STUDIES ON EFFECT OF FIBROSIN® IN EXPERIMENTALLY INDUCED MASTITIS OF BLACK BENGAL GOATS

Sponsor

LEGEND REMEDIES PRIVATE LIMITED

Dr. Tapan Kumar Mandal

Principal Investigator

WEST BENGAL UNIVERSITY OF ANIMAL AND FISHERY SCIENCES

FACULTY OF VETERINARY AND ANIMAL SCIENCE

DEPARTMENT OF PHARMACOLOGY & TOXICOLOGY

37, BELGACHIA ROAD, KOLKATA-700 037 * MOHANPUR-741 252, NADIA.

Studies on effect of Fibrosin®in experimentally induced mastitis of Black Bengal goats.

1.	Study	: To determine efficacy profile of "Fibrosin®" following single dose in experimentally induced mastitis of goats.
2.	Address of the conducting laboratory	: Deptt. of Pharmacology and Toxicology, West Bengal University of Animal & Fishery Sciences, Mohanpur Campus, Nadia, West Bengal.
3.	Sponsor	: Legend Remedies Private Limited, 101/102-D Mangaldeep Complex, 13/14 Sampatrao Colony, BPC Road, Baroda-390 005.
4.	Principal Investigator	: Dr. Tapan Kumar Mandal, Mandal 14/6/04 Reader, Deptt. of Pharmacology & Toxicology, W.B.U.A.F.S.

Introduction

Mastitis, a disease complex, is a sequel to interplay between microorganisms and the managemental practices. Indeed, its control and treatment has been a constant cause of worry for the farmers as well as the researchers. Indiscriminate use of antibiotics and the ubiquitous nature of mastitogens have led to the development of resistance and failure of antibiotic therapy. The use of micronutrients, herbal drugs and homeopathic drugs in modulating the immunity of mammary gland hold promising future in therapeutic management of mastitis. Micronutrients like selenium, zinc and copper and vitamins like vitamin A, Vitamin E and Vitamin C have been used successfully in the prevention and control of this disease. These micronutrients and vitamins function to maintain low tissue

concentration of reactive oxygen species and this prevent the development of oxidative stress.

Some of these are also involved in the maintenance of functional epithelium, which provides a physical barrier to the entrance of pathogens. The use of different herbs for therapeutic management of mastitis is now being viewed with great interest. Fibrosin® , a herbal product is used for the above purpose with some success, though the site and mechanism of action is not fully known. Therefore, in order to prevent and control of mastitis in animal, the detailed study of Fibrosin® is essential.

The objective of the present study is :

i) To evaluate the disposition kinetics of ceftriaxone in presence of Fibrosin® after intravenous administration in healthy and mastitis animals.

ii) To collect information about the level of vital enzymes in milk after using Fibrosin®) in presence of antibacterial drug (Ceftriaxone) in healthy and mastitic animals.

iii) To ascertain the intensity of damage of mammary gland, the status of vital enzyme in milk before and after experimentally induced mastitis are to be studied.

Experimental design:

a) A single dose of ceftriaxone dissolving in 5 ml of distilled water was administered to each 3 healthy goats through jugular vein at 50 mg kg^{-1} body weight. Blood samples were collected for estimation of ceftriaxone concentration to evaluate disposition kinetics of ceftriaxone in healthy goats.

b) A single dose of ceftriaxone dissolving in 5 ml of distilled water was administered to each animal of another 3 healthy goats through the jugular vein at 50 mg kg^{-1} body weight. A half bolus of fibrosin (1.9 gm) was administered orally to each goat before 1 hr of ceftriaxone administration. Blood samples were collected at different time

intervals for estimation of ceftriaxone concentration to evaluate disposition kinetics and to study the kinetic interaction in presence of fibrosin in healthy goats.

c) A single dose of ceftriaxone dissolving in 5 ml of distilled water was administered through the jugular vein at 50 mg kg^{-1} body weight to each of the induced mastitic goats. A half bolus of fibrosin was administered orally 1 hr prior to ceftriaxone administration. Ceftriaxone concentration was determined from collected blood samples to evaluate disposition kinetics in mastitic goats.

d) Milk samples were collected from all the groups to study vital milk enzymes like alkaline phosphatase and catalase. Reduced glutathion level was also determined. Lactoperoxidase activity was determined thoroughly in mastitic goats before and after administration of ceftriaxone intravenously in presence of fibrosin.

