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Short Communication

Use of Neem Preparations for the Treatment of Endometritis in Cows

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ABSTRACT

This study describes the effects of intrauterine infusion of methanol fraction of neem oil and neem seed powder in treatment of endometritis in cows. A total of forty six crossbred cows with endometritis were randomly divided into three groups. The animals of group I and II were treated with methanol fraction of neem oil and neem seed powder (25 mL each by intra–uterine route), respectively. Whereas, the control cows (III) were administered with groundnut oil at similar times. Efficacy of both neem preparations was assessed by whiteside test (color reaction to cervico–vaginal mucus) and bacterial load at subsequent estrus. The results indicate that the administration of neem preparations retrieved the cows from endometritis; majority (100 % in I and 62.5% in II) showed negative to whiteside test. There was a significant decrease in bacterial load in animal treated with neem–oil (96.02±2.02%) and seed–powder fraction (98.70±0.46%) compared to control (24.97±29.64 %). Further, a higher pregnancy rate (71.42%) was obtained in neem oil fraction–treated cows than the seed powder or control cows (25% each). In conclusion, the therapeutic efficacy of methanol fractionated neem oil appeared superior over the neem seed powder in endometritic cows.

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The local or systemic antibiotics, antiseptics, sulfonamides, hormones have been widely used for therapeutic management of endometritis in bovine with variable degree of success.(Hussain et al. 1991).However, the cost of treatments, frequency of administration, side effects on animal health and the milk disposal after treatment requires an alternate therapy. Several substances like bacterial endotoxin, serum, plasma or hyper immune serum, polymorphonuclear leucocytes (PMN) extracts and components, granulocyte macrophage colony stimulating factors, colostral whey and herbal extracts which stimulate natural uterine defense mechanism have also been tested as an alternative therapy for endometritis. Neem seeds have shown antimicrobial activity (Mitra 1963, Sharma et al 2011, Drabu et al. 2012, Mahima et al 2012), and even methanol fractionated neem oil shown to antibacterial activity comparable to standard antibiotics like enrofloxacin, gentamicin, oxytetracycline and streptomycin. Administration of crude neem oil intrauterine (Kumar et al. 2009) helped to recover a large proportion (88%) of endometritic cows; however, the therapeutic use of methanol fractionated of neem oil in endometritic cows has not been studied. This study was aimed to determine the effects of intrauterine infusion of methanol fractionated neem oil and neem seed powder in treatment of endometritis in cows.

This study involved crossbred (Vrindavani) cows maintained at Livestock Production and Management Section, IVRI, Izatnagar, Bareilly, U.P, India with similar feeding and managemental conditions during the entire period of study. For selection of endometritic cows, the cervico–vaginal mucus (CVM) was collected at estrus between 40–60 days post calving. It was examined grossly for the presence of white flakes, abnormal color and haziness and subjected to whiteside test (Popov 1969). The cows showing mucopurulent and hazy discharge and positive colour reaction to whiteside test were included in the experiment. A total of forty six cows with endometritis were selected and randomly divided into three groups. Animals of group I (n=14) received 25 mL methanolic fraction of neem oil diluted with groundnut oil (1:1 ratio) intrauterine twice at 48 hr interval after onset of estrus. Likewise, the animals of group II (n=16) were administered with methanolic extract of neem seed powder diluted with groundnut oil (1:1 ratio) following similar dose and schedule. The animals of group III (n=16) received refined groundnut oil in a similar manner and served as controls. For preparation of methanolic fraction the neem oil (Unjha Ayurvedic Pharmacy Agra, India) obtained from local market was subjected to fractionation by mixing equal volume of methanol. This mixture was vigorously shaken for 10 minutes and poured in a separating funnel and allowed to stand for another 10 minutes. The uppermost, methanol miscible fraction was collected and kept in a vacuum desiccator for complete evaporation of solvent and stored till use.

For methanolic extract of neem seed powder, seeds obtained from local market, cleaned properly, dried in a hot air oven overnight at 60°C and thoroughly crushed into powder form mechanically. A mixture of 500g seed powder and 2.5 litres methanol was prepared and subjected to hot extraction for 24 hrs. After cooling, the mixture was filtered using whatman filter paper no.30. The filtrate was heated on boiling water bath up to dryness. After complete evaporation of solvent, the residue fraction was collected and stored at room temperature till use.

