RESEARCH ARTICLE

Effect of Ashwagandha, Turmeric and Antibiotic on Blood Biochemical Profile of Repeat Breeding Gir Cows with Subclinical Endometritis

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Abstract

The present study was conducted to evaluate the influence of ashwagandha and turmeric on blood biochemical profile in subclinical endometritis (SCE) affected repeat breeding Gir cows. Thirty apparently healthy repeat breeder Gir cows, aged 4 to 8 years (>90 days in milk) and positive for SCE on cytobrush technique, were selected from the University Farm and nearby areas of Junagadh region. They were randomly divided into 5 equal groups. In Group A (positive control), cows were administered 30 mL of normal saline intrauterine (I/U) as a single dose. Group B received 30 mL of moxifloxacin I/U for 3 consecutive days. In Group C and D, cows were treated with 5 mL of ashwagandha and 5 mL of turmeric extract diluted in 30 mL of distilled water, respectively, while Group E received a combination treatment of 5 mL each of ashwagandha and turmeric extract, diluted in 30 mL of distilled water and administered I/U for 3 consecutive days. Blood samples collected at estrus before treatment (0 hr) and at subsequent estrus after treatment were compared for changes in blood biochemical profile. Following treatment, significant increase ($p<0.05$) in blood glucose, calcium, phosphorus, and TAC levels, and decrease in serum urea and SAA concentrations were noted across all SCE affected groups, whereas serum lipid peroxidation (LPO) decreased non-significantly ($p>0.05$). The highest first service conception rate was observed in the group treated with the combination of ashwagandha and turmeric extract (66.66%), followed by turmeric extract alone (50.00 %), ashwagandha extract alone (33.33%) sensitive antibiotic (33.33%), and control (0.00%). The study revealed that a combination of ashwagandha and turmeric extract has best therapeutic efficacy and can be used as an alternative therapy for the treatment of subclinical endometritis in repeat breeding cows.

Key Words: Gir cows, Biochemical profile, Subclinical endometritis, Therapeutic regimens.

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Introduction

India is agro-based country having agriculture and livestock husbandry and their management as a main occupation. The dairy industry's success hinges on maintaining a precise and optimal reproductive cycle of each animal in the herd. Any disruptions to this rhythm can result in significant economic losses, primarily due to infertility issues. Repeat breeding due to endometritis is one of the major gynaecological problems affecting the reproductive efficiency. Endometritis is localized inflammatory condition of endometrium, which is of two type: clinical and subclinical endometritis. SCE in dairy bovines is characterized by an elevated polymorphonuclear cells (PMN) proportion in endometrial cytology with clear cervico-vaginal discharge. That is why SCE is also called as cytological endometritis. During last decade, SCE and its impact on fertility have been intensely investigated. It is the most prevalent of all uterine diseases as it affects approximately 30% of lactating dairy cows, with the prevalence ranging from 11 to >70% in some herds (Pascottini *et al.*, 2017). The prevalence of SCE has been reported to be 12.7% (Pothmann *et al.*, 2015) to 40.2% (Janowski *et al.*, 2013) in repeat breeder cows.

The cytobrush or cytotape technique has been established as a recent diagnostic method to detect SCE in cows with no signs of endometritis. The therapeutic approach towards the subclinical endometritic cows involves either antibiotics/ antiseptics or hormonal therapy. It is acknowledged fact that the inadequate and indiscriminate use of antibacterial drugs may lead to partial or complete antimicrobial resistance

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(AMR) and high cost of antibiotics are other concerns. To overcome these problems, researchers are trying to develop various alternative therapies to treat SCE in cows. Plant-based remedies have served as a source of valuable medication due to their antimicrobial and anti-inflammatory properties, immune-modulatory activities, and precious therapeutic properties for maintenance of general health and healing potential (Wu *et al.,* 2018). Turmeric (*Curcuma longa*), one of such agents has proven anti-inflammatory, antioxidant, anti-mutagenic, and antibacterial pharmacological activities (Verma *et al.,* 2018). Similarly, ashwagandha (*Withania somnifera*) is also an important herb in the ayurvedic and indigenous medicinal system for over 3000 years. It is a sedative, diuretic, anti-inflammatory agent, generally used for increasing energy and endurance, and acts as an adaptogen that exerts a strong immune-stimulatory and anti-stress effect.

