RESEARCH ARTICLE

Therapeutic Efficacy of Silver Nanoparticles in Clinical Endometritis Affected Buffaloes

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Abstract

Bacterial invasion of the uterus during the postpartum period is a leading cause of clinical endometritis in dairy cattle and buffaloes. Excessive use of antibiotics has led to bacterial resistance, prompting the need for alternative therapies. Sixteen buffaloes diagnosed with clinical endometritis confirmed through detailed clinico-gynecological examination were selected for the current study and were divided into two groups of eight each. Group I (n=8) received intrauterine administration of 4 mg silver nanoparticles in 20 mL PBS for three consecutive days, while Group II (n=8) received a single dose of 100 µg *E. coli* LPS in 20 mL PBS. These animals were evaluated for haemato-biochemical parameters before and after treatment, which revealed significant reduction ($p<0.05$) in serum creatinine kinase at subsequent estrus post-treatment in both the groups. Haemoglobin and PCV increased non-significantly ($p>0.05$), whereas serum BUN decreased non-significantly at subsequent estrus after treatment. The recovery and conception rates were 75.0% and 66.7% in Group I and 85.5% and 71.4% in Group II, respectively, which did not differ significantly. Based on the present study, Silver nanoparticles are proved to be effective in managing clinical endometritis, demonstrating similar recovery and conception rate to those of *E. coli* LPS, thereby serving as a promising alternative to antibiotic therapies.

Key words: Clinical endometritis, Conception rate, *E. coli* LPS, Haemato-biochemical parameters, Silver nanoparticles, Recovery rate.

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Introduction

Clinical endometritis (CE) is a uterine disease which commonly affects postpartum dairy cows, leading to lower fertility and significant economic losses in the dairy industry (Sheldon *et al.,* 2009). This condition is characterized by the presence of purulent or mucopurulent discharge from the uterus, often accompanied by inflammation without any systemic signs of illness (LeBlanc, 2008). The primary causative agents of CE are pathogenic bacteria, including *Escherichia coli*, *Trueperella pyogenes*, and *Fusobacterium necrophorum*, which invade the uterine lining and disrupt its normal function (Williams *et al*., 2005). The severity of endometritis mostly depends on the immunity of the host and the virulence of invading microbes along with some poor managemental factors resulting in increased intercalving period and conception failure (LeBlanc, 2008).

The use of antibiotics remains a common therapeutic strategy for managing clinical endometritis which aims to eliminate bacterial infection and restore uterine health (Sheldon and Dobson, 2004). However, widespread and indiscriminate use of antibiotics has contributed to the development of antibiotic resistance, making treatments less effective over time (Singer *et al.,* 2003). Antibiotic resistance in uterine pathogens has been reported globally, raising concerns about the sustainability of current treatment practices (Swartz, 2002). Furthermore, antibiotic residues in milk and meat pose additional risks to public health and food safety, driving the need for alternative or more judicious treatment strategies (Oliver *et al.,* 2011). This raised the concern for many researchers to develop non-antibiotic agents to mitigate bacterial resistance to various antibiotics. *E. coli* lipopolysaccharide (LPS) has been widely used in treating endometritis, showing promising results in modulating uterine immune responses by enhancing the recruitment and activity of neutrophils and macrophages, accelerating the resolution of infection and improving uterine health (Singh *et al.,* 2003). Silver nanoparticles (AgNPs) are emerging as promising antimicrobial agents due to their broad-spectrum antibacterial and anti-inflammatory properties (Rai *et al.,* 2012). These nanoparticles exhibit potent

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antibacterial activity against both Gram-positive and Gram-negative bacteria, primarily through mechanisms such as disruption of bacterial cell membranes, generation of reactive oxygen species (ROS), and interference with bacterial DNA replication (Morones *et al.,* 2005). The anti-inflammatory properties of AgNPs are attributed to their ability to modulate inflammatory cytokine production, reduce oxidative stress, and inhibit the activation of inflammatory pathways, such as NF-κB (Gliga *et al.,* 2020). Based on this context, the current study hypothesized that the intrauterine infusion of silver nanoparticles could effectively eliminate bacterial infection responsible for endometritis compared with *E. coli* LPS, which is proved to be highly effective.

Materials And Methods

Sixteen buffaloes presented to the large animal Gynaecological ward, Department of Veterinary Gynaecology and Obstetrics, NTR College of Veterinary Science, Gannavaram (India), which had calved at least once and diagnosed positive for clinical endometritis depending on the clinic-gynaecological examination based on per-rectal examination, uterine mucus score, pH of uterine mucus, leucocyte, esterase activity in uterine discharge with presence of >18 % PMN cells in uterine cytology were selected for the present study. These animals were divided into two groups of 8 each, where Group I (n=8) received intrauterine administration of 4 mg silver nanoparticles solution (10 mg/mL, obtained from IgY Immunologix Hyderabad) in 20 mL PBS for three consecutive days, while Group II (n=8) received a single dose of 100 µg *E. coli* LPS (from Sigma-Aldrich, Germany) in 20 mL PBS.

