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Effect of feeding *Embilica officinalis* (Amla) on milk quality in cattle affected with subclinical mastitis

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Abstract
Embilica officinalis or Indian gooseberry is perhaps the single most often mentioned herb in "Charak Samhita", the Ayurvedic medicine literature (500 BC). Ayurveda, which is the oldest health system in the world, appreciates and uses amla to treat a host of diseases and promote positive health. Thirty two animals positive for subclinical mastitis were randomly divided into four different groups containing 8 animals each and supplemented with various doses of deseeded fresh amla. Changes in milk yield, fat%, solids not fat, total solids and total bacterial count were studied. Supplementation of amla @ 250 gram and 200 gram increased the milk yield by 14.58% and 14.28%, significantly decreased total bacterial count but did not have any significant changes in fat%, total solids and solid not fat, although an increase in fat was recorded. Supplementation at 150 gram too increased milk yield, decreased total bacterial count but the changes were statistically non significant. In unsupplemented group, an increase in somatic cell count, total bacterial count and decline in milk yield was recorded indicative of development of mastitis. Thus, amla can be used as an alternate to conventional therapy and can be supplemented to cattle in routine feeding especially in areas where it is surplus.

Keywords: Subclinical mastitis, cattle, *Embilica officinalis*, milk quality

Introduction
Embilica Officinalis or Indian gooseberry, Amla is a gift of nature to mankind with wide distribution in tropical and subtropical areas, and has therapeutic potential against harmful diseases (Kulkarni *et al*. 2018) [21]. The Sanskrit name, Amlaki, translates as the Sustainer or The Fruit where the Goddess of Prosperity Resides. It is perhaps the single most often mentioned herb in "Charak Samhita", the Ayurvedic medicine literature (500 BC). In Hindu religious mythology the tree is worshipped as the Earth Mother as its fruit is considered to be so nourishing as to be the nurse of mankind Ayurveda, which is the oldest health system in the world, appreciates and uses amla to treat a host of diseases and promote positive health. The active ingredient that has significant pharmacological action in amla is designated by Indian scientist as “Phyllemblin". The fruit is rich in quercetin, phyllaemblic compounds, gallic acid, tannins, flavonoids, pectin, and vitamin C and also contains various polyphenolic compounds. A wide range of phytochemical components including terpenoids, alkaloids, flavonoids, and tannins have been shown to posses’ useful biological (Kim *et al*. 2005; Arora *et al*. 2003 and Kumar *et al*. 2018) [18, 2, 20].
Embilica Officinalis is known to possess potent antibacterial activity against *Staphylococcus aureus* (Reghu and Ravindra, 2010; Dhale and Mogle, 2011; Patil *et al*. 2012, Varghese *et al*. 2013) [3, 7, 30, 46], *Escherichia coli*, *Klebsiella pneumoniae*, *K. ozaenae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. paratyphi* A, *S. paratyphi* B and *Serratia marcescens* (Saeed and Tariq, 2007) [35]. *Emblica* is an excellent antioxidant and free radical scavenger (Bhattacharya *et al*. 2002; Anila and Vijayalakshmi, 2003; Hazara, 2010) [3, 1, 12]. Vitamin C in *Embilica Officinalis* accounts for approximately 45-70% of the antioxidant activity (Scartezzini *et al*. 2006) [38]. Various investigators have reported that the fruits of *E. officinalis* have immune-modulatory activity (Rama Rao, 1998; Sairam *et al*. 2002; Ganju *et al*. 2003; Srikumar *et al*. 2007) [33, 36, 9, 43]. The anti inflammatory properties of *E. officinalis* is also established by numerous workers (Golechha *et al*. 2014; Yokozawa, 2000, Santosh Kumar *et al*. 2013) [37]. In addition it has a hepatoprotective effect (Pramyothin *et al*. 2006; Bhattacharya *et al*. 2000) [32]. Pharmacological research reports on amla reveals its analgesic

