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Haematological and biochemical restoration following *E. coli* lipopolysaccharide and silver nanoparticle therapy in cytological endometritic cows

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Abstract

Cytological endometritis is a prevalent postpartum uterine disorder that adversely affects fertility and reproductive performance in dairy cows. The present study was undertaken to evaluate the effect of intrauterine administration of *Escherichia coli* lipopolysaccharide (LPS) and silver nanoparticles (AgNPs) on haematological and biochemical parameters in cows diagnosed with cytological endometritis. A total of 86 postpartum cows were screened by endometrial cytology, and 24 cows exhibiting more than 5% polymorphonuclear neutrophils (PMNs) were selected and randomly allotted into two treatment groups. Group I cows received 100 µg of *E. coli* LPS intrauterinely at estrus, whereas Group II cows were administered 4 mg of silver nanoparticles intrauterinely for three consecutive days. Blood samples were collected before treatment and at the subsequent estrus following treatment for haematological and biochemical analyses. Significant ($p < 0.05$) increases in packed cell volume and lymphocyte percentage, accompanied by significant reductions in neutrophil and monocyte counts, were observed following treatment in both groups. Serum total protein concentration increased significantly, whereas creatinine and aspartate aminotransferase levels decreased following therapy. The observed changes indicate improvement in immune competence, metabolic status and resolution of uterine inflammation. The findings suggest that both *E. coli* lipopolysaccharide and silver nanoparticle therapies effectively restore altered haematological and biochemical profiles in cytological endometritic cows and may serve as promising non-antibiotic therapeutic alternatives for managing postpartum uterine infections.

Keywords: Cytological endometritis, *Escherichia coli* lipopolysaccharide, silver nanoparticles, haematology, biochemical parameters, non-antibiotic therapy, dairy cows

1. Introduction

Cytological endometritis also known as subclinical endometritis is one of the most important postpartum uterine disorders affecting reproductive efficiency in dairy cows. The condition is characterized by chronic inflammation of the endometrium without evident clinical signs and is diagnosed by the presence of increased polymorphonuclear neutrophils (PMNs) in endometrial cytology. Cytological endometritis has been associated with reduced conception rates, prolonged calving-to-conception intervals and substantial economic losses in dairy herds (Sheldon *et al.*, 2009; Nanda *et al.*, 2024) ^[31]. Following parturition, the uterus is frequently exposed to bacterial contamination. Failure of uterine defence mechanisms to eliminate invading pathogens predisposes cows to persistent uterine infections and endometrial inflammation. Among the bacterial pathogens involved, *Escherichia coli* is considered one of the major organisms implicated in postpartum uterine disease and altered uterine physiology (Ahmad *et al.*, 2003; Ahmad *et al.*, 2004) ^[1, 2]. Persistent uterine inflammation adversely affects reproductive performance and induces systemic physiological alterations in affected animals (Sarkar *et al.*, 2016) ^[28]. Haematological and biochemical parameters are valuable indicators of the health, metabolic and reproductive status of dairy cows. Alterations

in haemoglobin concentration, packed cell volume, leukocyte profile, serum proteins and metabolic biomarkers have been reported in cows affected with uterine infections (Heidarpour *et al.*, 2012; Perumal *et al.*, 2020; El-Sayed *et al.*, 2024a) ^[10, 11, 13, 24]. Furthermore, the proportion of PMNs within the postpartum uterus has been associated with ovarian activity and metabolic status which further highlights the importance of evaluating systemic responses during uterine disease (Krause *et al.*, 2014) ^[16]. Conventional treatment of endometritis has largely relied on antimicrobial therapy. However, increasing concerns regarding antimicrobial resistance and drug residues in animal products have stimulated interest in alternative therapeutic approaches. Immunomodulatory agents such as *Escherichia coli* lipopolysaccharide (LPS) have been shown to enhance uterine defence mechanisms and facilitate clearance of pathogenic microorganisms. Likewise, silver nanoparticles possess broad-spectrum antimicrobial activity and have emerged as promising non-antibiotic alternatives for the management of uterine infections (Salzano *et al.*, 2020; Nanda *et al.*, 2024) ^[27]. Assessment of haematological and biochemical changes following therapy can provide valuable information regarding the physiological recovery of affected animals and the efficacy of therapeutic interventions. Therefore, the present study was undertaken to evaluate the effect of intrauterine administration of *E. coli* lipopolysaccharide and silver nanoparticles on selected haematological and biochemical parameters in cows affected with cytological endometritis.