SCE is associated with a range of biochemical and physiological changes. These changes can be assessed through various blood biochemical parameters, which provide insights into the cow's systemic and reproductive health status. Understanding of these biochemical parameters helps in the timely diagnosis and treatment of SCE, thereby improving reproductive performance and overall health of animals. Therefore, in the present study, the blood biochemical profile of Gir cows affected by SCE was evaluated both before and after treatment.

Materials and Methods

The study was carried out following approval of experimental protocol (No. KU-JVC-IAEC-LA-117-2023) by the Institutional Animal Ethics Committee of the College. Apparently healthy repeat breeder Gir cows, aged 4 to 8 years, and more than 90 days postpartum were screened by endometrial cytobrush technique from Cattle Breeding Farm, Kamdhenu University, Junagadh and nearby areas of Junagadh region. These animals were maintained as per the standard feeding schedule followed at the farm or farmer's doorstep. Rectal palpation of the genital tract was conducted on each cow to exclude any genetic, hereditary or acquired defects. The SCE was declared in the cows on the basis of clear cervico-vaginal discharge and the cytobrush smears having ≥5% PMNs (Pothmann *et al.,* 2015). Thirty SCE affected Gir cows identified were randomly divided into five equal groups (6 animals/group) and were treated during current estrus with different medications intrauterine. In Group A (Positive control), 30 mL Normal Saline i/ut once; in Group B, Moxifloxacin (30 mL for 3 consecutive days i/ut); in Group C, Ashwagandha extract (5 mL in 30 mL distilled water for 3 consecutive days i/ut); in Group D, Turmeric extract (5 mL in 30 mL distilled water for 3 consecutive days i/ut); and in Group E ashwagandha and turmeric extract (5 mL each in 30 mL distilled water for 3 consecutive days i/ut) were administered. Animals were inseminated at subsequent estrus.

From each animal, blood samples were collected at estrus pre-treatment (0 h) and at subsequent estrus in clot activators vacutainer tubes. Blood glucose was estimated immediately after collection of blood sample using glucometer. Various biochemical constituents, *viz.*, serum urea, calcium, phosphorus, lipid peroxidation, total antioxidant capacity and serum amyloid-A were estimated with the help of automatic biochemical analyzer (Dia-Chem 240 plus, China) using standard diagnostic kits. The data were analyzed for Mean ±SEs by descriptive statistics. One-way analysis of variance (ANOVA) was used to see the treatment and period effect on the biochemical profile. The Duncan's MRT post-hoc test was used to compare pair-wise mean differences between the groups at $p<0.05$.

Results and Discussion

Blood Biochemical and Antioxidant Status

The group-wise findings on various blood biochemical attributes before and after treatment are presented in Table 1. Gir cows with SCE showed significantly reduced blood glucose profile. There was marked and significant ($p<0.05$) rise in the mean glucose concentrations in all experimental groups when assessed at the subsequent estrus following the administration of different treatments as compared to their pre-treatment values (0 hr). The therapeutic regimens employed were successful in eliminating uterine infections, which likely contributed to the observed rise in blood glucose levels during the subsequent estrus. The findings of the present study regarding the effect of antibiotics aligned with the reports of Chaudhari *et al.* (2020) and Parikh (2021). The observed increase in blood glucose levels following treatment may be attributed to elimination of microorganisms from the uterus with a reduction in PMN cell percentages after therapy, which restored the energy balance and resulted in an increase in glucose concentration (Singh *et al.,* 2023). A review of the literature however did not reveal any report on the effects of turmeric and ashwagandha extracts on various biochemical parameters in cows affected by SCE to compare our results.

Gir cows with SCE exhibited elevated urea concentrations. After treatment, all groups showed a non-significant reduction in urea levels compared to their pre-treatment values (0 hr). These findings stand in contrast to the results of Chaudhari *et al.* (2020), who identified a significant difference between pre- and post-treatment values. The increase in serum urea concentration in cows affected by endometritis could be attributed to muscle proteolysis, a process that occurs to compensate for the nutrient deficit in postpartum cows, as they often experience a negative energy balance. On the other hand, low urea levels might result from insufficient protein intake, potentially due to reduced feed consumption or the provision of low-quality forage. Serum urea levels are influenced by various parameters, including dietary protein intake and its rumen degradability, amino acid composition, protein intake relative to the cow's needs, liver and kidney function, muscle tissue breakdown, as well as the amount and rumen degradability of dietary carbohydrates.

