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Effect of low cost herbal combination and tri-sodium citrate treatment in subclinical mastitis affected crossbred dairy cow

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Abstract
Sub clinical mastitis affected cows were randomly selected by screening of milk sample and divided into three groups i.e., control, treatment-I and treatment-II on the bases of SCC and milk composition parameter. In control group no treatment applied, in treatment-I group TSC supplement orally given to rate of 35mg/kg BW for 10 days and treatment-II group herbal paste applied to udder and teat 8times a day for 10 day. Herbal paste prepared from turmeric, castor oil, lemon, aloe vera. Out of 10 cows and 15 quarter from treatment-I and 10 cows and 16 quarters from treatment-II group, 7(70%) cows and 11(73.33) quarters from treatment-I and 9 (90%) cows and 14 (87.50) quarters from treatment-II completely recovered on the bases of milk composition value, hematological value and palpation in udder and teat. Beat result obtained from in treatment-II group compare than treatment-I group.

Keywords: SCM, SCC, TSC, herbal paste, CMT

Introduction
Subclinical mastitis (SCM) causes a greater loss to dairy industry in term of reduction in milk production throughout the world. The disease is also characterized by biochemical changes in composition of milk, resulting in decreased keeping quality of milk and increased in somatic cell etc. less incidence occur in buffalo compare than crossbred cow due to thick and compact epithelium, thick muscle sphincter in streak canal of udder of buffalo (Saini *et al*.1994 and Mukesh *et al*. 2014) [13]. The milk production is the main expected purpose from dairy cattle. Milk quality is important for determining health and marketability of milk. Turmeric *(Curcuma longa)* is a flowering plant belongs to *Zingiberaceae* family. It is perennial, herbaceous plant native to the Indian subcontinent and Asia. Turmeric powder contain 60-70% carbohydrates, 6-8% protein, 6-13% water, 3-7% minerals, 5-10% fats, 1-6% curcuminoids, 2-7% fiber (KM Nelson *et al* 2017) [10]. Turmeric contains a substance with powerful anti-inflammatory and antioxidant properties. Curcumin is a natural anti-inflammatory compound. Castor oil (*Ricinus communis*) rich is a monounsaturated fatty acid, Ricinoleic acid and it has impressive anti-inflammatory properties. Animal and test-tube studies have found that ricinoleic acid reduces pain and swelling (Vieira *et al* 2000) [18]. Lemon (*Citrus limon)* fruit is a rich source of nutrients, antioxidants, and enzymes that help to flush out toxins present in animal body (Misharina *et al*). Aloe vera had antioxidant and antibacterial properties (Nejatzadeh-Barandozi 2013) [9]. Aloe vera (Aloe barbadensis miller) is most commonly used as a topical medication, rubbed onto the skin rather than eaten. Tri-sodium citrate might play important role against sub clinical mastitis. It has a role as a flavouring agent and an anticoagulant. Feeding citrate orally to the mastitic animals led to an increase in milk citrate levels and helped in improving clinical condition of animals (Renu *et al.,* 2016) [12]. Tri-sodium citrate administration increased the fat, S.N.F, Protein and milk yield over the respective values in mastitic milk (Prakash *et al.,* 2013). The aim of the present study was develop low cost indigenous medicine and check efficiency of TSC to control the clinical and subclinical mastitis. antibiotic therapy is implemented in treating both clinical and subclinical mastitis cases in field conditions. However, many bacterial strains are resistance to antibiotics used which is leading to serious hazard called Antimicrobial Resistance (AMR). WHO stated that 80% people of developing country depends on entho-veterinary medicine (Mooventhan *et al*. 2016) [7].

Material and Methods
The present investigation was carried out in the department of Animal Husbandry and Dairying and outdoor Veterinary hospital of C S A University of Agriculture & Technology, Kanpur. This study was done under conventional housing system. Different physical, clinical test applied in lactating cow and found thirty lactating cow suffering from subclinical mastitis. The experiment divided in to three groups (control, treatment-1, and treatment-2). In treatment-1 group cows were supplemented Tri-sodium citrate (TSC) @ 35mg/kg BW orally till 10 days and in treatment-2 herbal paste applied in udder 8 times a day for 10 days and in control group no supplementation was given to the lactating cow. Daily milk yield noted (morning and evening) and milk sample was collected at 0 day and after10 day from every group. Milk sample were collected for test of milk composition, somatic cell count, electrical conductivity etc.

Preparation of Herbal paste
In preparation of herbal paste these ingredients were required- Aloe vera – 3 leaf/300grams, Turmeric powder – 50g, Lemon - one and half, and Caster oil – 50g. Three Aloe vera leaves had chaffed with leave blade into a 2 X 2cm small piece and grinded to became a bubble mixed greenish paste without adulteration of water. Then turmeric powder along with tamarind seed size lime was added into the paste, further grinding of the ingredients to become reddish paste. This prepared paste was used for this study.

