RESEARCH ARTICLE

Therapeutic Efficacy of *Tinospora cordifolia* and Ceftiofur Sodium in Crossbred Cows Affected with Endometritis

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Abstract

Clinical research to evaluate the therapeutic efficacy of *Tinospora cordifolia* (Guduchi) and ceftiofur sodium in crossbred cows (n=20) affected with endometritis was undertaken. The cows were divided into two equal groups, Group I (n=10) consisted the treatment with ceftiofur sodium 1 gm intrauterine once, and Group II (n=10) with 1 g *Tinospora cordifolia* dissolved in 50 mL normal saline, intrauterine for 3 days. White side and leukocyte esterase tests were performed before and after treatment. All the cows were inseminated with frozen semen at subsequent estrus. Based on the reactivity to White side test following the treatment, 80 % (8/10) cows in Group I and 70 % (7/10) cows in Group II were found recovered. The overall conception rate was higher in Group I (60%) than in Group II (40 %).

Key words: Ceftiofur sodium, Endometritis, Leukocyte esterase test, *Tinospora cordifolia*, White side test.

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Introduction

Endometritis is described as a mild inflammation of the endometrium that usually occurs after calving, coitus or artificial insemination in cattle without any systemic or generalised problems (Sheldon *et al.* 2006). There are subclinical and clinical subtypes of endometritis. According to Sheldon *et al.* (2006), clinical endometritis is essentially a localised inflammation of the endometrium characterised by the presence of purulent or mucopurulent (>50% pus) material in the vagina 21 days postpartum that originates from the uterus but is unrelated to systemic illness. While subclinical endometritis is defined as having >18% polymorphonuclear (PMN) cells in uterine cytology samples taken 21 to 33 days after delivery or >5% PMNs in samples taken 34 to 47 days after delivery. Summer calving, high milk yield, parturient and postpartum complications like dystocia, multiple births, abortion, retained placenta, and metabolic disorders are among the risk factors associated with the development of endometritis (Gautam *et al.*, 2010).

Various substances, ranging from antibiotics to hormones have been tried earlier to counteract uterine infections. But these conventional therapies besides being costly also cause extra harm to animal in form of development of resistance, toxicity and side effects. Use of certain immunomodulatory substances as alternative therapeutic agents have been reported (Mandhwani *et al.*, 2017). The indigenous plant *Tinospora cordifolia* also known as Guduchi, used in Ayurveda, is well-known for its hepatoprotective, immunomodulatory, anti-stress, anti-neoplastic, anti-diabetic, anti-allergic, and anti-inflammatory properties (Saha and Ghosh, 2012). Considering the above points, the present study was designed with the objective to evaluate the comparative therapeutic efficacy of herbal (*Tinospora cordifolia*) and antibiotic (Ceftiofur sodium) infusions in endometritis affected crossbred cows.

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Materials and Methods

The study was conducted on clinical cases of endometritis in 20 crossbred cows (having abnormal cervico-vaginal discharge) belonging to the farmers of Dantiwada village brought to the Department of Veterinary Gynaecology and Obstetrics of the College of Veterinary Science and AH, Kamdhenu University, Sardarkrushinagar (Gujarat, India) during the period from February 2023 to July 2023. The cows (n=20) affected with endometritis were selected on the basis of White side test and leukocyte esterase test at estrum, and were randomly divided into two equal groups. The cows in group I were infused with 1 g of ceftiofur as 20

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mL of ceftiofur sodium solution (Resfur, Alivira Animal Health Ltd), intrauterine once, while the cows in group II received 1 g *Tinospora cordifolia* (sterile aqueous 100% natural extract of *Tinospora cordifolia*, Guduchi) dissolved in 50 mL normal saline, intrauterine once daily for 3 consecutive days.

Estrual cervical mucus samples were collected on the day of estrum before treatment and on next estrus post-treatment for White side test and leukocyte esterase test. Cows were inseminated at subsequent estrus following treatment. Cows those failed to conceive at 1st insemination were subjected to 2nd insemination in the next estrus. The pregnancy status was confirmed in non-return cows by rectal palpation on day 60 post-AI.

White Side Test and Leukocyte Esterase Test

White side test (WST) on estrual mucus was performed as described by Bhattacharyya *et al.* (2011) and the result was recorded in four categories: transparent (no infection), light yellow (mild infection), yellow (moderate infection) and dark yellow (severe infection). Leukocyte Esterase (LE) test was performed by putting a drop of the collected estrual mucus on LE commercial test strip (Multistix 10 SG, Bayer Corporation, Elkart, IN) with a pipette. A test result for every cow was read according to the manufacturer's colorimetric chart after 2 min, and the score was recorded as follows: 0 indicates no leukocytes, 0.5 indicates a very small quantity, 1 indicates a small amount, 2 indicates a moderate amount and 3 indicates a significant amount (Couto *et al.,* 2013).

