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Research article

Effect of essential oils of Eucalyptus globulus and Lavandula hybrida as teat dips to control subclinical mastitis in Friesian dairy cattle

Sadia Rasool[1,*], Muhammad Umar Farooq[2], Syed Saleem Ahmad[1], Muhammad Avais[1], Safdar Abbas[3], Nargis Ambreen[1]

[1]Department of Clinical Medicine and Surgery, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]Department of Meat Science and Technology, Faculty of Animal Production and Technology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[3]Veterinary Officer, I/C, Civil Veterinary Dispensary Ibrahimabad, Kasur, Pakistan

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*Corresponding Author:
Email:
sadiarasool764@yahoo.com

ABSTRACT

A clinical trial was conducted to evaluate the therapeutic efficacy of *Eucalyptus globulus* and *Lavandula hybrida* essential oils as teat dips in subclinical mastitis cows. In total, 24 cows screened positive for subclinical mastitis, out of 200, were selected for the study. Teat dip preparations included '98% *Eucalyptus* oil', '96% *Eucalyptus* oil', '98% *Lavender* oil', '96% *Lavender* oil', and '1% Iodine'. Essential oil teat dips were applied post-milking in 98:2 (98 parts oil + 2 parts Dimethyl Sulphoxide) and 96:4 (96 parts oil + 4 parts Dimethyl Sulphoxide) dilutions. The milk samples were collected for milk pH, somatic cell count, and colony forming unit, before initiating teat dipping and on the 14th and 28th day post-application of teat dips. After applications of essential oils, milk pH and Somatic Cell Counts of were significantly (P<0.05) reduced in all treated groups and was normalized at 28th-day post-application. The milk yield was increased by essential oil treated groups which was 10.98% in group A, 1.44% in group B, 36.18% in group C, and 7.29% in group Dat 28th day post-dipping. In conclusion, teat dipping with essential oils was proven effective to reduce the incidence of subclinical mastitis in dairy cows.

Keywords: Antiseptic, Milk Yield, Essential Oil, Somatic Cell Count, Subclinical Mastitis, Teat Dip

Introduction

Mastitis, defined as inflammation of the mammary gland, is considered an economically significant disease in dairy animals (cattle, buffalo, and goats) globally [1]. Mastitis has two major types: clinical mastitis (CM) and subclinical mastitis (SCM). The latter has more importance because of its 15 to 40 times higher prevalence than the clinical form, lack of inflammatory symptoms, challenge to diagnose, adverse effects to milk quality and production, and also remaining a reservoir of pathogens that can infect other animals due to its contagious nature [2: 3]. The significant loss that occurs because of subclinical mastitis includes reduction in milk yield [4].The subclinical form of mastitis has economic importance in reducing milk yield and an increase in medication expenses. Therefore, management control strategies of mastitis in a dairy herd are a critical approach to certify both animal health and food (milk) safety. Teat dipping is the most economical strategy to control subclinical mastitis [5].

Theory of teat decontamination after milking dates back to 1916, when diluting pineapple oil was used to reduce the spread of Streptococcus agalactiae. The practice was not however, implemented extensively for a longer period since supportive research data were not accessible on prevailing teat dip products. Now a day for teat dipping, Iodophors teat dip is used to prevent mastitis in dairy cattle, but it does not reduce the incidence of subclinical mastitis [6]. Herbal teat dips reduced subclinical mastitis; inhibit new intra-mammary infections with improved milk production. Essential oils are widely used in the medicine and pharmaceutical industries [7]. Plant essential oil comprises many ingredients that have antibacterial and antioxidant properties and are safe for use on teat skin. Antibacterial resistance has not been reported after the use of essential oils [8].

Post-milking teat dip has efficacy in reducing the incidence of contagious and opportunistic intramammary infections [9: 10]. Pre-milking teat dips are effective in reducing the incidence of environmental intramammary conditions [11]. Teat dipping is the most effective management strategy to control subclinical mastitis in dairy cows [12]. They reduce the risk of subclinical mastitis and reduce bacterial contamination in milk [13].

The basic objective along with hypothesis of study was to evaluate the antiseptic efficacy of plants' essential oils Eucalyptus globulus and Lavandula hybridainFriesian dairy cattle with subclinical mastitis.

Materials and Methods

This study was conducted at the University of Veterinary and Animal Sciences dairy farm, B-Block Ravi campus Pattoki, Punjab, Pakistan.

