Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 125-129
Received: 16-03-2019
Accepted: 18-04-2019

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Therapeutic efficacy of aerosolized moxifloxacin in respiratory tract infection in goats

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Abstract
A total of 24 goats age between 3 months to 4 years used in study were selected on the basis of clinical parameters. Deep nasal swabs collected for bacterial culture and sensitivity reviled more isolates of coagulase positive *Staphylococcus* spp. and maximum sensitivity to moxifloxacin. Goats were assigned to three groups of eight goats each. Group I was healthy control, group II treated with moxifloxacin @ 5 mg/kg body weight given intramuscularly and group III was treated with aerosolized moxifloxacin @ 5 mg/kg body weight given through nasal route. Hematological profile and oxidative stress indices were used for comparative efficacy of nebulizer therapy with standard therapy and parameters were compared to the healthy controls. There was improvement in the parameters (p<0.05) in group III than with group II. We concluded that aerosolized moxifloxacin @ 5 mg/kg body weight can be used as alternative therapeutic modality for respiratory tract infection in goats.

Keywords: Aerosolized, respiratory tract infection, moxifloxacin, haemato-biochemical, oxidative stress indices

1. Introduction
Small ruminants play an important role in nutrition and income of people around the world. They serve primarily as source of meet; also provide milk, skin and wool (Mbilu, 2007) [14]. Farm animals are commonly affected by respiratory tract infections (Mohamed and Abdelsalam, 2008) [16]. The respiratory affection is complex involving stress factors, bacteria and virus infection. Bacterial pneumonia is regarded as the most frequent and serious causes of mortality and economic losses associated with respiratory diseases (Andrawis, 2001) [3]. It is characterized by fever (40-41ºC), anorexia, painful coughing, dyspnea, mucopurulent nasal discharge and depression. It is one of the most common respiratory illnesses in goats throughout the world (Ackermann and Brogden, 2000) [1]. The severity of infection can be effectively reduced by use of antimicrobials, whch are generally given by oral and/or parenteral route (intramuscular, intravenous). The disadvantage of this treatment regimen is irritation at the site of delivery, systemic adverse side effect and long withholding period before consumption of meat and milk (Sustronck *et al*., 1995) [30]. By ananlysing theses problems, the application of aerosol therapy could provide an alternative therapeutic modality for treatment of respiratory tract infection. Aerosolized drug may maximize efficacy and reduce the adverse side effect of antibiotic resistance (Dalhoff, 2014) [7] and less systemic availability (Koning, 1985) [12].
Several studies have demonstrated the efficacy of aerosol therapy with bronchodilators and antimicrobial in respiratory disorders in cattle (Nuytten *et al* 1986, Desmecht *et al* 1992) [19, 8], but information regarding aerosol therapy for treating respiratory infection in goat is unavailable. Aerosol therapy plays an increasing role in the management of respiratory disorders. This alternative to systematic treatment permits a high concentration of medication to act locally while minimizing side effects and residues. In human medicine, literature in this field is prolific and continuously renewed, whereas in veterinary medicine, applications of aerosol therapy are less extensive. The present study was conducted to compare the therapeutic efficacy of moxifloxacin for the therapeutic management of respiratory tract infection administered intramuscularly and aerosolized form.

2. Materials and methods
2.1 Study animals
All the goats included in study were Indian non-descript breed reared at sheep and goat farm, Indian Veterinary Research Institute, Izatnagar. The selection criterion of goats was based on a thorough clinical examination performed on all the naturally infected animals.

Goats were visually evaluated for nasal or ocular discharge, anorexia and depression. When two or more of these signs were observed, body temperature was recorded. Deep nasal swabs, blood samples and sera from the cases were used in the present study, which were further analyzed by laboratory examination.

2.2 Chemicals and Reagents
Chemicals and reagents used in this study were of Hi-Media (Mumbai, India), Span Diagnostic Ltd (Surat, India).

