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Effect of Garlic (*Allium sativum*) Extract on Recovery and Conception Rate in Infectious Repeat Breeder Crossbred Cows

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Abstract

The study was undertaken to evaluate the therapeutic efficacy of Garlic (*Allium sativum*) extract in 20 infectious repeat breeder crossbred cows. Animals were divided into two groups of ten in each. The animals in first group were treated intrauterine with 30 ml 15% Garlic extract w/v dissolved in saline at 24 hrs interval for 7 days, while the animals in control group were infused with 30 ml saline for 7 days. Bacterial count in uterine flushing declined significantly ($p<0.05$) after treatment in Garlic group. A significant increase ($p< 0.05$) in total cellular count and PMNs % was found in Garlic group than in control group. An overall recovery rate of 80 vs 20 % and conception rate of 60 vs 10 % was found in Garlic treated cows over control placebo group proving its efficacy in infectious repeat breeders.

Introduction

One calf a year by cow is essential for economic viability in dairying. Repeat breeding, comprise a heterogeneous group of subfertile cows with a tune of 7.31 to 23.88 % among bovines. Though, antibiotics are quite effective in treating endometritis, but use of herbal medicines as antimicrobial and/or immunomodulator is becoming popular due to toxicity and side effects of allopathic medicines. Extract of Garlic (Kumar, 2013 and Singh, 2016) has been suggested as alternative therapy for repeat breeding cattle and buffaloes. The present study was carried out to evaluate the efficacy of Garlic extract I/Ut infusion in repeat breeding crossbred cows.

Materials and Methods

The study was conducted on clinical cases of endometritis in crossbred cows belonging to farmers brought to the AI centre of Department of Veterinary Gynaecology and Obstetrics of the College of Veterinary Science and A.H. Mhow and nearby Government AI centres during period from December 2015 to April 2017. All the cows

(> 90 days postpartum) were screened as per history, gynaecological examination, nature of estrual cervico-vaginal mucus and positive to whiteside test (WST) as per method of Popov (1969) to identify them as infectious repeat breeding crossbred cows. Which were divided into two groups with ten animals in each, T1 (treatment group and T2 was kept as negative control).

Fresh cloves of Garlic *(Allium Sativum)* were crushed in sterilized pestle-mortar and was first filtered through muslin cloth twice and fihnally through whatman filter paper. Alcoholic extract of Garlic filtrate was prepared using soxhlet extraction method. The residue obtained after evaporation of alcoholic extract was dissolved in saline to obtain 15 % w/v for intrauterine infusion.

Animals of T1 group were infused intrauterine with 30 ml of the above extract at 24 hrs interval for 7 days, whereas animals of control group were infused 30 ml saline for 7 days as placebo. The estrual cervico-vaginal mucus was examined for pH before and after treatment at subsequent estrus using pH paper strips. All animals (T1 and T2) were flushed before start of the therapy and after 24 hours of last administration of the Garlic extract (T1) and saline and 8-12 hours after they showed first signs of heat. Total bacterial count in uterine flushing was done as per Bauer *et al.* (1966). Total cellular count in the uterine flushing was determined by haemocytometeric technique (Jain, 1986). Polymorphonuclear (PMNs %) cell count in the uterine flushing was made in smears prepared from the cell suspension received by centrifugation (3000 rpm for five minutes) on clean grease free glass slides, and stained with Giemsa stain (Kasimanickam *et al*., 2004). Statistical analysis was carried out by using completely randomized design as per Snedecore and Cochran (1980).

Results and Discussion

The observations on the nature of the CVM revealed that during infection purulent discharge was observed in 30% cows and muco-purulent discharge in 60% cows, whereas the CVM of 10% cows was clear. Any alteration in the colour of estrual mucus will indicate genital infections (Bhat *et al.,* 2015). Following intrauterine infusion of Garlic extract, the discharge at subsequent estrus was observed as clear in 70% cows in comparison to untreated control group (10 %). The present findings are in agreement with the reports of Rahi (2011), Kumar (2013) and Singh (2016) following treatment with Garlic extract in crossbred cows.

In the present study, the higher values of pH in repeat breeder cows are in close proximity to the values reported earlier (Modi *et al*., 2011; Sudarshan Kumar *et al*., 2015 and Bhardwaz *et al*., 2018). In repeat breeding cows with endometritis due to infection, the metabolites of bacteria and inflammatory exudates may alter the pH of estrual cervical mucus to alkaline side resulting in failure of conception due to death of spermatozoa (Ravikumar *et al.,* 2007; Bhardwaz *et al*., 2018). Once the infection is eliminated, the pH of cervical mucus returns towards the normal neutral side.

