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RESEARCH PAPER

Effect of Intra-mammary infusion of *Moringa oleifera* on somatic Cell Count [SCC] and Total Bacterial Count [TBC] in cows with *Staphylococcus aureus* clinical mastitis

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Abstract : Therapeutic efficacy of hydro-methanolic extract of *M. oleifera* was evaluated in lactating cows with Staphylococcusaureus clinicalmastitis.Mastiticcows,screened and selected on the basis of CMT,SCC and cultural examination, were divided in two groups of five animals each.Intra-mammary infusion of Hydro-methanolic extract of *M.oleifera* @1 g / 10 ml of PBS intramammary once daily for 7 days resulted in reduction of 90.9%,93.04 % and 83.98% inCMT, SCC and TBC on day 14 of treatment which was comparable with that of group I with ceftriaxone.

Key Words : *M.oleifera, Staphylococcus aureus*, clinical mastitis, CMT, SCC,TBC

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INTRODUCTION

Mastitis is one of the major problems of dairy industry causing huge economic losses. The losses are mostly quantitative in the form of reduced milk production along with deterioration in physical, chemical and bacteriological quality of milk (Raza *et al.*, 2013). It has been documented those losses due to mastitis has increased 114-fold in about four decades from 529 million/ annum to 60532 million annum up to 2001 (Dua,2001). Nonetheless, *Staphylococcus aureus* is number one mastitis causing pathogen in India (Athar, 2007, Sharma *et al.*, 2007, Sumathi *et al.,*2008, Padhy *et al.,*2014).

Mastitis is caused by a whole consortium of more than 137 pathogenic micro-organisms. *Staph. aureus* forms micro-abscesses or granulomas in the mammary gland tissues and there is usually low response to orthodox antibiotic therapy due to intracellular localization of organism in the mammary gland epithelial cells.

Indiscriminate use of commonly used antibiotics such as Penicillin, Ampicillin, Streptomycin, Tetracycline and Oxytetracycline has led to development of drug

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resistance leading to treatment failure in mastitis (Owens *et al.,*1997, Tung, 2004). Methicillin resistant *S.aureus* has become a treatment challenge for veterinarians and has created additional public health concern due to its recent emergence as community acquired pathogen (Sato *et al.,*2017).With the increasing problems of drug resistance and other adverse effects,herbal plants are reviewed for their potential use as complementary and Alternative Medicine (Cowan,1999), Traditional healing system around the world that utilized herbal remedies were an important source for discovery of new antibiotics (Samy and Gopalkrishnakone, 2008).

Moringa oleifera commonly known as "Drumstick or Horse radish tree" is a member of the moringaceae family (Olson,2002) and a native of sub-Himalayan northern parts of India. A wide variety of nutritional and medicinal virtues have beenattributed to its roots, bark, leaves, flowers fruits and seeds (Ramchandran *et al*.,1980). The plant is rich in a unique group of compounds called glucosinolates and isothiocyanates reported to have potent hypotensive,anti-cancer,anti-oxidant and anti-bacterial properties against a variety of pathogens including *Staph. aureus*, *Staph. epidermidis*, *S.pyogenes*, *E.coli* etc. (Renitta *et al.,* 2009, Dewangan *et al.,* 2010). The hydro-ethanolic extract of *M.oleifera* is stated to possess high amount of flavonoids, polyphenols and tocoferol showing significant anti-oxidant potential (Siddiq *et al*., 2005, Sreelatha and Padma, 2009, Das and Kanodia, 2012 and Oybrinugafor *et al.,* 2012).

Inline with the anti-bacterial, anti-inflammatory and anti-oxidant properties of *M.oleifera*, the objectives of this research were to assess its therapeutic potential as an alternative / complementary therapy in clinical bovine mastitis.

MATERIAL AND METHODS

Collection of plant materials:

M. oleifera leaves were collected locally from the campus of Ranchi Veterinary College, Kanke, Ranchi, Jharkhand. The collected leaves were identified and authenticated by Professor, Department of Botany, Ranchi University, Jharkhand, India.

