Efficacy evaluation of *Bauhinia variegata* L. stem bark powder as adjunct therapy in chronic *Staphylococcus aureus* mastitis in goat

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Submitted: 05-02-2014 Revised: 24-02-2014 Published: 30-08-2014

ABSTRACT

Objective: The objective was to study the effect of *Bauhinia variegata* L. stem bark powder as adjunct therapy in chronic *Staphylococcus aureus* mastitis in goat. **Materials and Methods:** Mastitis was induced by intracisternal inoculation of coagulase positive *S. aureus* (J638) at the concentration of 2000 colony forming units. Group I animals were treated with repeated dose of ceftriaxone at 20 mg/kg intravenously, and Group II animals were treated with once daily oral administration of *B. variegata* L. stem bark powder at 6 g/kg for 7 days followed by maintenance dose at 3 g/kg for next 7 days along with repeated dose of the antibiotic at 20 mg/kg intravenously at 4 days interval. **Results:** No significant improvement in the clinical condition of the udder was noticed in the group treated with repeated dose of ceftriaxone alone. However, in the group treated with *B. variegata* L. stem bark powder along with repeated dose of ceftriaxone, no *S. aureus* colony was seen at 96 h and onwards in milk samples with a marked decrease in somatic cell count and milk alkaline phosphatase activity and increased lactoperoxidase activity. Further, plasma and milk concentration of ceftriaxone/ceftizoxime was increased, which indicated antibacterial, bioenhancing and antiinflammatory properties of the bark powder. The Group II animals also exhibited marked reduction in polymorphonuclear cells and fibrous tissue indicating antifibrotic property of *B. variegata* L. **Conclusion:** *B. variegata* L. stem bark powder can be considered as an effective adjunct therapy to intravenous ceftriaxone in *S. aureus* chronic mastitis in goat.

Key words: *Bauhinia variegata* L. ceftriaxone, chronic mastitis, goat, *Staphylococcus aureus*

Access this article online

Website: www.phcog.com

DOI: 10.4103/0973-1296.139786

Quick Response Code:

INTRODUCTION

Mastitis is a major problem in goats. The predominant bacterial species responsible for mastitis in dairy goats is *Staphylococcus aureus* and its prevalence in dairy goat herds varied widely from 7% to 40%.[1] Use of antibiotic is commonly practiced in mastitis therapy.[2] Selection of the antimicrobial agent and maintenance of adequate concentration of the drug at the site of infection are the most relevant problems in antibiotic therapy of mastitis. It has been reported earlier that an active metabolite of ceftriaxone that is, ceftizoxime is found in high concentration in milk following intravenous ceftriaxone administration in acute mastitis in goat.[3] However, fibrosis is a major problem in chronic mastitis which reduces the bioavailability of the drug at the site of infection. Hence, the antibiotic treatment with concomitant herbal therapy may be a possible strategy.

The aqueous and ethanol extracts of *Bauhinia variegata* L. has been shown significant antioxidant activity *in vitro*.[4] It has been reported that the bark is rich in the phenolic/flavonoid content.[5] Hence, the aim of the present work was to evaluate the potential of stem bark of *B. variegata* L. as adjunct therapy with intravenous ceftriaxone in chronic *S. aureus* mastitis in goat.

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MATERIALS AND METHODS

Drugs

Ceftriaxone (analytical grade, purity ≥90%) and ceftizoxime (analytical grade, purity ≥90%) were used as test drugs. The stem bark powder of *B. variegata* L. was used as an adjunct therapy.

Preparation of stem bark powder of *Bauhinia variegata* L.

The tree (*B. variegata* L.) having white colored flowers was identified and authenticated by Department of Botany, University of Calcutta. Barks were shade dried and made into powder.

