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Comparative efficacy of mucolytic agent (Acetyl cysteine) as nebulizer in conjunction with parenteral antibiotics against bacterial pneumonia in goats

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Abstract
In the present study, twenty-four goats suffering from bacterial pneumonia presented to Veterinary Clinical Complex, Veterinary College, Bidar from August 2016 to July 2017 were randomly divided into four groups consisting six in each. Group-1 was treated with Inj. Marbofloxacin @ 2 mg/kg b. wt./day for 5 day, Group-2 treated with Inj. Marbofloxacin @ 2 mg/kg b. wt./day for 5 day with nebulization of mucolytic agent (Acetyl cysteine)-2ml, for 30 mins/day for 3 days, Group-3 treated with Inj. Ceftiofur sodium @ 2 mg/kg b. wt./day for 5 day and Group-4 treated with Inj. Ceftiofur sodium @ 2 mg/kg b. wt./day for 5 day with nebulization of mucolytic agent (Acetyl cysteine)- 2 ml, for 30 mins/day for 3 days. To evaluate the efficacy of drugs alone or in conjunction with mucolytic agent as nebulizer, the blood was collected on day "0" and 5th day of post treatment from all the goats and both haematological and blood gas parameters were critically analysed. The results revealed that faster recovery in pulmonary function was observed in Group-2 (Marbofloxacin and Acetyl cysteine) followed by Group-4, Group-1 and Group-3. Thus, mucolytic agent (Acetyl cysteine) as a nebulizer in conjunction with parenteral antibiotic may be recommended as first line of treatment in bacterial pneumonia in goats.

Keywords: Goats, bacterial pneumonia, pulmonary function, mucolytic agent and nebulization

Introduction
Pneumonia evolves due to deterioration of pulmonary function through colonization of bacteria. To improve the pulmonary function in bacterial pneumonia cases many have used Penicillin, Ampicillin, Tetracycline, Gentamicin, Tyrosine, Ceftiofur sodium, Marbofloxacin, Florfenicol etc., (Hussein *et al.*, 2006; Balikci *et al*., 2008 and Skoufos *et al*. 2007) [6, 2, 22] alone or in combination with antihistamines, bronchodilators, steroids and mucolytic agents as supportive therapy. However, little data to indicate which treatment is optimal to enhance pulmonary function in bacterial pneumonia. Recently in the therapeutic modalities, nebulisation therapy was effectively used in treatment of respiratory diseases in different domestic animals (Sustronck *et al.*, 1995; Peter, 2008 and Joshi *et al.*, 2017) [20, 15, 7]. Hence, the present study envisaged to assess the efficacy of mucolytic agent (Acetyl cysteine) as nebulizer in conjunction with parenteral antibiotics against bacterial pneumonia in goats.

Material and Methods
Selection and grouping of goats
In the present study, a total of twenty-four goats (N=24) suffering from bacterial pneumonia presented to the Department of Veterinary Clinical Complex, Veterinary College, Bidar from August 2016 to July 2017, were selected randomly. For initial assessment all the cases were examined for pulmonary function as per the standard pulmonary function score card for inclusion or exclusion (Christodoulopoulos *et al.* (2002) [23]
The observations on certain pulmonary function parameters such as rectal temperature, conjunctival mucous membrane, respiratory rates, heart rates, lung auscultation, nasal discharge, ocular discharge, sneezing, coughing and feed intake were made by clinical and physical examination of bacterial pneumonia in goats and were scored accordingly as score 1, 2 and 3 which was compared with healthy goats. Later all the goats were allocated into four groups consisting six in each (n=6).
All the four groups (G-1, G-2, G-3 and G-4) were divided according to the use of antibiotics (Marbofloxacin and Ceftiofur sodium) with or without mucolytic agent (Acetyl cysteine) as

nebulizer to treat bacterial pneumonia in goats with common supportive therapy (Chlorpheneramine maleate, Meloxicam) as presented in table-1.

