EFFICACY OF GARLIC EXTRACT±ASHWAGANDHA FOR THE TREATMENT OF INFECTIOUS REPEAT BREEDING IN CATTLE

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ABSTRACT

The present research work was conducted to evaluate the efficacy of Garlic extract intrauterine (IU) *versus* Garlic extract (IU) plus Ashwagandha powder (oral) for the treatment of fresh (repeated ≤7 times) and chronic (repeated >7 times) infectious repeat breeding in cattle, respectively. Uterine infection was diagnosed by pH estimation, PMN cell count and White Side Test of cervico-vaginal mucus in both fresh and chronic cases while Immunoglobulin flocculation test with serum in chronic cases only. This indicated 92.8% cows having alkaline pH, PMN cell count >7% and 100% positive for White Side Test and increased immunoglobulin level in blood. Total 36 cows affected with endometritis were divided into three groups to undertake treatment on day of estrus *viz.* Group-I consisting of 12 fresh cases were treated with Garlic extract @10 µg in 30 ml PBS by intrauterine route once; Group-II consisting of 12 chronic cases were treated with Garlic extract as per Group-I plus Ashwagandha powder @ 15 gm with concentrates per oral for 5 days; and Group-III consisting of 12 cows as control were treated with placebo @ 30 ml PBS intrauterine. The clinical recovery of cows was confirmed by normal pH, negative white site test and PMN cell count <5% in cervico-vaginal mucus at subsequent estrus. Immunoglobulin flocculation test revealed raised immunoglobulin level in recovered cases of chronic endometritis. The recovered cows were inseminated with frozen-thawed semen at mid stage of estrus and pregnancy was confirmed at 50-60 day post insemination. The results of present investigation revealed highest recovery rate (83.3%) in group-I, whereas the highest conception rate (66.7%) and pregnancy rate (55.6%) was observed in group-II, thus suggesting the use of Garlic extract alomg with Ashwagandha for infectious repeat breeder cattle.

Keywords: Ashwagandha, Cows, Garlic extract, Infectious, Repeat breeder

INTRODUCTION

The success of dairy farming lies in ensuring the optimal reproductive efficiency as reproductive failure results in progressive economic losses. Among various reproductive disorders, repeat breeding is one of the major gynaecological problems (Kumar *et al*., 2017). Uterine infection implies adherence of pathogenic organisms to uterine mucosa and followed by colonization, penetration of the endometrial epithelium and release of bacterial toxins setting up uterine inflammation. Incidence of clinical and sub-clinical endometritis in cows is 12 % and 29.7%, respectively (Kumar *et al*., 2018). Several approaches including antibiotics, antiseptics and hormonal therapies were employed to treat repeat breeding cows with endometritis. However, there are certain limitations in the use of antibiotics like drug resistance, inhibition of normal uterine defense mechanisms and residual effect of antibiotics and hormones in the milk and meat (Rahi *et al*., 2013). Various herbs possess antibacterial, antiviral, antifungal, antioxidant and immunomodulatory properties ensuring prevention and cure of several diseases and disorders without side effects. Hence, different herbs were used as a source of valuable medicines globally (Gebreyohannes *et al*., 2013). Therefore, in the present study, herbal Garlic and Ashwagandha preparations were used for the treatment of repeat breeding due to infectious endometritis in cows.

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MATERIALS AND METHODS

The study was carried out in 36 infectious repeat breeder cows under field and farms. Infectious repeat breeding cows were selected on the basis of history and breeding records (repeated ≥3 times), results from laboratory investigation of cervico-vaginal mucus (CVM) *viz.* alkaline pH, positive white site test, presence of white flakes (Popov, 1969) and increased PMN cell count (>7%) in endometrial cytology smear and immunoglobuline flocculation test of serum (Deshpande *et al.,* 1991). Selected 36 endometritic cows were equally divided into 3 groups. Group-I repeat breeder cows (n=12) repeated seven times or less were administered with single intra uterine infusion of 10 mcg *Methanolic Garlic extract* dissolved in 30 ml PBS during mid-estrus stage. Group-II repeat breeder cows (n=12) repeated >7 times were administered with single intrauterine infusion of 10 mcg *Methanolic Garlic extract* as per group-I and 15 gm Ashwagandha powder in concentrate was fed additionally once daily for 5 days and Group-III repeat breeder cows (n=12) were administered placebo treatment @ 30 ml PBS intrauterine during mid estrus and were inseminated at next estrus only. The CVM and blood samples were collected from test animals at estrus before and after treatment to evaluate the treatment efficacy. The serum was separated and stored at 20^0C till laboratory investigation.