Preparation of extract:

The leaves of *M.oleifera* were shade dried and ground to powder. Hydro-ethanolic extract was prepared by dissolving 500 g of ground leaves in 2.0 litres of 80% methanol kept at room temperature with occasional shaking. After three days, extract was filtered off by using sterile whatmann filter paper no 1. The resultant methanolic extract was concentrated and evaporated to drynessbelow 40°C to yield a semisolid mass.The standard extract was reconstituted in sterile phosphate buffer saline (PBS, pH-7.4) 1 g extract/10ml of PBS. The reconstituted extract was filtered through a membrane filter (0.22um pore size, Millipore, Bangalore Pvt. Ltd.,India) and refrigerated in a sterile vial for intramammary infusion. The dose was selected on the basis of previous research.

Standard antibiotic:

Ceftriaxone was selected for the present study on the basis of *in vitro* sensitivity test by standard disc diffusion method.

Experimental design:

Animals :

The study was carried in a Military Dairy Farm, Namkum,Ranchi,Jharkhand in cross bred dairy cows maintained under identical feedingand management practices. Ten (10) cows, out of a total 95, selected on the basis of physical examination, CMT, SCC and cultural examination were divided into two groups of five animals each. The cows were subjected to the treatment schedule for seven days (Table A). The study was approved by International Animal Ethics committee as per CPCSEA guidelines.

Gr.	Treatment Schedule	Dose, Route and Duration
I	Hydro-methanolic extract of *M.oleifera*	1g / 10ml of PBS intramammary once daily for 7 days.
II	Ceftriaxone	500mg Intramammary once daily for7 days.

Milk sampling:

50 ml of milk sample was collected from each cow in sterile vial after cleaning the teat with 70% of ethanol and discarding a few streams of milk. Samples were collected on day 0 and thereafter on day 3,7 and 14. Somatic cell count was performed as per Schalm *et al.,*1971. California mastitis test was using CMT commercial reagent (Delavel) as per Schalm and Noorlander,1957. Total bacterial count (TBC) was performed per standard method described by Griffin *et al*., 1977. For bacteriological examination of milk samples

and the analysis of bacterial growth, milk samples were inoculated on blood agar plates incubated for 24 and 48 hours at 37°C. The organisms were identified on the basis of cultural and biochemical characteristics, grams staining and growth on selective media.

Statistical methods:

Statistical analysis of data was done as per the method suggested by Snedecor and Cochran (2004).

RESULTS AND DISCUSSION

M. oleifera hydro-methanolic extract showed good *in-vitro* anti-microbial activity against a number of micro-organisms including coagulase +ve*Staph.aureus*, coagulase-ve S*taph.*Sp., *Streptococcus* sp. and *E.coli,* commonly associated with mastitis in cows.In the present study, *S. aureus* was isolated as the major mastitis causing organism followed by *Streptococcus* sp. and *E. coli* etc. *Staph. aureus* is reported as one of most representative pathogenic bacteria causing bovine mastitis and is widely distributed in dairy cattle herds in several countries (Schmidt *et al.,* 2017). *Staph* sp. was enumerated as the major causative agent of clinical mastitis in bovines of Jammu region (Bhat*et al.,* 2017). Many studies from Asian countries have also reported that *Staph. aureus* is the chief etiological agent of mastitis in cattle and buffaloes (Kang -Hee *et al.,* 2001, Sharma *et al*., 2007, Abdul-Rady and Sayed, 2009; Rahman *et al.,* 2010, Sharma and Maiti, 2010).

Use of anti-biotics is a major drawback in the treatment of *Staph aureus* mastitis because of high resistance, poor response and chronic nature of infection. Also, there is limitation in antibiotic usagedue torise of antibiotic residues in milk. Furthermore, anti-biotic therapy can impede the normal defense mechanism of the udder by decreasing phagocytic function exacerbating inefficiency of phagocytes and subsequent relapse of function (Aboul-Ela., 2002). The obstacles in the control of *S. aureus* mastitis have paved the way for alternative approaches in its therapy and management.Antibiotic screening of natural products obtained from *M. oleifera* used in thecomplementary and alternative medicine (CAM) is amajor thrust area of research and development.