Nebulization

For nebulization, the apparatus called Compact nebulizer piston type (HOME MEDIX) was used with slight modification for the treatment of pneumonic goats with mucolytic agent (plate 1). The apparatus has capacity for medication of drug was 6ml, particle size was <5µm, >60% particle reached the alveoli, mass median aerodynamic diameter (MMAD) 3.5 µm, average nebulization rate 0.2 ml/min. The 2 ml of water soluble acetyl cysteine taken in a sterile syringe and mixed properly with 4 ml of distilled water and pour it in the nebulization cup and nebulization therapy given for 30 minutes once a day for three days.

Haematological and blood gas analyses

To evaluate the efficacy of drugs (Marbofloxacin and Ceftiofur sodium) alone or in conjunction with mucolytic agent (Acetyl cysteine) as nebulizer, the 2 ml whole blood was collected in K_3EDTA vials on day 0 and 5th day of post treatment from all the goats and both haematological and blood gas parameters were analysed and presented in table 2 and 3.

Haematological parameters including total erythrocyte count (TEC), Total leukocyte count (TLC), Packed cell volume (PCV), Haemoglobin content (Hb), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentrations (MCHC) were recorded using automatic blood cell counter (ERMA INC, PCE-210, Tokyo) and Differential leucocytic count (DLC) was done with Giemsa staining technique as per standard protocol.

The blood gas parameters such as venous partial pressure of carbon dioxide ($VpCO_2$), venous partial pressure of oxygen (VpO_2), venous Actual bicarbonate ion ($VHCO_3A$), venous total carbon dioxide content ($VtCO_2$) and venous base excess (VBE), venous hydrogen ion concentration (VH^+) and venous oxygen saturation (VO_2 sat) were recorded using blood gas analyser (ESCHWEILER, combi line, machine, CL1241, Germany)

Haematological and blood gas parameters recorded on '0' day and 5th day in each group were compared and critically analysed. After treatment recovery pathway was assessed based pulmonary function score recorded throughout the course of treatment in all the goats (Table 4).

Statistical analysis

The haematological and blood gas values obtained on day '0' and day '5' were analysed by two-way ANOVA using SPSS version 20. The results were demonstrated as means±SE. The results were considered statistical significant when $p \leq 0.05$.

Results and Discussion

Pneumonia is an inflammation of lung caused by mainly virus, bacteria and less commonly by other micro-organisms. The amount and type of bacteria involved determines the inflammatory reaction in airway spreading from upper respiratory tract to lower respiratory tract. In the present scenario increased morbidity and mortality due to bacterial pneumonia in goats common in rearing farmers. So, to reduce the economic loss due to death, there have been changes in the management of respiratory infection in recent years. Nebulisation is one such therapeutic modality that is used as a primary mode of drug delivery to the lungs (Mandal *et al.* 2016) [9]. Therefore, present study focus on the efficacy of acetyl cysteine which is water soluble mucolytic agent which mainly acts by liquefying the thickness of mucus and making airway passage clear (Bruce 2007) [1]. Airway inflammation has central role in the disease process and its progress.

A total of twenty four (N=24) bacterial pneumonia goats were selected for therapeutic trail and divided into four groups. They were treated with parenteral antibiotic (Marbofloxacin and Ceftiofur sodium) with or without conjunction of mucolytic agent along with supportive therapy. Hence, efficacy of acetyl cysteine as a nebulizing agent in conjunction with different parenteral antibiotics to reduce the airway inflammation was assessed by recording the pulmonary functions and also by analysing the haematological and blood gas parameters in goats.

Pulmonary functions

Pulmonary scoring was carried out by recording the certain pulmonary function parameters Viz., respiratory rates, heart rates, rectal temperature, conjunctival mucous membrane, lung auscultation, nasal discharge, ocular discharge, and sneezing, coughing and feed intake among goats suffering bacterial pneumonia and were compared with apparently healthy goats. The study suggests that goats suffering bacterial pneumonia were showing score-1 to 3 when compared to score-0 of apparently healthy goats.

Haematological examination

The haematological changes recorded before and after the treatment in the goats suffering from bacterial pneumonia (G-1, G-2, G-3 and G-4) and compared with apparently healthy control group of goats.