Sensitivity, Specificity and Predictive Value of Tests

Correct diagnosis in this study was defined either as (1) Cow diagnosed positive for endometritis upon WST or LE test but did not found pregnant at 2 month post-insemination, or (2) Cow diagnosed negative for endometritis upon WST or LE test and found pregnant at 2 month. An incorrect diagnosis was defined either as (1) Cow diagnosed positive for endometritis upon WST or LE test but found pregnant at 2 month, or (2) Cow diagnosed negative for endometritis upon WST or LE test but did not found pregnant at 2 month post-insemination. Diagnostic accuracy was defined as the percentage of correct diagnosis out of total number of WST or LE test. Sensitivity of the method was defined as percentage of cows found positive to endometritis by WST or LE test. Specificity of the method was defined as percentage of cows found negative to endometritis by WST or LE test. The positive predict value was defined as the percentage of actual endometritis cows out of total number of cow diagnosed with endometritis through WST or LE test. The negative predictive value was defined as the percentage of actual non-endometritis cows out of total number of cow diagnosed non-endometritis through WST or LE test.

Statistical Analysis:

The data generated were analysed using SPSS 20.0 software and significant differences among the treatments were determined using Duncan's Multiple Range Test (DMRT) as per Snedecor and Cochran (1994).

Results and Discussion

White Side Test

All the twenty crossbred cows were positive for endometritis by White side test (WST) before treatment. The severity or grade of infection in Group I (n=10) before treatment was mild in 10 %, moderate in 50 % and severe in 40 %, and after 20th day of treatment/next estrum it was only moderate and severe in 1 cow (10%) each. In Group II (n=10) before treatment WST grade was mild in 10 %, moderate in 40 % and severe in 50 %, and after 20th day of treatment was mild in 10 %, moderate in 20 % and severe in 0.00 % crossbred cows. Thus, the percentages of the cows that tested negative for WST in Group I and II after treatment were 80.00 and 70.00, respectively (Table 1). Detection rate of endometritis in crossbred cows using White side test was 100 %, which is in accordance with the results of Methai *et al.* (2005) and Krishnan *et al.* (2015).

Leukocyte Esterase Strip Test

The percentages of the cows that tested negative for LE in Group I and II after treatment were 70.00 and 50.00, respectively. The severity or grade of infection in endometritic crossbred cows with LE test in Group I (n=10) before treatment was grade 0.5 in 0.00 %, grade 1 in 20 %, grade 2 in 30% and grade 3 in 50 %, and after 20th day of treatment grade 0.5 was

Table 1: Reactivity to White side test (WST) in different groups of crossbred cows affected with endometritis

Parameter			Group I (n=10)		Group II (n=10)	
			Pre-treatment (Day 0)	Post-treatment (Day 20)	Pre-treatment (Day 0)	Post-treatment (Day 20)
White side test	Positive		10 (100%)	2 (20%)	10 (100%)	3 (30%)
	Grade	Mild	1 (10%)	-	1 (10%)	1 (10%)
		Moderate	5 (50%)	1 (10%)	4 (40%)	2 (20%)
		Severe	4 (40%)	1 (10%)	5 (50%)	-
	Negative		-	8 (80%)	-	7 (70%)

in 0 0.00 %), grade 1 in 10 %, grade 2 in 20% and grade 3 in 0.00 %. The result in Group II cows (n=10) before treatment was grade 0.5 in 0.00 %, grade 1 in 20 %, grade 2 in 40% and grade 3 in 40%, and after 20th day of treatment it was grade 0.5 in 20 %, grade 1 in 20 %, grade 2 in 10% and grade 3 in 0.00 % (Table 2). This suggested better health status post-treatment in Group I antibiotics treated than in group II herbal treated cows.

Leukocyte esterase is normally used to test the urine for the presence of white blood cells as an indication of inflammation process of the urinary tract. There is dearth of published evidence regarding the use of leukocyte esterase reagent strips in the diagnosis of endometritis.

The diagnostic accuracy of the LE and WST was calculated on the basis of prevalence of endometritis and reproductive performance of the cows under study (Table 3). It was revealed that the both the tests were nearly equally effective to diagnose the endometritis (80% vs 75%). The positive predictive value using the WST was 100 %, whereas, the negative predictive value was higher with LE (75%) as compared to that of the WST (66.66 %). The sensitivity and the specificity of LE observed in the present study agreed well with the findings of Santos (2006) who have found the sensitivity and specificity of LE test as 83% and 94%, respectively, in cows.