Method of Application
The udder which was affected was completely drained and was rubbed using coir pith to get rid of debris and stained infectious material present. Udder was washed with clean water. A paste was taken into a bowel which was then diluted with 100ml pure water to turn into a herbal solution. Thus, herbal solution formed was applied all over the affected as well as normal udder. After 3 hours of application, udder was cleaned and drained as stated earlier and the procedure continued again. This procedure was repeatedly carried out 8 times a day for continuous 10 days. The herbal paste was freshly prepared for everyday application by discarding the old one.

Sample of Milk
For analysis 100 ml, freshly drawn milk from each quarter of the cows was collected separately in clean, well sterilized, and previously dried sample bottle. The samples were taken from morning and evening milking at regular interval for laboratory analysis. Before withdrawing portion for chemical analysis milk samples were brought to the temperature of 68° F (room temperature) and mixed thoroughly into a clean receptacle in order to get a homogenous samples.

Analysis of Milk
Analysis of physico-chemical characteristics of cow's milk was done before and after treatment. Samples were done by on the basis of method (AOAC, 2005) [10]. pH were analyzed by (Beckman pH meter – Schmar and Company). Nitrogen content (N) in the milk samples was estimated by the Kjeldahl (1983)'s method and crude protein content was calculated as $N \times 6.25$, Electrical conductivity (meter Draminski® Electronics in Agriculture) was used to measure the electrical conductivity of milk. California Mastitis Test was done as per the standard procedures described by Pandit and Mehta

(1969) [11]. and Somatic cell count of milk was performed by the method as described by Schalm et al., (1971) [14]. The reactions occurring in CMT have been correlated with cell counts as follows.

|		
1+	400,000 – 1500,000	cells/ml
2+	800,000 – 5000,000	cells/ml
3+	> 500,000	cells/ml

Blood sample was analyzed in local private laboratory. Statistical analyses of data were done by two-way ANOVA and paired T test using described by Snedecor and Cochran (1989) [16] with SPSS 20 package software. A probability levels ($p<0.05$) was considered as statistically significant.

Result and Discussion
In present study, out of 15 SCM affected quarter from 10 cows belonging to treatment-I which were treated with Tri-sodium citrate, 11 quarters (73.33%) and 7 cows (70%) were completely recovered day 10 and in treatment-II 16 quarters affected of SCM from 10 cows, which were treated with herbal paste prepared from Aloe vera, turmeric, castor oil, lemon, 14 quarters (87.50%) and 9 cows (90%) were completely recovered by day ten. Recovered quarters and animals on the bases of reduced SCC, pH and increased the fat content in milk and increased blood hemoglobin, PCV and TEC. In milk composition pH, fat, SNF, protein and lactose was found significantly ($P<0.05$) increased in treatment-I same result also recovered by Prakash and Sharma (1994) [13] also recorded milk pH decreases with Tri-sodium citrate and oral therapy. Kumari et al. (2019) [5] also recovered same result with Tri-sodium citrate 30mg/kg body weight and significantly ($p<0.05$) decreased in Electrical conductivity, Chloride and Somatic Cell Count. Same result also recovered by Dhillon et al., (1995) [3] and Mbonwanayo et al., (2017) in somatic cell and Ilie et al. (2010) [4] in electrical conductivity. Electrical conductivity is an indicator of subclinical mastitis when it is above >5.5 mS/cm. In treatment-II herbal paste more significantly ($P<0.05$) increased compare than tri-sodium citrate. In milk composition significantly ($P<0.05$) increased in Fat, SNF, Protein and Lactose. Mooventhan et al. (2016) [7] also reported same result treated in clinical mastitis with tropical application of certain herbal ingredients and significantly ($P<0.05$) decreased in pH, SCC, EC, Chloride. Safangat et al. (2017) also reported significantly ($P<0.05$) decreased milk pH with juice of moringa leaves (Moringa oleifera) with chemical antiseptic of Iodips. Balakrishnan et al. (2017) also recovered same result treatment with a herbal preparation containing combination of Aloe vera leaves, Burn fruit, Curcuma longa leaves and calcium hydroxide. Hematological parameters of treatment-I significantly ($P<0.05$) increased in hemoglobin and MCV. In treatment-II significantly ($P<0.05$) increased in blood parameters of hemoglobin, TEC, MCV, PCV and Lymphocyte. These recovered are matching with finding of Shafi et al (2016) [15] treatment with Ocimum sanctum leaf powder, Mukherjee et al (2005) also observed reduced bacterial count and neutrophil and lymphocyte treatment with aqueous leaf extract of Ocimum sanctum and significantly ($P<0.05$) decreased in TLC, Neutrophils, Eosinophils, and Monocyte Same result also observed by Balakrishnan et al. (2017) who treated clinical mastitis with herbal therapy and reported good results in 6-7 days with two lemon fruits juice twice daily.
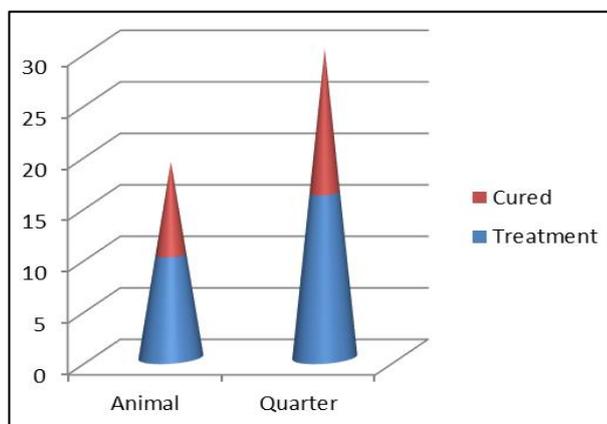
Table 1: No. of Animals