Haemogram

Alterations in the haemogram values suggests that, the mean and standard error (Mean±SE) TEC, HB and PCV level were found to be non-significant ($p \leq 0.05$) difference in before and after treatment groups of bacterial pneumonia in comparison with control group. The results of present study were in accordance with reports of Naik *et al.* (1991) [13] and Ole-mapenay and Mitema (1997) [14]. However, in contrast decreased in haemoglobin values, TEC and PCV have been reported in goats by Sadeghiana *et al*., 2011 and Ghanem *et al.*, 2015b) [16, 4] suffering from pneumonic condition.

Erythrocytic indices

The mean and standard error (Mean±SE) of MCV, MCH and MCHC in the present study showed no significant ($p \leq 0.05$) difference between before and after treatment except in the group-2, MCV value differ significantly ($p \leq 0.05$) after treatment. However, it was found to be within normal physiological range. Similar results were reported by Gutierrez *et al.* (1998) [5] and Soltesova *et al.* (2015) [19]. Whereas, slight decrease in the values of MCV, MCH and MCHC was noticed by Nahed and Tamer (2014) [11] in ewes suffering from respiratory infection.

Leucogram

Upon examination of the mean and standard error (Mean±SE) of total leukocyte count, neutrophils, monocytes, eosinophils, lymphocytes and basophils in the present study revealed that there was a significant ($p \leq 0.05$) increase in total leukocyte

count (Fig.1), neutrophilic (Fig.2), eosinophilic (Fig.3) and monocytic (Fig.4) count when compared to control group. However, the lymphocytic and basophilic count in this study remained non-significant ($p \leq 0.05$) in bacterial pneumonia goats. While, marginal decrease was observed in the lymphocytic count on the day of presentation in each group, after treatment, they resume back to normal as that of control group. The leucocytosis with neutrophilia, eosinophilia and monocytosis in the present study may be attributed to acute inflammatory changes in lower respiratory tract particularly due to bacterial infection and are in agreement with Nahed and Tamer (2014) [11] who reported that increased in TLC, Neutrophilic, eosinophilic and monocytic counts and decrease in lymphocytic count in the ewes suffering from respiratory diseases. Ghanem *et al.* (2015a) [3] also reported significant ($p \leq 0.05$) increase in WBC, neutrophils but no significant ($p \leq 0.05$) change in eosinophil and monocytic count in pneumonic Boer goats. The stress induced during respiratory illness that result in endogenous release of corticosteroids, which intern have a major role in regulating the concentration of leucocytes in mild to severe pneumonia in goats.

Blood gas analysis

The venous blood was collected from all the goats and immediately analysed in the blood gas analyser for $VpCO_2$, $VtCO_2$ and H^+ ion VpO_2, VO_2 sat, VBE and $VHCO_3A$ in bacterial pneumonia in goats. In the present study, mean and standard error (Mean±SE) of various blood gas parameters showed increase in the concentration of $VpCO_2$ (Fig.5), $VtCO_2$ (Fig.6) and H^+ ion (Fig.7) concentration and decreased blood gas parameters such as VpO_2 (Fig.8) and VO_2 sat (Fig.9) were found to be statistically significant ($p \leq 0.05$) as compared to control group. However, no significant changes were observed in the $VHCO_3A$ ions and VBE values. The results indicated that respiratory acidosis was predominant in bacterial pneumonia and was due to pulmonary hypoventilation. The results were in accordance with Ghanem *et al.* (2015a) [3] who has evaluated the pulmonary function test in Boer goats suffering with pneumonia and found significant ($p \leq 0.05$) decrease in blood pH and PO_2 and increased in PCO_2, bicarbonates, TCO_2 and BE in comparison with control group. Similar results were also reported by Nagy *et al.* (2006) [10], Kaneko *et al.* (2008) [8], Smith and Sherman (2009) [18] and Shakespeare (2012) [17]. The results conclude that, in respiratory acidosis there will be increased $VpCO_2$, $VtCO_2$ and H^+ ions, while decreased VpO_2 and VO_2 sat values due to hypoxic condition in pneumonic goats.

The overall analyses of both haematological and blood gas parameters on '0' day and 5th day of treatment revealed that alterations recorded in haematological and blood gas parameters due to bacterial infection and respiratory acidosis can be effectively used as a diagnostic indicators of bacterial pneumonia in goats. After treatment the values resume back to normal in all the study group as that of control group suggested that both Morbofloxacin and Ceftiofur sodium alone or in conjunction with mucolytic agent- acetyl cysteine may be effectively used against bacterial pneumonia in goats.