Induction of mastitis

Mastitis was induced by inoculating 35,000 c.f.u. of coagulase positive staphylococci intracisternally to the right quarters of the lactating goats. A confirmatory test was performed after inoculation of organisms using BTB (Bromothymol blue) paper test at 24 hr intervals. The following colour was observed.

Time (hr)	Colour of Milk	
	Left quarter	**Right quarter**
24	Light green	Light green
48	Light green	Bit darker green
72	Bit darker green	Dark deep green
96	Dark green	Deep bluish green
120	Greenish purple	Greenish purple

Level of vital milk enzymes level was estimated to ascertain the damage of mammary gland. After 120 hr of inoculation, both the quarters were swollen, hot and hard, though the right quarter was more harder than left quarter. The goats showed the signs of pain at both the quarters on touching. The goats also showed agalactia and defecated semisolid faeces with a slight increase of temperature from 103.4°F to 103.8°F.

Results

Plasma level of ceftriaxone

Mean values with SE of plasma concentration of ceftriaxone in healthy lactating goats, and with 1 hr presingle dose oral administration of fibrosin (1.9 gm) in healthy lactating and mastitic goats after single dose intravenous administration of ceftriaxone have been summarized in Table-1.

Maximum plasma concentration of ceftriaxone was achieved at 0.08 hr (135.00 ± 4.35 μg ml^{-1}) followed by sharp decline and the minimum plasma concentration was recorded at 1 hr pd (7.03 ±1.54 μg ml^{-1}) in healthy lactating goats without fibrosin. Ceftriaxone achieved its peak plasma concentration of 230.00 ± 11.54 μg ml^{-1} at 0.08 hr, followed by comparatively gradual decline and reached to its minimum plasma concentration of 16.60 ± 1.44 μg ml^{-1} at 2 hr pd in healthy lactating goats with 1 hr pre single dose oral administration of fibrosin. Centriaxone persisted at its maximum plasma concentration of 141.00 ± 14.22 μg ml^{-1} at 0.08 hr, followed by decline in concentration at a faster rate and was present at its minimum plasma concentration of 9.46 ± 3.26 μg ml^{-1} at 0.50 hr pd in mastitic goats with 1hr pre-single dose oral administration of fibrosin. The plasma concentration of ceftriaxone increased significantly ($P<0.05$) in fibrosin treated healthy lactating goats compared to healthy and fibrosin treated mastitic goats starting from 0.08 hr to 0.33 hr pd. Ceftriaxone persisted for 2 hr in fibrosin treated healthy goats while it was

present for 1 hr in healthy goats without fibrosin and 0.50 hr in mastitic goats with 1 hr pre-single dose oral administration of fibrosin.

Kinetics

The semilogarithimic plot of mean plasma level time profile of healthy lactating goats, and with 1 hr pre single dose oral administration of fibrosin in healthy lactating and mastitic goats obtained from computerized pharmacokinetic programme 'PHARMKIT' have been displayed in Fig. i, ii, & iii and disposition kinetic parameters have been presented in Table 2. The kinetic behaviour of ceftriaxone followed 'one compartment open model' in healthy lactating goats and fibrosin treated mastitic goats, while the kinetic pattern was fitted to 'two compartment open model' in fibrosin treated healthy lactating goats. It transpires from Table 2 that the mean B value increased significantly ($P<0.05$) in fibrosis treated mastitic goats. Significantly ($P<0.05$) lower $t_{½}\ \beta$ value (0.10 ± 0.10 hr) in mastitic goats with fibrosin was recorded compared to healthy goats without fibrosin (0.21 ± 0.01 hr).

The mean Vd_{area} value (0.28 ± 0.01 Lkg^{-1}) was significantly ($P<0.05$) higher in healthy goats than that of mastitic goats with fibrosin treatment (0.20 ± 0.01 Lkg^{-1}). The other kinetic parameters like AUC, Cl_B and Cl_H were not altered significantly between goats of both the groups. The kinetic behaviour of ceftriaxone in healthy lactating goats with 1 hr pre single dose oral administration of fibrosin followed 'two compartment open model'. The mean values with SE of B, β and $t_{½}\ \beta$ were 68.97 ± 9.66 $\mu g\ ml^{-1}$, 0.70 ± 0.04 hr^{-1} and 0.99 ± 0.06 hr respectively. The mean values of A, α and $t_{½}\ \alpha$ were respectively 275.20 ± 14.12 $\mu g\ ml^{-1}$, 5.39 ± 0.18 hr^{-1} and 0.12 ± 0.003 hr. Mean K_{12}, K_{21}, and K_{el} values were 2.14 ± 0.07, 1.64 ± 0.16 and 2.31 ± 0.14 hr^{-1} respectively, while mean values of fc and T~B were found to be 0.29 ± 0.02 and 2.34 ± 0.23 respectively. Mean Vd_c and Vd_{area} values were 0.14 ± 0.01 and 0.48 ± 0.04 L Kg^{-1}. The mean Cl_B, Cl_R and Cl_H values were $0.33 \pm$