Whiteside test (WST) was performed on the estrual cervico-vaginal mucus of control and treated animals, before and after treatment. Most of the cows became negative to Whiteside test following treatment with Garlic (80.00%) as compared to only 20.00 % cows in control group which reveals that the Garlic was most effective treatment for endometritis. The present findings corroborated with earlier study (Singh, 2016).

A significant decline ($p<0.05$) in bacterial count ($x10^4$/ml) was observed from pre-treatment to post-treatment estrus in uterine flushing of Garlic group (309.59 ± 2.53 to 1.17 ± 0.02) as compared to control group (328.62±0.17 to 296.96±0.22). Xiang (2009), Singh (2016) and Bhardwaz *et al*. (2018) reported significant drop in bacterial load in uterine flushings following treatment with ciprofloxacin. Reduction in bacterial load in control group may be due to natural uterine defense mechanisms. Besides this, uterine flushings might have also reduced bacterial load. A significant increase ($p<0.05$) in the total cellular count (10^4/ml) and (PMNs) % values was observed from pre-treatment to post-treatment in uterine flushing of Garlic group (0.461±0.01 to 3.50±0.05) and (27.91±0.14 to 43.05±0.1), respectively. A non- significant change was found in TCC in control cows after normal saline infusion. The non-significant increase in cellular count in control group might be due to natural uterine defense mechanism.

Table 1: Effect of Garlic (*Allium sativum*) extract on CVM, pH, Bacterial count, Total cellular count and PMN (%) in infectious Repeat Breeder Crossbred Cows

Parameters/Observations		Garlic (n = 10)		Control (n = 10)	
		Pre-treatment estrus	Post-treatment estrus	Pre-treatment estrus	Post-treatment estrus
CVM appearance	Purulent	30.00 (3)	10.00 (1)	60.00 (6)	50.00 (5)
	Muco-purulent	60.00 (6)	20.00 (2)	40.00 (4)	40.00 (4)
	Clear	10.00 (1)	70.00 (7)	0.00	10.00 (1)
Whiteside test	Positive	100.00 (10)	20.00 (2)	100.00 (10)	80.00 (8)
	Negative	0.00	80.00 (8)	0.00	20.00 (2)
CVM –pH (Mean ± SE)		8.11 ± 0.01^{A}	7.49 ± 0.01^{B}	8.36 ± 0.02^{X}	8.31 ± 0.02^{Y}
Bacterial count (10^4/ml)		309.59 ± 2.53^{A}	1.17 ± 0.02^{B}	328.62 ± 0.17	296.96 ± 0.22
Total cellular count (TCC)		0.461 ± 0.01^{A}	3.50 ± 0.05^{B}	0.433 ± 0.02^{X}	0.581 ± 0.01^{Y}
PMNs (%)		27.91 ± 0.14^{A}	43.05 ± 0.12^{B}	27.84 ± 0.12	25.57 ± 0.27

Means bearing different superscripts within the row for a group differ significantly ($p<0.05$).

Table 2: Recovery and conception rate in different groups of infectious repeat breeding crossbred cows after treatment

Groups	No. of cows	Recovery rate (%)	Conception rates (%)			
			1^{st} AI	2^{nd} AI	3^{rd} AI	Overall
Garlic	10	80.00 (8)	40.00 (4)	20.00 (2)	--	60.00 (6)
Control	10	20.00 (2)	--	10.00 (1)	--	10.00 (1)

The present findings are also in agreement with the finding of Davis and Kuttan (1999). Singh (2016) reported increase in TCC after treatment with Garlic. The herbal extract may act as attractant substance for PMNs and thus activates infiltration of PMNs into the uterine lumen.

Conception rate and Recovery rate

After treatment with Garlic extract, 80 % recovery rate and 60 % conception rate was recorded in the present study as compared with control group (20 and 10 %). Recovery rate was found to be significant ($p<0.05$). This could be due to the significant reduction of bacterial count in Garlic extract-treated animal, as Garlic extract is well known for its antimicrobial property against Gram positive and Gram negative bacteria (Chung *et al.*, 2003). The treatment with Garlic extract has been shown to stimulate the release of cytokines such as IL-2, IFN-α, IFNγ and increase the natural killer activity and enhances phagocytic activity of peritoneal macrophages (Kyo *et al.*, 1998).

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Conflict of Interest

All authors declare no conflict of interest.

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