Materials and methods

The present study was conducted on postpartum cows presented for reproductive examination at the Department of Veterinary Gynaecology and Obstetrics, NTR College of Veterinary Science, Gannavaram, Andhra Pradesh. A total of 86 postpartum cows were screened for cytological endometritis using endometrial cytology by the cytotype technique. Endometrial cytological smears were stained and examined

Microscopically for determination of polymorphonuclear neutrophil (PMN) percentage. Cows showed more than 5 per cent PMNs in endometrial cytology were diagnosed as positive for cytological endometritis and selected for the study. Twenty-four cytological endometritic cows were randomly allocated into two treatment groups comprising 12 animals each. Cows in Group I received 100 µg of *Escherichia coli* lipopolysaccharide (LPS) administered intrauterinely at estrus. Cows in Group II were treated with 4 mg of silver nanoparticles administered intrauterinely for three consecutive days.

Blood samples were collected from the jugular vein of all animals immediately before treatment and again at the subsequent estrus following treatment. Samples were collected into EDTA vacutainers for estimation of haematological parameters and into clot activator vacutainers for harvesting serum used in biochemical analysis. Haematological parameters including haemoglobin (Hb), packed cell volume (PCV), neutrophil, lymphocyte, monocyte, eosinophil and basophil counts were estimated using standard laboratory procedures. Biochemical parameters including total protein (TP), blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated using commercially available diagnostic kits following the manufacturer's instructions. The data obtained were expressed as Mean ± SE. Statistical analysis was performed to compare the pre-treatment and post-treatment values within each treatment group. Differences were considered significant at $p < 0.05$.

Results and discussion

Haematological parameters

The haematological parameters recorded in cows affected with cytological endometritis before treatment and at the subsequent estrus following treatment with *Escherichia coli* lipopolysaccharide (LPS) and silver nanoparticles (AgNPs) are presented in Table 1.

Table 1: Haematological parameters (Mean ± SE) in cows with cytological endometritis before and after treatment

Group	Treatment	Hb (gm/dL)	PCV (%)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
<i>E. coli</i> LPS (Group I; n=12)	Before treatment	8.42 ± 0.12	25.91 ± 0.74 ^b	53.25 ± 1.51 ^a	38.25 ± 1.64 ^b	5.25 ± 0.13 ^a	2.25 ± 0.13	1 ± 0.0
	After treatment	8.47 ± 0.10	29.75 ± 0.69 ^a	45.91 ± 1.23 ^b	46.08 ± 1.23 ^a	4.25 ± 0.13 ^b	2.75 ± 0.13	1 ± 0.0
Silver nanoparticles (Group II; n=12)	Before treatment	8.10 ± 0.14	25 ± 0.53 ^b	55.83 ± 2.05 ^a	34.51 ± 2.08 ^b	5.83 ± 0.11 ^a	2.83 ± 0.11	1 ± 0.0
	After treatment	8.49 ± 0.11	30.25 ± 0.66 ^a	47.66 ± 1.67 ^b	44 ± 1.67 ^a	4.83 ± 0.11 ^b	2.16 ± 0.11	1 ± 0.0

Different superscripts (a,b) within a column for the same treatment group differ significantly ($p < 0.05$).

The mean haemoglobin concentration increased marginally following treatment in both groups. In cows treated with *E. coli* LPS, haemoglobin concentration increased from 8.42 ± 0.12 to 8.47 ± 0.10 g/dL, whereas in AgNP-treated cows it increased from 8.10 ± 0.14 to 8.49 ± 0.11 g/dL. However, the differences were non-significant ($p > 0.05$). The slight increase observed following treatment may be attributed to improvement in general health status and reduction in inflammatory stress associated with uterine infection. Similar observations were reported by Heidarpour *et al.* (2012) ^[13], Perumal *et al.* (2020) ^[24], Kumar *et al.* (2023), Singh *et al.* (2023) ^[33] and El-Sayed *et al.* (2024a) ^[10, 11], who documented improvement in haemoglobin values following successful

treatment of uterine disorders. The packed cell volume (PCV) increased significantly ($p < 0.05$) following treatment in both groups. In the LPS-treated group, PCV increased from 25.91 ± 0.74 to 29.75 ± 0.69 per cent, while in the AgNP-treated group it increased from 25.00 ± 0.53 to 30.25 ± 0.66 per cent. Similar findings were reported by Heidarpour *et al.* (2012) ^[13], Sarkar *et al.* (2016) ^[28], Cui *et al.* (2019) ^[9], Perumal *et al.* (2020) ^[24], Thangamani (2022) ^[35] and Nanda *et al.* (2024), who observed lower PCV values in endometritic animals and improvement following therapeutic intervention. The increase in PCV observed in the present study may indicate restoration of physiological and nutritional status following elimination of uterine infection.

