THERAPEUTIC EFFICACY OF CRUDE *PHYLLANTHUS EMBLICA* (AMLA) EXTRACT IN THE MANAGEMENT OF SUB CLINICAL MASTITIS[#]

Ashish Pandey[1], S.V. Singh[2], Ramakant[3] and Naveen Kumar Singh[4]
Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry
Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad-224 229, Uttar Pradesh, India

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ABSTRACT

Mastitis is a complex multifactorial inflammatory reaction, which often results from an intramammary bacterial infection. It is the most common cause of antimicrobial use in dairy industry for more than fifty years, but most efficient, safe and economical treatment is still lacking. Problems associated with resistance or even ineffectiveness is quite real in the case of mastitis. So use of alternate medicines is now emphasized and is now considered a major thrust area in research on mastitis. Also in organic agriculture, the use of antibiotics is neither normally authorized nor desirable.

***Key words*:** Alternate medicines, Amla, bovine mastitis, antimicrobial resistance

Introduction

Mastitis is a complex multifactorial inflammatory reaction, which often results from an intramammary bacterial infection (Grohn *et al.,* 2004). It is also one of the most common dairy cow diseases that hold importance due to the considerable economic losses it causes to dairy farmers in terms of decrease in milk yield, marked compositional changes in milk that reduced milk quality, treatment and labour costs and increasing risk of early culling of cows. It is also the most common cause of antimicrobial use in dairy industry for more than fifty years, but most efficient, safe and economical treatment is still lacking. Despite the availability of several antibiotics with good *in vitro* activity, cure rates are poor, suggesting that inadequate concentrations of active antibiotic are coming into contact with the infecting bacteria for sufficient time and /or adequate concentrations to be effective (Nora and Jorge, 2012). Problems associated with resistance or even ineffectiveness is quite real in the case of mastitis caused by *Coliforms* and *Staphylococcus aureus* (Hill, 1986). So use of alternate medicines is now emphasized and is now considered a major thrust area in research on mastitis. Also in organic agriculture, the use of antibiotics is neither normally authorized, nor desirable.

In previous studies, authors postulated that phytochemicals present in fruits and vegetables could reduce various risks of diseases owing to prevent the oxidative damage produced by free radicals (Singletary *et al.*, 2005). *Phyllanthus emblica* (amla), one of the most common medicinal herbs has been widely used in ayurvedic medicines (Zhang *et al.,* 2000). Undoubtedly herb amla can be of immense use to combat subclinical mastitis. Still not much literature is available justifying the use of amla in treatment of subclinical mastitis. The present study was therefore conducted to study the therapeutic efficacy of Amla (*Emblica officinalis*) in treatment of subclinical mastitis in dairy cattle.

Materials and Methods

The present study was conducted from September 2015 to January 2016 on 32 cross breed lactating cows that were screened positive for subclinical mastitis.

Selection of animals

The cross breed cows were the crosses of Holestein Freisian of College of Veterinary Science and Animal Husbandry and Shiva Dairy Haliapur. The animals were housed, fed and managed under identical managemental conditions. The age of animals ranged between 4 and 8 years and were 1st to 3rd lactation. The physical and clinical examination of cows was carried out during lactation as suggested by Schalm *et al.* (1971) for exclusion of abnormality of udder and teat (e.g. size shape, consistency as udder possessing lumps, oedema, atrophy, fibrosis and misplacement of teats, blint teats induration etc). Such examination was continued until final selection of those cows, which revealed any visible clinical signs. (Radostits *et al*., 2007)

Place of work: Collection of *E. officinalis* fruits

Fresh fruits of amla were collected from the university farm. The compostion of the variety used has already been established by Pathak *et al.* (2003).

Variety of Amla used: Narendra Amla-10

Screening of animals

Milk samples were collected aseptically from each quarter before and after the therapy. The udder was initially washed with antiseptic solution for visible debris and teat ends were scrubbed with cotton soaked in 70 % ethanol and allowed to air dry. The first 3-4 streak of milk was discarded and next 10 ml of milk was collected into separate clean, dry and sterilized test tubes. The tubes were stoppered and brought immediately to the laboratory in ice packed container for mastitis screening tests. Milk was subjected to battery of tests namely California mastitis test, white side test, Mastrip test and somatic cell count.

