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Evaluation of ameliorative potential of antioxidants and/or immunomodulators in contagious caprine pleuropneumonia affected Himalayan Pashmina goats

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ABSTRACT

Contagious caprine pleuropneumonia causes oxidative stress and immune disturbances in affected goats. Hence these derogative alterations aggravate the pathogenesis and severity of this disease, thus, necessitating utilization of antioxidants and/or immunomodulators in the therapeutic regimes of contagious caprine pleuropneumonia. Present study evaluated ameliorative effects of N-acetyl cysteine (NAC) and alpha tocopherol (vitamin E) in association with primary antibiotic tylosin treatment in contagious caprine pleuropneumonia affected test goats in comparison to antibiotic only treated goats (n=6) and healthy goats (n=6). N-acetyl cysteine given at a dose of 12 mg per kg of body weight orally daily for 7 days, showed better antioxidant effect by significantly ameliorating oxidative stress (41.4%) compared to other treatments; besides, decreasing total oxidative status (TOS) and increasing total antioxidant status (TAS). Immunomodulatory potential of alpha tocopherol given at a dose of 10 mg per kg of body weight orally daily for 7 days, was found to be comparatively better than NAC in decreasing percentage of neutrophils and increasing percentage of lymphocytes; besides, decreasing the levels of tumor necrosis factor-α (TNF-α) and immunoglobulin G (IgG) when the modulation of total leukocyte count and differential leukocyte count was comparable between the two. Thus, it can be concluded from this study that NAC acts as a comparatively better antioxidant and alpha tocopherol as better immunomodulator when used in the therapeutic regimes of contagious caprine pleuropneumonia.

Keywords: Alpha tocopherol, Antioxidants, Contagious caprine pleuropneumonia, Immunomodulators, N-acetyl cysteine, Pashmina goats

Contagious caprine plueropneumonia (CCPP) is a serious respiratory disease of goats caused by *Mycoplasma capricolum* subspecies *caprineumonia* (Mccp) (Parray *et al*. 2019, Yatoo *et al*. 2019a). It causes heavy morbidity (100%) and mortality (80-100%) in affected goats (Yatoo *et al*. 2019a). Severe pathogenesis by Mccp including serofibrinous pleuropneumonia, congestion, fluid exudation, fibrinous deposition, and adhesion formation is responsible for disease severity and high mortality (Parray *et al*. 2019, Dhaygude *et al*. 2023). These molecular pathogenic alterations may include severe oxidative, inflammatory, immunogenic and allergic reactions in response to mycoplasma antigens (Jarikre *et al*. 2017, Yatoo *et al*. 2019a,b,c) thus envisaging multipronged therapeutic approaches involving safe and effective antibiotics, antioxidants, anti-inflammatory, antiallergic and immunomodulatory agents (Kizil *et al*. 2007, Xue *et al*. 2015, Jarikre *et al*. 2017, Yatoo *et al*. 2018a,b, 2019a,b,d,e, Darwish 2020). However, due to numerous constraints such as multi-drug approach, therapeutic protocols are marred by use of single convenient antibiotic (Yatoo *et al*. 2019a,b,c,d). Lack of vaccines for prophylaxis also renders herdsmen helpless for utilization of commonly available antibiotics (Yatoo *et al*. 2019a,b,c,d). Prolonged use of single type of antibiotic may lead to adverse effects and antibiotic resistance (Tatay-Dualde *et al*. 2017, Yatoo *et al*. 2018b, 2019a,d). Nevertheless, pathogen induced oxidative, inflammatory and immune alterations require specific therapeutics for amelioration of severity of pathogenesis (Yatoo *et al*. 2018b, 2019a,b,e). In our previous study, we have reported beneficial effects of anti-inflammatory and anti-allergic agents in the treatment of CCPP affected Himalayan Pashmina goats (Yatoo *et al*. 2019b). The present study aimed to evaluate ameliorative potential of antioxidants and immunomodulators in therapeutic regimes of CCPP. N-acetyl cysteine (NAC)

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and alpha tocopherol (vitamin E) have antioxidant (Jóźwik *et al*. 2010, Anugu *et al*. 2013, Jhambh *et al*. 2013, Yatoo *et al*. 2016) and immunomodulatory activities (Bagnicka *et al*. 2008, Anugu *et al*. 2013, Yatoo *et al*. 2018a) and thus, can be explored in the treatment of CCPP affected goats. This study can also help in deciphering the molecular basis of oxidative and immune disturbances in CCPP and the preliminary virulence of Mccp in the pathogenesis of this disease.