2.3 Haemato-biochemical assays
The hematological estimation of whole blood was done by standard methods as described by Coles, 1986 [6] for Hb, PCV, WBC and DLC. Erythrocytic lipid peroxides (LPO) were estimated as procedure followed by Placer *et al*. (1966) [23]. The concentration of malonaldehyde (MDA) in nanomoles per millilitre of erythrocytic hemolysate was derived using 1.56×10^5 L mol/cm as extinction coefficient (Utley *et al*., 1967) [33]. The Hb in the hemolysate was measured by the cyanmethemoglobin method and the LPO concentration in the erythrocytes was expressed in nmol MDA/mg Hb. The superoxide dismutase (SOD) activity was determined by the method of Minami and Yoshikawa (1979) [15] which is modified method given by Marklund and Marklund (1974) [13]. Blood samples were collected prior to treatment and also on 5th and 10th day of post-treatment.

2.4 Isolation and identification of bacteria
Deep nasal swabs were inoculated into brain- heart infusion (BHI) broth and incubated aerobically at 37 °C for 12 hours. A loopful of broth inoculum was streaked on blood agar plates (blood agar base supplemented with 7% sheep blood agar) and MacConkey's agar. Plates were incubated at 37 °C aerobically for 24-48 hours. After taking note of cultural growth characteristics, positive cultures were subjected to Gram's staining procedure for a broad classification of present bacteria in Gram positive and Gram negative. Pure culture of single colony type from both blood and McConkey agars were further transferred onto BHI agar slants for a series of standard biochemical tests for final identification by using the standard procedures defined by Quinn and co-workers (Quinn *et al*., 2004) [24].

2.5 Antibiotic Susceptibility Testing
The antibiotic sensitivity profile of recovered bacterial isolates was carried out as per the guidelines provided by Clinical Laboratory Standards Institute (CLSI, 2014). In brief, the bacterial isolates obtained in the present study were subjected to Disc diffusion assay as per the method described by Bauer *et al*. (1966) [4].

2.6 Therapeutic design
Group I healthy control animals were not given any treatment. Naturally infected goats in Group II were treated with standard therapy as moxifloxacin (Mofoi® Bovian Healthcare Pvt, India) @ 5 mg/kg body weight given intramuscularly. Group III naturally infected goats were treated with moxifloxacin (Mofoi® Bovian Healthcare Pvt., India) @ 5mg/kg body weight in aerosolized form administered intranasal using Info neb jet nebulizer. Meloxicam (Melonex®, Neovet Intas, India) @ 0.5 mg/kg body weight and Pheniramine maleate (Avil® Intervet India Pvt Ltd) @ 0.5 mg/kg body weight administered intramuscularly as common adjunctive therapy in group II and group III. Therapy was given till recovery of animals.

Fig 1: Goat with sign of nasal discharge

Fig 2: Goat receiving aerosl therapy

2.7 Data analysis
Data were analyzed by two way analysis of variance (ANOVA) using SPSS statistics 20.

3. Results and Discussion
3.1 Bacteria isolated from goats with respiratory disease
Amongst 16 clinical cases of respiratory tract infection in goats a total of 23 bacterial isolates were identified which included 16 bacterial isolates belonging to Gram's positive group and 7 bacterial isolates belonging to Gram's negative group. It was observed that out of total 23 isolates, 11 (47.83%) were coagulase positive *Staphylococcus* sp., 5 (21.47%) were coagulase negative *Staphylococcus* sp., and 7 (30.43%) were *E. coli*.

3.2 *In vitro* antimicrobial sensitivity
The antimicrobial sensitivity pattern in the identified 23 bacterial isolates were tested against the antibiotic such as moxifloxacin (MO, 5µg), gentamicin (G, 10 µg), enrofloxacin (EX, 5µg), Ceftriaxone (CTR, 30µg), Amoxyclav (AMC, 30µg) and Co-Trimoxazole (COT, 25µg) by the standard disc diffusion technique. 42.86% were found sensitive to Co-Trimoxazole. Overall 100% bacterial isolates were sensitive to moxifloxacin, 82.60% were sensitive to enrofloxacin, 69.57% were sensitive to amoxyclav and ceftriaxone and 60.86% were sensitive to gentamicin and Co-Trimoxazole.