Experimental design

In total, two hundred milking dairy cows were selected for the screening test. Out of these, twenty-four dairy cows found positive for subclinical mastitis, were selected for chemotherapeutical trials and divided into six groups (A, B, C, D, E, and F), with four animals each. Essential oils of *Eucalyptus* and *Lavender* have diluted with 2 and 4% Dimethyl Sulphoxide. Essential oils activity was enhanced using Dimethyl Sulphoxide (DMSO), which has good penetration activity through the teat skin. The use of essential oils improves the teat skin condition, and apparently, there were no teat skin damages or rashes observed during dipping trials [14]. Group A was treated with 98ml *Eucalyptus* oil diluted in 2ml Dimethyl Sulphoxide. Group B was treated with 96ml *Eucalyptus* oil diluted in 4ml Dimethyl Sulphoxide. Group C was treated with 98ml *Lavender* oil diluted in 2ml Dimethyl Sulphoxide. Group D was treated with 96ml *Lavender* oil diluted in 2ml Dimethyl Sulphoxide. Group E was treated with 1% Iodine. An additional group, “F,” was kept as a control with no treatment. These teat preparations were applied two times a day for 28 days.

Screening test

The milk samples were collected from experimental cows on days 0, 14, and 28. The screening test, the surf field mastitis test (SFMT), was performed to diagnose subclinical mastitis [15]. The surf field mastitis test interpretations were, N stands for (Negative, no clumps formation), T (stands for Traces of clumps), W (for Weak, formation of slight clumping), M (for Moderate, a precise gel formation), S (for Strong gel formation that adhere to the paddle).

Sample collection

Milk samples were collected from positive dairy cows. Each teat of animal was dipped in Pyodine antiseptic solution and was cleaned individually with tissue paper. The First 1-2 streaks of milk were discarded, and 10ml of milk sample was collected in a sterile sample collecting tube. These milk samples were labeled and placed in an icebox and brought to the Laboratory of Department of Clinical Medicine and Surgery University of Veterinary and Animal Sciences, Lahore, Pakistan. The milk samples were placed at 4-8°C in a refrigerator for somatic cell count, pH test, and bacteriological examination.

Parameters estimated

The parameter, measured include; somatic cell count (SCC), milk yield (Liter/day), milk pH (by Lacto scan), and colony forming units (CFUs) for bacteriological examination.

Somatic cell count

The positive milk samples were confirmed by milk somatic cell counts, which is a key indicator for health status of the udder and confirmation of subclinical mastitis in dairy animals. The somatic cells were counted at days 0, 14, and 28th by the Newman Lampert staining procedure [**16**]. Milk film was prepared on a glass slide and was stained with Newman Lampert stain. The stained milk film was examined under a compound microscope. Somatic cells were counted in 10 microscopic fields of a microscope. These were counted by moving the slide horizontally from one edge to the other. The somatic cell counting was calculated by the following formula;

The average numbers of somatic cells per microscopic field

=Total number of somatic cells counted/Total numbers of microscopic filed

Numbers of somatic cells per 0.01 millilitre (10 µL) = Average numbers of somatic cells per Field × 5000

Numbers of somatic cells per millilitre of the milk = Average numbers of the somatic cells per the Field × 5000 ×100

***Note* =** 5000 Microscopic fields were present in a 1cm² area of a glass slide.

Bacteriological Examination

For bacterial examination, the milk samples were cultured on the media blood agar and Nutrient agar. For further confirmation, mannitol salt agar and MacConkey agars were used. The cultural and morphological identification of primary growth were studied through colony characteristics. Secondary growth was identified by Gram staining.

Colony Forming Unit

A colony-forming unit is quantitative test for viable bacterial count in the milk that was used to calculate total number of bacteria present in the milk. Six test tubes containing 9ml of sterile normal saline were taken. A sterile micropipette was used to add 1ml of raw milk sample in the first test tube for 10 fold dilution. 1ml of the sample was discarded from the last test tube. Next, 6 nutrient agar petri plates were taken and 100ml sample from individual test tube was spread on these plates. These plates were incubated at 37ºC for 24 hours. Colonies were counted and calculated by formula;

Colony-forming units =

$$\textit{Number of colonies counted / Dilution factor} \times \textit{Amount poured (ml)}$$

Statistical Analysis

Repeated measured analysis of variance (ANOVA) on SPSS version 20.00 was used for statistical analysis of somatic cell count, milk pH, colony-forming units, and milk yield. *P*-values less than 0.05 were significant.