MATERIALS AND METHODS

Contagious caprine pleuropneumonia affected Himalayan Pashmina goats (N=18) diagnosed previously by clinical signs, culture, PCR and RFLP (Parray *et al*. 2019) were divided in three test groups as per treatment protocol and each group had 6 animals (n=6). The fourth group was kept as healthy control (n=6). All the goats were of similar age (1 year old), gender (female), breed (Pashmina goats), body weight (20 kg), clinical state (18 contagious caprine pleuropneumonia affected and 6 healthy), and metabolic state (non-pregnant). Sample size was calculated by resource equation method (Festing and Altman 2002) as explained by Charan and Biswas (2013).

In Group I, alpha tocopherol (Sigma Aldrich) was given at a dose of 10 mg per kg of body weight orally daily for 7 days (Anugu *et al*. 2013, Ramadan *et al*. 2018). In Group II, N-acetyl cysteine (Sigma Aldrich) at a dose of 12 mg per kg of body weight was given orally daily for 7 days (Jóźwik *et al*. 2010, Bagnicka *et al*. 2011, Jóźwik *et al*. 2010) in addition to antibiotic tylosin given at a dose of 20 mg per kg of body weight intramuscularly, repeated at the interval of 48 h four times (0 h, 48 h, 96 h, 144 h) in both the test groups. Group III was treated with tylosin only at a dose rate as given previously, and the healthy control Group IV did not receive any treatment. Blood samples (5 ml) were collected at 0 h, 48 h, 96 h, and 144 h of trial in clot activator vials under aseptic procedures and serum samples were harvested for estimation of oxidative and immune indices at respective intervals.

Oxidative status was evaluated by estimating total oxidant status (TOS) as per the method of Erel (2004) and total antioxidant status (TAS) as per the method of Erel (2005). Oxidative stress index (OSI) as the ratio of the total oxidant status (TOS) to total antioxidant status (TAS) levels was derived from TOS and TAS (Erel 2005).

Immune indices were evaluated through cellular and humoral immunity parameters. Cellular immunity (CI) including total leukocyte count (TLC) and differential leukocyte count (DLC) were calculated as per Jain (1995) and cytokine TNF-α by using commercial ELISA kit (Sincere Biotech Pvt. Ltd.). Humoral immunity (HI) parameter included detection of immunoglobulin G (IgG) by using commercial Goat IgG ELISA kit (Sincere Biotech Ltd. 2956-8-6) (Fig.1).

Statistical analysis: Normality of data was tested by Kolmogorov-Smirnov test and the data that were not normally distributed were transformed before further analyses. Data was analyzed by regular mixed model for comparative analysis of oxidative and immune indices in four groups of animals at four different intervals (0 h, 48 h, 96 h and 144 h) using SPSS software version 20 (IBM India). Values with P≤0.05 were considered as statistically significant.

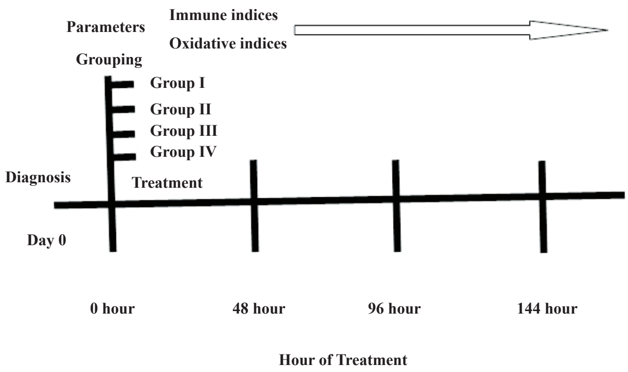

Fig. 1. Flowchart of treatment given.

RESULTS AND DISCUSSION

Oxidative stress and immune disturbances are considered as important mechanisms of pathogenesis of *Mycoplasma capricolum* subspecies *caprineumonia* and the cause of severity in contagious caprine pleuropneumonia (Soayfane *et al*. 2018, Liljander *et al*. 2019, Yatoo *et al*. 2019a,b). Hence interventions targeting these mechanisms can prove beneficial in managing this severe mycoplasma disease (Soayfane *et al*. 2018, Liljander *et al*. 2019, Yatoo *et al*. 2019a,b). The present study revealed trends of oxidative and immune disturbances and possible ameliorative effects of antioxidants and/or immunomodulators in contagious caprine pleuropneumonia affected Himalayan Pashmina goats.