The mean neutrophil percentage decreased significantly ($p < 0.05$) following treatment in both groups. Neutrophil

counts decreased from 53.25 ± 1.51 to 45.91 ± 1.23 per cent in the LPS-treated group and from 55.83 ± 2.05 to 47.66 ± 1.67 per cent in the AgNP-treated group. Increased neutrophil counts prior to treatment are indicative of active uterine inflammation and recruitment of inflammatory cells to the site of infection. The significant reduction following treatment suggests alleviation of uterine inflammation and recovery of normal physiological status. Similar findings were reported by Ahmad *et al.* (2003) [1], Kekan *et al.* (2005) [15], Sarma (2007) [29], Kumar *et al.* (2017) [17], Perumal *et al.* (2020) [24] and El-Sayed *et al.* (2024a) [10, 11].

The mean lymphocyte percentage increased significantly ($p < 0.05$) following treatment in both groups. In the LPS-treated group, lymphocyte count increased from 38.25 ± 1.64 to 46.08 ± 1.23 per cent, while in the AgNP-treated group it increased from 34.51 ± 2.08 to 44.00 ± 1.67 per cent. The lower lymphocyte counts observed before treatment may be attributed to stress and inflammatory conditions associated with endometritis. The significant increase following treatment indicates restoration of immune competence and recovery from infection. Similar observations were reported by Kekan *et al.* (2005) [15], Kumar *et al.* (2017) [17], Cui *et al.*

(2019) [9], Thangamani (2022) [35] and Singh *et al.* (2023) [33]. The monocyte percentage decreased significantly ($p < 0.05$) following treatment in both groups. The values declined from 5.25 ± 0.13 to 4.25 ± 0.13 per cent in the LPS-treated group and from 5.83 ± 0.11 to 4.83 ± 0.11 per cent in the AgNP-treated group. The reduction in monocyte count may be associated with regression of inflammatory processes and normalization of immune responses following successful treatment. The eosinophil and basophil counts remained within normal physiological limits and did not differ significantly before and after treatment in either group. Similar findings were reported by Ahmad *et al.* (2003) [1], Sahoo *et al.* (2014) [26] and Singh *et al.* (2023) [33], who observed no significant alterations in eosinophil and basophil counts following treatment of uterine infections.

Biochemical parameters

The biochemical parameters recorded in cows affected with cytological endometritis before treatment and at the subsequent estrus following treatment with *Escherichia coli* lipopolysaccharide (LPS) and silver nanoparticles (AgNPs) are presented in Table 2.

Table 2: Biochemical parameters (Mean $\pm$ SE) in cows with cytological endometritis before and after treatment

Group	Treatment	TP (gm/dL)	BUN (mg/dL)	CK (mg/dL)	ALT (IU/L)	AST (IU/L)
<i>E. coli</i> LPS (Group I; n=12)	Before treatment	6.96 ± 0.19^b	22.16 ± 0.19	1.68 ± 0.09^a	23.19 ± 0.13	89.41 ± 0.26^a
	After treatment	7.48 ± 0.13^a	22.91 ± 0.22	1.19 ± 0.07^b	23.75 ± 0.27	84.41 ± 1.58^b
Silver nanoparticles (Group II; n=12)	Before treatment	7.30 ± 0.13^b	21.39 ± 0.06^b	1.52 ± 0.05	24.67 ± 0.82^a	88.97 ± 0.26^a
	After treatment	7.50 ± 0.09^a	22.66 ± 0.22^a	1.40 ± 0.07	22.91 ± 0.31^b	80.50 ± 2.63^b

Different superscripts (a,b) within a column for the same treatment group differ significantly ($p < 0.05$)