0.01, 0.005 ± 0.0005 and 0.328 ± 0.01 L $Kg^{-1}hr^{-1}$ respectively which indicates that fibrosin, a polyherbal drug decreased the body clearance of ceftriaxone.

Milk enzyme

Alkaline phosphatase activity:

Mean milk alkaline phosphatase activity with SE in healthy, fibrosin treated healthy and fibrosin treated mastitic goats before and after single dose intravenous administration of ceftriaxone at 50 mg kg^{-1} have been presented in Table 3. Mean normal alkaline phosphatase activity in milk of healthy goats ranged between 4920.00±1062.35 and 7840.00±499.42nmole PNP produced hr^{-1} ml^{-1}. The enzyme activity increased significantly ($P<0.05$) to 12160.00 ± 831.40 n mole PNP produced hr^{-1} ml^{-1} in mastitic goats. In healthy goats treated with fibrosin, mean alkaline phosphatase activity increased non-significantly at 1 hr (5520.00 ± 1137.41 n mole PNP produced hr^{-1} ml^{-1}, while it decreased significantly ($P<0.05$) at 1 hr (5560.00 ± 669.74 n mole PNP produced hr^{-1} ml^{-1}) in mastitic goats treated with fibrosin. The enzyme activity was altered significantly ($P<0.05$) at 24, 48, 96 and 120 hr pd among the goats of three different groups after single dose intravenous administration of ceftriaxone at 50 mg kg^{-1}. The enzyme activity decreased in healthy goats and increased in fibrosin treated healthy goats while it maintained normal activity in fibrosin treated mastitic goats after single dose intravenous administration of ceftriaxone.

Catalase activity:

Mean milk catalase activity with SE in healthy, fibrosin treated healthy and fibrosin treated mastitic goats before and after single dose intravenous administration of ceftriaxone at 50 mg kg^{-1} have been presented in Table 4. Normal mean milk catalase activity in healthy goats ranged from 16.00 ± 0.86 to 22.76 ± 3.00 μ mole H_2O_2 hydrolysed min^{-1} ml^{-1}. Catalase

activity increased significantly ($P<0.05$), (66.94± 9.63 μ mole H_2O_2 hydrolysed min^{-1} ml^{-1}) in mastitic goats compared to healthy goats. Mean milk catalase activity decreased significantly ($P<0.05$), (40.25 ± 4.70 μ mole H_2O_2 hydrolysed min^{-1} ml^{-1}) in mastitic goats after 1 hr single dose oral administration of fibrosin. Mean milk catalase activity was not altered significantly ($P<0.05$) among all the three groups at 24, 48, 72, 96 and 120 hr pd after single dose intravenous administration of ceftriaxone at 50 mg kg^{-1}.

Reduced glutathione level:

Mean reduced glutathione level with SE in milk of healthy, fibrosin treated healthy and fibrosin treated mastitic goats before and after single dose intravenous administration of ceftriaxone at 50 mg kg^{-1} have been depicted in Table 5. Mean normal reduced glutathione level in milk of healthy goats was within a range of 319.90 ± 15.99 to 600.00 ± 51.36 n mole GSH ml^{-1}. Reduced glutathione level increased nonsignificantly (472.50 ± 25.98 n mole GSH ml^{-1} of milk) in mastitic goats compared to control value. Mean reduced glutathione level decreased nonsignificantly (306.00 ± 25.11 n mole GSH ml^{-1}) in mastitic goats after 1 hr of 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 hr of oral administration of fibrosin. Mean reduced glutathione level was altered significantly ($P<0.05$) among the goats of three different groups at 24, 48, 72, 96 and 120 hr post single dose intravenous administration of ceftriaxone.