Results and Interpretations

Subclinical mastitis is distributed worldwide and globally imposes a big threat to the dairy industry. Mostly, corporate dairy farms use commercially available antiseptic dipping solutions before and after milking to control subclinical mastitis. Commercially available antiseptic dipping solutions are very costly and they secrete toxic metabolites in the milk hazardous for both human and animal health. Keeping in view the importance of subclinical mastitis in the dairy sector, the present study was conducted at the University of Veterinary and Animal Sciences, dairy farm-B block; Pattoki, Pakistan intended to use *Eucalyptus* and *Lavender* oils as post-dipping solutions to control subclinical mastitis. The results were assessed based on the effect on milk pH, somatic cell count, colony-forming, and milk yield on.

Somatic cell count

In a normal healthy group somatic cell count of milk ranged between 3.39 ± 0.03 to 3.48 ± 0.03 × 10^5 cells/ml with an overall mean of 3.41 ± 0.03 × 10^5cells/ml. In subclinical mastitis group overall mean SCC was 7.23 ± 0.34 × 10^5cells/ml [**17**]. A very important parameter that increases in the case of subclinical mastitis-positive animals is somatic cell count which normally ranges from200,000 to 900,000 somatic cells per ml of healthy animals in Pakistan. Somatic cells are defending cells of the body and comprise neutrophils, lymphocytes, macrophages, and fewer amounts of epithelial cells [**18**]. The average values of somatic cell count in different groups at 0, 14^{th,} and 28th day of trial are shown in Table1. In essential oil-treated groups (A, B, C, and D) the percentage reduction for somatic cell count was found 57%, 47.9%, 35.9%, and 54.5% respectively on days 14 and 93.25%, 93.78%, 91.62%, and 87.84% respectively on day 28, whereas in groups E and F there was no percentage recovery. The results of the somatic cell count of the present study correlate with the findings of Zucali et al. [**19**]. Statistical analysis revealed significant variations in somatic cell count on different days. The standard error and mean comparison indicated a significant decrease in somatic cell count in different groups and restored to normal at day 28 of trial in plant essential oils treatments showing their effectiveness.

Table: 1-Effect of essential oils of *Eucalyptus* and *Lavender* on somatic cells per ml of milk

Days	Groups						*P*-Value
	Control	A	B	C	D	E	
0	42.8±2.3	53.00±2.2	46.25±4.4	43.87±1.1	49.55±0.4	39.75±4.9	0.001
14th	49.1±0.8	22.75±6.8	24.07±2.6	28.12±1.9	22.50±4.9	38.75±5.6	0.001
28th	49.9±0.8	3.575±1.0	2.87±0.49	3.675±1.6	6.025±0.1	45.60±4.1	0.001
Note = *P*-Value <0.05 considered significantly different							

Milk pH

The average values of milk pH in different groups at 0, 14th, and 28th day of the trial are shown in Table 2.

In essential oil-treated groups (A, B, C, and D) the percentage changes in milk pH were found 17.08, 4.96, 11.20, and 15.55%, respectively on day 14 and 19.93, 16.10, 17.10 and 15.54%, respectively on day 28, whereas in groups E and F there was no percentage recovery. These findings in accordance with those reported by Waghmare et al. [18], where the pooled mean value of pH of milk in group A was 6.56±0.01, in group B (treated with mastidip at 0, 7th, 14th, 21st and 30th day) was 6.66±0.01 and in group C (treated with mastidip at 0, 7th, 14th, 21st and 30thand 45th day) was 6.65±0.01. The result showed significant difference (P<0.05) in the pH of milk between different intervals. Plant essential oils application decreased milk pH and restored it to normal on day 28 of the trial showing the effectiveness of essential oils. The pH of milk is a very important parameter that increases in the case of subclinical mastitis. The normal milk pH ranges from 6.5 to 6.8 [20]. In the case of subclinical mastitis, pH increases from normal because of more concentration of bicarbonates and chloride ions in the udder cells [21: 22].

Table: 2- Effect of essential oils of *Eucalyptus* and *Lavender* on milk pH

Days	Groups						*P*-Value
	Control	A	B	C	D	E	
0	7.95±0.17	8.08±0.27	7.87±0.02	7.86±0.01	7.91±0.04	7.36±0.01	0.001
14th	8.12±0.16	6.7±0.07	7.48±0.05	6.98±0.16	6.68±0.07	7.71±0.09	0.001
28th	8.35±0.12	6.47±0.02	6.60±0.03	6.51±0.02	6.56±0.01	7.67±0.06	0.001
Note = *P*-Value <0.05 considered significant							