Isolation and identification of *Staphylococcus aureus*

Staphylococcus aureus (strain J638) was isolated from the mastitic milk sample from a Jamunapari goat in mannitol salt agar (MSA, HiMedia, India), which was confirmed by colony characteristics in MSA, Gram-staining and standard biochemical tests such as catalase, oxidase, indole, Methyl Red, Voges–Proskauer, urease, carbohydrate fermentation, and coagulase test.[6]

Antibiotic sensitivity test

The coagulase positive *S. aureus* isolate (J638) was tested for its sensitivity to ceftriaxone, ceftizoxime, and aqueous solution of *B. variegata* L. stem bark powder by the disc diffusion method.[7]

Inclusion and exclusion criteria

Before the start of the experiment, the animals were acclimatized for 7 days. Only apparently healthy lactating black Bengal goats aged 1½-2 year weighing between 10 and 12 kg yielding about 170-200 ml milk/day and containing negligible quantity of *S. aureus* were included in this study. All the experimental procedures were conducted as per the guidelines of the Institutional Animal Ethical Committee (IAEC) (IAEC approval number: EC/94, dated June 24, 2011).

Induction and confirmation of chronic mastitis

Twelve clinically healthy lactating female black Bengal goats after 21st day of parturition were inoculated with 2000 CFU of locally isolated coagulase positive *S. aureus* strain (J638). The inoculated animals were not milked out during the first 3 days postinoculation. The animals were closely observed for the development of any clinical sign for 4 weeks.

Confirmatory tests were conducted such as somatic cell count (SCC), California mastitis test (CMT), bromothymol blue (BTB) paper test and milk enzyme activity at every 5 days interval up to 30 days postinoculation (day 51 after parturition) with bacterial colony count at 0th, 15th, and 30th day postinoculation (i.e. day 21, 36, and 51 after parturition). Histomorphological examination of the mammary gland was conducted on 30th day postinoculation to confirm chronic mastitis.

Fixation of dose of the bark powder

Prepared stem bark powder was administered orally once daily at 6 g/kg body weight mixing with distilled water for consecutive 14 days. Biochemical parameters such as alanine transaminase (ALT), aspartate transaminase (AST) activity, plasma urea nitrogen (PUN), and creatinine (CRT) level were also monitored during this period. The dose level at 6 g/kg body weight did not alter the ALT, AST, PUN, and CRT level significantly. Hence, the dose rate was considered as nontoxic. ALT and AST activity, PUN level, plasma CRT level was determined as per the method described by Yatzidis (1960),[8] Wooton (1974)[9] and Varly (1975).[10]

Efficacy study

Twelve experimentally induced chronic mastitic goats were randomly divided into two equal groups (Groups I and II). Group I mastitic goats were treated with intravenous ceftriaxone alone at 20 mg/kg body weight at 4 days interval, whereas Group II induced chronic mastitic goats were administered stem bark powder of *B. variegata* L. orally once daily at 6 g/kg body weight at 24 h interval consecutively for 7 days followed by 3 g/kg body weight (maintenance dose) orally once daily for next 7 days along with intravenous ceftriaxone at 20 mg/kg body weight at 4 days interval. The dosing of intravenous ceftriaxone was repeated when either the parent drug or its metabolite came to below the detectable level in milk of mastitic goats. Blood samples (2 ml) were collected at 0 (predosing), 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, and 144 h postdosing. Milk samples (2 ml) were collected from both the teats into the test tubes at 0 (predosing), 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312, 336, 360, 384, 408, and 432 h postdosing. One milliliter of milk or plasma was taken for estimation of drug concentration by high performance liquid chromatography. Fresh milk sample (10 μL) was used for SCC and rest of the samples was stored at −20°C for enzymatic assay.

Somatic cell count

The SCC of the milk samples were conducted at 48 h interval postdosing by modified method of Petersson *et al.* (2011) for direct microscopic SCC with pyronin - Y-methyl green stain.[11]

Bacterial colony count, California mastitis test, bromothymol blue test and milk enzyme activity

The colony count of *S. aureus* was conducted as per the standard technique.[6] CMT and BTB test were conducted as per Chakrabarti (2007).[12] Milk alkaline phosphatase (ALP), lactoperoxidase (LPO) activity and reduced glutathione (rGSH) level were estimated by the method described by Bernt, 1974,[13] Makinen and Tenovuo, 1982[14] and Pecker, 1994.[15]

High performance liquid chromatography analysis of plasma or milk

The extraction of the drugs (ceftriaxone and/or ceftizoxime) was done according to the modified method of Sar *et al*., 2011.[16] The mobile phase was prepared according to the method of United States Pharmacopoeia.