Lactoperoxidase activity:

Mean lactoperioxidase activity (15200.00 ± 4541.91μ mole min^{-1} ml^{-1}) in milk of mastitic goats was decreased compared to 27400.00 ± 5860.27 μ mole $min^{-1}L^{-1}$ in milk of healthy goats (Table 6). Mean lactoperoxidase activity was increased at a faster rate at different time intervals after single dose intravenous administration of ceftriaxone in presence of fibrosin

except at 144 hr (61066.66 ± 17705.92 μ mole min^{-1} L^{-1}) when it decreased compared to activity produced at 120 hr.

Conclusion

From the above study, it could be concluded that Fibrosin® changed the disposition kinetics of ceftriaxone significantly in healthy and mastitic goats after intravenous administration of ceftriaxone. Ceftriaxone undergoes more hepatic clearance in both healthy and mastitic goats. Fibrosin® decreased the body clearance of ceftriaxone in healthy goats resulting into longer persistence of ceftriaxone. So, repeated dosing of Fibrosin® may interfere to hepatic clearance of ceftriaxone. But single oral dose administration of Fibrosin® did not interfere to body clearance of ceftriaxone in mastitic goats. Fibrosin® helped to recover normal activity of milk alkaline phosphatase and catalase and also maintained a more or less normal level of reduced glutathione in mastitic affected goats, while it stimulated lactoperoxidase activity, a natural antimicrobial system of milk in mastitic goats in presence of ceftriaxone.

Table 1: Mean plasma concentration (μg ml^{-1}) of ceftriaxone in healthy lactating goats, and with 1 hr pre single dose oral administration of fibrosin (1.9 gm) in healthy lactating and mastitic goats after single dose intravenous administration at 50 mg kg^{-1}.

(Mean of 3 replicates with SE)

Time (hr)	Healthy lactating	Fibrosin treated healthy lactating	Mastitic
0.08	$135.00^{b} \pm 4.35$	$230.00^{a} \pm 11.54$	$141.00^{b} \pm 14.22$
0.16	$106.91^{b} \pm 7.42$	$180.00^{a} \pm 8.08$	$80.58^{b} \pm 13.03$
0.25	$76.08^{b} \pm 2.70$	$135.33^{a} \pm 4.33$	$47.00^{b} \pm 10.11$
0.33	$54.50^{b} \pm 1.44$	$103.00^{a} \pm 2.51$	$29.86^{b} \pm 7.56$
0.50	$33.75^{NS} \pm 3.03$	$60.26^{NS} \pm 0.37$	$9.46^{NS} \pm 3.26$
0.66	19.16 ± 1.92	40.58 ± 4.75	BDL
1	7.03 ± 1.54	33.66 ± 3.71	BDL
2	BDL	16.60 ± 1.44	BDL
3	BDL	BDL	BDL
4	BDL	BDL	BDL
6	BDL	BDL	BDL
8	BDL	BDL	BDL
12	BDL	BDL	BDL
24	BDL	BDL	BDL

Means in row bearing at least one common superscript do not differ significantly ($P<0.05$)
a,b – higher superscript, lower superscript
NS – Non-significant
BDL- Below detection limit

Table 2: Mean kinetic parameters of ceftriaxone in healthy lactating goats, and with 1 hr pre single dose oral administration of fibrosin (1.9 gm) in healthy lactating and mastitic goats after single dose intravenous administration at 50 mg kg^{-1}
(Mean of 3 replicates with SE)

