Therapeutic Efficacies of Self-Formulated Anti-Oxidant Trace Minerals Formulation in Bovine Mastitis

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A B S T R A C T

The study was undertaken on clinical cases of bovine mastitis presented to Teaching Veterinary Clinical Complex (TVCC), F.V.Sc & A.H, SKUAST-K and adjoining areas during the course of study August 2014-May 2016. For therapeutic trials, the clinical cases of mastitis were divided into two groups. In Animals of Group I therapeutic regimen I was used conducted and included 15 clinical cases of mastitis, in Group II animal’s therapeutic regimen II was used and included 12 clinical cases of mastitis. The animals of group II were given self-formulated anti-oxidant trace mineral mixture. Group II animals were not given any anti-oxidant trace mineral mixture. The requirements of trace minerals like Cu, Zn, Mn and Se are increased in mastitis as evidenced by their decreased levels in clinical mastitis and improved clinical response on their supplementation. Anti-oxidant trace minerals like Cu, Zn, Mn and Se significantly reduce oxidative stress and augment clinical recovery and may play a significant role in treatment of mastitis in lactating animals.

Keywords
Therapeutic efficacies, self-formulated anti-oxidant

Introduction

Mastitis is a parenchymal inflammation of the mammary gland, which is caused by microorganisms, usually bacteria that invade the udder, multiply and produce toxins which are harmful to the mammary gland (Sharma et al., 2006). Mastitis could induce increased formation of free radicals in milk leading to oxidative stress (Gu et al., 2009). Substantial amounts of free radicals are produced during an inflammatory response such as that which occurs when the mammary gland becomes infected. Antioxidant supplementation could decrease the duration; incidence and severity of clinical mastitis as the formation of increased free radicals during mastitis are mostly associated with decreased antioxidant defence. Trace elements with anti-oxidant function include copper, zinc, manganese, iron and selenium which act as co-factors for anti-oxidant enzymes superoxide dismutase, catalase and glutathione peroxidase which are directly or indirectly involved in quenching or
neutralising the excess free radicals generated due to oxidative stress. The present study was thus undertaken to evaluate the therapeutic efficacy of self-formulated anti-oxidant trace mineral formulation in bovine mastitis.

**Materials and Methods**

A total of 39 multiparous cows in the age group of 4-8 years with BCS 3.0 were included for the study. The study was undertaken on clinical cases of bovine mastitis presented to Teaching Veterinary Clinical Complex (TVCC), F.V.Sc & A.H, SKUAST-K and local animal husbandry dispensaries in Ganderbal, Manasbal, Shuhama, Gulab bagh and Shalimar areas during the course of study August 2014-May 2016. 27 animals were found to be affected with clinical mastitis and 12 apparently healthy lactating animals served as control group. For therapeutic trials, the clinical cases of mastitis were divided into two groups. In Animals of Group I therapeutic regimen I was used conducted and included 15 clinical cases of mastitis, in Group II animals therapeutic regimen II was used and included 12 clinical cases of mastitis. The animals of group II were given self-formulated anti-oxidant trace mineral mixture at therapeutic doses as recommended by NRC, 2001. Group II animals were not given any anti-oxidant trace mineral mixture.

Both the groups of animals were given antibiotic enrofloxacin at the dose rate of 6.6mg/kg per animal twice daily for 5 days. The therapeutic trial design for cows with clinical mastitis is provided in Table 1. Trace minerals like copper(Cu), zinc (Zn), manganese (Mn) and selenium(Se) were estimated from the plasma of normal as well as mastitic animals on day 0 and day 10 of treatment after acid digestion of samples. The samples were analysed at Kashmir University, Hazratbal in the Department of University Scientific Instrumentation Centre (USIC) using Polarised Zeeman Atomic Absorption Spectrophotometer (Z-2300, Hitachi).

**Results and Discussion**

The therapeutic efficacy of two treatment regimens with mean days of recovery in both the groups of animals is depicted in Table 2. In group I, 15 clinical cases of mastitis were given self-formulated mineral mixture once daily for 7 days. In this group of animals, eleven animals recovered within mean of 4±0.5 days as was evidenced by clinical recovery (CMT negative) and return of milk SCC and milk chemistry parameters to normal. The percent recovery was 73.33%. The rest of the animals (four) did not recover by the treatment regimen I provided to these animals as evidenced by CMT positive scores, constantly higher milk SCC values and abnormal milk chemistry. Group II constituted 12 clinical cases of mastitis and were not given any mineral mixture. In group II, twelve animals were treated out of which eight animals recovered completely within mean of 4.5±0.8 days as evidenced by clinical recovery (CMT negative) and return of milk SCC and milk biochemical parameters to normal. The per cent recovery was 66.66%. The rest of the animals (four) did not recover by the treatment regimen II provided to these animals as evidenced by CMT positive scores, constantly higher milk SCC values and abnormal milk chemistry. The mean values of copper and manganese in the mastitic group were statistically significant from the mean values of copper in the normal control group (p<0.05%). The mean values for Cu (µmol/L) and Mn (µmol/L) for group I animals in the mastitic group receiving therapeutic regimen I on day 0 and day 10 in group I animals differed significantly from each other (p<0.05) while no significant difference was observed in animals of group II (Table 3).
**Table 1** Therapeutic trial design for cows with clinical mastitis

| Group                        | No. of animals treated | Treatment                                | Dosage         | Route | Frequency | Duration of treatment |
|------------------------------|------------------------|------------------------------------------|----------------|-------|-----------|----------------------|
| Group I (Therapeutic regime I) | 15                     | Inj. Enrofloxacin and Anti-oxidant trace mineral mixture | 6.6 mg/Kg      | I/M   | BID       | 5 days               |
|                              |                        |                                          | 5.005 gms      | Oral  | OD        |                      |
| Group II (Therapeutic regime II) | 12                     | Inj. Enrofloxacin                        | 6.6 mg/Kg      | I/M   | BID       | 5 days               |