Further assessment of recovery path way of each group using the observations on pulmonary score revealed that in all the groups 100% complete recovery was achieved on 5th day of treatment. Among parenteral antibiotic therapy alone, faster recovery was observed in marbofloxacin (Group-1) than ceftiofur sodium (Group-3). Whereas, parenteral antibiotic therapy in conjunction with mucolytic agent- acetyl cysteine as nebulizer showed faster recovery in marbofloxacin in conjunction acetyl cysteine (Group-2) than ceftiofur sodium in conjunction acetyl cysteine (Group-4). This study suggested that water soluable acetyl cysteine may be effectively used as an adjuvant therapy to minimise the oxidative stress induced in bacterial pneumonia of goats.

Table 1: Treatment group

Group	Treatment
1 (n=6)	Treated with Inj. Marbofloxacin @2mg/kg b. wt./day for 5day,
2 (n=6)	Treated with Inj. Marbofloxacin @ 2 mg/kg b. wt./day for 5 day with nebulization of mucolytic agent (Acetyl cysteine)- 2 ml, for 30 mins/day for 3 days,
3 (n=6)	Treated with Inj. Ceftiofur sodium @ 2 mg/kg b. wt./day for 5 day and
4 (n=6)	Treated with Inj. Ceftiofur sodium @ 2 mg/kg b. wt./day for 5day with nebulization of mucolytic agent (Acetyl cysteine)- 2 ml, for 30 mins/day for 3 days.
Supportive therapy	Chlorpheniramine maleate @ 0.5 mg/kg b. wt./ day for 5 days Meloxicam@ 0.5 mg/kg b.wt./ day for 5 days

Table 2: Results showing haematological parameters of before and after treatment groups in comparison with control group.

Parameters Groups	Control group	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
		Group1		Group-2		Group-3		Group-4	
MCHC (g/dl)	38.83±0.48^{c}	35.02±0.81^{b}	34.27±0.59^{b}	26.93±1.69^{a}	25.83±1.27^{a}	36.00±1.05^{bc}	34.28±0.36^{b}	33.85±1.36^{b}	34.60±0.54^{b}
MCH (pg)	6.05±0.19^{b}	6.32±0.38^{b}	6.77±0.20^{b}	4.47±0.23^{a}	4.9±0.62^{a}	6.48±0.07^{b}	6.22±0.07^{b}	5.95±0.23^{b}	6.35±0.06^{b}
MCV (fl)	15.62±0.36^{a}	18.02±0.91^{ab}	19.87±0.70^{ab}	16.77±0.59^{ab}	24.08±6.64^{b}	18.13±0.63^{ab}	18.15±0.37^{ab}	23.33±2.69^{ab}	18.45±0.36^{ab}
PCV (%)	28.43±0.75^{ab}	28.98±1.79^{ab}	31.5±1.55^{b}	28.32±3.63^{ab}	30.9±4.24^{b}	28.20±1.79^{ab}	29.20±1.60^{ab}	28.33±0.48^{ab}	27.03±0.39^{a}
Hb (g/dl)	9.13±0.34^{abc}	9.57±0.74^{bc}	10.74±o.55^{c}	7.50±0.77^{a}	9.83±1.20^{bc}	10.05±0.59^{ab}	10.63±0.54^{c}	9.47±0.44^{abc}	9.60±0.11^{bc}
TEC (10^6/µl)	14.92±0.15	12.94±1.01	14.82±0.88	12.50±1.11	15.47±1.73	15.40±0.88	15.61±0.16	12.52±1.81	14.62±0.24

* Mean±SE values with different superscripts with in a column differ significantly (P≤0.05)

Table 2: Results showing haematological parameters of before and after treatment groups in comparison with control group