Histomorphological examination

Mammary gland tissues were collected from left and right half of the udder of chronic mastitis induced animals on 30 days postinoculation and after completion of treatment period from Group II animals with biopsy needle and fixed in 10% formalin.[17] The fixed tissues were further processed[18] and stained with Van-Gieson's stain for collagen fibers.[19]

Statistical analysis

The data were analyzed by paired *t*-test and *t*-test of independent sample assuming equal variance using SPSS 10.0 (Manufactured by SPSS Inc., USA).

RESULTS

Confirmation of chronic mastitis

Toward the end of the 2nd week, the milk became watery and negligible amount of milk yield from left teat was recorded in animals of both Groups I and II. However, the right half of the udder in both groups yielded reduced amount of milk [Table 1]. By 30th day postinoculation, shrinkage of both halves of the udder with marked hardness of the gland was noticed in all the induced mastitic goats with increased SCC [Table 2] and bacterial colony count [Table 3]. Milk ALP activity was increased significantly, while LPO activity was reduced by 30th day postinoculation. Histomorphological findings revealed profuse infiltration of polymorph nuclear cells along with excess proliferation of fibrous tissue [Figure 1a and b] in both half of the udder.

Antibiotic sensitivity test

The *S. aureus* (J638) isolate was found to be highly sensitive to ceftizoxime and intermediate sensitive to ceftriaxone. Whereas, aqueous extract of stem bark powder showed a zone of inhibition having a small diameter.

Plasma level of ceftriaxone and ceftizoxime

In Group II mastitic animals treated with repeated dose of intravenous ceftriaxone along with concurrent oral administration of stem bark powder, the concentration of the drug and its metabolite was increased, and their persistence also found to be prolonged [Table 4].

Milk level of ceftriaxone and ceftizoxime

The drug concentration could not be determined in the left half of Group I animals due to scanty milk secretion at all-time intervals. In the right half of the udder, ceftriaxone could not be detected in milk even after the second dose. Instead, only its metabolite that is, ceftizoxime was detected in milk at 24 h postdosing

Table 1: Average milk yield (ml) before and after intracisternal inoculation of *S. aureus* and during/after treatment in Group I animals (treated with intravenous ceftriaxone at 20 mg/kg at 4 days interval) and in Group II animals (treated with intravenous ceftriaxone at 20 mg/kg at 4 days interval with daily oral administration of stem bark powder of *Bauhinia variegata* L. for 14 days)

