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Immunomodulatory effect of Neem (*Azadiracta indica*) Extract on Recovery and Conception Rate in Infectious Repeat Breeder Crossbred Cows

Anavil Bhardwaz[1], S.P. Nema, S.S. Mahour[3] Daljeet Chabbra, R. Aich and Sudarshan Kumar*

[1]Department of Veterinary Clinical Complex, Khalsa Veterinary College, Amritsar

Department of Veterinary Gynaecology, College of Veterinary Science and AH, Mhow (MP)

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Corresponding author:

drsudarshandogra@yahoo.com

Abstract

The study was undertaken to evaluate the therapeutic efficacy of Neem (*Azadiracta indica*) 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 with 15% concentration of Neem extract w/v dissolved in saline to make 30 ml, Intra Uterine at 24 hrs intervals 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 Neem group. A significant increase ($p<0.05$) in total cellular count and PMNs % was found in Neem group than in control group. An overall recovery rate of 80% vs 20% and conception rate of 50% vs 10% was found in Neem treated cows over control group proving its efficacy in infectious repeat breeder cows.

Introduction

Decreased reproductive efficiency of dairy animals drastically affects the economy of dairy farming. Repeat breeding alone is a major cause of infertility to the tune of 7.31 to 23.88 % among bovines. Endometritis have been recognized to cause repeat breeding (Jainudeen and Hafez, 2008). Antibiotics are quite effective in treating endometritis, but *in vitro* sensitivity, accurate dosage regimens and compulsory milk disposal make herbal medicines, most viable preposition. Kumar (2013) and Singh (2016) studied immunomodulatory and therapeutic efficacy of Neem on endometritis in repeat breeding crossbred cows and reported that hydro-alcoholic extract of the Neem has a better antibacterial and immuno-modulatory effect and hence can be used as a therapy for endometritis in repeat breeding crossbred cows. The present study was carried out to evaluate the efficacy of intrauterine infusion of Neem extract 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 College of Veterinary Science and A.H., Mhow (India) and nearby Government AI centres during 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 reaction to Whiteside test (WST) to identify them as infectious repeat breeders. Twenty crossbred cows were identified as repeat breeders based on cycling normally without any apparent genital abnormalities and repeated estrus after three or more consecutive services, and were divided into two groups with 10 animals in each (T1 treatment group and T2 negative control).

Fresh leaves of Neem (*Azadiracta indica*) were dried and crushed in sterilized pestle-mortar and was first filtered through muslin cloth twice and finally through Whatman filter paper. Alcoholic extract of Neem leaf (*Azadiracta indica*) filtrate was prepared using Soxhlet extraction method. The residue obtained after evaporation of alcoholic extract was dissolved in saline to obtain 15 % w/v solution of Neem extract 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 Neem extract (T1) or saline, and again 8-12 hrs after they showed first signs of heat. Total bacterial count in uterine flushing was done as per routine standard method. Total cellular count in the uterine flushing was determined by haemocytometeric technique. 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 and Bhardwaz *et al*., 2018). Statistical analysis was carried out by using completely randomized design as per Snedecor and Cochran (1980).

Results and Discussion

The observations (Table 1 and 2) on the nature of CVM revealed that during infection purulent discharge was observed in 40% cows and muco-purulent discharge in 50% 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 Neem extract, the discharge at subsequent estrus was observed as clear in 70% cows in comparison to untreated control group (10 %). The present findings were in agreement with the earlier findings following treatment with Neem extract in crossbred cows (Kumar, 2013; Singh, 2016).

In the present study, the higher values of pH observed in repeat breeder cows were 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 Neem (80.00%) as compared to control group (20.00%) which reveals that the Neem was most effective treatment for endometritis. The present findings corroborated with earlier study (Singh, 2016).

A significant decline ($p<0.05$) in bacterial count (x 10^4/ml) was observed from pre-treatment to post-treatment estrus in uterine flushing of Neem group (310.30 ± 2.73 to 1.25 ± 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 and garlic. Reduction

Table 1: Effect of Neem (*Azadiracta indica*) extract on CVM, pH, bacterial count, total cellular count and PMNs (%) in infectious repeat breeder crossbred cows

Parameters/Observations		Neem (n = 10)		Control (n = 10)	
		Pre-treatment estrus	Post-treatment estrus	Pre-treatment estrus	Post-treatment estrus
CVM appearance	Purulent	40.00 (4)	20.00 (2)	60.00 (6)	50.00 (5)
	Mucopurulent	50.00 (5)	10.00 (1)	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)		8.17 ± 0.01^{A}	7.50 ± 0.01^{B}	8.36 ± 0.02^{X}	8.31 ± 0.02^{Y}
Bacterial count (10^4/ml)		310.30 ± 2.73^{A}	1.25 ± 0.02^{B}	328.62 ± 0.17	296.96 ± 0.22
Total cellular count (TCC)		0.563 ± 0.01^{A}	2.18 ± 0.17^{B}	0.433 ± 0.02^{X}	0.581 ± 0.01^{Y}
PMNs (%)		27.40 ± 0.16^{A}	56.08 ± 0.18^{B}	27.84 ± 0.12	25.57 ± 0.27