Parameters Groups	Control group	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
		Group1		Group-2		Group-3		Group-4	
Eosinophils (%)	1.00±0.51b	1.65±0.37 a	0.83±0.31 b	1.85±o.21 a	0.67±0.21^{b}	1.67±0.42 a	0.50±0.22 b	1.83±0.40 a	1.17±0.17 b
Basophils (%)	0.33±0.24	0.33±0.21	0.5±0.34	0.5±0.22	0.33±0.21	0.33±0.21	0.50±0.22	0.17±0.17	0.33±0.21
Monocytes (%)	0.67±0.37 ^{a}	2.33±0.49^{bc}	1.33±0.49 ^{ab}	2.67±o.49^{bc}	1.33±0.49^{ab}	3.00±0.45^{c}	1.50±0.34^{ab}	2.50±0.43^{bc}	1.83±0.48^{abc}
Lymphocyte (%)	56.67±1.36^{bc}	43.5±1.73^{abc}	54.5±1.82^{abc}	41.17±2.21 ^{a}	59.17±0.40^{ab}	44.33±2.53^{ab}	56.83±2.09 ^{ab}	39.17±1.54^{c}	55.17±1.85^{abc}
Neutrophils (%)	40.83±1.30 ^{a}	52.67±1.31^{b}	42.83±1.76 ^{a}	55.33±1.84^{b}	38.5±0.76 ^{a}	51.67±1.76^{b}	40.67±1.67 ^{a}	56.33±1.54^{b}	41.50±2.05 ^{a}
TLC (10^3/µl)	10.92±1.08^{a}	16.22±0.94 ^{b}	8.95±0.63 ^{a}	18.33±2.08^{bc}	7.77±0.73 ^{a}	18.90±2.03^{bc}	8.92±0.56 ^{a}	21.52±1.87 ^{c}	8.33±0.36 ^{a}

* Mean±SE values with different superscripts with in a column differ significantly ($p \leq 0.05$)

Table 3: Results showing blood gas parameters of before and after treatment groups in comparison with control group.

Parameters Groups	Control group	Before Treatment	After treatment	Before Treatment	After treatment	Before treatment	After Treatment	Before Treatment	After Treatment
		Group-1		Group-2		Group-3		Group-4	
VBE (mmol/l)	-4.06±0.36^{bc}	-5.51±0.86^{ab}	-3.56±0.38^{bc}	-4.53±0.49^{bc}	-2.67±0.38^{c}	-6.66±1.12^{a}	-3.73±0.53^{bc}	-3.90±0.45^{bc}	-3.60±0.45^{bc}
VH+ (nmol/l)	53.28±6.47^{ab}	70.06±8.25^{bcd}	56.68±4.32^{abc}	81.03±6.53^{cd}	41.83±5.71^{a}	93.48±15.73^{d}	54.22±6.34^{ab}	60.93±5.14^{abc}	46.18±5.14^{ab}
$VHCO_3$ A (mmol/l)	17.87±2.32	20.75±1.73	17.73±1.65	20.68±0.95	19.07±0.74	20.33±0.58	18.17±1.43	20.92±1.95	18.83±1.95
$VtCO_2$ (mmol/l)	18.02±1.86^{ab}	23.35±1.90^{cd}	14.52±2.42^{a}	21.37±1.74^{bc}	20.83±0.66^{bc}	22.35±0.96^{bd}	20.13±1.46^{bc}	26.85±0.27^{d}	20.50±0.27^{bc}
$VpCO_2$ (mmHg)	36.70±6.59^{ab}	61.21±7.66^{c}	33.95±5.97^{a}	58.21±4.04^{c}	32.94±3.21^{a}	47.36±4.80^{ac}	36.86±1.89^{ab}	52.06±4.89^{bc}	37.56±5.65^{ab}
VO_2 sat (%)	88.58±2.50^{de}	63.47±2.17^{a}	73.80±4.00^{ac}	67.87±6.77^{ac}	93.17±2.40^{e}	65.18±6.92^{ab}	76.55±3.03^{bd}	78.85±2.87^{cd}	94.10±2.43^{e}
VpO_2 (mmHg)	95.13±11.56^{cd}	55.57±1.51^{a}	105.40±17.48^{cd}	76.93±7.72^{abc}	106.23±3.66^{cd}	67.22±3.03^{ab}	95.38±13.26^{bcd}	60.00±3.61^{abc}	124.00±22.91^{d}

*Mean±SE values with different superscripts with in a column differ significantly (P≤0.05)