The mean total protein concentration increased significantly ($p < 0.05$) following treatment in both groups. In cows treated with *E. coli* LPS, total protein concentration increased from 6.96 ± 0.19 to 7.48 ± 0.13 g/dL, whereas in AgNP-treated cows it increased from 7.30 ± 0.13 to 7.50 ± 0.09 g/dL. Similar findings were reported by Srivastava and Sahni (2000) [34], Pandey *et al.* (2009), Amle *et al.* (2014) [3], Patel *et al.* (2020) [22] and El-Sayed *et al.* (2024b) [10, 11], who observed lower serum total protein concentrations in endometritic animals compared to healthy animals. The increase in total protein concentration following treatment may be attributed to restoration of uterine health, improved metabolic activity and reduction in inflammatory stress. The mean blood urea nitrogen (BUN) concentration increased slightly following treatment in both groups. In the LPS-treated group, BUN concentration increased from 22.16 ± 0.19 to 22.91 ± 0.22 mg/dL, whereas in the AgNP-treated group it increased significantly ($p < 0.05$) from 21.39 ± 0.06 to 22.66 ± 0.22 mg/dL. The increase in BUN concentration may be associated with restoration of normal protein metabolism and improvement in nutritional status following recovery from uterine infection. Similar observations were reported by Ghanem *et al.* (2016) [12], Jan *et al.* (2021) [14] and Safak *et al.* (2023) [25].

The mean creatinine concentration decreased following treatment in both groups. In the LPS-treated group, the reduction was significant ($p < 0.05$), declining from 1.68 ± 0.09 to 1.19 ± 0.07 mg/dL, whereas in the AgNP-treated group it decreased from 1.52 ± 0.05 to 1.40 ± 0.07 mg/dL. Elevated creatinine concentrations prior to treatment may be associated with metabolic disturbances and inflammatory stress accompanying uterine infection. The reduction observed following treatment indicates improvement in metabolic status and physiological recovery. Similar findings were

reported by Shabunin *et al.* (2020) [30], Torres *et al.* (2020) [36], Shinde (2022) [32] and El-Sayed *et al.* (2024b) [10, 11]. The mean alanine aminotransferase (ALT) activity showed minimal variation following treatment. In the LPS-treated group, ALT activity increased slightly from 23.19 ± 0.13 to 23.75 ± 0.27 IU/L, whereas in the AgNP-treated group it decreased significantly ($p < 0.05$) from 24.67 ± 0.82 to 22.91 ± 0.31 IU/L. The values remained within normal physiological limits, suggesting absence of severe hepatic damage. Similar observations were reported by Boonprong *et al.* (2007) [6], Mondal *et al.* (2012) [19], Biswal *et al.* (2013) [5] and Chaudhari *et al.* (2020) [7]. The mean aspartate aminotransferase (AST) activity decreased significantly ($p < 0.05$) following treatment in both groups. In the LPS-treated group, AST activity declined from 89.41 ± 0.26 to 84.41 ± 1.58 IU/L, while in the AgNP-treated group it decreased from 88.97 ± 0.26 to 80.50 ± 2.63 IU/L. Elevated AST levels prior to treatment may be associated with tissue damage and inflammatory stress resulting from uterine infection. The reduction following treatment indicates recovery of tissue integrity and improvement in physiological status. Similar findings were reported by Azawi *et al.* (2008) [4], Heidarpour *et al.* (2012) [13], Chaurasia *et al.* (2016) [8], Chaudhari *et al.* (2020) [7] and Shinde (2022) [32]. Overall, the biochemical findings of the present study indicate that successful treatment of cytological endometritis with either *E. coli* lipopolysaccharide or silver nanoparticles was associated with improvement in metabolic status, reduction in inflammatory stress and restoration of normal physiological functions.

Conclusion

The present study demonstrated that cytological endometritis is associated with significant alterations in haematological

and biochemical parameters, reflecting the inflammatory and metabolic disturbances accompanying uterine infection in cows. Treatment with either *Escherichia coli* lipopolysaccharide (LPS) or silver nanoparticles resulted in favourable changes in haematological indices, including significant increases in packed cell volume and lymphocyte percentage, along with significant reductions in neutrophil and monocyte counts. Similarly, biochemical evaluation revealed significant increases in total protein concentration and reductions in creatinine and aspartate aminotransferase activity following treatment. These changes indicate improvement in immune competence, metabolic status and physiological recovery of affected animals. The findings suggest that both *E. coli* lipopolysaccharide and silver nanoparticles are effective non-antibiotic therapeutic alternatives for the management of cytological endometritis and contribute to restoration of normal haematological and biochemical profiles. Therefore, these therapeutic approaches may be considered promising options for improving uterine health and reproductive performance in postpartum dairy cows.

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