The dosage of silver nanoparticles to be administered was calculated based on the growth inhibition effect (EC50) of nano-silver on *E. coli* (Fig. 1), which is one of the major causative agent for uterine infections. The Fig. 1 is a graphical representation of the effect of silver nanoparticles at various concentrations on *E.coli*

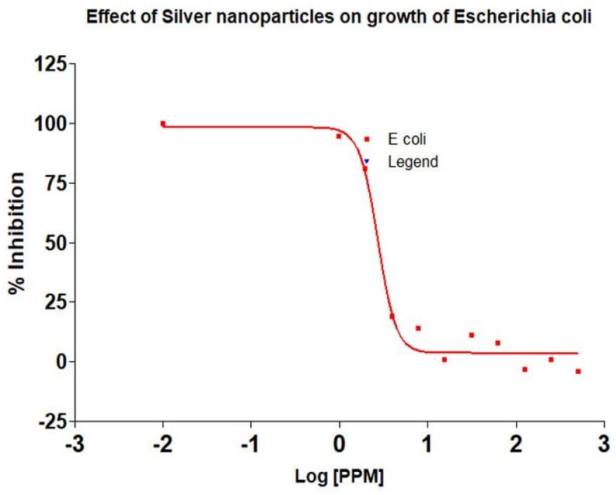

Fig. 1: Sigmoidal dose-response cure for effect of siver nanoparticles on growth of *E.coli*

Blood and serum samples were obtained before the treatment and at the subsequent estrus after the treatment. The serum was separated from jugular blood samples by centrifugation at 1000 xg and was stored at -20 °C for subsequent analysis. Whole blood samples were analysed for haemoglobin (Hb) and packed cell volume. Serum biochemical profiles such as blood urea nitrogen (BUN) and serum creatinine were estimated by IFCC method using Multiskan GO, Thermo Scientific Instruments with the help of commercially available kits manufactured by Erba Diagnostics Pvt. Ltd. The animals were re-examined for recovery based on pH and PMN cells in uterine discharge, and ultrasonographic examination at the subsequent estrus.

Statistical differences in the mean values of the tested parameters before and after treatment were analysed using paired sample t-test and the percentage recovery and conception rate between the groups were analysed using Chi-Square test with the support of Windows XV (version 22.0, SPSS IBM Corp), and Microsoft Excel (version 2019).

Results and Discussion

Changes in Haemato-Biochemical Parameters

At the subsequent estrus a significant reduction ($p>0.05$) in serum creatinine levels was observed after the treatment with silver nanoparticles (Group I) and *E. coli* LPS (Group II). Haemoglobin and PCV levels increased non-significantly ($p>0.05$), whereas serum BUN levels decreased non-significantly at subsequent estrus after treatment with silver nanoparticles and *E. coli* LPS (Table 1).

Thrall (2004) stated that inflammatory diseases interfered with erythropoiesis, primarily through the action of cytokines which suppressed the production and function of erythropoietin. These mechanisms collectively resulted in reduced PCV, reflecting anaemia of inflammation or chronic disease. It was suggested that the immunomodulatory properties of the treatment molecules might contribute to a non-significant increase in Hb and PCV levels. The improvement was likely due to the enhanced uterine health and effective elimination of bacterial infection from the uterine lumen.

Endometritis induces a systemic inflammatory response, which may alter renal perfusion and glomerular filtration rates, leading to impaired creatinine clearance (Azawi *et al.,* 2008). The non-significant decrease in mean serum BUN in the present study might be due to low grade clinical endometritic buffaloes used for the present study and also Moore and Varga (1996) stated that the rise in blood urea may also be due to muscle proteolysis, which occur to meet a nutrient deficit, caused by low feed consumption or provision of poor-quality forage.

Recovery and Conception Rate

The recovery rate was based on the pH and PMN cells (Fig. 2) before treatment and at the subsequent estrus after treatment. The mean pH and PMN cell were highly

Table 1: Haemato-biochemical values of clinical endimetric buffaloes before treatment and at subsequent estrus after treatment (Mean ±SE, n=8)

Group	Treatment	Hb (g/dL)	PCV (%)	BUN (mg/dL)	CK (mg/dL)
Silver nanoparticles (Group I)	Before treatment	11.43±0.26	35.02±0.36	21.30±0.82	1.51±0.11
	After treatment	12.06±0.13	35.76±0.19	21.03±0.81	1.40±0.11
E. coli LPS (Group II)	Before treatment	11.15±0.25	34.10±0.34	21.52±0.80	1.38±0.11
	After treatment	11.71±0.22	34.57±0.23	21.26±0.72	1.27±0.09

Data did not vary statistically between periods (p>0.05).