(Sharma *et al*. 2004) [40], anti-tussive (Nosalova *et al*. 2003) [29], anti-atherogenic (Kumar *et al*. 2013) [19], adaptogenic (Muruganandam *et al*. 2002) [26], gastroprotective (Chatterjee *et al*. 2010), nephro-protective (Yokozawa *et al*. 2007) [49], neuro-protective (Vasudevan and Parle, 2007) [47] and anticancer (Madhuri, 2008) [22] properties. The potential biological properties of *Embilica Officinalis* remain untrapped in the animal health sector. The complete package of antibacterial, antioxidant, anti inflammatory, free radical scavenging, hepato-protective properties in one wonder drug can thus be of immense use in the prevention and treatment of innumerable health disorders, mastitis being one of them. Mastitis is globally recognised as the most common and costly disease affecting dairy herds causing heavy financial losses to dairy industries by reducing yield and milk quality, and associated treatment costs (Mushtaq *et al*. 2018) [27]. Subclinical mastitis or hidden mastitis is the most commonly occurring form of mastitis that ensues when the animal's immunity or udder immunity gets compromised and mastitogens proliferate and succeed in establishing themselves to some extent. Subclinical mastitis causes considerable changes in milk composition and serum, which may contribute to the impaired immune defence (Megalia *et al*. 2001).

There is dearth of literature regarding the effect of amla in treatment of subclinical mastitis. Therefore, the present research focuses on the effect of feeding crushed raw amla on the changes in milk composition and production status in subclinically affected cows.

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 Animal

The cross breed lactating cows aged between 3-5 years in their first to third lactation and managed under identical managemental conditions were included in this study. The physical and clinical examination of all cows was carried out during lactation as suggested by Schalm *et al*. (1971) [39] 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: College of Veterinary Science & Animal Husbandry

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). The fruit extract was prepared by the method described by Dash *et al*. 2011.

Variety of Amla Used: Narendra Amla-10

Screening of animals

Milk samples were collected aseptically from each quarter before on day 0 and thereafter on days 3, 7 and 15. 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 & 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. Somatic cell count of milk was performed as per the method described by Schalm *et al*. (1971) [39].

Experimental design

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. Animals 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 cows were left untreated as control. Milk samples were collected before and after supplementation for determining the alterations in milk quality- milk yield, fat (%), solid not fat and total solids. The total bacterial count (TBC) was carried out as per the standard method (Griffin *et al*. 1977).

Statistical analysis

Statistical analysis was done using t-test as described by Snedecor and Cochran (1994) [42].

Result

Animals in A -250 group supplemented with amla @ 250 gm PO for 15 days showed 75% recovery. Two animals remained in sub clinically affected state. A significant decrease in the mean CMT, WST and MST score points were recorded after treatment. 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-1).

A significant decrease in total bacterial count was observed in group 1 animals from $4.4x10^5$±1.13cfu/ml to $2.3x10^5$ ± 0.93 cfu/ml (Table-2). The milk yield increased significantly from 8.23 ±0.96 lts to 9.43 ± 0.82 with increase by 14.58% (Table-3). An increase in fat (%) 4.23± 1.13to 4.56±1.72, solid not fat and total solids was noticed but was statistically insignificant (Table -4).

Animals in A-200 group showed 62.5% recovery. 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 too decreased but was statistically non significant. 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 -1).

A significant decrease in total bacterial count was observed in group II animals from $4.0x10^5$ ±0.96 cfu/ml to 2.7 x 10^5 1.07 cfu/ml (Table-2). The milk yield increased significantly from 7.92 ±0.94 lts to 8.80 ± 1.86 lts with an increase of 14.28% (Table-3). An increase in fat% from 4.43 ±1.35 to 4.58±1.17, solid not fat and total solids was noticed but was statistically insignificant (Table-4).