	Group I (antibiotic treated), *n*=6		Group II (herbal+ antibiotic treated), *n*=6	
	Left half	Right half	Left half	Right half
Before inoculation				
Day 15	177.50±2.47	182.16±1.35	191.00±2.11	187.33±2.89
Day 16	183.50±3.22	183.50±2.47	185.66±2.71	186.50±1.54
Day 17	189.66±1.76	187.83±3.52	183.83±3.56	189.66±2.84
Day 18	189.16±2.38	190.50±4.28	191.50±4.32	191.66±2.81
Day 19	185.16±3.53	194.50±2.82	191.33±2.44	190.16±1.77
Day 20	179.00±1.61	188.00±3.08	185.16±2.44	187.00±1.52
Day 21	185.50±1.60	190.66±1.64	183.66±3.25	185.00±1.86
After inoculation				
Day 22	182.66±2.78	187.83±3.04	188.16±1.51	187.83±3.04
Day 27	178±2.16	180.5±1.83	178.5±1.85	175.00±3.09
Day 32	108.83*±3.17	121.00*±5.44	100.33*±3.59	130.16*±3.71
Day 37	31.16*±2.93	51.16*±3.86	46.83*±5.22	60.50*±4.40
Day 42	ADYM	28.16*±1.93	21.33*±2.51	26.33*±2.26
Day 47	ADYM	14.50*±1.72	2.28*±0.27	11.16*±0.94
Day 52	ADYM	10.15*±1.19	2.21*±0.21	7.21*±0.56
During/after treatment				
Day 53	ADYM	7.66*±0.88	2.15*±0.16	9.10*±0.86
Day 54	ADYM	6.25*±0.30	2.13*±0.14	15.35*±2.43
Day 55	ADYM	6.91*±0.62	2.00*±0.09	14.18*±2.04
Day 56	ADYM^{2nd}	7.16*±0.86^{2nd}	2.08*±0.13^{2nd}	10.71*±0.66^{2nd}
Day 57	ADYM	9.20*±0.45	4.13*±0.23	15.70*±1.85
Day 58	ADYM	6.83*±0.47	5.46*±0.33	14.49*±0.82
Day 59	ADYM	5.75*±0.47	4.43*±0.24	14.05*±1.00
Day 60	ADYM	7.58*±0.30	4.88*±0.57^{3rd}	21.21*±2.20^{3rd}
Day 61	ADYM	5.00*±0.56	4.53*±0.26	18.88*±1.19
Day 62	ADYM	5.86*±0.33	4.50*±0.18	17.63*±0.64
Day 63	ADYM	5.91*±0.35	4.98*±0.33	18.01*±0.89
Day 64	ADYM	5.66*±0.61	4.90*±0.12^{4th}	19.65*±1.13^{4th}
Day 66	ADYM	5.91*±0.23	4.33*±0.73	16.21*±1.41
Day 67	ADYM	6.05*±0.36	4.73*±0.38	17.50*±0.80
Day 68	ADYM	3.73*±0.39	3.96*±0.24	16.48*±1.28
Day 69	ADYM	5.11*±0.30	3.93*±0.37	16.11*±0.75
Day 70	ADYM	4.95*±0.18	4.46*±0.23	17.35*±1.02

S. aureus: Staphylococcus aureus; B. variegata: Bauhinia variegata; n: Number of animals in each group; ADYM: Animal produced milk yield <0.5 ml. 2nd, 3rd, and 4th are the repetition of the doses of ceftriaxone at 20 mg/kg intravenously at 4 days interval. *Significantly (P<0.05) reduced milk yield compared to average milk yield of "day 21" before inoculation

Table 2: SCC (mean±SD×10^3 cells/ml) before and after intracisternal inoculation of *S. aureus* and during/after treatment in Group I animals (treated with intravenous ceftriaxone at 20 mg/kg at 4 days interval) and in Group II animals (treated with intravenous ceftriaxone at 20 mg/kg at 4 days interval with daily oral administration of stem bark powder of *Bauhinia variegata* L. for 14 days)

	Left half of the udder		Right half of the udder	
	Group I, *n*=6 (antibiotic)	Group II, *n*=6 (herbal+antibiotic)	Group I, *n*=6 (antibiotic)	Group II, *n*=6 (herbal+antibiotic)
Preinoculation				
Day 21	692.17±45.65	772.17±72.55	554.00±45.93	594.17±36.43
Postinoculation				
Day 51	ADYM	3948.17*±326.07	3786.66*±155.70	3453.00*±280.12
Postdosing/during treatment				
Day 53	ADYM	2781.17*±166.04	3639.67*±196.58	2714.50*±202.91
Day 55	ADYM	1832.50*±170.16	3257.67*±65.90	1582.50*±94.15
Day 57	ADYM^{2nd}	1168.00±110.30^{2nd}	2310.33*±216.70^{2nd}	999.67*±31.69^{2nd}
Day 59	ADYM	1035.83±151.82	1727.33*±135.25	865.83±31.52
Day 61	ADYM	959.33±25.18^{3rd}	1795.33*±141.91	974.33±35.84^{3rd}
Day 63	ADYM	861.33±17.40	1478.67*±120.04	841.33±23.04
Day 65	ADYM	940.33±64.11^{4th}	1026.67*±89.50	988.67±81.42^{4th}
Day 67	ADYM	1037.17±131.66	1253.33*±184.72	862.17±98.18
Day 69	ADYM	949.67±166.93	1448.83*±187.53	986.33±159.57
Day 71	ADYM	1185.17±236.94	1353.33*±217.19	918.50±75.65