Table 2: pH of uterine mucus discharge and PMN cells in cytology in clinical endometritic buffaloes before and after treatment (at subsequent estrus) (Mean ±SE)

Group	No. of animals	pH of cervico-vaginal mucus		Percent of PMN cells	
		Before treatment	At subsequent estrous	Before treatment	At subsequent estrous
G-I	8	8.7±0.18	6.5 ± 0.25**	25.56±1.03	5.3±0.75**
G-II	8	8.8±0.15	6.5 ± 0.14**	25.62±1.31	5.5±0.54**

**p<0.01 between periods

Table 3: Clinical recovery rate and conception rate in clinical endometritis affected buffaloes based on uterine discharge cytology

Treatment Groups	No. of animals treated	No. of animals recovered	Rate of recovery (%)	No. of animals conceived	Conception rate (%) among recovered
G-I	8	6	75.00	4	66.66
G-II	8	7	87.50	5	71.43

significantly reduced at subsequent estrus post-treatment in both the groups, with no group difference at either of the stage (Table 2).

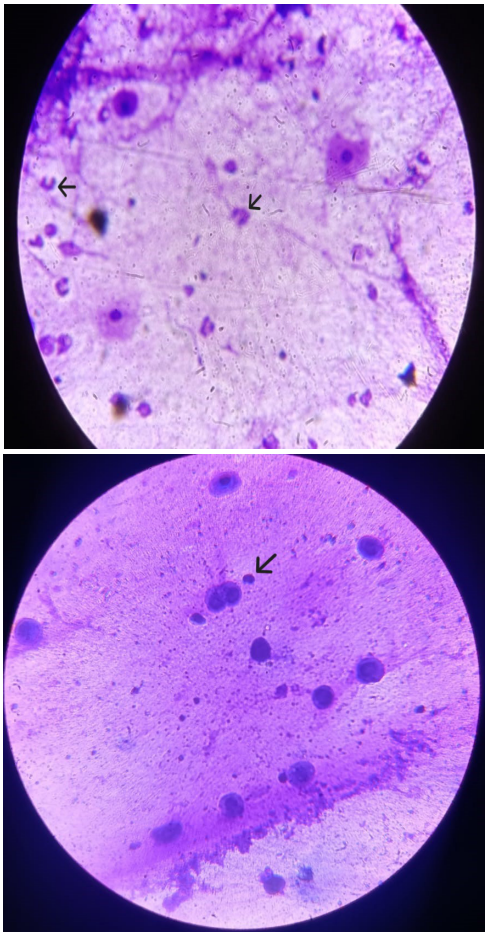

Fig. 2: PMN cells in endometrial cytology before and after treatment

Clinical recovery and conception rate of Group I buffaloes which received intrauterine AgNPs therapy was 75.00% (6/8) and 66.66% (4/6), respectively. The corresponding rates observed in the *E. coli* LPS group (Group II) were 87.50% (7/8) and 71.43% (5/7), respectively (Table 3). The results indicate that buffaloes in Group I exhibited a slightly lower recovery and first-service conception rate compared to Group II, with no statistically significant difference. This suggests that the efficacy of silver nanoparticles (AgNPs) is comparable to that of *E. coli* LPS, making them effective alternatives to antibiotic therapies for improving recovery and conception rates in buffaloes with clinical endometritis.

Postpartum endometritis is a prevalent reproductive condition in dairy buffaloes and cows, then in other domestic animals. It is categorized into clinical and subclinical forms based on rectal examination of the reproductive organs, the properties of cervico-vaginal mucus, and endometrial cytology (Kasimanickam *et al.,* 2004; Singh *et al.,* 2017). Clinical endometritis is an inflammation of the endometrial lining, often occurring in postpartum dairy cattle and other animals typically marked by the presence of purulent or mucopurulent discharge which can lead to compromised fertility, prolonged calving intervals, and substantial economic loss due to reduced reproductive efficiency (Sheldon *et al.,* 2009). Silver nanoparticles are renowned for their broad-spectrum antimicrobial efficacy against a variety of pathogens. They operate by disrupting cell membranes, generating reactive oxygen species, and interacting with microbial DNA, which prevents replication and leads to cell death (Rai *et al.,* 2012). The nanoscale of AgNPs enhances their bioavailability and retention within tissues, making them suitable for intravaginal or intrauterine administration in livestock (Fayyaz, 2021). Studies have found that AgNPs can inhibit the growth of

common endometritis pathogens and prevent biofilm formation, which is crucial for treating persistent infections. AgNPs have also demonstrated anti-inflammatory effects by modulating cytokine expression, reducing inflammatory responses in tissues (Natan and Banin, 2017).

This study in general suggests that the efficacy of silver nanoparticles (AgNPs) is comparable to that of *E. coli* LPS, making them an effective alternative to antibiotic therapies for improving recovery and conception rates in buffaloes with clinical endometritis without any antibiotic resistance. Moreover, the treatment cost for clinical endometritis in buffaloes could be reduced by utilizing readily available AgNPs in place of *E. coli* LPS, which is the gold standard for treatment of endometritis, disadvantages being difficulty to procure with high initial cost.

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