**Table 2** Therapeutic efficacy of treatment regimes in animals with clinical mastitis

|                          | Group II | Group III |
|--------------------------|----------|-----------|
| Animals treated          | 15       | 12        |
| Animals Recovered        | 11       | 8         |
| % Recovery               | 73.33%   | 66.66%    |
| Recovery time (Days±S.E) | 4±0.5    | 4.5±0.8   |

**Table 3** Comparison of milk SCC and trace mineral profile between healthy lactating (control group) and mastitic animals

| Parameters      | Control Group | Mastitic Group |
|-----------------|---------------|----------------|
| SCC(×10^5/mL)   | 2.83±.05^a    | 9.94±0.22^b    |
| Copper (µmol/L) | 8.25±.07^a    | 7.01± 0.06^b   |
| Zinc (µmol/L)   | 14.47±.31^a   | 11.71±0.16^b   |
| Mn (µmol/L)     | 3.78±.11^a    | 2.94±0.04^b    |
| Se (ng/mL)      | 38.47±1.67^a  | 31.29±1.14^b   |

Values within a row having superscript (a, b, c) with at least one common alphabet do not differ significantly at 5% level (p<0.05) from each other.

**Table 4** Effect of therapeutic regimens on trace mineral profile of animals with clinical mastitis

| Parameters | Days of treatment | Group I (Antibiotic + Antioxidant trace minerals) | Group II (Antibiotic only) |
|------------|------------------|--------------------------------------------------|---------------------------|
| Se (ng/mL) | Day 0            | 29.94±1.80^a                                     | 32.98±1.16^a              |
|            | Day 10           | 35.39±1.46^b                                     | 31.67±1.08^b              |
| Mn (µmol/L)| Day 0            | 2.94±.06^a                                       | 2.94±.04^a                |
|            | Day 10           | 3.30±.03^b                                       | 2.93±.05^a                |
| Zinc (µmol/L)| Day 0         | 11.86±.22^a                                      | 11.52±.22^a               |
|            | Day 10           | 13.85±.21^b                                      | 11.13±.16^b               |
| Copper (µmol/L)| Day 0         | 6.88±.06^a                                       | 7.18±.10^a                |
|            | Day 10           | 7.65±.06^b                                       | 7.12±.13^a                |
| SCC(×10^5/mL)| Day 0          | 9.88±.31^a                                       | 10.03±.33^a               |
|            | Day 10           | 3.19±.09^b                                       | 4.43±.13^b                |

Values within a column having superscript (a,b) with at least one common alphabet do not differ significantly at 5% level (p<0.05) from each other.
The present study revealed decreased levels of Mn in clinical mastitis as compared to normal control group. The mean values of Zn (µmol/L) and Se (ng/mL) for normal healthy control group and the mastitic group (Table 4) differed significantly from each other (p<0.05). The mean values of zinc and selenium on day 0 and day 10 in group I and group II animals differed significantly from each other (p<0.05).

Copper is an important co-factor of superoxide dismutase, an enzyme, which protects cells from the pro-oxidative influence of free radicals (Kleczkowski, 2003). Our findings are in agreement with Kleczkowski, 2008 who also found decreased concentrations of copper in animals with clinical mastitis. Our findings of decreased values of Mn in clinical mastitis are in agreement with Erskine et al., 1997 and Yang and Li, (2015) who also reported that the plasmatic levels of minerals decrease in clinical mastitis. In our study, the zinc levels in dairy cows with clinical mastitis were low compared with healthy cows. Ibrahim et al., 2016 also found a significant decrease in the values of zinc in cows with clinical mastitis. Low Zn status leads to low quality milk with high SCC and increased incidence of mastitis (Gaafar et al., 2010). The present study revealed decreased levels of Se in clinical mastitis as compared to normal control group. Our findings of low Se values in clinical mastitis are in agreement with Weiss et al., (1990), Erskine et al., (1987), Grasso et al., (1990) and Kommisrud et al., (2005). Selenium is an essential micronutrient present in tissues throughout the body and is important physiologically because it is an integral component of the enzyme glutathione peroxidase. Weiss et al., (1990), found that high serum Se concentrations are associated with reduced rates of clinical mastitis and low bulk tank milk SCC. Trace elements as copper, iron, zinc, selenium and manganese play important roles in several biochemical processes as they are essential component in the antioxidant enzymes as superoxide dismutase and catalase (Yang and Li, 2015).

The requirements of trace minerals like Cu, Zn, Mn and Se are increased in mastitis as evidenced by their decreased levels in clinical mastitis and improved clinical response on their supplementation. Anti-oxidant trace minerals like Cu, Zn, Mn and Se significantly reduce oxidative stress and augment clinical recovery and may play a significant role in prophylaxis of mastitis in lactating animals.

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