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EFFECT OF COMBINED THERAPY OF INTRAVENOUS CEFTRIAXONE AND ORAL POLYHERBAL DRUG ON MILK ENZYME ACTIVITY IN HEALTHY AND MASTITIC GOATS

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ABSTRACT

Fibrosin®, a poyherbal drug, helps in cleansing udder tissue and let down of milk. The present study was aimed to evaluate milk enzyme activities in mastitic goats following single dose intravenous administration of ceftriaxone, with 1 h pre-single dose oral administration of polyherbal drug. Eighteen clinically healthy lactating black Bengal goats were divided into 3 groups (group I, II and III). Mastitis was artificially induced by inoculating 35,000 cfu of coagulase positive *Staphylococci* intracisternally to the right teat of group-III goats. Diagnosis of mastitis was confirmed by bromothymol blue paper test and manifestation of clinical signs. A single intravenous dose of ceftriaxone was administered at 50 mg kg^{-1} body weight to each goat of group-I while a half bolus of polyherbal drug (1.9 g) was administered orally to each goat in group-II and III before 1 h of ceftriaxone administration. The milk alkaline phosphatase and catalase activities significantly increased in mastitic goats as compared to the healthy ones. Mean reduced glutathione level in milk of mastitic goats was increased while milk lactoperoxidase activity showed a decreased activity. The intravenous administration of ceftriaxone with 1 h pre-single dose oral administration of polyherbal drug in mastitic goats resulted in decrease in milk alkaline phosphatase and catalase activities; and also reduced glutathione to normal level while lacto-peroxidase activity, a natural antimicrobial system of milk, significantly increased in mastitic goats. The polyherbal drug Fibrosin® may be a better supportive therapy with intravenous cetriaxone to treat mastitis.

Keywords: Ceftriaxone, fibrosin, mastitis, milk enzyme, polyherbal drug

INTRODUCTION

Goat is unique versatile animal known as 'poor man's cow' in India. It supplies both meat and milk. Goat milk is cheap, whole-some, easily digestible and nutritious. India has nearly 15% of world goat population (Pal *et al.,* 2011). The goats in India produce around 2.76 million tonnes of milk which is 22% of world's milk production (FAO, 2004). Moreover, the goat milk is recommended for infants, old and convalescent people because it has good nutritional and medicinal qualities (Ohiokpehai, 2003). West Bengal has its own goat breed 'Black Bengal goat' which produces excellent quality meat.

Mastitis is a global problem and denotes inflammatory condition of udder irrespective of their cause. It is characterized by physical, chemical and microbiological changes in milk and pathological changes in glandular tissues of udder. Mastitis annually causes a loss of about ₹6100 crore to Indian dairy sector of which ₹1700 crore are due to clinical mastitis alone (Dua, 2001). Though many herbal drugs are often used as adjunct therapy in mastitis but little is known about their pharmacological properties and mechanism of action. Fibrosin® (Legend Remedies, Vadodara, India) is a poyherbal drug which

helps in cleansing the udder tissue and let down of milk. However, Fibrosin increases the bioavailability of antibiotic (ceftriaxone/ceftizoxime) in both plasma and milk, and acts as bio-enhancer (Sar *et al*., 2006; 2011). Therefore, Fibrosin has been selected as an adjunct therapy with intravenous ceftriaxone in the present study. Due to the pathological changes in udder and microbiological changes in milk, normal enzyme activities are often altered. Further, antibiotics and herbal drugs being foreign substances to the body may alter the enzymatic activity. So, the study of some milk enzyme activities have been made to assess the intensity of damage to mammary gland in mastitis and possibility of protecting tissue damage after combined therapy of antibacterial and herbal drug. Lactoperoxidase, a goat milk protein, has been found to be effective against *Staphylococcus aureus* (Anonymous, 1998). On the other hand, reduced glutathione plays an important role in various biochemical processes including lipid peroxidation and provides a defense mechanism for tissues against reactive oxygen species. Catalase in milk plays a major role in decreasing the level of hydrogen peroxide, a reactive oxygen species, leading to the stabilization of cellular membrane of mammary gland. Ceftriaxone is a broad spectrum cephalosporin resistant to various types of β-lactamases with potent activity against both Gram positive and Gram negative bacteria including Enterobacteriaceae, *Haemophilus influenzae*, *Streptococcus pneumoniae* and other non-enterococcal streptococci. Methicillin resistant staphylococci, Enterococci, *Pseudomonas aeruginosa* and *Bacteroides fragilis* are typically resistant (Neu *et al.,* 1981). Ceftriaxone undergoes hydrolysis by inducing Cytochrome P450 and is converted into an active metabolite i.e. ceftizoxime (more resistant to β-lactamases) which persists for a longer period in plasma and milk following intravenous administration (Sar *et al.,* 2006). The present study was undertaken to evaluate the effect of combined herbal and antibiotic (ceftriaxone) therapy on altered milk enzyme activities in goat mastitis and to assess the efficacy of polyherbal drug as an adjunct therapy with intravenous ceftriaxone.