Cervico–vaginal mucus (CVM) was collected before treatment and at subsequent estrus as described by Nair and

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Kharche (1988). The CVM was subjected to whiteside test (Popov 1969) to detect endometritis and the pH of the CVM was also assessed using pH meter (Tsiligianni et al. 2001).Prior to bacteriological examination, the CVM was mucolysed and bacterial load was determined by standard plate count method using 10–fold dilution of CVM.. One hundred µl of diluted sample was inoculated in triplicate on nutrient agar plates and incubated at 37°C for 24 hrs and individual colonies counted.. The bacterial load per ml of CVM was calculated by multiplying with the dilution factor. The clinical recovery of the cows was assessed by change in pH, nature of white side test of estrual mucus and total bacterial count at subsequent estrus. The cows during subsequent estrus were inseminated twice, 12 hrs apart using 0.50 ml of frozen / thawed semen from fertile crossbred bulls. Pregnancy was confirmed by rectal palpation at 60 days following insemination.

Data were analyzed as per standard statistical procedure. Treatment means were tested using "t" test, whereas pre and post–treatment means of same parameters were compared using paired "t" test (Snedecor and Cochran 1994).

At pre–treatment estrus, all the endometritic cows had either mucopurulent or hazy discharge with moderate to dark colour reaction to white side test (Table 1). The CVM of endometritic cows has been shown to possess large number of leucocytes, which was responsible for change in appearance (hazy or cloudy) and development of colour reaction to whiteside test (Popov 1969). Whereas, at post–treatment estrus, the CVM of all the cows treated with methanol fraction of neem oil and 62.5% of animals treated with neem seed powder discharged clear CVM with negative colour reaction. In contrast, most of control animals were having hazy or mucopurulent discharge with light to moderate colour reaction post–treatment. These results are in agreement with the earlier reports (Kumar et al. 2009). Clarity of CVM in higher proportion of cows, following administration of neem preparations might be due to antibacterial activity of neem compounds fractions (Satyavati et al. 1976), which increases phagocytosis or elimination of pathogens due to stimulation of uterine defense mechanism (Talwar et al. 1993).

Table1: Physico–chemical characteristics of cervico–vaginal mucus in endometric crossbred cows treated with different neem preparations *Figures in the parentheses indicate percentage.*

Parameter		Group I (Methanolic fraction of neem oil) n= 14		Group II (Metahanolic fraction of neem seed powder) n=16		Group III (Control; Groundnut oil) n= 16	
		Pre–treatment estrus	Post–treatment estrus	Pre–treatment estrus	Post–treatment estrus	Pre–treatment estrus	Post–treatment estrus
Nature of CVM							
1.	Mucopurulant	8 ((57.10))	0 (0.00)	10 (62.50)	0 (0.00)	8 (50.00)	4 (25.00)
2.	Hazy	6 (42.80)	0 (0.00)	6 (37.50)	6 (37.50)	8 (50.00)	12 (75.00)
3.	Clear	0 (0.00)	14 (100)	0 (0.00)	10 (62.50)	0 (0.00)	0 (0.00)
White side test							
1.	Positive	14 (100)	0 (0.00)	16 (100)	6(37.50)	16 (100)	16 (100)
	Dark	2 (14.28)	0 (0.00)	2 (12.50)	0 (0.00)	4 (25.00)	0 (0.00)
	Moderate	12 (85.70)	0 (0.00)	14 (87.50)	0 (0.00)	12 (75.00)	4 (25.00)
	Light	0 (0.00)	0 (0.00)	0 (0.00)	6 (37.50)	0 (0.00)	12 (75.00)
2.	Negative	0 (0.00)	14 (100)	0 (0.00)	10 (62.50)	0 (0.00)	0 (0.00)

Table 2: Mean (±SE) bacterial load (x106/ml) in cervico–vaginal mucus and conception rate of endometritic–crossbred cows treated with different neem preparations

No. of Animals	Pre–Treat Estrus	Post–Treat Estrus	Percent Reduction	Overall Conception rate
Group I (n = 14) (Meth. frac. of neem oil)	43.61a** (±13.45)	1.01bB (±0.38)	96.02 (±2.02)	10 (71.42%)
Group II (n = 16) (Meth. frac. of neem seed powder)	78.19a** (±18.52)	0.55bB (±0.11)	98.70 (±0.46)	4 (25.00%)
Group III (n =16) (Control, groundnut oil)	105.34 (±59.06)	32.33A+ (±15.16)	24.97 (±29.64)	4 (25.00%)

Means bearing different superscripts within rows (a, b) and columns (A, B) differ significantly.
** = P<0.01, significance as compared in rows.
+ = *P*<0.05, significance as compared in columns.