SCC: Somatic cell count; *S. aureus: Staphylococcus aureus, n:* Number of animals in each group; SD: Standard deviation; *B. variegata: Bauhinia variegata;* ADYM: Animal produced milk yield <0.5 ml. 2^{nd}, 3^{rd} and 4^{th} are repetition of dose of ceftriaxone at 20 mg/kg intravenously. *Significantly (P<0.05) increased SCC compared to SCC of "day 21"

Table 3: Bacterial colony count (mean±SD×10^{10} cfu/ml) before and after intracisternal inoculation of *S. aureus* and during/after treatment in Group I animals (treated with intravenous ceftriaxone at 20 mg/kg at 4 days interval) and in Group II animals (treated with intravenous ceftriaxone at 20 mg/kg at 4 days interval with daily oral administration of stem bark powder of *Bauhinia variegata* L. for 14 days)

	Left half of the udder		Right half of the udder	
	Group I, *n*=6 (antibiotic)	Group II, *n*=6 (herbal+antibiotic)	Group I, *n*=6 (antibiotic)	Group II, *n*=6 (herbal+antibiotic)
Preinoculation				
Day 21	1.03±0.08	1.00±0.08	1.05±0.08	1.01±0.08
Postinoculation				
Day 36	11.83*±1.51	13.17*±0.79	9.67*±0.80	10.50*±0.67
Day 51	18.95*±0.78	15.67*±0.33	19.83*±0.90	28.33*±0.95
Postdosing/during treatment				
24 h	9.45*±0.78	8.33*±0.21	7.23*±0.23	6.50*±0.22
48 h	7.23*±0.24	1.83*±0.31	6.78*±0.15	1.33±0.21
96 h	6.5*±0.14	Nil	6.20*±0.25	Nil
144 h	5.5*±0.13	Nil	5.56*±0.16	Nil

*Significantly (P<0.05) increased bacterial colony count compared to bacterial colony count of "day 21". Nil: No colony; *n*: Number of animals in each group; SD: Standard deviation; *S. aureus: Staphylococcus aureus*

[Table 5]. Following the second dose at 96 h, ceftizoxime was again detected in milk from 120 h and was available up to 216 h, whereas in Group II animals, along with ceftizoxime, ceftriaxone was also detected in milk of both right and left half of the udder and persisted for a longer time [Table 5].

Milk somatic cell count

There was no significant improvement in mean SCC in Group I mastitic animals treated with ceftriaxone alone [Table 2]. Whereas in Group II animals, average SCC was found to be decreased in left and right half from day 57 to 59, respectively [Table 2].

Bacterial colony count

In Groups I and II animals, the mean bacterial colony count of both half of the udder was found to be decreased and in Group II no single colony was detected in the milk samples from 96 h postdosing in either half of the udder [Table 3].

Milk enzyme activity

The mean ALP activity, LPO activity and rGSH level were recorded to be in the range of 5872.35 ± 457.09–6438.14 ± 349.81 nmole ρNP/h/ml, 27942.92 ± 5426.64–39745.76 ± 2607.03 μmole/min/L and 344.40 ± 20.82–362.60 ± 7.84 ηmole GSH/ml, respectively. By 30th day postinoculation (day 51 after parturition) ALP activity and rGSH level were increased to 18778.67 ± 841.70–20628.99 ± 1958.53 nmole ρNP/h/ml and 394.30 ± 13.66–421.20 ± 18.99 ηmole GSH/ml, respectively, whereas LPO activity was decreased (18185.66 ± 1801.35–19886.57 ± 2846.29 μmole/min/L). In Group I animals treated with antibiotic alone, enzyme activity of the left half could not be determined due to almost cessation of milk while, in the right half, no significant change in ALP activity, LPO activity and rGSH level was noticed. In contrast, significant clinical improvement in animals of Group II has been evident from a significant reduction in ALP activity (8530.28 ± 957.97-9167.96 ± 532.05 nmole ρNP/h/ml) and remarkable increase in LPO activity (40711.06 ± 3935.50-40991.26 ± 3904.79 μmole/min/L) by 18th day postdosing (day 71 after parturition).