MATERIALS AND METHODS

Eighteen clinically healthy lactating "Black Bengal" goats were divided into 3 groups (group I, II, and III). The animals were caged individually in custom's made up of stainless steel metabolic cage and provided with *ad libitum* drinking water and standard feed. The coagulase positive *Staphylococcus* sp., obtained from the Department of Veterinary Microbiology, WBUAFS, was inoculated in the right quarter for induction of mastitis in all the members of group III goats as per Sar *et al*. (2006). Ceftriaxone at a concentration of 100 mg ml^{-1} distilled water was administered through jugular vein at dose rate of 50 mg kg^{-1} body weight in all the goats. A half bolus of fibrosin (1.9 g) was administered orally 1 h prior to intravenous ceftriaxone administration in group II and III goats. Milk samples were collected at 0, 1, 12, 24, 48, 96, 120 h post-dosing for determination of milk enzyme activities. Reduced glutathione level and alkaline phosphatase, catalase and lactoperoxidase activities in milk were estimated as per the method of Pecker (1994), Bernt (1974), Maehly and Chance (1954) and Makinen and Tenovuo (1964), respectively. The data obtained was analyzed statistically.

RESULTS AND DISCUSSION

Both the quarters of group III goats after 120 h were swollen, hot and hard, though the right quarter was harder than left quarter. The goats showed the signs of pain at both the quarters on touch. The goats also showed agalactia and defaected semisolid-faeces with a slight increase in temperature from 39.6°C to 39.8°C. Chakrabarti (1997) reported that unaffected quarter became swollen due to diffusion of bacterial toxin in acute mastitis.

All the mastitic goats recovered on 5^{th} day following single dose intravenous administration of ceftriaxone with concomitant administration of polyherbal drug. Bromothymol blue paper test showed normal light green colour following exposure to the milk of recovered animals. Normal range of lacto-peroxidase activity was found to be 27400.0±5860.27 µ mole $min^{-1} L^{-1}$. Mean normal alkaline phosphatase activity in

Table I: Mean milk alkaline phosphatase activity (n mole ρNP produced h^{-1} ml^{-1}) in healthy lactating goats, and with 1 h pre-single dose oral administration of fibrosin (1.9 g) in healthy lactating and mastitic goats after single dose intravenous administration of ceftriaxone at 50 mg kg^{-1}

Time (h)	Group I (Healthy lactating)	Group II (Fibrosin treated healthy lactating)	Group III (Mastitic)
0	$4940.0^{b} \pm 1027.71$	$4920.0^{b} \pm 1062.35$	$6600.0^{b} \pm 1385.68$
Mastitis	-	-	$12160.0^{a} \pm 831.40$
1	-	$5520.0^{b} \pm 1137.41$	$5660.0^{b} \pm 669.74$
24	$1100.0^{c} \pm 323.32$	$10800.0^{a} \pm 1495.38$	$5070.0^{b} \pm 473.44$
48	$1740.0^{c} \pm 311.77$	$10800.0^{a} \pm 1685.91$	$6084.0^{b} \pm 159.35$
96	$1540.0^{c} \pm 31.77$	$8755.0^{a} \pm 1599.30$	$6272.0^{b} \pm 68.12$
120	$1280.0^{c} \pm 219.39$	$7500.0^{a} \pm 528.29$	$5180.0^{b} \pm 496.53$

Means in the same row bearing same alphabet as superscript do not differ significantly from each other at $P_{0.05}$

milk of healthy goats ranged between 4920.0±1062.3 and 6600.0^{b} ± 1385.68 n mole ρNP produced h^{-1} ml^{-1}. Normal mean milk catalase activity in healthy goats ranged from 23.49^{b} ± 3.51 to 27.76 ± 3.00 µ mole H_2O_2 hydrolyzed min^{-1} ml^{-1} and reduced glutathione level in milk of healthy goats was within a range of 319.90 ± 15.99 to 351.0^{b} ± 33.77 n mole GSH ml^{-1}.