In the present study, the mean serum calcium levels showed an increase post-treatment in all groups but the difference was statistically significant ($p<0.05$) in Groups C, D, and E only, compared to their pre-treatment levels (Table 1). The current increase in serum calcium post-treatment compared to pre-treatment values across all treated groups concurred well with the findings reported by Chaudhari *et al.* (2020) and Parikh (2021) with different therapies. Pandey *et al.* (2009) and Das *et al.* (2012) also reported that the mean serum calcium concentration was significantly lower ($p<0.05$) in repeat breeder cows compared to normal healthy cows. A low plasma calcium profile in repeat breeder cows may be attributed to metabolic disturbances that impair calcium absorption from the gut. Inflammatory condition can disrupt the regulation of serum calcium, potentially leading to hypocalcemia (Bradford *et al.,* 2015). After ashwagandha, turmeric and ashwagandha plus turmeric extract treatment, which subsequently decreases the risk of infectious diseases such as metritis and endometritis, the calcium level gets increased. Calcium plays a crucial role in neuromuscular excitability, muscle contractions, and nerve impulse transmission at the cellular level. Calcium is crucial for sensitizing the female genital tract to hormones like oxytocin, and its deficiency may predispose cows to uterine inertia, leading to dystocia, retained fetal membranes, and endometritis.

In the current study, Gir cows with SCE also exhibited reduced phosphorus levels (0 day), which increased markedly and significantly ($p<0.05$) in all experimental treatment groups when assessed at the subsequent estrus post-treatment. These findings concurred well with the reports of Chaudhari *et al.* (2020) and Parikh (2021) using different therapeutic modalities in SCE cows. Pandey *et al.* (2009) and Das *et al.* (2012) also reported that the mean serum phosphorus concentration was significantly lower ($p<0.05$) in repeat breeders compared to normal healthy cows. Phosphorus plays a crucial role in the transfer of biological energy via ATP, and its deficiency can disrupt normal enzymatic reactions, impacting the proper function of reproductive organs. Inadequate phosphorus intake not only affects reproductive hormones, but also influences the immune system, potentially increasing susceptibility to infections like metritis and SCE. Furthermore, it has been observed that the phosphorus-to-calcium ratio in the diet is crucial for maintaining optimal reproductive health. An imbalanced ratio, particularly with excess calcium relative to phosphorus, may exacerbate issues related to fertility and uterine health in cattle.

The mean serum LPO levels were found to be quite elevated in SCE cows. Following treatment, it showed a significant decrease ($p<0.05$) in groups C, D and E, however, in Group B, the decrease in post-treatment serum LPO level was not statistically significant ($p>0.05$) compared to pre-treatment level. According to Parikh (2021), the average serum LPO levels decreased significantly ($p<0.05$) after treatment in all the groups. Similarly, Heidarpour *et al.* (2012) found that MDA levels were notably higher ($p<0.001$) in cows with SCE, measuring 29.76±12.36 nmol/l, compared to the control group (17.03±4.83 nmol/l). Antioxidant systems play a crucial role in both the development and persistence of endometritis, as well as in protecting the organism from the harmful effects of free radicals (Heidarpour *et al.,* 2012). It is well established that inflammatory conditions, such as endometritis, compromise the body's antioxidant defenses. Serum MDA levels can serve as a biochemical marker to assess the intensity of endometritis in cows. Kaya *et al.* (2017) showed that blood serum MDA concentrations were linked to the severity of endometritis. In Gir cows with SCE, elevated LPO levels can compromise the integrity of uterine cells, impairing their function and contributing to poor reproductive outcomes. Additionally, LPO byproducts like malondialdehyde (MDA) can further exacerbate the inflammation and cause tissue damage, prolonging the condition and delaying recovery.

The mean serum TAC levels were also observed to be reduced in all SCE cows (0 day), which rose markedly and significantly ($p<0.05$) in all experimental groups when assessed at the subsequent estrus post-treatment (Table 1). Kaya *et al.* (2017) found that endometritis was linked to a significant decrease ($p<0.05$) in TAC levels. Our findings also support the results of Perumal *et al.* (2020), who reported significantly lower antioxidant profiles in cows with endometritis compared to healthy cows. In cows with SCE, the inflammatory response may lead to an imbalance between pro-oxidant and antioxidant systems, contributing to oxidative stress.