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. A non significant decrease in the mean CMT, WST and MST score points were recorded after treatment. The SCC also decreased significantly from 6.24± 1.84 to 5.16 ± 1.88 after the treatment. (Table -1). A

significant decline in total bacterial count was observed in group 1 animals from 3.8 x 10^5 ±1.19 cfu/ml to 3.1 x 10^5 ±1.04 cfu/ml(Table-2). The milk yield increased insignificantly from 8.28 ±1.23 lts to 8.46 ± 1.22 (Table-3). An increase in fat% (4.70 ± 1.52 to 4.82± 1.21), solid not fat and total solids was noticed but was statistically non significant (Table-4).

In control un supplemented i.e. group IV no recovery was observed. All the animals remained sub clinically affected. The mean CMT score of 2.44± 0.24 on day 1 increased non significantly to 2.72 ± 0.22. An increase in mean WST and MST score points and SCC were also observed but was statistically insignificant (Table -1). An increase in total bacterial count was recorded from 4.2 x 10^5 ±1.24 cfu/ml to 5.1 x 10^5±1.16 cfu/ml (Table-2). The milk yield decreased insignificantly from 8.98±1.42lts to 7.82±1.86 (Table-3).

Amongst the amla treated groups maximum recovery was obtained in Group I (@ 250gm PO OD for 15 days) followed by group II (@ 200gm PO OD for 15 days) and group III (@ 150gm PO OD for 15 days). The recovery recorded in Amla treated groups can be attributed to the synergistic effect of the bioactive principles i.e. antibacterial property antibacterial, anti-inflammatory and immunopotentiating property of *Emblica Officinalis*. Golechha *et. al.* (2014) at the dose of 700mg/kg, exhibited maximum anti-inflammatory activity in all experimental models and the effects were comparable to that of the standard anti-inflammatory drugs. Savala *et. al.*, 2012 prescribed the concentration for human dosage as 0.75 gms/kg body weight.

Discussion

Sharma *et al*. (2014) [41] 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. An increase in the severity of mastitis leads to a significant increase in milk SCC (Dang *et al*. 2010) [6] and hence widely used as marker to determine the mammary health and quality of milk (Swain *et al*. 2014; Eberhart *et al*. 1982) [44, 8]. Depending on the size of the inflammation one may observe a reduction in milk yield and unfavourable changes in the milk composition (Jozwik *et al*. 2004) [15]. Milk production loss per affected quarter due to sub-clinical mastitis was estimated to be 17.6% on average (Graaf and Dwinger, 1996) [11]. Mungube *et al*. (2005) [25] recorded a reduction by 1.2%, 6.3% and 33% in quarters with CMT scores 1+, 2+ and 3+ scores respectively. The increase in milk production should thus indicate suppression of inflammatory changes and improvement in udder health.

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) [13, 31, 45], antifungal (Hossain, *et al*. 2012; Mehmood *et al*. 1999) [13, 23] and antioxidant properties (Golechha *et al*. 2012) [10]. The potent anti inflammatory activity of *Emblica Officinalis* was earlier established by Golechha *et al*. 2014; Mishra, 2004; Yokozawa, 2007 [49]; Kumar *et al*. 2013) [19]. Reghu and Ravindra, (2010) [3] had revealed the inhibitory activity of Amla extracts to the growth of *S. aureus.* Saeed and Tariq, (2007) [35] 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.* Varghese *et al*. (2013) [46] also observed maximal antibacterial activity against *S. aureus* for the fruit extract, comparable with that of the commonly used antibiotics having varied mode of action and were of the view that 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) [43]. 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; Jyothi and Rao, 2011) [14, 16]. *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) [3]. The potent antioxidant properties of Amla has also been confirmed by Hazara, (2010) [12]. 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 **(**Kumar *et al*. 2006; Nampoothiri *et al*. 2011). Vitamin C in *Embilica Officinalis* accounts for approximately 45-70% of the antioxidant activity (Scartezzini, *et al*. 2006) [38]. Chawla and Kaur (2004) [5] showed that the elevated content of antioxidants in the blood of cows to a considerable degree protected them from metabolic diseases, including mastitis. Although ruminants can synthesize vitamin C in the liver and it is not considered to be an essential nutrient for healthy cattle, a large reduction in plasma vitamin C concentration was reported in lactating cow with artificially induced mastitis (Weiss *et al*. 2004) [48]. Khopde *et al*. (2001) [17] reported that ascorbic acid and other polyphenols present in the natural formulation of amla showed much superior antioxidant activity compared to their equivalent amounts in pure isolated form.