Non-significantly higher (P>0.05) TOS levels were noted initially in infected groups than the healthy group. A non-significant (P>0.05) decrease in TOS levels was observed in all test groups at 144th hour of trial however decrease was more prominent in N-acetyl cysteine treated group (2.95 to 2.12) followed by alpha tocopherol treated group (2.62 to 2.2) and tylosin only treated group (2.53 to 2.05). Between test groups, non-significantly (P>0.05) more decrease in TOS level in N-acetyl cysteine treated group (28.13%) compared to alpha tocopherol treated group (16.03%) and tylosin only treated group (18.97%) was noted. The TOS levels were significantly (P≤0.05) lower in healthy control group compared to other groups as shown in Table 1.

Lower (P>0.05) TAS levels were noted initially in infected groups than the healthy group. TAS levels showed non-significant (P>0.05) increase in all test groups at 144th interval of trial however magnitude of increase was more in N-acetyl cysteine treated group (0.78 to 1.77) followed by alpha tocopherol treated group (0.89 to 1.02) and tylosin only treated group (0.74 to 0.78). Between treated groups non-significant (P>0.05) difference in TAS levels

Table 1. Effect of different therapeutic regimes on TOS levels (Mean±SE) (µmol H_2O_2 equivalent/L)

Hours post treatment	Alpha tocopherol and tylosin treated group	N-acetyl cysteine and tylosin treated group	Tylosin only treated group	Healthy control group
0	2.62 ± 0.20^{aAB}	2.95 ± 0.38^{aA}	2.53 ± 0.48^{aAB}	1.37 ± 0.25^{aB}
48	2.28 ± 0.30^{aA} (1)*	2.20 ± 0.26^{aA} (1)*	2.28 ± 0.24^{aA} (1)*	1.30 ± 0.20^{aA} (1)*
96	2.53 ± 0.61^{aA} (1)*(1)**	2.03 ± 0.46^{aA} (0.17)*(1)**	2.53 ± 0.46^{aA} (1)*(1)**	1.20 ± 0.29^{aA} (1)*(1)**
144	2.20 ± 0.37^{aA} (1)*(1)**(1)***	2.12 ± 0.15^{aAB} (0.78)*(1)**(1)***	2.05 ± 0.34^{aAB} (1)*(1)**(1)***	1.04 ± 0.16^{aB} (1)*(1)**(1)***

[a] Values with similar superscript did not differ significantly (P>0.05) in a column. [AB] Values with different superscript differ significantly (P≤0.05) in a row. *,**,***P values of the treatment at each time point.

were observed at various intervals however more increase in N-acetyl cysteine treated group (56.00%) compared to alpha tocopherol treated group (12.74%) and tylosin only treated group (5.12%) was noted. The levels were usually higher in healthy control group compared to other test groups as shown in Table 2.

Non-significantly higher (P>0.05) OSI levels were noted in infected Groups I, II, and III than the healthy control group. OSI showed non-significant (P>0.05) decrease in all test groups at 144th interval of trial however magnitude of decrease was more in Group II (4.1 to 2.4) followed by Group I (3.0 to 2.4) and Group III (3.4 to 2.7). Between groups, significant (P≤0.05) decrease in Group II (41.4%) compared to Group I (26.6%) and Group III (20.5%) was noted however the indices were significantly (P≤0.05) higher than control Group IV (Supplementary Table 1).

Higher total oxidant status (TOS) and oxidative stress index (OSI) and lower total antioxidant status (TAS) in infected groups can be attributed to oxidative stress induced by mycoplasma pathogens in affected goats and consumption of antioxidants for scavenging free radicals. This is in corroboration with Kizil *et al*. (2007) who have reported oxidative disturbances in *Mycoplasma* pneumonia. Increase in levels of TOS and decrease in levels of TAS in contagious caprine pleuropneumonia affected goats has also been noted by Yatoo *et al*. (2019b). This may be due to ability of *Mycoplasma capricolum* subspecies *capripneumonia* to induce oxidative stress through production of reactive oxygen species like hydrogen peroxide and superoxide (Razin *et al*. 1998, Liljander *et al.* 2019). Increase in levels of pro-oxidants (malondialdehyde, hydrogen peroxide and myeloperoxidase) and decrease in levels of antioxidants (superoxide dismutase and reduced glutathione) have been noticed in pneumonic goats (Jarikre *et al*. 2017, Soayfane *et al*. 2018).