CMT score, SCC and total bacterial count:

The therapeutic efficacy of hydro-methanolic extract of *M.oleifera* was evaluated in terms of CMT,SCC and TBC intwo experimental groups I and II, respectively. The initial mean CMT point score was 2.2±0.20 and 2.4±0.24 on day 0 in group I and II, respectively (Table 1 and 2). The initial mean SCC was recorded as 43.12±5.76X10^5cells/ml and 44.50±3.47X 105 cells/ml for group I and II, respectively. Group I cow treated with *M.oleifera* extract showed reduction of 90.9% in CMT score and 93.04 % in SCC onday 14 of treatment which was comparable with respective score in antibiotic treated group (91.67% and 92.18% reduction in CMT and SCC). Complementary results were observed with TBC as bacterial count was reduced to 4.19 ±6.08 X10^3/ml with 83.98% inhibition in group I while a lower inhibition (69.24%) was observed in group II.

Numerous studies have identified compounds within herbal plants that are effective as antibacterial agents (Parekh and Chanda, 2007). The hydro-methanolic extract of *M. oleifera* showedgood *in -vitro* anti-microbial activity against a number of micro-organisms including *Staph.aureus*. Accordingly, *in vivo* efficacy of the extract was evaluated by monitoring CMT score and SCC indices of treated cows. The amount of gel formation in CMT test corresponds to increase in number of leucocytes present during inflammation of mammary gland which is characteristic of mastitis.Also, high CMT score indicates high SCC value.

In the present study, the anti-inflammatory activity of the extract was not independently tested but the reduction in mastitis score (CMT and SCC) suggests an anti-inflammatory activity along with anti-microbial

Table 1: Therapeutic effect of *Moringa oleifera* extract on the basis of average CMT point score, SCC (10^5 cells/ml of milk) and TBC (10^3 cells/ml of milk)

Parameters	Days post treatment			
(n= 5)	0	3	7	14
CMT	2.20±0.20^C	2.00±0.00^C	1.40±0.24^B	0.2±0.20^A
SCC	43.12±5.76^C	24.10±4.18^B	13.90±0.91^B	3.0±0.79^A
TBC	26.08±1.54^D	18.04±1.61^C	8.46±133.43^B	4.2±6.08^A