Milk yield

One of the most important economic losses of subclinical mastitis is decreases in milk yield. The average values of milk yield in different groups at 0, 14, and 28 days of trial are shown in Table 3. In essential oil-treated groups (A, B, C, and D) the percentage increase in milk yield was 2.32, 5.07, 9.24and 8.60%, respectively at day 14th and 11.0, 1.44,36.18 and 7.29%,respectively on28 day. There was no percentage recovery in groups E and F. These results are in agreement with findings of Vala et al. [23], where mean values of milk yield were recorded in group I (control), II (an herbal drug used at days 0, 7, 14, 21, 28 and 35) and III (an herbal drug used at days 0, 7, 14, 21, 28, 35, and 49). In group I (control), the pooled mean value of milk yield was 16.5 litres per day at day 0, in group II was 21.48 litres per day and in group III was 14.1 litters per day. The milk yield increased by 1.9% and 6.3% in groups II and III after applying the herbal tea dipping solution on day 49 as compared to day 0. The results indicated non-significant difference in milk yield. After the application of plant essential oils, the milk yield showed an increasing trend on different days.

Table: 3- Effect of essential oils of *Eucalyptus* and *Lavender* on milk yield (Liter/day)

Days	**Groups**						***P*-Value**
	Control	**A**	**B**	**C**	**D**	**E**	
0	11.4±0.5	25.7±1.4	23.4±0.5	12.6±2.1	24.6±1.8	15.6±1.2	0.30
14th	11.3±0.6	26.3±1.5	24.6±0.8	13.7±1.5	22.5±2.7	13.3±0.8	0.30
28th	8.2±0.4	28.5±1.5	23.8±2.0	17.2±1.9	26.4±2.6	11.7±0.7	0.30

Note = *P*-Value <0.05 considered significant

Colony-forming unit

Teat dipping is the most effective management strategy for controlling the spread of contagious mastitis pathogens [24]. In essential oil-treated groups (A, B, C, and D) the percentage decrease in milk yield was found 73.95%, 32.39%, 31.57%, and 27.97% respectively on day 14 and 95.30%, 81.69%, 79.76%, and 80.76% respectively on day 28. While in groups E and F there was no percentage recovery. The values of colony-forming units are under the findings of Kamal and Bayoumi [25] in which they found the average values of colony-forming units for *Staphylococcus aureus;* $6.6\pm2.3\times10^4$ in the control group, $4.7\pm0.53\times10^4$ in pre-milking teat dips group, and $4.4\pm0.06\times10^2$ in the post-milking teat dip group. For *E.coli*, in the control group, their mean values were $3.5\pm0.38\times10^5$, in the pre-milking teat dip group were $8.2\pm0.7\times10^4$ and for the post-milking teat dip group, the values were $9.8\pm0.02\times10^2$. Statistical analysis showed a significant decrease in colony-forming units at different intervals. The mean comparison showed a significant decrease in colony-forming units in different groups and restored to normal at day 28th of trial in plant essential oils treatments showing the effectiveness of essential oils.

The *Eucalyptus globulus* and *Lavandula hybrida* essential oils are well known to have anti-inflammatory, antibacterial, and immunomodulatory properties. These properties are responsible for normalizing the somatic cell count, pH of milk, and colony-forming units thus leading to the increase in milk yield.

Table: 4- Effect of essential oils of *Eucalyptus* and *Lavender* on colonyforming units per ml of milk.

Days	Groups						*P*-Value
	Control	A	B	C	D	E	
0	38.75±7.38	53.75±3.49	35.50±5.66	42.75±3.35	35.75±4.32	40.50±4.69	0.001
14th	40.00±7.58	14.00±0.070	24.00±1.95	29.25±5.30	25.75±4.62	35.00±2.04	0.001
28th	41.00±7.53	2.50±0.50	6.50±1.16	8.65±4.73	6.87±1.51	33.50±3.92	0.001
Note = *P*-Value <0.05 considered significant							

Conclusion

Based on findings of the present study, it is concluded that plants essential oils used as a teat dips have better results for the reduction in somatic cell count, pH, milk yield and colony forming units and increased the milk yield in Friesian dairy cattle. So they should use as pre and post milking teat dips to control the sub-clinical mastitis in commercial dairy farms. Teat end scorings and condition of skin improved by application of plant essential oils and they also act as a fly repellent. The microbial population also decreased on teat skin to prevent future infections. After application of plant essential oils milk yield increased resulting in economic benefit to the farmers. The important aspect of this study is an economic viability of these oil preparations; hence they reduce management cost for the farmers.

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