Post treatment, decrease in oxidative indices like TOS and OSI indicates minimization of oxidative stress and increase of TAS indicates building up of antioxidant capacity. This might be due to effect of antioxidants used in addition to prevention reactive oxygen species formation from mycoplasma pathogenesis and neutralization of free radicals. More prominent decrease in oxidative indices (28.13% TOS, 41.4% OSI) and increase in antioxidant status (56.00% TAS) in N-acetyl cysteine treated group than alpha tocopherol treated group (16.03%, 20%, 12.74% respectively) indicates better antioxidant effect of N-acetyl cysteine than alpha tocopherol in contagious caprine pleuropneumonia affected goats. Jóźwik *et al*. (2010) and Omara *et al*. (1997) have also reported the antioxidant role of N-acetyl cysteine in goats affected with respiratory diseases. Increase in levels of antioxidants may be due to exogenous supply of antioxidants or endogenous generation as a defense mechanism against oxidative stress. N-acetyl cysteine has potent antioxidant activity and can supplement TAS levels (Yatoo *et al*. 2016). Supplementation of antioxidants from external sources, generation of internal antioxidants and neutralization or prevention of generation of free radicals can ultimately decrease OSI (Aktas *et al*. 2017, Yatoo *et al*. 2019b). N-acetyl cysteine helps in neutralizing reactive oxygen species and increases antioxidant capacity (Bagnicka *et al*. 2008, Jóźwik *et al*. 2010, Yatoo *et al*. 2016).

Immune disturbances in contagious caprine pleuropneumonia can be due stimulation of host defense mechanisms by *Mycoplasma capricolum* subspecies *capripneumoniae* resulting in immune activation and hence cellular and humoral immune responses (Abdelsalam *et al*. 1988, Vanden Bush and Rosenbusch 2003, Hermeyer *et al*. 2012, Mulongo *et al*. 2014, Maritim *et al*. 2018, Yatoo *et al*. 2019a,b,c). Antigens of *Mycoplasma capricolum* subspecies *capripneumoniae* including capsular polysaccharides, lipoproteins, enzymes,

Table 2. Effect of different therapeutic regimes on TAS levels (Mean±SE) (µmol Trolox equivalent/L)

Hours post treatment	Alpha tocopherol and tylosin treated group	N-acetyl cysteine and tylosin treated group	Tylosin only treated group	Healthy control group
0	0.89 ± 0.03^{aA}	0.78 ± 0.09^{aA}	0.74 ± 0.06^{aAB}	1.37 ± 0.25^{aB}
48	0.90 ± 0.02^{aA} (1)*	0.96 ± 0.30^{aA} (1)*	0.99 ± 0.23^{aA} (1)*	1.38 ± 0.24^{aA} (1)*
96	0.96 ± 0.10^{aA} (1)*(1)**	1.93 ± 0.59^{aA} (0.64)* (1)**	0.74 ± 0.09^{aA} (1)*(1)**	1.38 ± 0.24^{aA} (0.17)* (1)**
144	1.02 ± 0.14^{aA} (1)*(1)**(1)***	1.77 ± 0.95^{aA} (1)* (1)** (1)***	0.78 ± 0.08^{aA} (1)*(1)**(1)***	1.39 ± 0.24^{aA} (0.35)* (1)** (1)***

[a] Values with similar superscript did not differ significantly (P>0.05) in a column. [AB] Values with different superscript differ significantly (P≤0.05) in a row. *,**,***P values of the treatment at each time point.

