *et al*. [24] reported decreased milk density in bovine subclinical mastitis. The alteration in milk density is caused by the invasion of microbes of udder causing inflammatory changes in the udder parenchymaand these changes may be directly proportional to the extent of inflammation in the udder parenchyma [24]. Since, the present study dealt with subclinical mastitis in goats in which inflammatory changes might not have change the milk consistency.

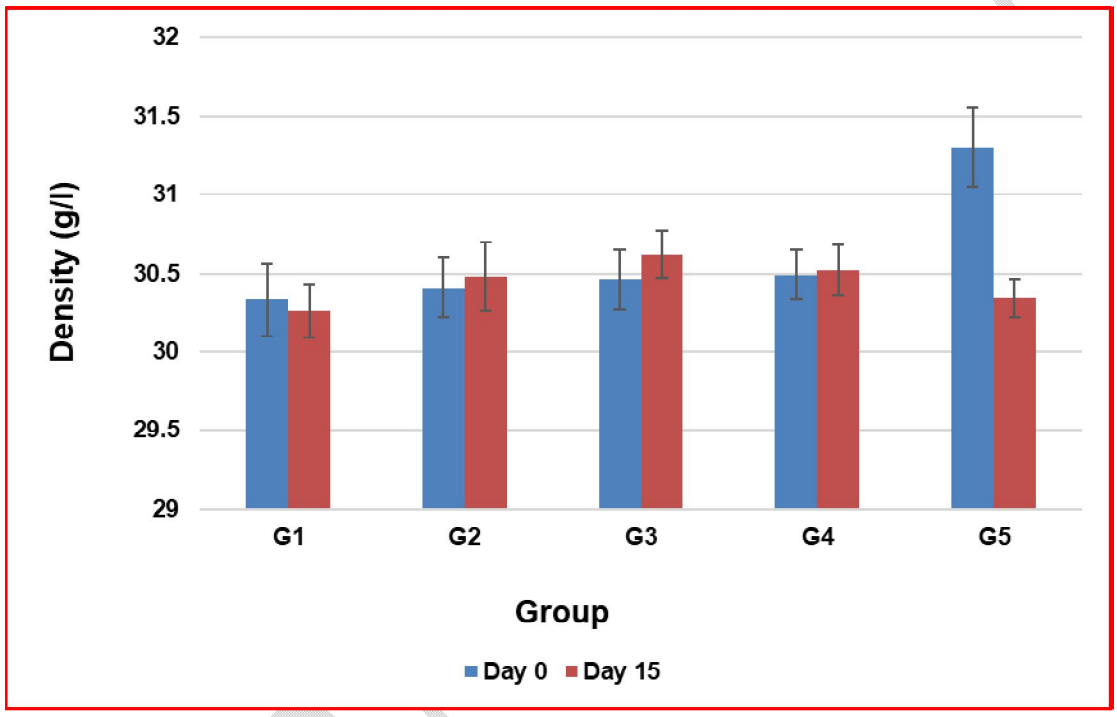

Figure 04: Mean milk density (g/L) in goats in different treatment groups at differentintervals

3.2 Standard plate count (SPC)

SPC in all the 24 lactating goats on day 0 (pre-treatment) and day 15 (post treatment) were recorded under therapeutic trial and compared with the healthy control goats.

Results showed that mean SPC $\log_{10}$on day 0 before treatment in goats of groups G1, G2, G3 and G4 were 1.03±0.01 cfu/ml, 1.02±0.01 cfu/ml, 1.03±0.01 cfu/ml and 1.04±0.01 cfu/ml, respectively. On day 15 (post treatment) the mean SPC $\log_{10}$in goats of groups G1, G2, G3 and G4 were 0.85±0.01 cfu/ml, 0.87±0.02 cfu/ml, 0.78±0.01 cfu/ml and 0.84±0.02 cfu/ml, respectively. The mean SPC $\log_{10}$in healthy control group (G5) on day 0 and day 15 was 0.02±0.01cfu/ml and 0.03±0.01 cfu/ml, respectively.

The mean SPC $\log_{10}$ on day 0 (pretreatment) was significantly higher in goats of all treatment groups in comparison to healthy control group. A significant improvement in mean SPC $\log_{10}$ of goats was reported in all treatment groups on day 15 (post treatment).However,maximum improvement was seen in goats of group G3 (Table 07; Figure 05).

