



Evaluation of Therapeutic Potential of Poly Herbal Formulation on Sub Clinical Mastitis

Padma Nibash Panigrahi*, Arzoo Nisha, Mukesh Kumar Srivastava, Ashish Srivastava, Alok Chaudhury and Arvind Kumar Tripathi

Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, UP, INDIA

* Corresponding author: PN Panigrahi; E-mail: pnpvetmed@gmail.com

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ABSTRACT

The present study was conducted to determine efficacy of poly herbal formulation (combination of *Moringa oleifera*, *Ocimum sanctum* and *Azadirachta indica* leaves and *Curcuma longa* rhizome) on sub clinical mastitis in cows. For this study, Three cows (12 quarters) were considered apparently healthy control group (group A) after thorough physical examination and various diagnostic tests. A total of nine cows (36 quarters) found positive for sub clinical mastitis on screening were randomly divided into three groups with three animals (12 quarters) each. Poly herbal formulation was administered orally for seven days twice daily at 10 mg/kg in group B animals. Group C animals were treated with conventional treatment for seven days. Group D animals were treated with combination of Poly herbal formulation and conventional treatment. Therapeutic efficacy of poly herbal formulation was evaluated based on alteration of somatic cell count of milk and qualitative changes in milk. A significant increased concentration of SCC and pH of milk and significantly decreased concentration of fat and solid not fat (%) was reported in SCM affected animals as compared to healthy control before treatment. Poly herbal formulation significantly reduced the SCC and pH as well as significantly increased the fat and SNF (%) as that of conventional therapy. The combination of poly herbal formulation and conventional treatment accelerate the recovery. Hence, the present poly herbal formulation may be advised along with conventional treatment for early recovery in sub clinical mastitis.

HIGHLIGHTS

- Study focused on efficacy of poly herbal preparation on sub clinical mastitis as alternative treatment.
- Alteration of somatic cell count of milk and qualitative changes in milk considered for therapeutic efficacy.
- The present poly herbal formulation may be advised along with conventional treatment for early recovery in sub clinical mastitis.

Keywords: Sub clinical mastitis, *Moringa oleifera*, *Ocimum sanctum*, *Azadirachta indica*, *Curcuma longa*, somatic cell count

Mastitis is a common disease of dairy animals that causes huge economic losses in developing countries like India. The sub clinical mastitis is characterized by having no visible signs either in the udder or in the milk, but the milk production decreases. Timely detection of subclinical mastitis (SCM) by the most accurate test is the first requisite to effectively control the mastitis (Sharma *et al.*, 2008). Treatment and prevention of mastitis are dependent on antibiotics. Unfortunately, due to abuse of antibiotics, the development of resistant pathogens has become a global concern to veterinary and public health. Therefore, the present situation demands a newer approach of therapy.

Several recent studies have highlighted the effectiveness of medicinal plants and natural compounds for treatment and management of mastitis (Mushtaq *et al.*, 2018; Mooventhan *et al.*, 2016). *Moringa oleifera* is a tropical plant native to India. It is also known as Miracle Tree due to its high nutrient contents. Aqueous extracts of *Moringa oleifera*

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were found to be inhibitory against many pathogenic bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* in dose dependent manner (Saadabi and Abu Zaid, 2011). Curcumin, a polyphenol derived from rhizome of *Curcuma longa*, has been successfully used in animals and humans due to its antibacterial and anti-inflammatory properties (Sarkar *et al.*, 2016; Sharma *et al.*, 2007). Recently, a study demonstrated that curcumin ointment reduced pain, mammary tension and erythema in humans with mastitis 72 h after the treatment (Afshariani *et al.*, 2014). *Azadirachta indica* possess profound antibacterial activity as well as anti-inflammatory activity to reduce udder inflammation (De and Mukharjee, 2009). *Ocimum sanctum* was found to possess significant analgesic (Singh and Majumdar, 1994), anti-inflammatory, immunomodulatory (Sadekar *et al.*, 1998) and antimicrobial activity (De *et al.*, 1999). Therefore, the above cited project is being proposed to evaluate therapeutic potential of poly herbal formulation against sub-clinical mastitis.

MATERIALS AND METHODS

Ethical approval

The guidelines for animal experimentation followed in this study was approved by Institutional Animal Ethics Committee and the ethical guidelines of UP Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Viswavidyalaya evam Go-Anusandhan Sansthan (DUVASU), Mathura were followed during all the experiments.

Animals

The present study was conducted in Department of Veterinary Medicine, DUVASU, Mathura in association with Teaching Veterinary Clinical Complex, DUVASU, Mathura and University dairy farm. Lactating cows of any breed presented to the TVCC and/ or University dairy farm was included in this study. Owner's complaint and/or history suggestive of sub clinical mastitis i.e., gradual loss of milk yield, salty taste, slimy or watery consistency etc. was taken into consideration for preliminary screening.

Screening of animals for sub clinical mastitis

Approximately 10 ml milk was collected from each

animal for screening the sub clinical mastitis by California Mastitis Test (CMT) as per procedure given by (Schalm and Noorlander, 1957) using a modified CMT reagent (Sharma and Rajani, 1969).