Table 3. Effect of different therapeutic regimes on total leukocyte count (Mean±SE)

Hours post treatment	Alpha tocopherol and tylosin treated group	N-acetyl cysteine and tylosin treated group	Tylosin only treated group	Healthy control group
0	13900.00±278.99[a]	14008.33±247.46[a]	13925±137.08[a]	5458.33±707.39[aA]
48	11703.33±402.86[b] (0.03)*	10925.00±795.69[b] (0.05)*	11450±548.78[b] (0.03)*	5458.33±671.24[aA] (1.00)*
96	9781.67±300.73[c] (0.00)* (0.02)**	8233.33±594.65[b] (0.00)* (0.09)**	9575±630.31[c] (0.01)* (0.03)**	5508.33±693.71[aA] (1.00)* (1.00)**
144	6600.00±844.39[dA] (0.00)* (0.00)** (0.02)***	5691.67±518.88[cA] (0.00)* (0.01)** (0.03)***	7150±869.29[dA] (0.00)* (0.02)** (0.02)***	5450.00±685.20[aA] (1.00)* (1.00)** (0.80)***

[abcd]Values with different superscript differ significantly (P≤0.05) in a column. [A]Values with different superscript differ significantly (P≤0.05) in a row. *,**,***P values of the treatment at each time point.

metabolites or reactive oxygen species may cause immune cell infiltration, release of cytokines, inflammatory mediators and immunoglobulins (March *et al*. 2002, Yatoo *et al*. 2019a,b,c,e). This disrupted immune response in contagious caprine pleuropneumonia can be regulated by immunomodulators. Alpha tocopherol and N-acteyl cysteine are known for their immunomodulatory activities (Bagnicka *et al*. 2008, Anugu *et al*. 2013, Ramadan *et al*. 2018).

Significantly (P≤0.05) higher TLC was noted in all the test Groups I, II, III compared to healthy control Group IV on all time intervals. Post treatment, a significant (P≤0.05) decrease in TLC was observed in all the test groups at 144th hour of trial however decrease was more in Group I (13900 to 6600) and Group II (14008.33 to 5691.67). Between treated Groups I, II and III non-significant (P>0.05) difference in TLC was noted on all time intervals as shown in Table 3. Non-significantly (P>0.05) more decrease in Group I (52.51%) and Group II (59.36%) as compared to Group III (48.65%) was noted.

All the test groups showed significantly (P≤0.05) higher percentage of neutrophils and lower percentage of lymphocytes compared to healthy control group at 0th h, however, percentage of eosinophils were significantly (P≤0.05) higher in N-acetyl cysteine treated group only. Monocytes and basophils did not differ significantly (P>0.05) between various groups on 0th h. Post treatment, a significant (P≤0.05) decrease in percentage of neutrophils was observed at 96th h in alpha tocopherol treated group and N-acetyl cysteine treated group and at 144th h in tylosin only treated group. A significant increase (P≤0.05) in percentage of lymphocyte was observed in all tests groups at 96th and 144th h and in tylosin only treated group also at 48th h. Monocytes showed significant (P≤0.05) increase in tylosin only treated group at 144th h. However, only N-acetyl cysteine treated group showed significant (P≤0.05) decrease in percentage of eosinophils on 48th h. Basophils showed non-significant (P>0.05) change at various intervals. Between treated groups, DLC values did not differ significantly (P>0.05) at 48th, 96th and 144th h (Supplementary Table 2).

The test groups showed significantly (P≤0.05) higher TNF-α levels compared to healthy control group at 0th h. There was non-significant (P>0.05) decrease in TNF-α levels in all the test groups post treatment as shown in

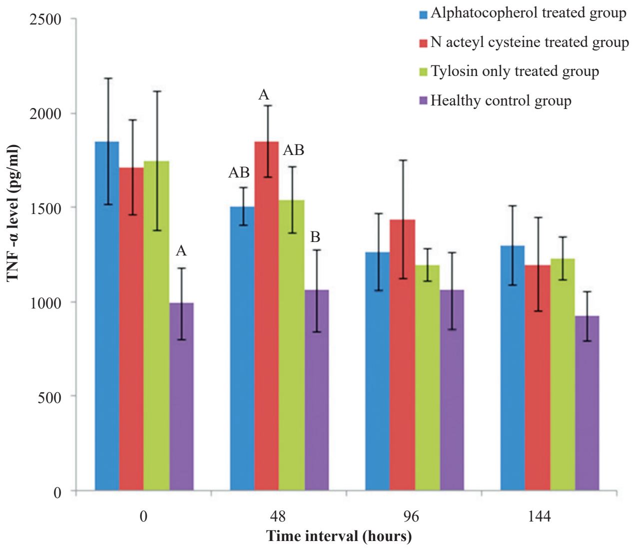

Fig. 2. Effect of different therapeutic regimes on TNF-α levels (pg/ml) ([A,B]Values with different superscript differ significantly (P≤0.05) between different Groups at a particular time).