**Values with different superscript in rows differs significantly ($p \leq 0.05$)

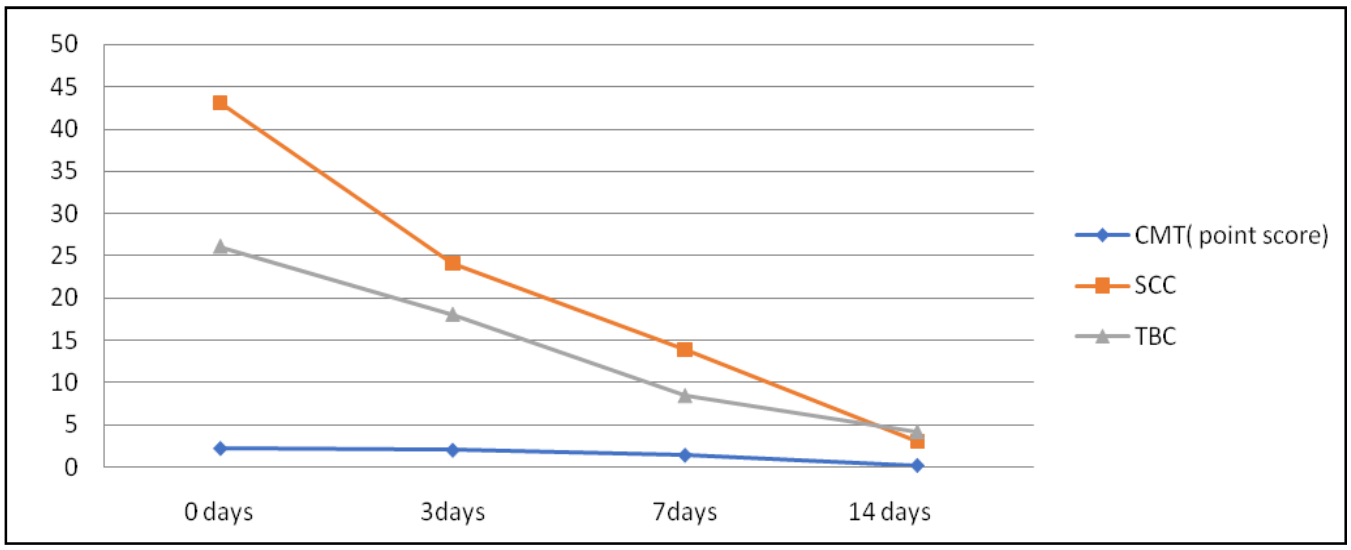

Fig. 1: Graph showing therapeutic effect of *M. oleifera* extract on the basis of average CMT point score, SCC and TBC

Table 2: Therapeutic effect of *Ceftriaxone*on the basis of average CMT point score, SCC (10^5 cells/ml of milk) and TBC (10^3 cells/ml of milk)

Parameters	Days post treatment			
(n= 5)	0	3	7	14
CMT	2.40±0.24^{C}	2.00±0.00^{C}	1.00±0.00^{B}	0.20±0.20^{A}
SCC	43.50±5.27^{C}	18.60±1.70^{B}	13.60±0.89^{B}	3.40±0.66^{A}
TBC	20.94±2.60^{B}	16.60±2.52^{B}	10.66±41.55^{A}	6.44±7.99^{A}

**Values with different superscript in rows differs significantly (p? 0.05)

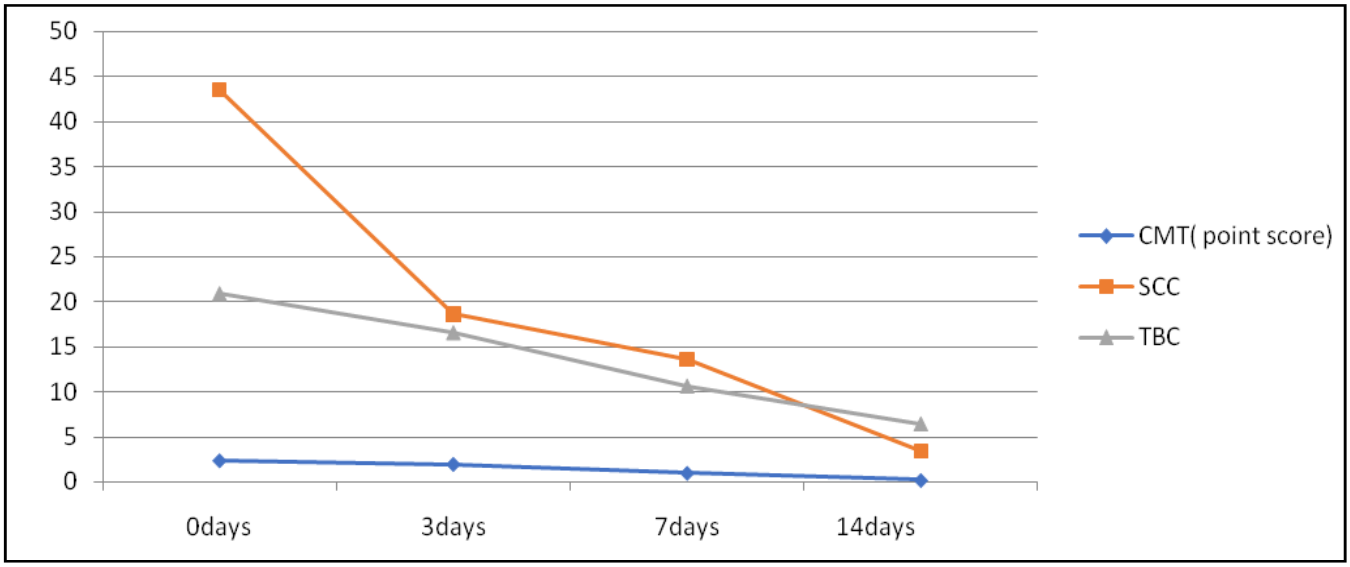

Fig. 2 : Graph showing therapeutic effect of ceftriaxone on the basis of CMT point score, SCC and TBC