Plant material

Combination of *Moringa oleifera* leaves, *Ocimum sanctum* leaves, *Azadirachta indica* leaves and *Curcuma longa* rhizome was taken for this study as poly herbal preparation. Fresh green leaves of *Moringa oleifera*, *Ocimum sanctum* and *Azadirachta indica* and rhizome of *Curcuma longa* was collected from medicinal herb garden, DUVASU, Mathura. The plant material was cut into pieces, wash with distilled water to remove the dirt, air dried and uniformly powdered separately using an electric grinder. Combination of all four plant materials was given orally.

Therapeutic trail

Three cows (12 quarters) were considered apparently healthy control group (group A) after thorough physical examination and various diagnostic tests. A total of nine cows (36 quarters) found positive for sub clinical mastitis on screening were randomly divided into three groups (group B, group C and group D) with three animals (6 quarters) each. Polyherbal formulation was administered orally for seven days twice daily at 10 mg/kg (EC₅₀) in group B animals. Group C animals were treated with conventional treatment (combination of ceftizoxime @ 20 mg/kg, intravenously once; flunixin meglumine @ 1.1-2.2 mg/kg intramuscularly if required and supportive therapy as per requirement) for seven days. Group D animals were treated with combination of Polyherbal formulation and conventional treatment. Therapeutic efficacy of polyherbal formulation was evaluated based on alteration of somatic cell count of milk and qualitative changes in milk (milk fat, solid not fat, pH, density etc). Therapeutic efficacy was evaluated at weekly interval for 14 days.

Analysis of milk

Approximately 5-10 ml of milk was collected from each quarter of each animal in clean test tube on day-0 (before treatment), day-7 (7th day of treatment) and day-14 (14th day of treatment). 1-2 ml was taken for MCMT and rest of the milk sample was taken for milk analysis and somatic

cell count in automatic Lactoscan Milk analyzer. Somatic cell count (SCC), pH of milk, fat percentage, solid not fat (SNF), specific gravity, milk protein content, lactose content etc was calculated in automatic Lactoscan Milk analyzer as per manufacturer's instructions.

STATISTICAL ANALYSIS

The data was expressed as mean \pm SEM. Standard error of mean and p-values were used to determine whether there is any significant difference among different treatment groups using one-way analysis of variance (ANOVA) following standard protocol (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Table 1 summarizes the alteration in somatic cell count (SCC) in cows of different groups at different interval of study. A significant ($p < 0.05$) increase in SCC was recorded in milk of cows effected with sub clinical mastitis (group B, group C and group D) as compared to healthy control before treatment (day 0). Both poly herbal formulation (group B) and conventional treatment (group C) reduced the SCC significantly at day 14 but values were statistically similar to that of healthy control animals in combination therapy (group D).

The increased in Somatic cell count (SCC) in SCM affected cows can be attributed to increase polymorph nuclear cells and neutrophils. Whereas, after treatment with polyherbal combination there was significant reduction in somatic cell count (SCC) at different interval of study indicated reduction of inflammation as a result of treatment. It might be due to potent anti-inflammatory and anti-bacterial effect

of *Moringa oleifera*, *Ocimum sanctum* and *Azadirachta indica* leaves and *Curcuma longa* rhizom (Nikkon *et al.*, 2003; Dangi *et al.*, 2002; De *et al.*, 1999; Sarkar *et al.*, 2016).

The milk fat and solid not fat (SNF) % reduced significantly in SCM cows as compared to healthy control at day 0 (table 2). These results are in accordance with Souza *et al.* (2009), but disagreement with Leitner *et al.* (2004), Merin *et al.* (2004) and Min *et al.* (2007). Poly herbal formulation, conventional treatment as well as combination therapy significantly increased the above concentration. The observed high fat content in milk could be attributed to increased fermentation efficiency, enhanced by phytochemicals rich *Moringa oleifera*, *Ocimum sanctum* and *Azadirachta indica* leaves and *Curcuma longa* rhizome in conjunction with amino acids and P, Ca, and Mg which are essential for high milk synthesis (Kholif *et al.*, 2015).

No Significant differences were recorded in mean values of milk density, milk protein concentration and milk lactose concentration either between the groups or within groups at different observation periods of study (table 2). The density of milk and lactose were decreased non significantly in animals affected with SCM and these results were closed to the findings of Merin *et al.* (2004) and disagreement with that of Moroni *et al.* (2005). Lactose is synthesized in the gland cells of udder from glucose and galactose. Milk of infected glands has a significantly lower concentration of lactose, which is accompanied by significantly higher whey and albumin concentrations. The accumulation of whey proteins probably results from a breakdown of the caseins and other milk proteins, which down-regulate milk secretion (Silanikove *et al.*, 2000; Shamay *et al.*, 2003) and explain the lower lactose concentration.