Fig. 2. Between groups, non-significant (P>0.05) change was noted in TNF-α levels at various intervals however non-significantly (P>0.05) more decrease in alpha tocopherol treated group (42.04%) compared to N-acetyl cysteine treated group (29.96%) and tylosin only treated group (29.37%) was noted.

Higher total leukocyte count, differential leukocyte count, and TNF-α in contagious caprine pleuropneumonia affected goats may be due to cellular immune response of the host against *Mycoplasma capricolum* subspecies *capripneumoniae* pathogen whereas humoral immune response may have resulted in higher IgG levels. This is in corroboration with Yatoo *et al*. (2019b) who also noted elevated total leukocyte count, differential leukocyte count and TNF-α in affected goats. Increase in total leukocyte count has also been reported by Liljander *et al*. (2019) in experimental contagious caprine pleuropneumonia infected goats. Higher percentage of neutrophils and lower percentage of lymphocytes in affected groups may be due to acute type of infection (Abdelsalam *et al*. 1988). Increased TNF-α levels indicate inflammatory nature of the disease resulting in elevation of proinflammatory cytokine levels (Yatoo *et al*. 2019b). These alterations reveal cellular immune response in affected goats. However humoral immunity (IgG and IgM) is also important in infections of *Mycoplasma capricolum* subspecies *caprineumonia* (Samiullah 2013). Significantly (P≤0.05) higher IgG levels were observed in alpha tocopherol treated group and tylosin only treated group at 0th h compared to healthy control group. Post treatment IgG levels decreased (P≤0.05) significantly in alpha tocopherol treated group upto 96th h. Between treated groups, significantly (P≤0.05) higher levels of IgG were noted in alpha tocopherol treated group at 48th and 144th intervals and in tylosin only treated group at all intervals compared to healthy control group as shown in Fig. 3. Non-significantly (P>0.05) more decrease in alpha tocopherol treated group (68.86%) compared to N-acetyl cysteine treated group (51.23%) and tylosin only treated group (55.36%) was noted. IgG is involved in humoral immune response to mycoplasma pathogens (March *et al*. 2002). Hence significant increase in IgG levels in infected goats than the healthy ones can be attributed to induction of antibody mediated immune response by mycoplasma antigens. This is in corroboration with March *et al*. (2002) who also reported significant rise in antibody titre of IgG in goats following the experimental infection with *Mycoplasma capricolum* subspecies *caprineumonia*.

Changes in values of neutrophils, lymphocytes, monocytes, eosinophils and basophils in affected animals post treatment showed a trend of returning towards normal values as noted in healthy animals. This can be due to prevention of mycoplasma infection by tylosin or modulation of immune response by immunomodulators (Bagnicka *et al*. 2008, Jhambh *et al*. 2013, Yatoo *et al*. 2019a,b). Jhambh *et al*. (2013) and Bagnicka *et al*. (2008) have also reported decrease in somatic cell count, a neutrophil based count following vitamin E and N-acteyl cysteine supplementation in mastitis cows and goats respectively. Regaining of near normal TNF-α and IgG levels after treatment can be due antimycoplasma action of tylosin (Yatoo *et al*. 2019a,b) and immunomodulatory action of alpha tocopherol (Yatoo *et al*. 2018a) stabilizing

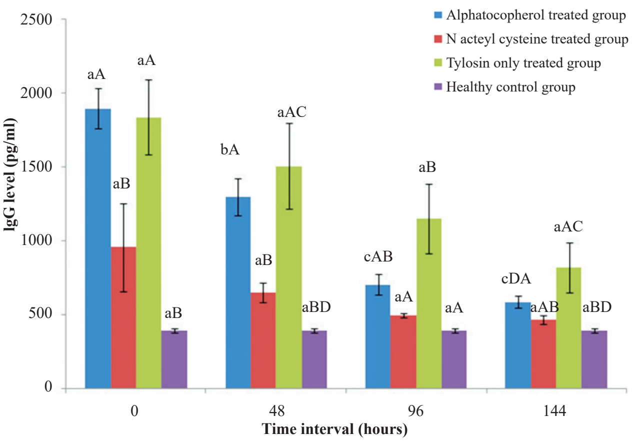

Fig. 3. Effect of different therapeutic regimes on IgG levels (pg/ml). (^{a,b,c,d}Values with different superscript differ significantly (P≤0.05) in a Group between various time intervals; ^{A,B,C,D}Values with different superscript differ significantly (P≤0.05) between different Groups at a particular time).