Collection of blood for haematology

Blood sample were collected for haematology from each animal in vials containing EDTA by regular venepuncture.

[#]Part of M.V.Sc. Thesis: Corresponding author and Guest faculty in Veterinary Clinical complex, College of Veterinary Science and Animal Husbandry, NDUA & T, Kumarganj, Faizabad-224 229, UP; [2,3]Assistant Professor; [4]Assistant Professor, Veterinary Clinical complex, College of Veterinary Science and Animal Husbandry, NDUA & T, Kumarganj, Faizabad-224 229, UP

Complete blood count was conducted as per the standard techniques in mastistic animal on the day of blood collection prior to and after herbal therapy.

Total erythrocyte count (TEC)

Total erythrocyte count was determined with improved Neubaur's counting chamber using Gower's solution as the diluting fluid (Jain, 1986). The values were expressed in millions per cube mm of blood (10^6cu mm).

Haemoglobin (Hb)

Haemoglobin content was estimated using Sahli's haemoglobinometer with double prism standard as described by Jain (1986). The results were expressed in gm/dl of blood.

Packed cell volume (PCV)

Packed cell volume was determined by micro haematocrit method and the values were read with Adams micro haematocrit reader. The results were expressed in percentage.

Total leukocyte count (TLC)

Total leukocyte count was made with improved Neubaur's counting chamber using N/hydrochloric acid as diluting fluid (Jain, 1986). The values were expressed in thousands per cu mm of blood (10^3 cu mm).

Differential leukocyte count (DLC)

Different leukocyte count was done as per method of Jain (1986). Blood smear were prepared immediately after blood collection. The smears were stained with Leishman stain. The values were expressed in percentage.

Treatment protocol

Thirty two animals positive for subclinical mastitis were supplemented with various doses of deseeded fresh amla. All the cows were randomly divided into four different groups containing 8 animals each.

Animal of group 1 were given crushed deseeded fresh amla at the dose rate 250 gm (A-250) for 15 days. Cows of group II were supplemented with crushed deseeded fresh amla at the dose rate of 200 gm (A-200) for period as in group 1. Cows in group III were supplemented with crushed deseeded fresh amla at the dose rate of 150 gm (A-150) for period as in group 1. Group IV animals were administered standard antibiotic treatment i.e. inj Cefotaxime @ 3 gm i/m for 5 days.

Groups	No. of animals	Treatment
I	8	Amla @ 250 gms PO s.i.d. for 15 days
II	8	Amla @ 200 gms PO s.i.d. for 15 days
III	8	Amla @ 150 gms PO s.i.d. for 15 days
IV	8	Inj Cefotaxime @ 3 gm i/m for 5 days

Statistical analysis

Statistical analysis was done using t-test as described by Snedecor and Cochran (1994).

Results and Discussion

Group I

Animals in group 1 (A -250) supplemented with amla @ 250 gm PO for 15 days showed 75% recovery (Table 1). Two animals remained in sub clinically affected state. The mean CMT score of 2.37 ± 0.18 on day 1 decreased significantly to 1.52^{*} ± 0.26 after treatment. The mean WST and MST score point decreased significantly from 2.52 ± 0.19 and 1.87 ± 0.45 to 1.66^{*} ± 0.38 and 1.26^{*} ± 0.52, respectively. A similar trend was noticed in SCC ($x10^{5)}$ also as 0 days count decreased significantly from 5.78± 0.89 to 2.86^{*} ± 0.95 after the treatment (Table 2).

Complete blood count

The level of haemoglobin increased significantly from 9.22 ± 1.39 to 10.91^{*}± 1.46. The level of total erythrocyte count (x 10^6) increased in significantly from 4.91 ± 1.20 to 5.28 ± 1.27 and the total leukocyte count (x 10^3) decreased significantly from 14.71 ± 1.20 to 10.08^{x} ± 1.42. A significantly decline in Neutrophils count (%) from 58.38 ±1.72 to 40.63^{*} ±0.24 was recorded. The level of lymphocyte (%) increased significantly from 38.83 ±1.45 to 56.53^{x}± 1.34 (Table 3).