In fibrosin treated healthy goats (group II), the mean alkaline phosphatase activity in milk showed insignificant increase to 9640.0 ± 918.01 n mole ρNP produced h^{-1} ml^{-1} at 1 h (Table 1). The enzyme activity increased significantly to 12160.0 ± 831.40 n mole ρNP produced h^{-1} ml^{-1} milk in mastitic goats (group III). In healthy goats treated with fibrosin, mean alkaline phosphatase activity increased insignificantly at 1 h (5520.0 ± 1137.41 n mole ρNP produced h^{-1} ml^{-1} milk), while it decreased significantly at 1 h (5660.0 ± 669.74 n mole ρNP produced h^{-1} ml^{-1} milk) in mastitic goats (group III) treated with fibrosin. The enzyme activity was altered significantly at 24, 48, 96 and 120 h pd among the goats of 3 different groups after single dose intravenous administration of ceftriaxone at 50 mg kg^{-1}.

Catalase activity increased significantly (66.94 ± 9.63 µ mole H_2O_2 hydrolyzed min^{-1} ml^{-1} milk) in mastitic goats (group III) as compared to healthy goats (Table 2). Catalase activity showed significant decrease (40.25±4.70 µ mole H_2O_2 hydrolyzed min^{-1} ml^{-1} milk) in mastitic goats after 1 h single dose oral administration of fibrosin (1.9 g). The catalase activity was insignificantly altered among all the 3 groups at 24, 48, 72, 96 and 120 h pd after single dose intravenous administration of ceftriaxone at 50 mg kg^{-1}.

Mean reduced glutathione level was 472.50 ± 25.98 n mole GSH ml^{-1} milk in mastitic goats of group III (Table 3). Mean reduced glutathione level decreased non-significantly (306.0 ± 25.11 n mole GSH ml^{-1}) in mastitic goats of group III after 1 h pre-single dose oral administration of fibrosin, while it increased non-significantly (359.94 ± 22.77 n mole GSH ml^{-1}) in healthy goats after 1 h of fibrosin

Table 2: Mean milk catalase activity (µ mole H_2O_2 hydrolysed min^{-1} ml^{-1}) in healthy lactating goats, and with 1 h pre-single dose oral administration of fibrosin (1.9 g) in healthy lactating and mastitic goats after single dose intravenous administration of ceftriaxone at 50 mg kg^{-1}

Time (h)	Group I (Healthy lactating)	Group II (Fibrosin treated healthy lactating)	Group III (Mastitic)
0	$23.49^{b} \pm 3.51$	$23.64^{b} \pm 3.77$	$27.76^{b} \pm 3.00$
Mastitis	-	-	$66.94^{a} \pm 9.63$
1	-	$23.55^{b} \pm 3.81$	$40.25^{b} \pm 4.70$
24	$20.13^{b} \pm 3.22$	$25.65^{b} \pm 3.89$	$12.50^{b} \pm 2.45$
48	$18.43^{b} \pm 3.35$	$22.58^{b} \pm 3.06$	$14.55^{b} \pm 2.48$
72	$22.55^{b} \pm 2.97$	$18.35^{b} \pm 2.28$	$17.68^{b} \pm 2.78$
96	$24.36^{b} \pm 2.73$	$21.50^{b} \pm 3.03$	$20.14^{b} \pm 2.90$
120	$27.80^{b} \pm 3.47$	$25.45^{b} \pm 2.86$	$22.38^{b} \pm 2.99$

Means in the same row bearing same alphabet as superscript do not differ significantly at $P_{0.05}$

Table 3: Mean reduced glutathione level (n mole GSH ml^{-1}) in milk of healthy lactating goats, and with 1 h pre-single dose oral administration of fibrosin (1.9 g) in healthy lactating and mastitic goats after single dose intravenous administration of ceftriaxone at 50 mg kg^{-1}

Time (h)	Group I (Healthy lactating)	Group II (Fibrosin treated healthy lactating)	Group III (Mastitic)
0	$325.00^{b} \pm 23.09$	$319.90^{b} \pm 15.99$	$351.00^{b} \pm 33.77$
Mastitis	-	-	$472.50^{b} \pm 25.98$
1	-	$359.94^{b} \pm 22.77$	$306.00^{b} \pm 25.11$
24	$240.75^{b} \pm 17.46$	$870.25^{a} \pm 80.68$	$315.00^{b} \pm 21.65$
48	$298.75^{c} \pm 25.11$	$777.00^{a} \pm 50.23$	$516.05^{b} \pm 29.01$
72	$292.50^{c} \pm 12.55$	$997.50^{a} \pm 81.12$	$473.25^{b} \pm 30.74$
96	$310.75^{c} \pm 11.69$	$1120.00^{a} \pm 63.51$	$654.00^{b} \pm 74.48$
120	$260.50^{b} \pm 11.69$	$802.50^{a} \pm 44.60$	$341.25^{b} \pm 45.46$

Means in the same row bearing same alphabet as superscript do not differ significantly from each other at $P_{0.05}$

administration. The reduced glutathione level was altered significantly among the goats of 3 groups at 24, 48, 72, 96 and 120 h post-single dose intravenous administration of ceftriaxone at 50 mg kg^{-1}.