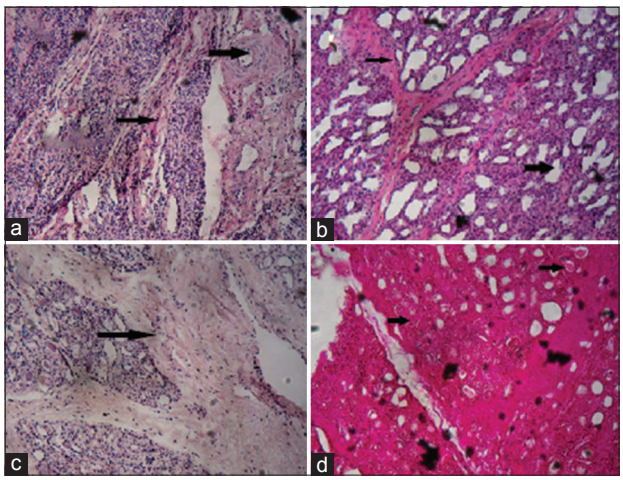

Figure 1: (a) Mammary gland tissue (left half) after 30th day postinoculation revealing the presence of excess proliferation of fibrous tissue with disorganization of alveolar structures (×40), (b) mammary gland tissue (right half) after 30th day Postinoculation showing extensive fibrous tissue proliferation (×40), (c) mammary gland tissue (left half) after treatment exhibiting reduction of fibrous tissue (×40) in Group II, (d) mammary gland tissue (right half) after treatment revealing toward healthy glandular structures with little fibrous tissue surrounding (×40) in Group II

California mastitis test

California mastitis test was found positive up to 144 h postdosing following first and second dose of ceftriaxone in Group I animals and in Group II animals, CMT was found negative in milk samples of 96 h postdosing and onwards from either half of the udder.

Clinical signs after treatment

The treated animals of Group I showed shrinkage of the left half with marked hardness and a negligible amount of

Table 4: Mean concentration (μg/ml) of (a) ceftriaxone and (b) ceftizoxime in Group I animals (treated with intravenous ceftriaxone at 20 mg/kg at 4 days interval) and in Group II animals (treated with intravenous ceftriaxone at 20 mg/kg at 4 days interval with daily oral administration of stem bark powder of *Bauhinia variegata* L. for 14 days) in plasma

Ceftriaxone (μg/ml)			Ceftizoxime (μg/ml)		
Time (h)	Group I, *n*=6 (antibiotic)	Group II, *n*=6 (herbal+antibiotic)	Time (h)	Group I, *n*=6 (antibiotic)	Group II, *n*=6 (herbal+antibiotic)
0.08	76.57^{b}±6.33	91.17^{a}±2.16	0.08	BDL	BDL
24	0.48^{b}±0.26	7.50^{a}±1.32	24	0.45±0.04	0.46±0.03
48	BDL	6.38±1.16	48	0.38^{b}±0.03	1.51^{a}±0.16
72	BDL	5.07±1.14	72	BDL	1.26±0.19
96	BDL^{2nd}	3.94±0.85^{2nd}	96	BDL^{2nd}	0.50±0.09^{2nd}
96.08	83.18^{b}±4.76	154.83^{a}±5.19	120	0.33^{b}±0.03	0.47^{a}±0.06
120	13.71^{b}±0.33	57.55^{a}±8.92	144	0.53^{a}±0.03	0.41^{b}±0.008
144	3.11^{b}±0.19	15.25^{a}±1.80	168	0.19^{b}±0.015	0.91^{a}±0.04
168	BDL	1.63±0.16	192	BDL	BDL^{3rd}
192	BDL	BDL^{3rd}	216	BDL	3.38±0.15
192.08	BDL	61.73±4.56	240	BDL	0.57±0.05
216	BDL	1.83±0.17	264	BDL	0.70±0.02
240	BDL	BDL	288	BDL	0.36±0.009^{4th}
264	BDL	BDL	312	BDL	0.48±0.01
288	BDL	BDL^{4th}	336	BDL	1.87±0.08
288.08	BDL	55.13±3.45	360	BDL	0.32±0.007
312	BDL	3.82±0.20	384	BDL	BDL
336	BDL	BDL	408	BDL	BDL