Group II

Animals in group II (A-200) supplemented with amla @ 200 gm PO for 15 days showed 62.5% recovery (Table1). Three animal remained sub clinically affected. The mean CMT score of 2.50 ± 0.19 on day 1 decreased significantly to 1.85^{*} ± 0.31 after treatment. The mean WST and MST score point decreased in significantly from 2.25 ± 0.35 and 1.87 ± 0.81 to 1.37 ± 0.14 and 1.38 ± 0.83, respectively. A declining trend was noticed in SCC also as 0 days count decreased significantly from 6.25± 0.78 to 4.24^{*} ± 0.54 after the treatment (Table 2).

Complete blood count

The level of haemoglobin increased from 8.85 ± 1.43 to 9.83 ± 1.61. The level of total erythrocyte count (x 10^6) increased from 6.07 ± 1.20 to 6.83 ± 1.87 and the total leukocyte count (x 10^3) decreased from13.06 ± 0.78 to 9.39^{*} ± 0.33. The decline in TLC was statistically significant. A significant increase in lymphocyte from 40.51± 1.51 to 57.18*± 1.63 and decline in Neutrophils from 56.46±1.72 to 39.50*±1.06 was observed. The changes in, eosinophil count and monocyte counts were statistically insignificant (Table 3).

Group III

Animals in group III (A-150) supplemented with amla @ 150 gm PO for 15 days showed 25% recovery (table-1). Six animal remained sub clinically affected. The mean CMT score of 2.51 ± 0.19 on day 1 decreased non significantly to 2.12 ± 0.22 after treatment. The mean WST and MST score point decreased in significantly from 2.18 ± 0.80 and 1.98 ± 0.58 to 1.86 ± 0.88 and 1.72 ± 0.48, respectively. The SCC also decreased from 6.24± 1.84 to 5.96 ± 1.88 after the treatment but the decrease was statistically insignificant (Table 2).

Complete blood count

An insignificant increase was recorded in level of haemoglobin from 8.31 ± 1.37 to 8.83± 1.33. The level of total erythrocyte count (x 10^6) increased from 5.11± 1.24 to 5.93 ± 1.22 and the total leukocyte count (x 10^3) decreased from12.60± 1.48 to 11.68 ± 0.50. The changes in neutrophil, lymphocyte,

eosinophil count and monocyte counts were statistically insignificant (Table 3).

Group IV

Highest recovery was noticed in group IV animals supplemented with Cefotaxime 3 gms i/m for 5 days i.e. 87.5% recovery (Table 1). Only one animal remained sub clinically affected. The mean CMT score of 2.25 ± 0.25 on day 1 decreased significantly to 1.37* ± 0.18 after treatment. The mean WST and MST score point recorded significant decrease from 1.98 ± 0.21 and 1.84 ± 0.51 to 1.11*± 0.84 and 0.88*± 0.28, respectively. Somatic cell count ($x10^{5}$) decreased significantly from 6.48 ± 0.80 to 3.84 ± 0.48 after the treatment (Table 2).

Complete blood count

The level of haemoglobin (%) decreased insignificantly from 8.88 ± 1.54 to 8.61 ± 1.41. The level of total erythrocyte count ($x\ 10^{6}$) increased from 4.73 ± 1.33 to 5.21 ± 1.84 and the total leukocyte count ($x10^{3}$) decreased from14.82 ± 1.65 to 10.39* ± 0.88. The decline in total leucocyte count (%) was statistically significant. The neutohphil count (%) declined significantly from 53.56 ± 1.94 to 38.25*±1.69. A significant increase in lymphocyte (%) was recorded from 42.61±1.61 to 59.18*±1.68 (Table 3).

