RESEARCH PAPER -



Various factors responsible for sub clinical and clinical mastitis and pharmacotherapeutic role of tri-sodium citrate

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ABSTRACT : In the present investigation, 300 cows milk samples were collected and these samples were screened out on the basis of California mastitis test (CMT) for estimation of clinical and subclinical mastitis in cow. Total 64.66 per cent prevalence rate was found, out of which 53 per cent and 11.66 per cent were found sub clinical and clinical mastitis, respectively. Most of the samples were positive to bacteriological examination and various type of bacteria were isolated, the most prevalent being *Streptococcus aureus*, followed by *S. agalactiae, E. coli, Micrococcus* sp., *Enterobater aerogen, Bacillus* sp. and *Corynebacterium*. Administration of trisodium citrate creates unfavourable pH medium in the quarter/udder retarding the infection. It was also observed that trisodium citrate administration increased the fat, S.N.F, milk yield, over the respective values of in mastitic milk.

KEY WORDS : Clinical, Sub-clinical mastitis, Trisodium citrate, Bacteria, Cow

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INTRODUCTION

Mastitis in milch animals is a big problem in our country. Mastitis means inflammation of the parenchyma of the mammary gland. Many infective agents have been implicated as causes of mastitis. It is responsible for major financial losses in dairy farming and great nutritional and technological impacts can be resulted. Because valuable components of the milk like lactose, fat and casein are decreased while undesirable components like ions and enzymes are increased making the milk unfit for processing technology (Girma, 2001; Shitandi *et al.*, 2004). When infection has become established, it is difficult to remove and the infected animals are potential source of infection to other animals. Hence, keeping in view the above points, a study on the role of trisodium citrate in pharmacotherapeutic of mastitis was undertaken.

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MATERIAL AND METHODS

In the present study, 300 cow's milk samples were collected on the basis of California mastitis test (CMT). Approximately 10 ml of milk was collected in a sterile test tube and brought to the laboratory and stored at 4°C for a maximum of 24 hour until inoculated on a standard bacteriological media (Biru, 1989; N.M.C., 1990).

Bacteriological isolation :

Milk samples were bacteriologically examined according to the procedures employed by Brown (1969). In refrigerated milk samples, bacteria may be concentrated in the cream layer and held with in clumps of fat globules (N.M.C., 1990). Hence, dispersion of fat and bacteria was accomplished by warming the samples at 25°C for 15 minutes and shaking before plating on a standard bacteriological media. The estimation of chloride content was done as per the standard volumetric procedure (Prakash, 1979). The analysis of milk samples for fat, S.N.F. (solid not fat) milk yield, citric acid concentration were carried out. The role of trisodium citrate in pharmacotherapeuties of mastitis was calculated on 10th day post treatment of 10 mastitic cows through oral route 30 mg/kg body weight. The identification of mastitis treatments was done by carrying out

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physical examination of milk and udder.

RESULTS AND **D**ISCUSSION

Total 300 cow's milk samples were screened out on the basis of California mastitis test (CMT). 194 (64.66%) samples were found to be positive for mastitis, out of which 159 (53%) and 35 (11.66%) were found sub clinical and clinical mastitis, respectively which were positive for bacteriological examination. From culture positive samples, a total seven strains of bacteria were also isolated (Fig. 1), the most prevalent being *Streptococcus aureus*, followed by *Streptococcus agalactiae*, *E. coli, Micrococcus, Enterobater arogen, Bacillus* and *Corynebacterium*.



The prevalence rate was observed higher in cows with unwashed udder and injured teat. The result of the administration of trisodium citrate @ 30 mg/kg bady weight once daily, orally in 250 ml distilled water continued for 10 days, gradually decreased the milk pH. It was recorded $7.18 \pm$ 0.07 in case of sub clinical mastitis cows and 7.23 ± 0.09 in clinical mastitic cow. The cases were treated with trisodium citrate and pH of milk was found normal from third day. Previously, Dhillon et al. (1989) observed that chemotherapeutic treatment of mastitis has not been successful to desirable extent. Since normal milk pH is considered unsuitable for the growth of common bacterial pathogens and mastitic milk has an alkaline pH. It was hypothesized that administration of trisodium citrate orally might correct/optimize milk pH. Prakash and Sharma (1994) also recorded a gradual decrease in milk pH after the trisodium citrate and oral therapy. Fat percentage was an important parameter in this

investigation. In the market, fat percentage is indicative of quality of milk. In sub-clinical and clinical cases, fat percentage was recorded 2.51 ± 0.04 and 2.56 ± 0.21 , respectively and after treatment with trisodium citrate, it was found increased 3.21 ± 0.04 (Table 1). It is in closed agreement with Singh *et al.* (1998) who also observed that fat content of milk in sub-clinically and clinically infected quarters was reduced to 2.89 ± 0.78 and 2.78 ± 0.22 g/dl, respectively against normal value of 3.01 ± 0.78 g/dl.

Table 1 also shows the solid not fat (S.N.F.) was below the normal range in both the cases and it was observed $7.81\pm$ 0.03 and $6.82\pm$ 0.02 in sub-clinical and clinical mastitic milk, respectively. It was found increased ($8.55\pm$ 0.04) after the treatment and the recorded results had similarity with finding of Ashworth *et al.* (1967).

Climatic condition also influences milk citrate concentration. However, the mean of citric acid concentrations in mastitic milk of cows during pre-treatment were recorded 45.83±0.60 and 43.00±0.94, respectively. Comparatively the citrate content in the mastitic milk samples was much lower than the citric acid content of normal milk. It is also evident from the data of Table 1 that the extent of decrease in citrate content in mastitic milk samples was proportionate to the degree of mastitis. On administration of trisodium citrate orally to cows suffering from different degrees of subclinical/clinical mastitis, marked increase in the citrate contents in mastitic milk was observed. It is evident that trisodium citrate significantly increased the citric acid content of mastitic milk samples and replenished the citrate contents of mastitic milk to the extent that contents once again became equivalent to the citric acid contents of the normal milk sample. It may well be inferred from the above observations and results that mastitis caused severe drop in the concentration of citric acid which may be brought to the normal level by oral feeding of trisodium citrate @ 30 mg/kg body weight daily for ten days.

The rise in milk yield in the animals, suffering from mastitis may be explained keeping in view the role of citrate in the synthesis and secretion of milk. Fleet *et al.* (1975) called milk citrate a harbinger of lactogenesis. Moreover, restoration of trisodium citrate in animals suffering from mastitis decreases pH of milk and perhaps acts against Ca⁺⁺ injury and reduces the inflammatory process in the quarters of the udder, which in turn makes mammary secretary cells more efficient and ultimately the milk yield is increased.

Table 1: Biochemical parameters of milk from sub-clinical, clinical and treated cows			
Biochemical parameters	Sub-clinical mastitis cow	Clinical mastitis cow	Treated cow with trisodium citrate
pH	7.18 ± 0.07	7.23±0.09	6.51±0.17
Fat	2.51±0.04	2.56±0.21	3.21±0.04
S.N.F %	7.81±0.03	6.82±0.02	8.55±0.04
Milk yield kg/day	2.50±0.34	2.08±0.03	3.00±0.34
Citric acid con.	45.83±0.60	43.00±0.94	86.00±1.63

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