levels of inflammatory cytokines and immunoglobulins (Yatoo *et al*. 2019b, Finch and Turner 1996, Bagnicka *et al*. 2008, Anugu *et al*. 2013, Das *et al*. 2013, Ramadan *et al*. 2018, Yatoo *et al*. 2019b). Combination therapy facilitates regaining normal levels of immune indices (Yatoo *et al.* 2019b). Bednarek *et al*. (2003) have also reported supportive effect of anti-bacterial and anti-inflammatory agents in infections. More pronounced effect on immune indices in alpha tocopherol treated group than N-acteyl cysteine treated group (decrease in TNF-α 42.04% Vs 29.96%; IgG 68.86% Vs 51.23%) indicated better immunomodulatory role of alpha tocopherol in contagious caprine pleuropneumonia affected goats. Effective role of vitamin E in mediating cellular and humoral immune response has been reported (Shinde *et al*. 2007, Anugu *et al*. 2013, Yatoo *et al*. 2018a, Ramadan *et al*. 2018). Role of vitamin E in modulation of IgG response has also been reported (Gentry *et al*. 1992, Anugu *et al*. 2013, Ramadan *et al*. 2018). Vitamin E helps in immunomodulation through improvement in immune cell health and function, and antioxidant effect (Pollock *et al*. 1994, Hamam and Hala, Abou-Zeina 2007, Anugu *et al*. 2013, Ramadan *et al*. 2018) however regulation of IgG levels are determined by the phase of infection, vaccination or physiological stage of animal (Anugu *et al*. 2013, Yatoo *et al*. 2018a).

In conclusion, antigens of *Mycoplasma capricolum* subspecies *caprineumonia* stimulate oxidative and immune disturbances in contagious caprine pleuropneumonia. This results in increase in oxidants and decrease in antioxidants on one side and infiltration of inflammatory cells, production of inflammatory mediators, cytokines and immunoglobulins on the other side. Alpha tocopherol and N-acteyl cysteine as antioxidant and immunomodulator in conjunction with tylosin as antibiotic resulted in restoration of oxidative indices and immune indices to near normal levels. However, future thrust should be on exploring molecular mechanisms of this oxidative and immune interplay.

This study revealed that the oxidative stress in contagious caprine pleuropneumonia affected Pashmina goats may be due to elevated total oxidant status and decreased total antioxidant status. Further disturbances in immune response in these affected animals was indicated by altered cellular (TLC, DLC, TNF-α) and humoral (IgG) immune indices resulting in increased percentage of neutrophils, and TNF-α levels, and decreased percentage of lymphocytes and IgG levels. These oxidative and immune disruptions can aggravate the pathogenesis of this disease, thus necessitating intervention of antioxidants and/or immunomodulators for the therapeutic management of this disease in addition to antibiotics. Better antioxidant effect of N-acetyl cysteine in ameliorating oxidative disturbance in contagious caprine pleuropneumonia affected Pashmina goats by decreasing total oxidant status, oxidative stress index and increasing total antioxidant status. Similarly, alpha tocopherol showed better immunomodulatory effects by normalising total leukocyte count, percentage of neutrophils, lymphocytes, concentration of tumor necrosis factor-α and immunoglobulin G. Role of various antioxidants in ameliorating bacterial pneumonias have been described including acorbic acid (Zhao *et al*. 2021), vitamin D (Asgharpour *et al*. 2020), and vitamin A (Green *et al*. 2016). with antibiotics (Kashyap *et al*. 2019).

Thus, it can be concluded that for minimizing pathogenic alterations of contagious caprine pleuropneumonia, supplementation of antioxidants and immunomodulators in the therapeutic management of contagious caprine pleuropneumonia is imperative. Besides, this study establishes basis of oxidative and immune disturbances in contagious caprine pleuropneumonia; however, for elucidating molecular mechanisms and correlating these to virulence of *Mycoplasma capricolum* subspecies *capripneumonia* or the pathogenesis of contagious caprine pleuropneumonia, further studies are warranted.

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