The mean SAA levels were also found elevated in SCE cows, which showed a significant decrease ($p<0.05$) post-treatment in Groups B, D and E compared to pre-treatment levels. However, in Group C, the decrease in post-treatment SAA levels was not statistically significant ($p>0.05$). Parikh (2021) while evaluating Gir cows for SCE found lower mean concentration of SAA level in healthy Gir cows compared to those with SCE as we observed. According to Biswal *et al.* (2014), Ahmadi *et al.* (2018) and Parikh (2021), the average SAA levels decreased significantly ($p<0.05$) after treatment in all the groups. Brodzki *et al.* (2015) demonstrated that SAA levels were significantly ($p<0.001$) higher in cows with SCE compared to healthy controls. Salah *et al.* (2021) however reported that SAA levels significantly ($p<0.05$) decreased in both the SCE and healthy groups in postpartum buffaloes.

SAA is an acute phase protein primarily synthesized by liver parenchymal cells in response to inflammation, particularly during uterine infections. Chapwanya *et al.* (2013) suggested that endometrial cells in cows may also contribute to SAA secretion, highlighting its role in reproductive health. SAA levels typically rise in conjunction with various infections and inflammatory conditions, making it a valuable biomarker for assessing inflammatory responses in cattle (Bannikov *et al.,* 2011). In addition to its role in cholesterol metabolism, it also exhibits immunological functions. It binds directly to Gram-negative bacteria, facilitating their opsonization, which enhances phagocytosis by immune cells. Furthermore, SAA can modulate inflammatory responses by inhibiting the phagocytic burst and activating platelets, contributing to the body's overall defence mechanism. In the present study, the therapeutic regimens, which included sensitive antibiotic, turmeric extract, and ashwagandha combined with turmeric extract, effectively reduced bacterial infection in the uterus. This led to a decrease in the inflammatory response in cows, as evidenced by the reduction in SAA concentrations post-treatment. The present findings regarding the effect of sensitive antibiotic treatment on SAA concentrations in cows with SCE aligned well with the reports of Biswal *et al.*(2014) and Parikh (2021), who noted a significant ($p<0.05$) reduction in SAA concentrations following treatment with sensitive antibiotics in subclinical endometritic cows.

Table 1: Blood biochemical changes before and after treatment in SCE affected Gir cows (Mean ± SE)

Parameters	Treatment groups (n=6 each)	Before Treatment (0 h)	After Treatment (Next estrus)
Blood glucose (mg/dL)	Gr A	52.67±2.16^{a}	59.33±0.71^{b}
	Gr B	53.67±2.94^{a}	59.17±1.38^{b}
	Gr C	49.33±2.14^{a}	56.17±1.30^{b}
	Gr D	58.83±2.55^{a}	60.67±2.30^{b}
	Gr E	51.00±1.15^{a}	57.00±2.03^{b}
Serum urea (mg/dL)	Gr A	34.61±2.62^{a}	33.21±1.42^{a}
	Gr B	32.30±1.94^{a}	30.24±2.39^{a}
	'Gr C	37.15±1.03^{a}	34.97±1.13^{a}
	Gr D	32.36±1.65^{a}	30.18±1.93^{a}
	Gr E	30.85±1.57^{a}	29.33±1.32^{a}
Serum calcium (mg/dL)	Gr A	7.04±0.25^{a}	7.31±0.19^{a}
	Gr B	7.38±0.29^{a}	7.78±0.34^{a}
	Gr C	7.78±0.15^{a}	8.12±0.10^{b}
	Gr D	7.17±0.26^{a}	7.53±0.24^{b}
	Gr E	7.76±0.09^{a}	8.08±0.06^{b}
Serum phosph-orus (mg/dL).	Gr A	4.77±0.06^{a}	5.08±0.09^{b}
	Gr B	4.70±0.10^{a}	5.16±0.09^{b}
	Gr C	4.97±0.05^{a}	5.23±0.08^{b}
	Gr D	4.76±0.09^{a}	5.10±0.06^{b}
	Gr E	4.90±0.08^{a}	5.20±0.07^{b}
Serum LPO (µMol/L)	Gr A	4.90±0.10^{a}	5.11±0.14^{a}
	Gr B	5.02±0.21^{a}	4.84±0.13^{a}
	Gr C	4.88±0.06^{b}	4.40±0.13^{a}
	Gr D	4.46±0.15^{b}	3.80±0.16^{a}
	Gr E	4.74±0.13^{b}	3.78±0.11^{a}
Serum TAC (µMol/L)	Gr A	4.08±0.50^{b}	4.99±0.56^{a}
	Gr B	4.05±0.45^{b}	4.99±0.43^{a}
	Gr C	3.32±0.54^{b}	5.13±0.68^{a}
	Gr D	3.88±0.56^{b}	4.85±0.36^{a}
	Gr E	3.60±0.35^{b}	4.78±0.41^{a}
Serum amyloid A (µg/mL)	Gr A	28.10±2.01^{a}	28.52±2.14^{Aa}
	Gr B	29.89±1.52^{a}	24.68±1.20^{ABb}
	Gr C	25.49±1.23^{a}	19.82±1.55^{Ca}
	Gr D	28.34±1.32^{a}	22.98±0.88^{BCb}
	Gr E	28.05±1.47^{a}	22.05±1.11^{BCb}