3.3 Haematology in response to therapy

Parameters	Group	Period(in day)		
		Day 0	**Day 5**	**Day 10**
Hb (g %)	I	9.66 ± 0.13^{aB}	9.7 ± 0.13^{aB}	9.69 ± 0.13^{aA}
	II	8.75 ± 0.24^{aA}	8.89 ± 0.20^{aA}	9.31 ± 0.15^{aA}
	III	8.53 ± 0.34^{aA}	8.68 ± 0.32^{aA}	9.24 ± 0.27^{aA}
PCV (%)	I	27.54 ± 0.38^{aB}	27.70 ± 0.35^{aB}	27.71 ± 0.35^{aA}
	II	25.02 ± 0.70^{aA}	25.33 ± 0.58^{aA}	27.44 ± 0.43^{bA}
	III	24.31 ± 0.98^{aA}	24.75 ± 0.92^{aA}	26.32 ± 0.78^{aA}
TLC (per/µL)	I	9918.75 ± 151.46^{aA}	9899.88 ± 139.48^{aA}	9906.25 ± 144.68^{aA}
	II	17218.75 ± 963.67^{cB}	14501.25 ± 686.54^{bC}	11192.50 ± 553.94^{aB}
	III	17090 ± 953.11^{cB}	11947.50 ± 408.90^{bB}	9912.5 ± 358.41^{aA}
Neutrophil (%)	I	36.63 ± 1.6^{aA}	36.88 ± 1.3^{aA}	37 ± 1.1^{aA}
	II	69.25 ± 2.06^{cB}	57.25 ± 1.44^{bB}	42.13 ± 1.82^{aB}
	III	68.88 ± 1.87^{cB}	52 ± 1.21^{bC}	38.75 ± 0.94^{aB}
Lymphocyte (%)	I	57.75 ± 1.13^{aB}	$57.5 \pm 1.13^{a,C}$	57.5 ± 1.04^{aA}
	II	25.13 ± 1.8^{aA}	37.63 ± 1.02^{bA}	53.75 ± 2.1^{cA}
	III	25.13 ± 1.69^{aA}	44.25 ± 1.08^{bB}	57.75 ± 0.9^{cA}
Monocyte (%)	I	3.75 ± 0.68^{aA}	3.75 ± 0.56^{aA}	3.63 ± 0.57^{aA}
	II	4.38 ± 0.68^{aA}	4 ± 0.65^{A}	3 ± 0.27^{aA}
	III	4.5 ± 0.46^{bA}	$2.75 \pm 0.37^{a,A}$	2.5 ± 0.27^{aA}
Eosinophil (%)	I	1.88 ± 0.23^{aA}	1.88 ± 0.35^{aA}	1.88 ± 0.52^{aA}
	II	1.25 ± 0.25^{aA}	1.13 ± 0.23^{aA}	1 ± 0.27^{aA}
	III	1.5 ± 0.33^{aA}	1 ± 0.33^{aA}	1 ± 0.27^{aA}

[Superscript small alphabet- different sign shows that value differs significantly ($p<0.05$) row wise, capital alphabet- different sign shows that value differ significantly ($p<0.05$)]

3.4 Oxidative stress indices in response to therapy

Parameters		Period (in day)		
		0	**5**	**10**
LPO (nmol MDA/mg Hb)	I	3.67 ± 0.09^{aA}	3.70 ± 0.09^{aB}	3.70 ± 0.08^{aA}
	II	4.75 ± 0.11^{cB}	4.38 ± 0.10^{bA}	4.10 ± 0.12^{aB}
	III	4.79 ± 0.11^{bB}	4.21 ± 0.07^{aA}	3.72 ± 0.06^{aA}
SOD (U/mg Hb)	I	5.89 ± 0.10^{aB}	5.90 ± 0.10^{aB}	5.91 ± 0.08^{aB}
	II	4.41 ± 0.22^{aA}	4.71 ± 0.25^{aA}	4.95 ± 0.31^{bA}
	III	4.39 ± 0.22^{aA}	4.90 ± 0.18^{aA}	5.88 ± 0.10^{bB}

Superscript small alphabet- different sign shows that value differs significantly ($p<0.05$) row wise, capital alphabet- different sign shows that value differ significantly ($p<0.05$)