Table 4: assessment of recovery pathway of bacterial pneumonia in goats

Groups Days	Group1	Group2	Group3	Group4
Day1	0.00	0.00	0.00	0.00
Day2	0.00	0.00	0.00	0.00
Day3	16.67	33.33	16.67	16.67
Day4	50.00	66.67	33.33	66.67
Day5	100.00	100.00	100.00	100.00

Plate 1: Nebulization with mucolytic agent (acetyl cysteine) among treatment groups in pneumonic goats

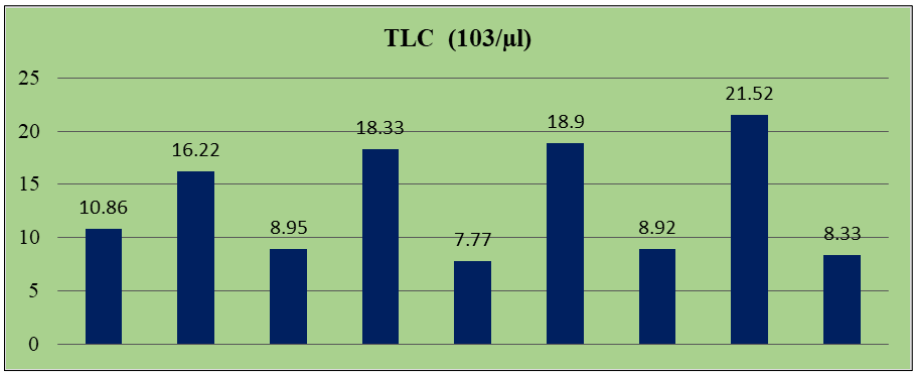

Fig. 1: Mean values of total leucocyte count of different treatment groups (before and after treatment) in comparison with control group.

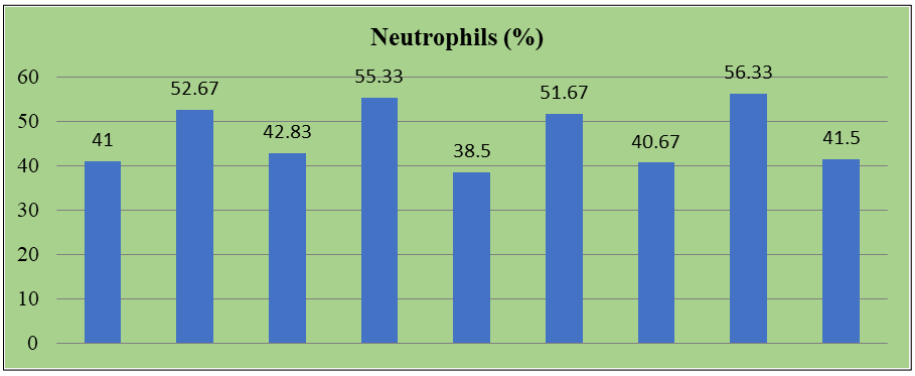

Fig 2: Mean values of neutrophil of different treatment groups (before and after treatment) in comparison with control group.

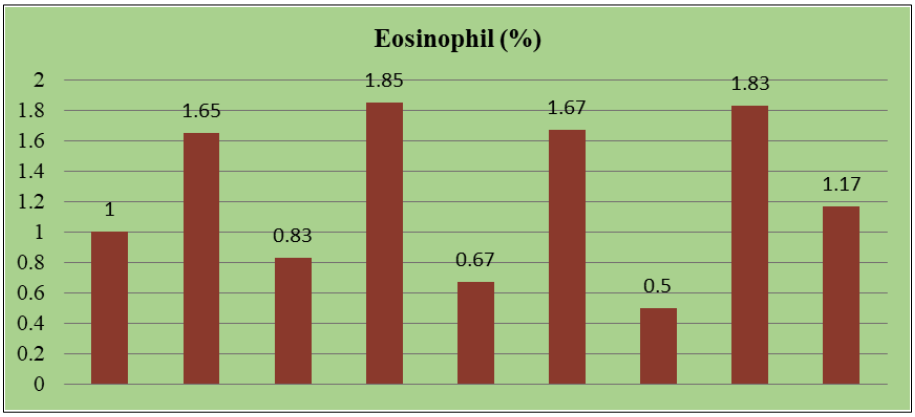

Fig 3: Mean values of eosinophil of different treatment groups (before and after treatment) in comparison with control group.