Means bearing different superscripts within the row for a group differ significantly ($p<0.05$). Fig in parenthesis represent %

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

Groups	No. of cows	Recovery rate (%)	Conception rate (%)			
			1st AI	2nd AI	3rd AI	Overall
Neem	10	80.00 (8)	30.00 (3)	10.00 (1)	10.00 (1)	50.00 (5)
Control	10	20.00 (2)	--	10.00 (1)	--	10.00 (1)

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 Neem group (0.563±0.01 to 2.18±0.17) and (27.40±0.16 to 56.08±0.18), 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. The present findings are in agreement with the finding of Davis and Kuttan (1999). Kumar (2013) and Singh (2016) reported increase in TCC after treatment with Neem. These herbal extracts 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 Neem leaf extract, 80 % recovery rate and 50 % conception rate was recorded in the present study as compared with control group (20 and 10 %). Recovery rate was found to be significantly ($p<0.05$) better in treated group. This could be due to the significant reduction of bacterial count in Neem extract-treated animal, as Neem extract is well known for its antimicrobial property against Gram positive and Gram negative bacteria (Chung *et al.*, 2003). The treatment with Neem 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.

References:

Bhardwaz, A., Nema, S.P., Sudarshan, K., Chabbra Daljeet, Shukla, S. and Madhwani, R. (2018). Effect of ciprofloxacin on recovery and conception rate in infectious repeat breeder crossbred cows. *Indian J. Vet. Sci. & Biotech.,* **14**(1): 71-74.

Bhat, F.A., Bhattacharya, H.K..Fazili, M.R., Hussain, S.A. and Khan, M.Z. (2015). Studies on oestrual cervical mucus of repeat breeding cows with special reference to ovularory disturbance and genital infection. *Theriogenology*, **5**(2): 113-123.

Chung, K.S., Kang, S.Y. and Kim, J.Y. (2003). The antibacterial activity of garlic juice against pathogenic bacteria and lactic acid bacteria. *Korean J. Microbiol. Biotech.*, **31**(1): 32-35.

Davis, L. and Kuttan, G. (1999). Effect of *Withania somnifera* on cell mediated immune response in mice. *J. Exptl. Clin. Cancer Res.*, **21**(4): 585-590.

Jainudeen, M.R. and Hafez, B. (2008). Reproductive failure in females. In "Reproduction in Farm Animals". 7th edn. Blackwell publishing Co., Australia, pp 265.

Kasimanickam, R., Duffielda, T.F., Fosterb, R.A., Gartleya, C.J., Lesliea, K.E., Walton, J.S. and Johnsona, W.H. (2004). Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. *Theriogenology*, **62:** 9-23.

Kumar, A. (2013). Evaluation of immunomodulatory and therapeutic efficacy of turmeric (*Curcuma longo*) neem (*Azadirachta indica*) and garlic (*Allium sativum*) on endometritis in repeat breeding crossbred cows. M.V.Sc. Thesis, G.B. Pant Univ. of Agric and Technol., Pantnagar, India.

Kyo, E., Uda, N., Suzuki, A., Kakimoto, M., Ushigina, M., Kasuga, S. and Itakura, Y. (1998). Immunomodulation and antitumour activities of aged garlic extract. *Phytomedicine*, **5**(4): 259-267.

Modi, L.C., Suthar, B.N., Nakhashi, H.C., Sharma, V.K. and Panchasara, H.H. (2011). Physical characteristics of oestrual cervical mucus and conception rate in repeat breeder Kankrej cattle. *Intl. J. Agril. Sci.& Vet. Med.,* **5**(4): 416-423.

Ravikumar, B.P., Devaraj, M. and Jayakumar, K. (2007). Certain biochemical studies on the uterine flushings of normal and endometritis cows. *Indian J. Anim. Reprod.*, **28**(2): 101-103.

Singh, G. (1991). Studies on incidence of various reproductive disorders in bovines with special reference to mycotic infections in repeat breeding animals. M.V.Sc. thesis, Punjab Agricultural University, Ludhiana, India.

Singh, S. (2016). Phytotherapeutic measures for endometritis in crossbred cows. M.V.Sc & A.H. thesis. Nanaji Deshmukh Veterinary Science University, Jabalpur, India.

Snedecor, G.W. and Cochran, W.G. (1980). *Statistical Methods*, 14th edn. Oxford and IBH Publishing House, New Delhi, India.

Sudarshan Kumar, Bhardwaz, A. and Srivastava, A.K. (2015). Whiteside test-a field test on the cervical mucus of cows for diagnosis of endometritis. *Intas Polivet*, **16**(2): 207-213.

Xiang, C. H. (2009). Observation on the curative effect of integrated traditional Chinese and western medicine on endomertitis sterility in cows and analysis on its function mechanisms. *J. Anhui Agric. & Sci.*, **25**: 83.

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