Table 1: Effect of poly herbal formulation on somatic cell count (SCC) in sub clinical mastitis (SCM)

Parameters	Group	Day 0	Day 7	Day 14
SCC ($\times 10^3$ /ml)	Group A	90.34 \pm 28.67 ^{aA}	99.17 \pm 7.44 ^{aA}	97.3333 \pm 11.15 ^{aA}
	Group B	355.17 \pm 35.71 ^{bB}	306.67 \pm 30.84 ^{abC}	258.33 \pm 27.49 ^{aB}
	Group C	365.00 \pm 35.94 ^{bB}	203.33 \pm 9.25 ^{aB}	198.17 \pm 19.45 ^{aB}
	Group D	336.67 \pm 40.80 ^{bB}	189.33 \pm 26.09 ^{aB}	165.17 \pm 15.67 ^{aAB}

Group A = healthy control, group B = poly herbal formulation, group C = conventional treatment and group D = combination of poly herbal formulation and conventional treatment. Values (Mean \pm SE) within same column for a particular parameter (capital letters) and in same row (small letter) bearing similar superscript do not differ at $P < 0.05$.

Table 2: Effect of poly herbal formulation on milk parameters in sub clinical mastitis (SCM)

Parameters	Group	Day 0	Day 7	Day 14
Fat (%)	Group A	4.62±0.26 ^{aB}	4.47±0.24 ^{aB}	4.69±0.22 ^{aA}
	Group B	3.15±0.46 ^{aA}	3.86±0.24 ^{aA}	4.15±0.17 ^{bA}
	Group C	3.17±0.29 ^{aA}	4.04±0.27 ^{bB}	4.20±0.18 ^{bA}
	Group D	3.13±0.25 ^{aA}	4.16±0.22 ^{bB}	4.50±0.17 ^{bA}
SNF (%)	Group A	8.40±0.20 ^{aB}	8.41±0.13 ^{aA}	8.58±0.26 ^{aA}
	Group B	7.30±0.33 ^{aA}	7.84±0.22 ^{abA}	8.08±0.18 ^{bA}
	Group C	7.37±0.28 ^{aA}	8.04±0.13 ^{bA}	8.17±0.05 ^{bA}
	Group D	7.15±0.32 ^{aA}	8.13±0.15 ^{bA}	8.25±0.28 ^{bA}
Protein (%)	Group A	3.18±0.05	3.15±0.16	3.17±0.15
	Group B	3.72±0.10	3.39±0.20	3.25±0.25
	Group C	3.68±0.17	3.24±0.23	3.10±0.08
	Group D	3.61±0.15	3.29±0.18	3.14±0.13
Lactose (%)	Group A	4.72±0.17	4.70±0.23	4.81±0.35
	Group B	4.28±0.30	4.28±0.33	4.40±0.81
	Group C	4.30±0.03	4.31±0.07	4.61±0.24
	Group D	4.38±0.09	4.39±0.11	4.56±0.21
Density	Group A	32.22±0.90	30.36±0.63	29.82±0.50
	Group B	26.24±0.46	26.25±1.56	25.55±2.56
	Group C	26.54±0.30	25.49±0.48	27.68±0.61
	Group D	26.36±0.91	26.30±1.02	26.48±0.71
pH	Group A	6.37±0.03 ^{aA}	6.28±0.03 ^{aA}	6.33±0.02 ^{aA}
	Group B	6.73±0.04 ^{bB}	6.53±0.06 ^{aB}	6.45±0.07 ^{aA}
	Group C	6.73±0.05 ^{bB}	6.43±0.06 ^{aAB}	6.48±0.07 ^{aA}
	Group D	6.82±0.04 ^{bB}	6.55±0.07 ^{aB}	6.52±0.06 ^{aA}

Group A = healthy control, group B = poly herbal formulation, group C = conventional treatment and group D = combination of poly herbal formulation and conventional treatment. Values (Mean ± SE) within same column for a particular parameter (capital letters) and in same row (small letter) bearing similar superscript do not differ at P<0.05.

A significantly ($p < 0.05$) increased concentration of milk pH was recorded in SCM affected cows on day 0 as compared to untreated healthy control (table 2). Increased pH of SCM milk was also reported by other researchers (Hassan, 2013; Hussain *et al.*, 2012; Batavani *et al.*, 2007). The rise in pH was due to the leakage of blood bicarbonate into the milk following damage to the mammary epithelium (Kumhar *et al.*, 2018). The pH of milk decreased significantly post therapy in all three treatment groups on day 7 as well as day 14 and the values had no significant difference as that of healthy control. The decrease in pH after treatment might be due to reduction in alkalinity resulted due to inflammation by increased number of Na⁺ and Cl ions in the milk (Ahmed *et al.*, 2005). The present curative results are in agreement

with (Mooventham *et al.*, 2016) who studied the effect of *Moringa oleifera* leaves in cow reported reduction in pH of milk after Moringa supplementation. Similar type of study was also reported by Jaguezeski *et al.* (2018) on Curcumin supplementation.

Based on the results of the study it was concluded that, poly herbal formulation was found effective against sub clinical mastitis as evidenced by restoration of somatic cell count and other milk parameters. The combination of poly herbal formulation and conventional treatment accelerate the recovery as evidenced in the present study. Hence, the present poly herbal formulation may be advised along with conventional treatment for early recovery in sub clinical mastitis.

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