Maximum recovery was recorded in group IV in which the animals were treated with antibiotic Inj Cefotaxim 3 gms i/m for 5 days. Amongst the amla treated groups maximum recovery was obtained in Group I (@ 250 gm PO OD for 15 days) followed by group II (@ 200 gm PO OD for 15 days) and group III (@ 150 gm PO OD for 15 days). The recovery recorded in Amla treated groups can be attributed to the antibacterial, anti-inflammatory and immunopotentiating property of *Emblica officinalis*. Sharma *et al.* (2014) had earlier recorded better udder health of cows in groups supplemented with polyherbal preparation of which *Emblica officinalis* was a component, as indicated by decline in SCC, prevalence of subclinical mastitis, and incidence of clinical mastitis can be attributed to their better immune status due to polyherbal supplementation.

The changes in mastitis markers CMT score point, WST score point, MST score point and somatic cell count reflect the severity of mastitis. The decline observed reflects the recovery. Somatic cells of milk are white blood cells and epithelial cells, which slough off from the lining of the mammary gland during the normal course of milking (Harmon, 1994). They are widely used as marker to determine the mammary health and quality of milk (Swain *et al.,* 2014; Eberhart *et al.,* 1982). Milk SCC increase significantly in mastitic cows and act as a protective mechanism to kill the invading pathogens in the mammary gland. An increase in the severity of mastitis leads to a significant increase in milk SCC (Dang *et al.,* 2010*)*. The changes in hematology may be due to the erythropoetic effect of *Emblica officinalis*. Lohar *et al*. (2009) recoreded highest RBC count and Hb% for fruit extract *Phyllanthus emblica* in comparison to control in the test animals and were of the view that the plant extracts boost synthesis of haemoglobin and formation of RBCs. Flavonoids derived from EO exhibit maximum beneficial action by elevating the haemoglobin levels in rats (Anila and Vijaylakshmi, 2000).

The varied literature on the medicinal plant reveals that the plant *E. officinalis* have the antibacterial (Hossain *et. al.,* 2012*;* Philip *et al*., 2012*;* Usha *et al.,* 2012), antifungal (Hossain, *et al.,* 2012; Mehmood *et al.,* 1999) and antioxidant properties (Golechha *et al*., 2012). The potent anti inflammatory activity of *Emblica officinalis* was earlier established by Golechha *et. al. (*2012), Mishra (2004), Yokozawa, 2007 and Santosh Kumar *et. al. (*2013). Golechha *et al. (*2012) at the dose of 700 mg/kg, HAEEO exhibited maximum anti-inflammatory activity in all experimental models, and the effects were comparable to that of the standard anti-inflammatory drugs. Reghu and Ravindra, (2010) had revealed the inhibitory activity of Amla extracts to the growth of S. *aureus.* Saeed and Tariq, (2007) also observed potent antibacterial activity of aqueous infusion and decoction of *Emblica officinalis* against *Escherichia coli*, *Klebsiella pneumoniae*, *K. ozaenae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. paratyphi* A, *S. paratyphi* B and *Serratia marcescens.* A potent antibacterial activity against *E. coli*, *K. pneumonia*, *K.ozaenae*, *Proteus mirabilis*, *P. aeruginosa*, *S. paratyphi* A and *S. paratyphi* B and *Serratia marcescens was also observed by* Srikumar *et al*. (2007). Maximal antibacterial activity was obtained for *S. aureus* for the fruit extract with zone of growth inhibition of diameter 19 mm, followed by leaf, seed and stem extracts in the descending order (Varghese *et al.*, 2013). They also observed bactericidal activity of the fruit, seed, stem and leaf extracts of *Emblica officinalis* comparable with that of the commonly used antibiotics having varied mode of action. None of the antibiotics were superior to the *Emblica* extracts against *Pseudomonas.* The antimicrobial properties of *P. emblica* were also studied by Srikumar *et al.* (2007). The bactericidal activity of *E. officinalis* could be attributed to the bioactive compounds present in *E. officinalis* namely flavonoids, phenols, saponins and tannins such as emblicanin A and B which could be effectively employed as effective chemotherapeutic agents in antibacterial treatment and therapy (Javale and Sabnis, 2010 and Jyothi and Subba Rao, 2011) . *Emblica* is an excellent antioxidant and free radical scavenger. The antioxidant activity of fruits of *E. officinalis* has been traced to its tannoid principles both *in vitro* and *in vivo* (Bhattacharya *et al.,* 2002). The potent antioxidant properties of amla have also been confirmed by Hazara (2010). The ability of the extracts of the fruits in exhibiting their antioxidative properties and flavonoid content follow the order: *T. chebula*>*E. officinalis*>*T. belerica*, whereas with respect to phenolic content the order was observed as *E. officinalis*>*T. belerica*>*T. chebula*. The free radical-scavenging activity of plants extract and individual compounds in the extracts of *P. emblica* were also recorded in several *in vitro* studies (Nampoothiri *et al.,* 2011). Vitamin C in *Embilica officinalis* accounts for approximately 45-70% of the antioxidant activity (Scartezzini *et al.,* 2006).