Gr A= Positive control, Gr B = Sensitive Antibiotic, Gr C =Ashwagandha extract, Gr D = Turmeric extract, and Gr E = Ashwagandha & Turmeric extract. Means with different superscripts within group /row (a,b) and between groups/within the column (A,B,C) differ significantly ($p<0.05$).

First Service Conception Rates

The first service conception rates for Group A, B, C, D, and E were 0.00, 33.33, 33.33, 50.00, and 66.66 %, respectively, which however did not differ statistically by the chi-square test. The highest conception rate was observed in Group E, which received the ashwagandha + turmeric combination. In contrast, Group A, the positive control, recorded no conception. The current findings on the effect of ashwagandha and turmeric combination and turmeric extract alone were consistent with the results (66.66 and 50.00%) reported by Kumar *et al.* (2018) in SCE cows. However, a higher conception rate (83.33%) was reported by Mahour *et al.* (2021), and a lower one (30.00%) by Gopikrishnan *et al.* (2022) following treatment with turmeric extract. The current finding on the effect of ashwagandha treatment on the first service CR (33.33 %) in SCE Gir cows was consistent with the result of Kumar *et al.* (2018). Lawange *et al.* (2019) obtained highest conception rate of 66.70% when garlic extract was administered intrauterinely in combination with ashwagandha powder orally in infectious repeat breeding in dairy cattle. The current findings on the effect of sensitive antibiotic treatment on the first service CR (33.33%) in SCE Gir cows aligned with those of Gopikrishnan *et al.* (2022), who reported a 40.00% conception rate following intrauterine infusion of strepto-penicillin (2.5 gm). However, Kumar *et al.* (2018) and Mahour *et al.* (2021) observed significantly higher conception rates of 83.33% and 100% after treatment with levofloxacin and ciprofloxacin antibiotics, respectively. Raje *et al.* (2023) reported a significant decrease ($p<0.05$) in the percentage of PMNs starting from the first day of treatment, with a sustained reduction over the next four days, suggesting recovery from uterine infection following intrauterine administration of moxifloxacin. In the positive control group, a 0.00% FSCR was observed. In contrast, higher FSCRs of 14.20% by Barlund *et al.* (2008), 10.00% by Singh *et al.* (2018), and 8.33% by Chaudhari (2018) were reported.

In cows with SCE, turmeric (*Curcuma longa*) may offer potential benefits due to its strong anti-inflammatory and immune-regulating properties. By reducing the activation of immune cells like macrophages, neutrophils, and dendritic cells in the uterus, curcumin could help to restore immune balance, lower inflammation, and improve uterine health in cows affected by SCE. Ashwagandha (*Withania somnifera*) is a well-known adaptogen and herbal remedy with strong immunomodulatory and anti-inflammatory properties, which could potentially offer benefits in managing SCE in cows. By promoting a balanced immune response, ashwagandha could help to reduce uterine inflammation, support reproductive health, and improve fertility outcomes in cows affected by SCE. However, further studies are needed to confirm its potential therapeutic role in SCE cows. Thus in view of significant drawbacks associated with antibiotic use, such as the development of bacterial resistance, weakening of the uterine defence mechanism, high treatment costs, and the risk of milk residue, herbal treatments, such as the use of ashwagandha and turmeric, are emerging as promising options to combat uterine infections effectively.

Conclusions

From the study, it can be concluded that the repeat breeder Gir cows with subclinical endometritis show significantly lower levels of blood glucose, serum calcium, phosphorus and serum total antioxidant capacity, with elevated serum urea, lipid peroxidation and serum amyloid-A levels. These altered biochemical profile was found to be normalized at subsequent estrus in all the treatment groups including antibiotic and herbal extracts, with improved first service conception rates. The combination of ashwagandha and turmeric extract showed best therapeutic efficacy with 66.66% first service conception rate and hence can be used as an alternative therapy for the treatment of subclinical endometritis in repeat breeding Gir cows.

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