Mean lacto-peroxidase activity (15200.0 ± 4541.91 µ mole min^{-1} L^{-1}) in milk of mastitic goats was decreased compared to 27400.0 ± 5860.27 µ mole min^{-1} L^{-1} milk of healthy goats (Table 4). Mean lactoperoxidase activity showed increase at different time intervals, except at 144 h (61066.7 ± 17705.92 µ mole min^{-1} L^{-1}) after single dose intravenous administration of ceftriaxone at 50 mg kg^{-1} in presence of fibrosin. Due to the unavailability of reagents we could not determine the lactoperoxidase activity in healthy and Fibrosin treated healthy groups. So milk sample collected at 0 h was considered as control. Mean milk lactoperoxidase activity decreased in mastitic goats which is a natural antimicrobial system of milk. Sandholm *et al.* (1988) reported that lactoperoxidase does not have sufficient substrate (thiocyanate, hydrogen peroxide) to produce an effective antibacterial system in milk as one of the potential causes of failure of endogenous antibacterial factors to operate effectively. Baskaran *et al.* (1999) reported a direct correlation between glutathione level and lipid peroxidation. Mean milk lactoperoxidase activity in mastitic goats decreased while reduced glutathione level non-significantly increased. In mastitic goats, milk alkaline phosphatase activity increased significantly which indicated substantial tissue damage in mammary gland of mastitic goats. Patil *et al.* (2003) reported that mammary epithelial cells synthesize lactoperoxidase at a concentration of 2-35 mg ml^{-1}. Synthesis of lactoperoxidase was interfered in these mastitic goats due to substantial tissue damage of mammary gland. Reduced glutathione plays very important role in various biochemical processes including lipid peroxidation and it provides a defense mechanism for tissues against the reactive oxygen species. Cytotoxicity of molecular oxygen is held in check by the delicate balance between the rate of generation of the partially reduced oxygen species and the rate of their removal by the different defense mechanisms and any shift in this delicate balance can lead to cellular damage. The increased activity of catalase in milk suggests decreased level of hydrogen peroxide, a reactive oxygen species leading to stabilization of cellular membrane of mammary gland. Therefore, the increased activity of catalase in milk may stimulate the defense mechanism during mastitis in goats. Glutathione is a significant

Table 4: Mean lactoperoxidase activity (µ mole min^{-1} L^{-1}) in milk of mastitic goats with 1 h pre-single dose oral administration of fibrosin (1.9 g) after single dose intra-venous administration of ceftriaxone at 50 mg kg^{-1}

Time (h)	Mastitic (Gr III)
0 (Control)	27,400.0 ± 5,860.27
Mastitis	15,200.0 ± 4,541.91
1	19,800.0 ± 3,425.51
24	27,464.0 ± 6,534.64
48	60,666.6 ± 4,234.01
96	64,560.0 ± 8,175.51
120	68,000.0 ± 16,474.20
144	61,066.7 ± 17,705.92

component of the collective antioxidant defenses and is highly potent antioxidant and antitoxin in its own right. The thiol group of reduced glutathione (GSH) is important for many facets of cell function and GSH plays multiple regulatory roles at the cell level. GSH is essential both to the functional and structural integrity of the cells, the tissues and the organ system. The glutathione status of a cell (that is the excess of reduced over oxidized glutathione) will perhaps turn out to be the most accurate single indicator of the health of a cell. Increased reduced glutathione in mastitis condition suggests possible role in stabilization of cellular membrane during mastitis.

Intravenous administration of ceftriaxone with 1 h pre single dose oral administration of polyherbal drug in mastitic goats returned increased mean milk alkaline phosphatase activity, catalase activity to normal level while mean lactoperoxidase activity, a natural antimicrobial system of milk, significantly increased in mastitic goats. Concomitant administration of polyherbal drug (Fibrosin) and Ceftriaxone having excellent antibacterial action with increased lactoperoxidase activity may be an effective therapy for goat mastitis.

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