The pH of dark yellow coloured CVM was greater than the CVM with moderate yellow colour (9.00±00 vs 8.25±0.20). The overall mean pH of CVM at pre–treatment estrus was similar among groups (8.50, 8.67 and 8.27), which declined significantly (P<0.01) following treatments at subsequent estrus in grI (7.51±0.20) and gr II (7.63±0.25). However, in control the mean pH value did not differ significantly either at pre– or post–treatment estrus. The mean pH of CVM in endometritic cows, at pre–treatment estrus was towards alkaline side. Alkaline CVM in endometritic animals is caused by metabolites of bacteria and inflammatory exudates (Salphale et al. 1993).Sarkar (2004) reported similar pH in estrual mucus of endometritic cows. It appears that the pH drops towards the neutral side, once the infection from the uterus is eliminated (Markusfeld 1984). In contrast, the higher pH in control animals at pre–treatment estrus (8.27±0.12) and post (8.38±0.11) treatment indicated the persistence of uterine infection.

The mean bacterial load at pre–treatment estrus was statistically similar (P>0.05) among all the three groups and ranged from 43.61±13.45 to 105.34±59.06 X 10^6 per ml (Table 2). A significant decline (P<0.01) in bacterial load was observed at post–treatment estrus in both treatment groups. However, in

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controls, the decline at post–treatment was of lower magnitude and statistically insignificant (P>0.05) as compared to their bacterial load at pre–treatment estrus. The percent reduction in bacterial load in animals treated with methanol fraction of neem oil (96.02±2.02) and neem seed powder (98.70±0.46) was greater than that of control cows (24.97±29.64) (Table 2). Bacterial load has been used to assess the level of infection and as a diagnostic indicator to assess health status of organs including uterus (Dhaliwal 2001). The bacterial load in our study in CVM of endometritic cows is in accordance with the findings of others (Sarkar 2004, Barman et al. 2009). The perusal of literature revealed, no information addressing effects of such therapies on uterine bacterial infection. A similar decline in bacterial load was recorded with about 88% recovery in endometritis cows, and buffaloes which were given 50 ml crude neem oil, twice at 24 hr interval (Kumar et al. 2009). Greater reduction in bacterial load in our animals might have occurred, due to antibacterial activity of methanol fractionated neem oil and seed powder. This was further supported by the findings of (Barman et al. 2009), who reported that methanolic fraction of neem oil showed antibacterial sensitivity against 95% bacterial isolates belonging to CVM of endometritic cows that was comparable to antibiotics such as enrofloxacin and gentamicin. Further, it was also observed that 1 mg of nimbidine – a principal compound of neem oil, was equivalent to 800 units of penicillin and 0.5 mg of streptomycin sulphate (Singh and Sastry 1981). The decline in bacterial load appears to have occurred due to suppressive action of neem preparations on growth of both gram positive and gram negative bacteria as has been observed by (Koul et al. 1990) and (Barman et al. 2009).The percent reduction in bacterial load in controls was only 24.97±29.64 which indicated presence of uterine infection in these animals. This decrease in control animals can be attributed to expulsion of uterine infection with the mucus flow that takes place under the influence of estrogens during transition from one estrous cycle to another.

The overall conception rate was higher in animals treated with methanol fractionated neem oil (71.42%, Table 2) than the neem seed powder and control. Extracts of neem has been found to contain non–specific immunostimulant, antimicrobial, anti–viral and antifungal properties (Talwar et al. 1993). Our findings are in agreement with the results of (Kumar et al. 2009) who reported no adverse effect on cyclicity in cows following treatment with neem oil. Intrauterine infusion of neem oil also caused an infiltration of neutrophils in rats (Upadhyay et al. 1990), which is considered to be responsible for the clearance of bacteria from the uterus. Perusal of literature revealed that no work has been conducted to assess the efficacy of different fractions of neem in treatment of uterine infections in cows.

It can be concluded that the therapeutic efficacy of methanol fractionated neem oil appeared to be superior over neem seed powder as all cows recovered from endometritis, caused drastic decline in bacterial load and also allowed higher proportion of cows to conceive.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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