Embilica officinalis can be potentially used in feed to reduce the incidence of disease through improving nonspecific immunity of periparturient cows and require further studies on standardization, formulation, and mode of delivery to explore more beneficial effects.

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Table 1: Therapeutic efficacy of *Emblica officinalis (*amla) supplementation in cows having sub clinical mastitis

Group	Total Number of animals	Post supplementation status of cows		Percentage
		Recovered	Unrecovered	Recovered animals
I	8	6	2	75.00%
II	8	5	3	62.50%
III	8	2	6	25.00%
IV	8	7	1	87.50%

Table 2: Screening test profile before and after treatment.

	CMT		WST		MST		SCC x10^5 cells/ml	
Groups	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
I	2.37±0.18	1.52^{x}±0.26	2.52±0.19	1.66^{x}±0.38	1.87±0.45	1.26^{x}±0.52	5.78±0.89	2.86^{x}±0.95
II	2.50±0.19	1.85^{x}±0.31	2.25±0.35	1.87^{x}±0.14	1.87±0.81	1.38^{x}±0.83	6.25±0.78	4.24^{x}±0.54
III	2.51±0.19	2.12±0.22	2.18±0.80	1.86±0.88	1.98±0.58	1.72±0.48	6.24±1.84	5.96±1.88
IV	2.25±0.25	1.37^{x}±0.18	1.98±0.21	1.11^{x}±0.84	1.84±0.51	0.88^{x}±0.28	6.48±0.80	3.84^{x}±0.48

*Values differs significantly P<0.05

Table 3: Mean ± SE of pre and post treatment values of haematological parameters

Parameters		GROUP I	GROUP II	GROUP III	GROUP IV
Hb (gm/dl)	Pre treatment	9.22±1.39	8.85±1.43	8.31±1.37	8.88±1.54
	Post treatment	10.91^{X} ±1.46	9.83±1.61	8.83±1.33	8.61±1.41
PCV (%)	Pre treatment	28.36±1.03	26.56±1.74	25.38±1.59	29.56±1.73
	Post treatment	30.81±1.48	29.50±1.42	26.19±1.48	30.50±1.42
TECx (10^6/µl)	Pre treatment	4.91±1.20	6.07±1.20	5.11±1.24	4.73±1.33
	Post treatment	5.28±1.27	6.83±1.87	5.93±1.22	5.21±1.84
TLC x 10^3/µl)	Pre treatment	14.71±1.20	13.06±1.78	12.60±1.48	14.82±1.65
	Post treatment	10.08^{x}±1.42	9.39^{x}±1.33	10.68±1.50	10.39^{x}±1.88
N (%)	Pre treatment	58.38±1.72	56.46±1.72	54.72±1.48	53.56±1.94
	Post treatment	40.63^{x}±1.24	39.50^{x}±1.06	47.31±1.33	38.25^{x}±1.69
L (%)	Pre treatment	38.83±1.45	40.51±1.51	41.23±1.32	42.61±1.81
	Post treatment	56.53^{x}±1.34	57.18^{x}±1.63	48.94±1.81	59.18±1.68
E (%)	Pre treatment	2.59±0.39	2.32±0.34	2.85±0.35	2.60±0.34
	Post treatment	1.84±0.24	2.13±0.15	2.35±0.32	2.09±0.16
M (%)	Pre treatment	2.10±0.21	1.75±0.17	2.02±0.24	2.35±0.19
	Post treatment	1.70±0.25	1.50±0.14	2.03±0.19	1.60±0.14

*Values differs significantly P<0.05

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