Table 07:Mean milk Standard Plate Count $\log_{10}$ (cfu/ml) in goats in different treatmentgroups at different intervals

Groups	**SPC $\log_{10}$ (Mean±SE)**	
(n=6)	**Day 0**	**Day 15**

G1	1.03^{aA}±0.01	0.85^{abB}±0.01
G2	1.02^{aA}±0.01	0.87^{aB}±0.02
G3	1.03^{aA}±0.01	0.78^{bB}±0.01
G4	1.04^{aA}±0.01	0.84^{abB}±0.02
G5	0.02^{bA}±0.01	0.03^{cA}±0.01

Mean values with different superscripts between group (lowercase) and between days (uppercase) differ significantly ($p<0.05$)

The result of present study is in accordance with the findings of Dik *et al*. [25] who reported increase in SPC in goats affected by mastitis. The udder health is positively influenced by SPC as there is increase in colonization of pathogens in the udder during mastitis which might result directly in elevating the total bacterial count thus leading to increase the SPC. Hence SPC can be considered as useful tool for evaluation of udder health.

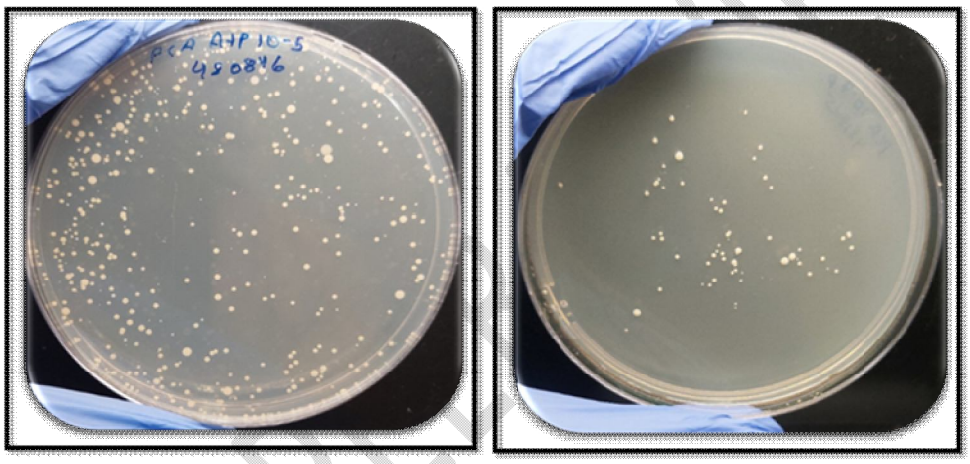

Plate 01: Colonies on plate count agar (standard plate count)

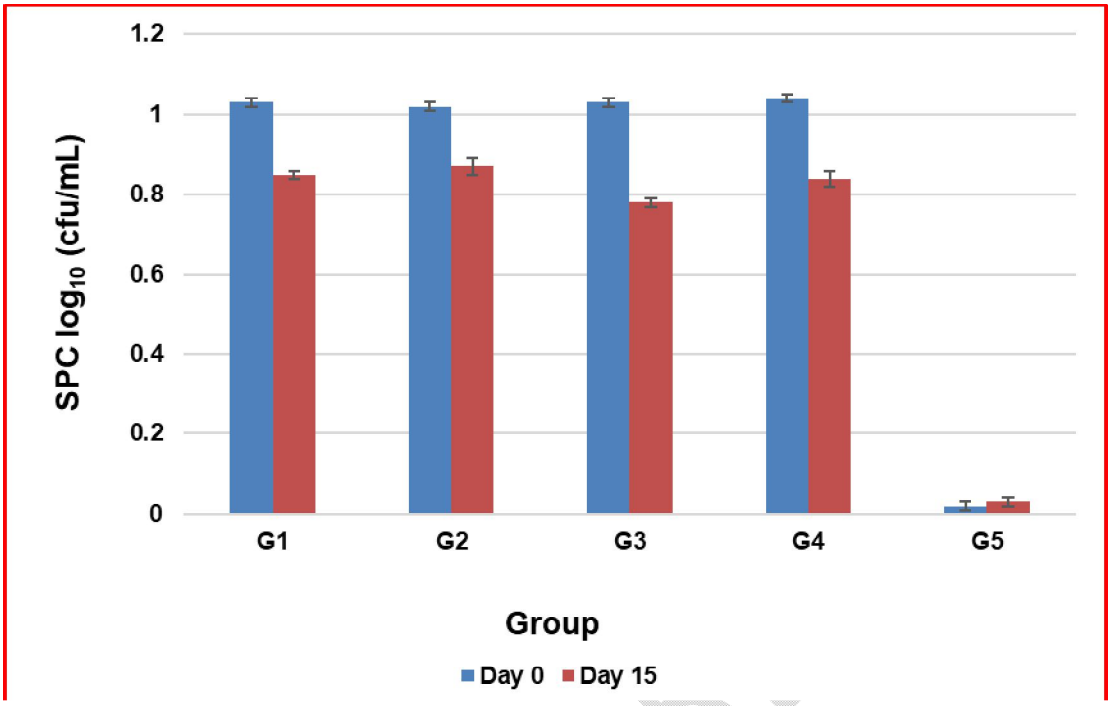

Figure 05:Mean milk SPC log10 (cfu/ml) in goats in different treatment groups at different intervals

3.3 Therapeutic response evaluation

The therapeutic response in different treatment groups was evaluated on the basis of improvement in milk parameters and Standard plate count (SPC) on day 15 post treatment in goats of all the treatment groups.