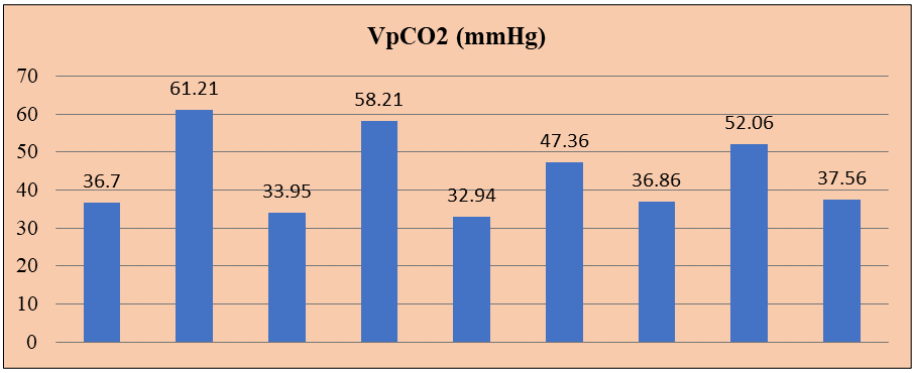

Fig 4: Mean values of monocyte of different treatment groups (before and after treatment) in comparison with control group**.**

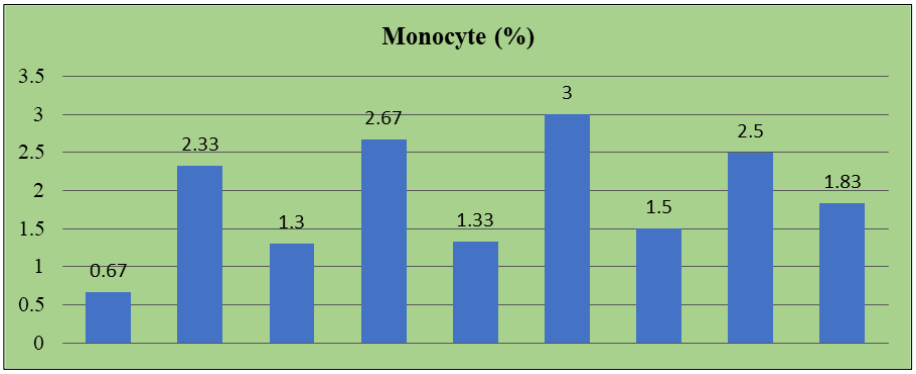

Fig 5: Mean values of venous partial pressure of carbon dioxide of different treatment groups (before and after treatment) in comparison with control group.

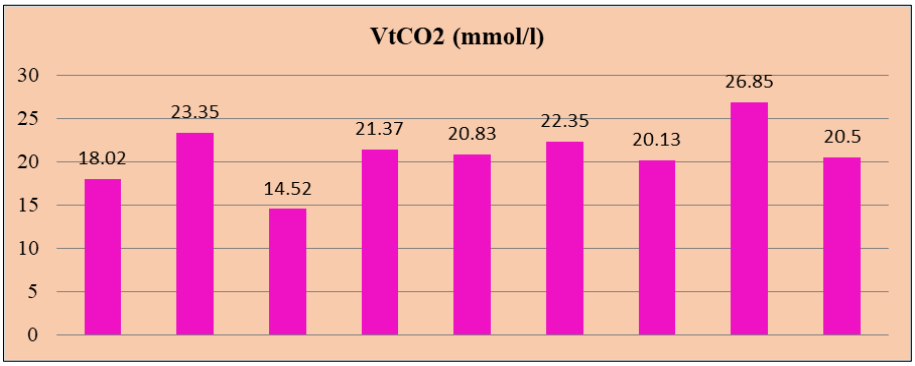

Fig 6: Mean values of venous total carbon dioxide of different treatment groups (before and after) in comparison with control group.