Kinetic parameters	Healthy lactating	Fibrosin treated healthy lactating	Mastitic
B($\mu g\ ml^{-1}$)	$172.25^{b} \pm 7.66$	68.97 ± 9.66	$237.23^{a} \pm 12.26$
β (hr^{-1})	$3.27^{b} \pm 0.20$	0.70 ± 0.04	$6.67^{a} \pm 0.71$
$t_{½}\beta$ (hr)	$0.21^{a} \pm 0.01$	0.99 ± 0.06	$0.10^{b} \pm 0.01$
AUC ($\mu g\ hr\ ml^{-1}$)	52.89 ± 2.82	149.04 ± 7.60	36.69 ± 5.45
Vd_{area} (Lkg^{-1})	$0.28^{a} \pm 0.01$	0.48 ± 0.04	$0.20^{b} \pm 0.01$
Cl_B ($L\ kg^{-1}hr^{-1}$)	$0.93^{NS} \pm 0.04$	0.33 ± 0.01	$1.39^{NS} \pm 0.23$
Cl_R ($L\ kg^{-1}hr^{-1}$)	-	0.005 ± 0.0005	$0.008^{NS} \pm 0.002$
Cl_H ($L\ kg^{-1}\ hr^{-1}$)	$0.93^{NS} \pm 0.04$	0.328 ± 0.01	$1.38^{NS} \pm 0.40$
MRT (hr)	$0.30^{a} \pm 0.01$	0.88 ± 0.06	$0.14^{b} \pm 0.014$
C^{o}_{P}($\mu g\ ml^{-1}$)	-	344.18 ± 16.34	-
A ($\mu g\ ml^{-1}$)	-	275.20 ± 14.12	-
α (hr^{-1})	-	5.39 ± 0.18	-
$t_{½}\alpha$ (hr)	-	0.12 ± 0.003	-
Vd_c($L\ kg^{-1}$)	-	0.14 ± 0.01	-
Vd_B($L\ kg^{-1}$)	-	1.14 ± 0.30	-
Vdss ($L\ kg^{-1}$)	-	0.32 ± 0.03	-
K_{12} (hr^{-1})	-	2.14 ± 0.07	-
K_{21} (hr^{-1})	-	1.64 ± 0.16	-
K_{el} (hr^{-1})	-	2.31 ± 0.14	-
f_C	-	0.29 ± 0.02	-
T~B	-	2.34 ± 0.23	-

Means in row bearing at least one common superscript do not differ significantly ($P<0.05$)
a,b – higher superscript, lower superscript
NS – Non-significant
BDL- Below detection limit

Table 3: Mean milk alkaline phosphatase activity (n mole PNP produced hr^{-1} ml^{-1}) in healthy lactating goats, and with 1 hr pre single dose oral administration of fibrosin (1.9 gm) in healthy lactating and mastitic goats after single dose intravenous administration of ceftriaxone at 50 mg kg^{-1}.

(Mean of 3 replicates with SE)

Time (hr)	Healthy lactating	Fibrosin treated healthy lactating	Mastitic
0	4940.00^{b} ± 1027.71	4920.00^{b} ± 1062.35	6600.00^{b} ± 1385.68
Mastitis	-	-	12160.00^{b} ± 831.40
1	-	5520.00^{b} ± 1137.41	5660.00^{b} ± 669.74
24	1100.00^{c} ± 323.32	10800.00^{a} ± 1495.38	5070.00^{b} ± 473.44
48	1740.00^{c} ± 311.77	10800.00 ± 1685.91	6084.00^{b} ± 159.35
96	1540.00^{c} ± 31.77	8755.00^{a} ± 1599.30	6272.00^{b} ± 68.12
120	1280.00^{c} ± 219.39	7500.00^{a} ± 528.29	5180.00^{b} ± 496.53

Means in row bearing at least one common superscript do not differ significantly (P<0.05)
a, b, c – Superscripts

Table 4: Mean milk catalase activity (µ mole H_2O_2 hydrolysed min^{-1} ml^{-1}) in healthy lactating goats, and with 1 hr pre single dose oral administration of fibrosin (1.9 gm) in healthy lactating and mastitic goats after single dose intravenous administration of ceftriaxone at 50 mg kg^{-1}.

(Mean of 3 replicates with SE)

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

Means in row bearing at least one common superscript do not differ significantly (P<0.05)
a, b – Superscripts

Table 5: Mean reduced glutathione level (n mole GSH ml^{-1}) in milk of healthy lactating goats, and with 1 hr pre single dose oral administration of fibrosin (1.9 gm) in healthy lactating and mastitic goats after single dose intravenous administration of ceftriaxone at 50 mg kg^{-1}.

(Mean of 3 replicates with SE)

Time (hr)	Healthy lactating	Fibrosin treated healthy lactating	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 row bearing at least one common superscript do not differ significantly ($P<0.05$)
a, b, c – Superscripts

Table 6: Mean lactoperoxidase activity (μ mole min^{-1} L^{-1}) in milk of mastitic goats with 1 hr pre single dose oral administration of fibrosin (1.9 gm) after single dose intravenous administration of ceftriaxone at 50 mg kg^{-1}.

(Mean of 3 replicates with SE)

Time (hr)	Mastitic
Control	27400.00 ± 5860.27
Mastitis	15200.00 ± 4541.91
1	19800.00 ± 3425.51
24	27464.00 ± 6534.64
48	60666.60 ± 4234.01
96	64560.00 ± 8175.51
120	68000.00 ± 16474.20
144	61066 ± 17705.92