

EFFICACY OF NEEM SEED EXTRACT±ASHWAGANDHA FOR THE TREATMENT OF ENDOMETRITIS IN BUFFALOES

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ABSTRACT

Repeat breeder buffaloes (n=36) with subclinical uterine infection tested positive by pH evaluation and White side test of cervico-vaginal mucus (CVM) and endometrial cytology were divided equally into three groups. Group-I containing fresh endometritic buffaloes (repeated ≤7 times) was administered once with Methanolic Neem seed extract (100 mcg/ml) @ 30 ml by intrauterine (IU) route, Group-II consisting of chronic endometritic buffaloes (repeated >7 times) was administered with Methanolic Neem seed extract (100 mcg/ml) @ 30 ml IU once only plus 15 gm Ashwagandha powder by oral route for 5 days and Group-III as a control group was administered with placebo as 30 ml PBS through IU. In both treated groups, pH of CVM decreased (p<0.05) after treatment and white side test turned negative in all recovered buffaloes. Polymorphonuclear cell count was reduced (p<0.05) post-treatment in recovered cases. Immunoglobulin level in serum of chronic endometritic buffaloes increased (p<0.05) post-treatment. A higher recovery rate was observed in Group-II as 75% against 66.7% in Group-I and nil recovery in control group. Pregnancy rates were recorded as 62.5%, 55.5% and nil in group-I, II and III, respectively.

Keywords: Ashwagandha, Buffaloes, Efficacy, Endometritis, Methanolic Neem seed extract

INTRODUCTION

Antimicrobial drugs hamper the uterine defense mechanism by inhibiting the phagocytic activity of polymorphonuclear leucocytes (PMNs). Besides, antimicrobial drugs have many disadvantages like high cost of treatment, reduced milk yield, development of resistance and varying success rate (Shukla and Pandit, 1989). Burgeoning literature cite the antimicrobial potential of many herbs like Neem, Garlic, Ginger, Tulsi, Turmeric and Aloe vera. Neem (*Azadirachta indica*) was extensively used in India as traditional Ayurvedic medicine for the treatment of various diseases. It was demonstrated that Neem has immuno-modulatory, anti-inflammatory, antifungal, antibacterial, antiviral, and antioxidant properties (Kumar, 2014). Recent studies have proved the efficacy of aqueous Neem seed extract to cure endometritis in both cows and buffaloes (Thombre, 2017).

MATERIALS AND METHODS

Repeat breeder buffaloes were examined transrectally for the confirmation of normal genitalia. The buffaloes having poor body condition score and pathology of genitalia like cervicitis, kinked cervix and ovaro-bursal adhesions were excluded. The buffaloes with alkaline pH (>7.5) of CVM, positive white side test and increased PMN cell count (>4%) in endometrial cytology were considered as positive for endometritis. Total 36 buffaloes were selected for present research trial. On the basis of insemination record from owners, the cases were classified into fresh and chronic endometritic. The cases with history of repeat subsequent inseminations at seven times or less were considered as fresh endometritic cases and more than seven times were considered as chronic endometritic cases.

The CVM samples were collected in petridish with help of 20 ml syringe and AI sheath on the day of oestrus (pre and post treatment) for pH estimation by digital pH

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meter and White side test. The positive and negative CVM samples by White side test were scored as '1' and '0', respectively. Blood samples were collected and serum was separated for estimation of general serum immunoglobulin level. General immunoglobulin levels were estimated using immuno-flocculation test (Deshpande *et al.*, 1991) which is quantitative and rapid test. Endometrial smear prepared by cytobrush technique and smear was prepared and stained with Geimsa stain.

The selected buffaloes with endometritis were equally divided into three groups *viz.* Group-I consisting of fresh endometritic buffaloes (n=12) were treated with 100 mcg/ml Methanolic Neem seed extract @ 30 ml by IU route once on the day of estrus; Group-II comprising of chronic endometritic buffaloes (n=12) were at par with Group-I plus oral Ashwagandha powder 15 gm in feed for 5 days. Group-III buffaloes as control were administered with placebo treatment as 30 ml PBS solution by IU route on the day of oestrus.

Data pertaining to pH, PMN cell count, recovery and pregnancy rate was analyzed statistically by using complete randomized design and ANNOVA.

RESULTS AND DISCUSSION

Buffaloes were diagnosed for endometritis on the basis of alkaline pH of CVM, positive White side test and PMN cell count >4% (Gilbert *et al.*, 2005; Barlund *et al.*, 2007; Singh *et al.*, 2016) and selected for the experiment to evaluate comparative efficacy of two herbal protocols. White side test was effectively used previously also for the diagnosis of endometritis in buffaloes (Puro, 2016; Thombre, 2017).