Means in a row bearing unlike superscript indicates significant difference at 5% level (P<0.05). 2nd, 3rd, and 4th are repetitions of dose of ceftriaxone at 20 mg/kg intravenously. BDL: Below detectable level; *B. variegata: Bauhinia variegata*

Table 5: Mean concentration (μg/ml) of (a) ceftriaxone and (b) ceftizoxime in Group I animals (treated with intravenous ceftriaxone at 20 mg/kg at 4 days interval) and in Group II animals (treated with intravenous ceftriaxone at 20 mg/kg at 4 days interval with daily oral administration of stem bark powder of *Bauhinia variegata* L. for 14 days) in milk

Time (h)	Ceftriaxone				Ceftizoxime			
	Left half		Right half		Left half		Right half	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
0.08	ADYM	BDL	BDL	BDL	ADYM	BDL	BDL	BDL
24	ADYM	3.68±0.57	BDL	4.90±0.44	ADYM	0.12±0.01	2.82^{a}±0.43	0.29^{b}±0.09
48	ADYM	1.30±0.20	BDL	2.24±0.35	ADYM	0.02±0.003	BDL	0.30±0.03
72	ADYM	0.22±0.04	BDL	1.10±0.06	ADYM	0.63±0.08	BDL	1.26±0.26
96	ADYM^{2nd}	BDL^{2nd}	BDL^{2nd}	BDL^{2nd}	ADYM^{2nd}	BDL^{2nd}	BDL^{2nd}	BDL^{2nd}
120	ADYM	7.51±0.23	BDL	9.80±0.38	ADYM	0.79±0.03	2.04^{b}±0.20	3.79^{a}±0.12
144	ADYM	17.03±0.38	BDL	21.94±0.92	ADYM	1.69±0.14	2.08^{b}±0.21	11.57^{a}±0.69
168	ADYM	2.72±0.24	BDL	4.56±0.26	ADYM	0.09±0.03	2.36^{b}±0.07	5.63^{a}±0.26
192	ADYM	BDL^{3rd}	BDL	BDL^{3rd}	ADYM	BDL^{3rd}	1.06^{a}±0.04	0.25b±0.005^{3rd}
216	ADYM	14.72±0.62	BDL	25.12±0.43	ADYM	5.72±0.24	0.60^{b}±0.08	5.63^{a}±0.11
240	ADYM	2.15±0.29	BDL	1.92±0.11	ADYM	4.51±0.18	BDL	3.72±0.18
264	ADYM	0.28±0.003	BDL	BDL	ADYM	0.57±0.22	BDL	0.65±0.18
288	ADYM	BDL^{4th}	BDL	BDL^{4th}	ADYM	BDL^{4th}	BDL	BDL^{4th}
312	ADYM	14.05±1.07	BDL	15.78±1.05	ADYM	18.91±0.60	BDL	14.04±0.79
336	ADYM	12.06±0.46	BDL	7.32±0.13	ADYM	15.97±0.66	BDL	9.07±0.06
360	ADYM	2.14±0.07	BDL	1.36±0.07	ADYM	0.85±0.04	BDL	0.15±0.01
384	ADYM	BDL	BDL	BDL	ADYM	0.27±0.008	BDL	BDL

Means in a row bearing unlike superscript indicates significant difference at 5% level (P<0.05). 2nd, 3rd and 4th are repetitions of dose of ceftriaxone at 20 mg/kg intravenously. ADYM: Animal produced milk yield<0.5 ml; *B. variegata: Bauhinia variegata*; BDL: Below detectable level

milk production [Table 1]. However in Group II animals, following treatment milk secretion increased two-fold in both left and right half of the udder [Table 1].