effect of the extract. The observation could be corelated by the known anti-inflammatory activity of *M.oleifera* against carrageenan induced paw edema in mice (Medhi *et al.,*2003), carrageenan induced paw edema and cotton pellet granuloma formation in rats (Kumbhare and Thangaveel, 2011, Bhattarcharya *et al.,*1982).

Rajnarayana *et al*., 2001 reported flavonoids potently inhibit biosynthesis of prostaglandins which are

mediators of inflammation through cyclooxygenase and lipoxygenase pathways. Manthey *et al.,* 2001 suggested that flavonoids inhibit inflammatory processes through inhibition of regulatory enzymesprotein kinases that mediate adhesion of circulating leukocytes to sites of injury. Analgesic, and anti-inflammatory effect of *M.oleifera* might be due to flavonoids, steroids and tannins as reported by Bhujbal *et al.*, 2008.

Total bacterial count of milk sample is an index of the quality of milk, herd health, efficacy of farm sanitation, milk handling and storage/transportation temperature. Gunawardana *et al.,* 2011 stated thatcows with mastitis contribute to high TBC with shedding of 1,000,000 bacteria/ ml. According to Gianina *et al.* 2003, the anti-bacterial activity of *M. oleifera* might be attributed to the presence of alkaloids such asmoringine, moringinine and spirachin, as well as pterygospermin.Bukar *et al.*, 2010 argued that the presence of a short polypeptide named 4-(alpha-L-rhamnosyloxy) benzyl-isothiocyanate in *M.oleifera* extract might exert its anti-microbial action through growth inhibition by disrupting cell membrane synthesis, a mechanism of action similar to the β-Lactam and cephalosporin antibiotics.Flavonoids exhibit their antibacterial activity against a large number of microorganisms by inhibition of the membrane-bound enzymes (Pretorious, 2003). Tannins are polyphenols with pronounced ability to suppress bacterial cell proliferation by blocking essential enzymes of microbial metabolism such as the proteolytic macerating enzymes (Kamba and Hassan, 2010). Saponins exert some antibacterial activity by combining with cell membranes to elicit changes in cell morphology leading to cell lysis (Moyo *et al.*, 2012).Terpenoids and steroids were detected in *M. oleifera* which were reported to be active against *Staphylococcus aureus* (Cowan,1999). A number of authors has observed good antibacterial effect of aqueous and ethanolic leaf extracts of *Moringa oleifera* on the growth of gram-positive and negative bacteria (Dewagan *et al*., 2010, Devendra *et al.,* 2011, Peixoto *et al*., 2011). The phytochemical analysis of *M. oleifera leaf* extract is reported to contain an array of bio-active compounds such as alkaloids, glycerides, flavonoids, steroids, terpenoids, saponins, tannins and anthraquinone. (Faizi *et al*.,1994 and 1995; Bennett *et al*., 2003; Price, 2007; Singh *et al.,* 2009; Sreelatha *et al*., 2011). The major flavonoids found in *M.oleifera* are myrecetin, quercetin and kaempferol (Coppin *et al.,* 2013). The significant inhibition of *M. oleifera* in the present study might be due to plethora of phyto-constituents present in the leaf fraction of the plant.

Conclusion:

Intramammary administration of hydro-methanolic extract of *M. oleifera* reduced bacterial count in milk, CMT score and SCC exhibiting both anti-bacterial and anti-inflammatory activity. Therefore, it may be concluded that the hydro-methanolic extract of *M. oleifera* possesses good therapeutic potential against bovine mastitis and can be used as suitable alternative therapy.

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