The majority of respiratory tract infections are endogenous, caused by the normally resident bacteria on the upper respiratory tract, although exogenous infections can also occur by direct contact with sick animals or through infected aerosols. In our present study amongst 16 naturally infected goats a total of 23 bacterial isolates were identified which included 16 bacterial isolates belonging to Gram positive group and 7 bacterial isolates belonging to Gram negative group. It was observed that out of total 23 isolates, 11 (47.83%) were coagulase positive *Staphylococcus* sp., 5 (21.47%) were coagulase negative *Staphylococcus* sp, and 7 (30.43%) were *E. coli*. Similar bacterial isolation were reported by Vihan and Singh (1992) [34] and Aher *et al*. (2013) [2]. Yimer and Asseged (2007) [35] also reported *Staphylococcus* spp, *E. coli*, in the respiratory tract of sheep. Bacterial isolation in our study is in agreement with the findings of Oros *et al*. (1997) [20], Ozbey and Muz (2004) [21] and Shafarin *et al*. (2007) [27]. The isolation of *S. aureus* from the nasal passage is consistent with other findings obtained from goat by Ugochukwu (1985) [32]. Robbins *et al*. (1981) reported that *S. aureus* resides in the upper respiratory tract and is involved in disease, processes only when stress conditions prevail. The detection of *E. coli* in nasal samples of goats is also consistent with findings by Pelczar *et al*. (1986) [22]. Overall 100% bacterial isolates were sensitive to Moxifloxacin, 82.60% were sensitive to Enrofloxacin, 69.57% were sensitive to Amoxyclav and Ceftrixone and 60.86% were sensitive to Gentamicin and Co-Trimoxazole. In the present study we found that mean Hb, PCV, Lymphocyte counts and A:G ratio were significantly reduced and mean TLC, Neutrophil, were significantly increased in diseased animals as compared to healthy control. Subacute to chronic bacterial disease causes a significant increase in TLC and neutrophil count (Smith, 2009) [29]. Our findings were supported by previous reports from Mondal *et al*., 2004 [17]. Chronic inflammatory disease causes mild to moderate anemia (non-regenerative anemia) in all species of large animals (Kaneko *et al*., 2008) [11]. TLC and DLC are more reliable indications for the presence of the lesion than clinical assessment (Radostits *et al*., 2007) [25]. Free radicals are produced continuously by normal metabolic processes, but their rate of production increases during certain inflammatory or other disease conditions (Bernabucci *et al*., 2005) [5]. Oxidative stress occurs either due to excess production of free radicals or inadequate availability of antioxidants or a combination of both. Free radicals and oxidative stress has been reported to contribute development of inflammation (Trouba *et al.*, 2002) [31] also impair immune system function (Sies, 1991) [28]. Malonyldialdehyde concentration assess the degree of peroxidative damage to cell membrane, as it is the most abundant aldehyde formed as a by-product during this process (Gurer *et al*., 1998) [10]. Due to abundance of

polyunsaturated fatty acids and presence of powerful transition-metal catalyst erythrocytes are highly susceptible to peroxidative damage (Ranjan *et al*., 2005) [26]. SOD catalyses the formation of O_2 from reactive oxygen species. A coproduct of SOD activity is H_2O_2, which is converted to H_2O by catalase (Fang *et al*., 2002) [9].

The total leukocyte counts of infected goats with nebulizer therapy were significantly lower than the standard therapy by day 5th post treatment. Neutrophil count decreased significantly in goats treated with nebulizer therapy as compared to standard therapy by day 5th post treatment. Lymphocyte count was increased significantly in goats treated with nebulizer therapy as compared to goats treated with standard therapy by day 5th post treatment. In respiratory tract infections, neutrophils are involved in removal of invading microorganisms by their phagocytic activity. However, in some cases of excessive response of neutrophils results in a number of tissue damaging products. A significant reduction in LPO in goats treated with nebulizer therapy as compared to goats treated with standard therapy by day 10 post treatment. A significant increment in SOD was observed in goats treated with nebulizer therapy as compared to goats treated with standard therapy day 10 post treatments. Sustronck *et al*. (1995) [30] reported better therapeutic efficacy of antibiotic for treatment of respiratory disease through aerosolized ceftofur. Neiderman *et al*. (2012) [18] opinioned that aerosolized antibiotic has better efficacy in amelioration of respiratory disease since it facilitates high concentration of the delivered drug at the site of action.

Conclusion

Respiratory tract infection in goats causes marked changes in haematological and produces oxidative stress. Aerosolized moxifloxacin can be used efficiently to treat respiratory tract infection and can be used as an alternative therapeutic modality.

Acknowledgement

The authors would like to thank Joint Director, I.V.R.I. and Incharge, Sheep and goat farm Livestock Production management I.V.R.I. for providing research facility.

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