Table 1: Screening test profile before and after treatment

Group	CMT		WST		MST		SCC x10^5 cells/ml	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
I	2.37±0.18	1.52*±0.26	2.52±0.19	1.66*±0.38	1.87±0.45	1.26x±0.52	5.78±0.89	2.86x±0.95
II	2.50±0.19	1.85x±0.31	2.25±0.35	1.67x±0.14	1.87±0.81	1.38x±0.83	6.25±0.78	4.24x±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	5.16*±1.88
IV	2.44±0.24	2.72±0.22	1.98±0.21	2.12±0.84	1.84±0.51	2.28±0.26	6.48±0.80	7.16±0.48

*Values differs significantly $P<0.05$

Table 2: Total bacterial count before and after treatment

	Before treatment (x 10^5 cfu/ml)	After Treatment (x 10^5 cfu/ml)
Group-1 (A-250)	4.4 ±1.13	2.3* ±0.93
Group-2 (A-200)	4.0±0.96	2.7 *±1.07
Group-3 (A-150)	3.8±1.19	3.1 ±1.04
Group-4(un supplemented)	4.2±1.24	5.1 ±1.16

*Values differs significantly $P<0.05$

Table 3: Milk yield (lt.) before and after treatment in sub clinical mastitis positive cows

Days	**Group I PHM (250gm)**	**Group II PHM (200gm)**	**Group III (150gm)**	**Group IV (untreated group)**
0 day	8.23±0.96	7.92±0.94	8.28±1.23	8.98±1.42
5th day	8.42±0.64	8.13±0.06	8.30±1.07	8.64±1.51
10th day	9.33±0.22	8.57±0.97	8.24±1.06	8.33±1.22
15th day	9.43*±0.82	8.80*±1.86	8.46±1.22	7.82*±1.86
% variation	14.58	14.28	3.38	-12.91

Table 4: Changes in values of FAT, SNF, TOTAL SOLIDS before and after therapy

	I		**II**		**III**		**IV**	
	Before	**After**	**Before**	**After**	**Before**	**After**	**Before**	**After**
Fat (%)	4.23±1.13	4.56±1.72	4.43±1.35	4.58±1.17	4.70±1.52	4.82±1.21	4.63±1.43	4.90±1.27
SNF (%)	7.90±1.26	8.16±1.32	8.18±1.74	8.23±1.87	8.20±1.63	8.26±1.52	8.13±1.81	8.63±1.92
Total solids (%)	12.38±2.23	12.92±2.24	12.20±2.02	12.26±2.17	12.86±1.73	13.08±1.96	12.86±2.11	13.13±2.61

Conclusion

Embilica officinalis can be potentially incorporated in feeding schedule of lactating cattle to reduce the incidence of disease especially mastitis through improving nonspecific immunity of periparturient cows especially in areas where *Embilica officinalis* is in abundance, but it require further studies on standardization, formulation and mode of delivery to explore more beneficial effects. Plant products such as *A. indica* could be used as an anti-inflammatory and antibacterial arsenal against the disease to reduce the burden of antibiotics.

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Declaration of Interest

The authors declare that this work has not been submitted anywhere for publication.

Conflict of interest

The authors report no conflict of interest.

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