Mean pH of CVM in all the selected buffaloes was 8.5 ± 0.1 before treatment (Table 1). The current observation regarding pH of CVM in repeat breeder buffaloes corroborates with earlier findings (Kumar *et al.*, 2004). After treatment, average pH, white side test and PMN cell count differed ($p < 0.05$) in recovered animals from both treatment groups. However, the same in control group as well as non-

recovered buffaloes was similar ($p > 0.05$, Table 1). The immunoglobulin flocculation test in serum yielded mean immunoglobulin level as 10.3 mg/ml in group-II and control buffaloes before treatment and that same increased ($p < 0.05$) in Group-II buffaloes.

The buffaloes with fresh endometritis recovered after intrauterine infusion of methanolic Neem seed extract with a recovery rate of 66.7% in Group-I. Higher recovery rates as 75-80% were reported in previous studies for the treatment of endometritis in buffaloes using Neem seed oil through IU route (Kumar *et al.*, 2009, 2013c). However, 87.5% and 75% recovery rates were documented earlier after treatment with Methanolic and acetic Neem bark extract, respectively, in endometritic cows (Kumar *et al.*, 2013b). Further, in the current study, the mean pH value reduced ($p < 0.05$) from 8.6 ± 0.1 to 7.5 ± 0.1 in recovered animals while, the same was similar ($p > 0.05$) in non-recovered and control buffaloes (Table 1). The mean PMN cell count in endometrial cytology reduced ($p < 0.05$) from $14.8 \pm 1.9\%$ to $2.5 \pm 0.6\%$ in Group-I, however, it remained almost similar in non-recovered and control cases ($p > 0.05$, Table 1).

The combined herbal therapy in Group-II buffaloes with chronic endometritis yielded 75% recovery rate as against nil recovery in control group. No report of similar herbal combination for the treatment of endometritic buffaloes and even cows could be traced from published literature for the comparison of current findings. However, the efficacy of herbal blends like oral Ashwagandha powder with IU Garlic extract (Rahi *et al.*, 2013) and IU Ashwagandha extract with oral Gilloy extract (Kumar *et al.*, 2017) in cows was studied with 75% recovery rate. The mean pH value in this group declined ($p < 0.05$) from 8.5 ± 0.1 to 7.5 ± 0.01 after treatment and the same was similar ($p > 0.05$) in non-recovered and control buffaloes. Further, the average PMN cell count reduced ($p < 0.05$) from $13.7 \pm 2.1\%$ to $2.3 \pm 0.6\%$ after treatment in recovered cases, whereas it remained almost unchanged in non-recovered and control cases (Table 1). The mean

Table 1: Mean pH and PMN cell count before and after treatment in endometritic buffaloes

Treatment	Before treatment		After treatment			
	pH	PMN, %	Recovered		Non-recovered	
			pH	PMN, %	pH	PMN, %
Neem extract	8.6±0.1 ^a	14.8±1.9 ^a	7.5±0.1 ^b	2.5±0.6 ^b	8.5±0.1	14.0±2.9
Neem + Ashwagandha	8.5±0.1 ^a	13.7±2.1 ^a	7.5±0.1 ^b	2.3±0.6 ^b	8.4±0.3	12.0±3.1
Control	8.1±0.1	14.5±1.5	-	-	8.3±0.1	13.7±1.6

^{a, b}p<0.05

serum immunoglobulin level increased ($p<0.05$) in recovered cases from 10.5 ± 0.9 to 15.3 ± 0.7 mg/ml after treatment, whereas, it declined slightly ($p>0.05$) in non-recovered cases (9.1 ± 0.1 mg/ml) and in control group (9.5 ± 0.07 mg/ml).

Pregnancy rate in Group-I and II was 62.5 and 55.5%, respectively as against nil pregnancy in control group. The pregnancy rate in present study was higher than earlier studies who recorded only 25% pregnancy rate in endometritic repeat breeder cows treated with Methanolic Neem seed extract (Kumar *et al.*, 2013a), whereas, the same was reported by as 44.4% in endometritic buffaloes treated with aqueous Neem seed extract (Thombre, 2017).

Thus, the combination therapy (Group-II) yielded higher recovery rate (75%) as against with bare Neem seed extract (Group-I, 66.7% recovery). Conversely, the pregnancy rate was recorded higher in Group-I (62.5%) as against Group-II (55.5%).

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