The antibiotic used in different treatment groups in the present study i.e. combination of amoxicillin and sulbactam @ 10 mg/kg b.wt. intramuscularly for 3 days was found effective in treating subclinical mastitis. The efficacy of amoxicillin and sulbactam against subclinical mastitis has been extensively studied by Amarwal*et al*. [26] and Sharma *et al*. [27] who reported in vitro antibiotic sensitivity of amoxicillin and sulbactam as more than 90% and 96.87%, respectively in goats affected with subclinical mastitis. Further, Jeph *et al*. [28] studied in vitro antibiotic sensitivity of 21 isolates and reported the efficacy of amoxicillin and sulbactam to be 87.50% in clinical mastitis. Pangprasit*et al*. [29] also stated that amoxicillin is highly efficacious in treating CM and SCM particularly caused by environmental Streptococcus spp. in dairy animals. Bergonier*et al*. [30] stated that the intramuscular route of drug administration is a preferable route for the treatment of mastitis as it is of easier application and iatrogenic risk of udder contamination is reduced.

Subclinical mastitis causes decrease in milk yield and lowers the milk quality by altering various milk parameters. Various strategies have been adopted for its cure among which post teat dip has been proved to be an essential tool in mastitis treatment as it is effective in preventing intramammary infections against different mastitis causing pathogens. The use of teat dips viz. eucalyptus oil 98 % + DMSO 2% (in group G1 and G3) and 5% povidone iodine (in group G2 and G4) in the present study remediate the milk parameters and SPC in subclinical mastitis in goats.

Eucalyptus oil, used in present study possess antibacterial, antioxidant and anti-inflammatory properties. The efficacy of eucalyptus oil as teat dip against subclinical mastitis is well documented by Rasool *et al*. [9] who evaluated the efficacy of *Eucalyptus globulus* essential oil as teat dip in cows with SCM and reported decrease in the SCC, pH of milk and colony-forming units thus, increasing the milk yield. Adida *et al*. [31] also studied in vitro antibacterial activity of *Eucalyptusglobulus* essential oil and aqueous extracts of *Thymus fontanesii* against subclinical mastitis pathogens and concluded that both showed moderate activity. Eucalyptus oil being lipophilic in nature, crosses the cell membrane, alter

membrane composition and increases its permeability leading to leakage of ions and loss of mitochondrial potential resulting in cell lysis of microorganism [32].

Povidone iodine used in the present study has low toxicity and excellent antibacterial and is used as teat disinfectant commonly in dairy farms. Literature regarding use of povidone iodine as teat dip is well described by Thapa and Joshi [33] and Singh *et al*. [34]. However, povidone-iodine has weak antibacterial persistence as an external use medicine. Hence, a long-term antibacterial effect against mastitis-causing bacteria can be achieve by developing a film-forming ingredients and disinfectants-based barrier teat dip [35].

In the present study Eucalyptus oil 98% + 2% DMSO as teat dip in combination with antibiotic amoxicillin sulbactam (in group G3) was proved as better teat dip as compared to 5% povidone iodine because its role can be better understood on subclinical mastitis in a way that it acts as antibacterial, anti-inflammatory and antioxidant by being lipophilic in nature and leading to cell death in mastitis causing pathogen [36].

4. CONCLUSION

Significantly higher values of somatic cell count, standard plate count, PH and significantly lower value of lactose were observed in subclinical mastitis affected goats. The mean density values varied within normal physiological range. In the present study therapeutic efficacy was evaluated on the basis of restoration of milk parameters and standard plate count, the most effective therapy for subclinical mastitis was elicited by Eucalyptus oil 98% + 2% DMSO as teat dip in combination with antibiotic amoxicillin sulbactam (G3). Hence, Eucalyptus oil was proved as a better teat dip because of its antibacterial, anti-inflammatory and lipophilic in nature properties in comparison of povidone iodine for subclinical mastitis in goats.

FUTURE RESEARCH

Further work may be emphasized on comparison of various essential oils as teat dip in subclinical mastitis in does. The study may be designed by involving other factors that affect somatic cell count, quality of milk etc. and the extension activities may be intensified to educate goat owners for the prevention of subclinical mastitis, maintaining udder hygiene and health coverage of lactating does.

Disclaimer (Artificial intelligence)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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