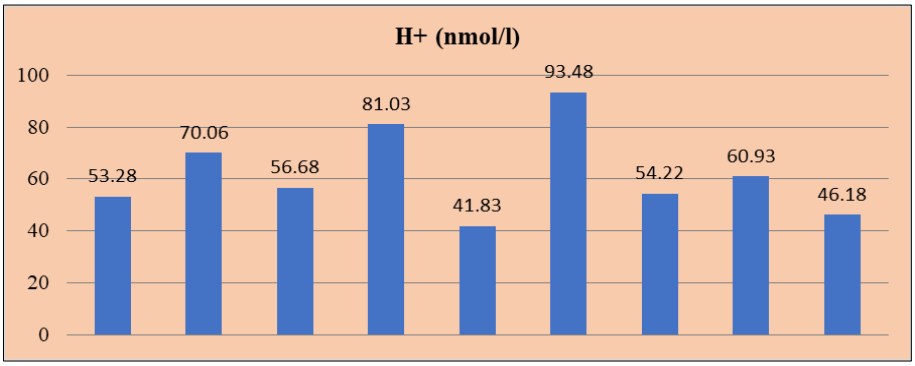

Fig 7: Mean values of venous hydrogen ions of different treatment groups (before and after treatment) in comparison with control group.

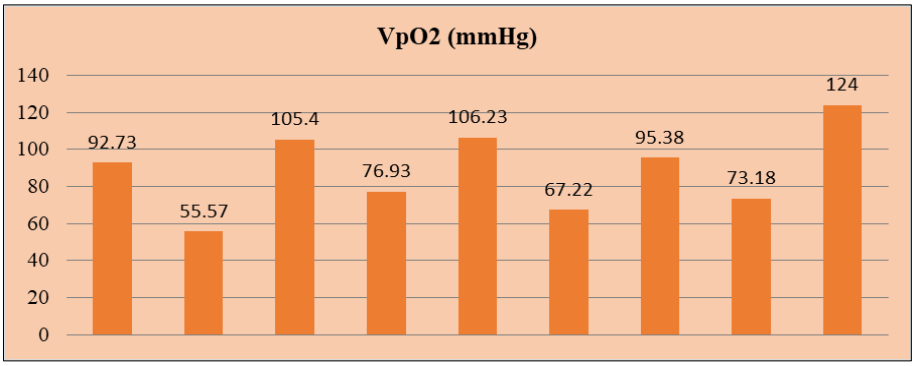

Fig 8: Mean values of venous partial pressure of oxygen of different treatment groups (before and after treatment) in comparison with control group**.**

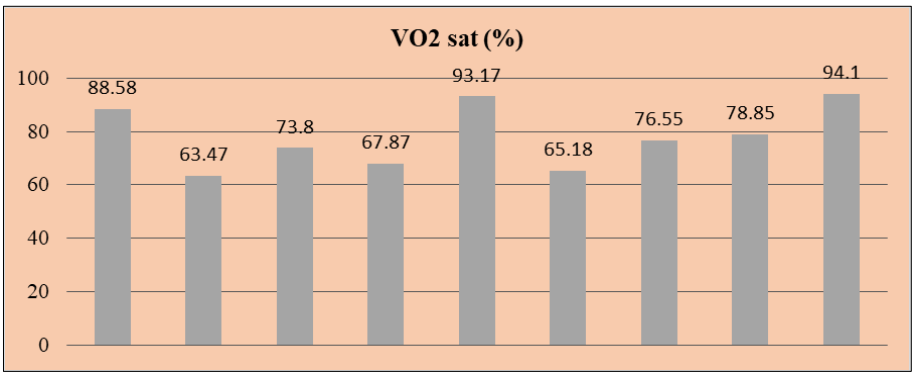

Fig 9: Mean values of venous oxygen saturation of different treatment groups (before and after treatment) in comparison with control group.

Conclusion

The study suggested that haematological parameters and blood gas parameters could be used as a diagnostic indicator to assess the efficacy of drug and concluded that the faster recovery was achieved when antibiotics are used parenteraly in conjunction with mucolytic agent as nebulizer for treatment of bacterial pneumonia in goats. Thus, mucolytic agent (Acetyl cysteine) as a nebulizer in conjunction with parenteral antibiotic may be recommended as first line of treatment in recouping the pulmonary function of bacterial pneumonia in goats.

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