Histomorphological examination

Histomorphological study following completion of treatment revealed reduction of the fibrous tissue in the left half of the udder [Figure 1c] whereas in the right side, more amount of columnar epithelial cells with multiple alveoli were found showing progress toward normal glandular structure of the mammary gland [Figure 1d] in Group II.

DISCUSSION

In this study, SCC varied between 554.00 ± 45.93 and 772.17 ± 72.55 × 10^3 cells/mL in milk of healthy goats prior to challenge, which was increased significantly by 30th day postinoculation of *S. aureus* [Table 2]. These findings corroborated with the findings of Pettersen (1981)[20] and Kozacinski *et al.* (2002).[21] Bacterial colony count confirmed increased number of *S. aureus* on 30th day postchallenge [Table 3]. Declined milk yield and significant increase (P < 0.05) in ALP activity in milk from both half of the udder in all the groups inoculated with *S. aureus* along with decrease in LPO activity is an indication of mastitis.[22] Histomorphological examination exhibited excess proliferation of fibrous tissue in both half of the udder [Figure 1a and b] indicating chronic mastitis.

The concentration of ceftriaxone/ceftizoxime in plasma was significantly higher (P < 0.05) in the Group II animals compared to the Group I animals which indicated bio-enhancing effect of *B. variegata* Linn. Both ceftriaxone and its metabolite were detected in milk samples of Group II animals for a longer time and with a considerably higher concentration, which substantiated the possible bio-enhancing action of the bark powder.

Significant decrease in SCC from 96 h postdosing in the left half and from 144 h postdosing in the right half in Group II animals during treatment compared to SCC in chronic mastitic condition along with a significant reduction in the bacterial colony count indicated both antiinflammatory and enhanced antibacterial activity of intravenous ceftriaxone in presence of stem bark powder of *B. variegata* L. It was further supported by the results of CMT.

In all animals of both Groups I and II, postchallenge milk ALP activity was increased significantly (P < 0.05), which suggested substantial tissue damage in mammary gland.[22] Synthesis of LPO was interfered in these goats due to substantial tissue damage, which was also evident from significantly reduced LPO activity in mastitic

condition. A significant increase in LPO activity with a marked reduction in milk ALP activity in Group II animals suggested possible recovery of the animals from chronic mastitis. Hence, it can be concluded that stem bark powder of *B. variegata* L. as an adjunct therapy potentiated the antibacterial effect of intravenous ceftriaxone. The milk production was also increased in Group II animals following treatment compared to Group I animals, which received antibiotic only.

Histomorphological evaluation exhibited marked a reduction of the fibrous tissue in the left half and more number of active columnar epithelial cells in the right half of the udder in Group II animals, which indicated antifibrotic activity of *B. variegata* L.

CONCLUSION

Hence, bark powder of *B. variegata* L., having bioenhancing, antibacterial, antiinflammatory, antioxidant and antifibrotic properties might be an effective adjunct therapy with antibiotic treatment in chronic mastitis.

ACKNOWLEDGMENT

We acknowledge Dr. Katherine Petersson, Assistant Professor, University of Rhode Island, Department Fisheries, Animal and Veterinary Science, Kingston, USA for his generous assistance during this study.

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Cite this article as: Dash JR, Sar TK, Samanta I, Pal S, Khan M, Patra NC, Sarkar U, Maji AK, Mandal TK. Efficacy evaluation of *Bauhinia variegata* L. stem bark powder as adjunct therapy in chronic *Staphylococcus aureus* mastitis in goat. Phcog Mag 2014;10:512-8.

Source of Support: Nill, **Conflict of Interest:** None declared.