Occurrence of First Post-Treatment Estrus and Conception Rate

There was no significant influence of ceftiofur on first post-treatment estrus and fertile estrus, though it was quite shorter in ceftiofur treated group than *Tinospora cordifolia* treated group (Table 4). The findings of the first post-treatment expressed estrus in the present study are in accordance with Davoodian *et al.* (2020). On the contrary, Kumar *et al.* (2022) observed less interval from treatment to estrus induction in herbal group as compared to ceftiofur group. The first service conception rate (FSCR) was 40 and 20 % in Group - I and II, respectively (Table 4). The statistical analysis revealed a non-significant difference between the groups, though the FSCR in Group I was apparently higher than in Group II. The present findings also supported the observations of Syed (2016), who noted the higher FSCR using Closprostenol sodium than *Tinospora cordifolia* in crossbred cows. These results were however in contrast with the findings of Hajibemani *et al.* (2016) and Yildiz and Balikci (2016) in crossbred cow.

The overall conception rates in group I and II cows were 60% and 40%. The difference between the groups was statistically non-significant although the conception rate was higher in the group of cows treated with ceftiofur sodium as compared to *Tinospora cordifolia* treated group II. These

Table 2: Diagnosis of endometritis in crossbred cows by leukocyte esterase strip test

Parameter			Group I (n=10)		Group II (n=10)	
			Pre-treatment (Day 0)	Post-treatment (Day 20)	Pre-treatment (Day 0)	Post-treatment (Day 20)
LE test	Positive		10 (100%)	3 (30%)	10 (100%)	5 (50%)
	Grade	3	5 (50%)	-	4 (40%)	-
		2	3 (30%)	2 (20%)	4 (40%)	1 (10%)
		1	2 (20%)	1 (10%)	2 (20%)	2 (20%)
		0.5	-	-	-	2 (20%)
		0	-	7 (70%)	-	5 (50%)

Table 3: Sensitivity, specificity, positive predictive value, negative predictive value and diagnosis accuracy of endometritis by leukocyte esterase (LE) and White side test (WST) in crossbred cows

Diagnosis results / Predictive value	LE at 20th day post-treatment	WST at 20th day post-treatment
Diagnosis endometritis correct (A)	7	5
Diagnosis endometritis incorrect (B)	1	0
Diagnosis non-endometritis correct (C)	9	10
Diagnosis non-endometritis incorrect (D)	3	5
Sensitivity - 100 x A/(A+D)	70.0%	50.0%
Specificity - 100 x C/(C+B)	90.0%	100%
Positive predictive value - 100 x A/(A+B)	87.5%	100%
Negative predictive value - 100 x C/(C+D)	75.0%	66.66%
Diagnosis accuracy - 100 x (A+C)/(A+B+C+D)	80.0%	75.0%

Table 4: Occurrence of the first post treatment estrus, fertile estrus and conception rate in different groups of crossbred cows affected with endometritis

Groups	First estrus post-treatment (days)	Fertile estrus Post-treatment (days)	Conception rate (CR %)		
			I Service CR	II Service CR	Overall CR
I	21.1±1.865	27.167±4.238	4/10 (40)	2/6 (33.33)	6/10 (60)
II	22.6±1.74	36.5±6.397	2/10 (20)	2/8(25)	4/10 (40)

results were in agreement with the earlier report in crossbred cows (Kumar *et al.,* 2022). Contrary to our findings, Davoodian *et al.* (2020) and Kadivar *et al.* (2022) observed a lower overall conception rate in crossbred cows. According to Reppert (2015), intrauterine infusion of ceftiofur hydrochloride to cows with subclinical and clinical endometritis enhances reproductive performance. The variations in the post-treatment conception rate in infectious repeat breeder cows might be due to type of antibiotic, degree of sensitivity to antibiotic, route of administration, dose and severity of uterine infection and time of breeding following treatment (Mosaferi *et al.,* 2013). Improved recovery rate and conception rate were obtained by Kumar *et al.* (2004) and Bhardwaj *et al.* (2018) in crossbred cows treated with *Tinospora cordifolia* and garlic extract. Saha and Ghosh (2012) suggested that *Tinospora cordifolia* possesses antibacterial, anti-inflammatory and immunomodulation properties. Looking to the effect of *Tinospora cordifolia* in the treatment of clinical endometritis, the findings suggest the implication of immunity boosting mechanisms in clearance of the infection, and it being the non-significantly differing with the ceftiofur group as far as the conception rate is concerned, can be considered as an alternative treatment.

Conclusion

The present study demonstrated that the intrauterine treatments with *Tinospora cordifolia* and ceftiofur were found nearly equal effective in terms of clearance of uterine infection, percent conception rate in endometritic crossbred cows. *Tinospora cordifolia* being less costly, can be recommended for the intrauterine use in clinical cases of endometritis in crossbred cows.

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