No. of Animals (n=20)		
Treated	Cured	% Cure
10	07	70.00
No. of Quarter (n=38)		
Treated	Cured	% Cure
15	11	73.33
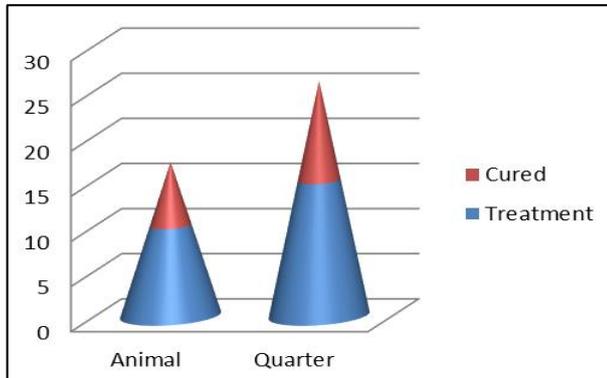
Fig 1: Animal and Quarter

Table 2: No. of Animals

No. of Animals (n=20)		
Treated	Cured	% Cure
10	09	90.00
No. of Quarter (n=30)		
Treated	Cured	% Cure
16	14	87.50

Fig 2: Animal and Quarter

Table 3: Means ± of S.E. of several milk composition parameters in experimental group of crossbred dairy cow

Milk parameters	Control	Treatment-1	Treatment-2
pH	6.9 ± 0.1^a	6.71 ± 0.14^b	6.45 ± 0.02^c
Fat (%)	2.42 ± 0.04^c	3.18 ± 0.07^b	3.35 ± 0.01^a
SNF (%)	7.72 ± 0.03^c	8.41 ± 0.05^b	8.52 ± 0.02^a
Protein (%)	3.44 ± 0.12^c	3.51 ± 0.04^b	3.56 ± 0.05^a
Lactose (%)	2.26 ± 0.04^c	3.60 ± 0.03^b	3.80 ± 0.01^a
Electrical conductivity	5.70 ± 0.02^a	4.62 ± 0.11^b	4.51 ± 0.04^c
Chloride	0.15 ± 0.37^a	0.14 ± 0.12^b	0.13 ± 0.10^c
CMT	Clumping noticed	No clump	No clump
Somatic Cell Count (cells/ml) in lakhs	2.75 ± 2.20	1.95 ± 2.01	1.93 ± 1.20

Means having different superscripts within the same row differs significantly (*P*<0.05)

Table 4: Means ± of S.E. of several Hematological parameters in experimental group of crossbred dairy cow

Parameter	Control	Treatment-1	Treatment-2
TLC	10722 ± 160.8^a	7074 ± 172.3^b	7060 ± 180.5^c
TEC	4.80 ± 0.10^c	5.36 ± 0.32^b	5.61 ± 0.14^a
Hb (gm/dl)	8.8 ± 0.17^c	10.42 ± 1.42^b	10.66 ± 0.41^a
MCV (fl)	56.42 ± 0.17^c	57.71 ± 0.14^b	58.57 ± 0.14^a
PCV (%)	25.9 ± 0.21^c	30.94 ± 1.24^b	32.24 ± 0.62^a
MCHC (g/dl)	32.52 ± 0.07^c	32.80 ± 0.04^b	32.95 ± 0.01^a
Lymphocyte (%)	46.32 ± 0.74^c	58.36 ± 1.02^b	61.84 ± 1.72^a
Monocyte (%)	1.52 ± 0.07^a	1.51 ± 0.18^b	1.50 ± 0.18^c
Eosinophils (%)	3.38 ± 0.25^a	2.14 ± 0.13^b	1.68 ± 0.28^c
Neutrophils (%)	49.96 ± 0.71^a	37.24 ± 1.21^b	36.14 ± 1.52^c

Means having different superscripts within the same row differs significantly (*P*<0.05)

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