Methanolic Garlic extract (20% W/V) was prepared in advance by adding 200 gm Garlic seeds pest in 100% methanol to make final volume 1000 ml in a conical flask. The solution was kept at room temperature for 48 h with constant shaking followed by sieving with muslin cloth and then filtration through ordinary filter paper. The filtrate was poured in wide stainless-steel plate and allowed to evaporate under room temperature till the formation of semi-solid pest which took around one month. From the prepared Garlic pest, each dose was prepared by dissolving 10 mcg pests in 5 ml PBS as stock solution. Thus, the prepared stock doses were stored in refrigeration temperature (4^0C). Transportation of doses to field was attempted in proper thermos flask on ice packs. Each dose was reconstituted with addition of 25 ml PBS for administration through intrauterine route. Ashwagandha powder available in market was used for therapeutic purpose and the powder was fed in concentrate @ 15 gm/day to the chronic cases for 5 days as an immuno-booster.

The clinical recovery of cows was confirmed by normal pH, negative white site test and PMN cell count <5% at subsequent oestrus. The recovered cows were inseminated with frozen-thawed semen at mid stage of estrus. Conception was ensured by non-return of estrus in subsequent cycle. Pregnancy was confirmed by trans-rectal palpation after 50-60 days of insemination. The data was statistically analyzed using analysis of variance (ANOVA) to find out the significant differences of mean values (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

The present study was conducted to evaluate the antimicrobial property of Garlic and immunomodulatory effect of Ashwagandha herbs. Uterine infection in repeat breeder cows was diagnosed on the basis of pH estimation and White Side Test of cervico-vaginal mucus, uterine cytology of endometrial contents and Immunoglobulin Flocculation Test in serum. The diagnosis was made on the basis of cumulative results of all tests and no single test result was considered.

The pH observations in infectious repeat breeder cows were recorded as mildly acidic in 2.8%, normal in 13.9%, moderately alkaline in 61.1% and highly alkaline in 22.2% cases. A highly significant ($p<0.05$) number of cases were carrying pH from 8.1 to 8.5. White Side Test showed variable grade of severity of endometritis viz. 27.8% mild, 36.1% moderate and 19.5% severe. Endometrial cytology revealed PMN cell count as >5% in all selected cases. The PMN cell count ranged between 05-18% with an average of 9.8±0.3% and, thus diagnosed as subclinical stage

of endometritis (Sheldon *et al*., 2009; Bajaj *et al*., 2017). The average immunoglobulin concentration in endometritic cows was 6.9-7.0 mg/ml in 24 cases.

On treatment of Garlic extract by intrauterine route in infectious repeat breeding cows from Group-I, 10 cows (83.3%) responded to treatment against nil response in control group. Efficacy of Garlic extract was studied and reported previously with variable recovery rate from 50-100% in cases of infectious repeat breeder cows (Sarkar *et al*., 2006; Rahi *et al*., 2014; Yildiz, 2016; Kumar *et al*., 2018; Sharma *et al*., 2018). However, the current findings regarding recovery rate as 83.3% by garlic extract corroborate with others (Kumar *et al*., 2018; Sharma *et al*., 2018).

Mean pH value before treatment was 8.3±0.1 which changed to 7.3±0.1 in recovered cows after treatment; however, the same remained almost similar in non-recovered (8.4±0.0). The pH value of CVM was 7.8±0.2 before treatment which slightly increased to 8.4±0.1 after treatments in control cases which may be due to progression of uterine infection in untreated animals. Negative White Side Test was confirmed in all responded cases, but it remained positive in non-recovered and control group. Average PMN cell count was recorded as 9.4±0.5% before treatment, which reduced ($p<0.05$) to 2.9±0.3 in recovered cows, whereas, the same decreased ($p>0.05$) to 8.0±0.0 in non-recovered cows after treatment.

Conception rate was recorded as 60.0% in inseminated (only recovered) cows after Garlic extract treatment in the present study, which was slightly higher than earlier findings (Sarkar *et al*., 2006; Kumar *et al*., 2011; Rahi *et al*., 2014; Yildiz, 2016; Sharma *et al*., 2018; Kumar *et al*., 2018) who reported same (range: 50.0-52.4%). Further, the pregnancy was confirmed by trans-rectal palpation after 50-60 days of insemination and it was recorded as 50.0% as against nil pregnancy in control group.

Endometritis is basically caused by non-specific bacterial agents and moreover the infection is mostly undulant. Mixed infection evident in endometritis indicates body stress and lowered immune status. Garlic is natural protection even against physiological threats like oxidative stress, cardiovascular complexities and immune dysfunction. Garlic was recommended for immune-modulatory effect as it increases T-lymphocyte blastogenesis and phagocytosis with modulation of cytokine production (Mukherjee *et al*., 2014).

Combined treatment of Garlic extract with Ashwagandha in infectious repeat breeder cows from Group-II showed 75% recovery rate as against nil recovery in control group. Conception rate was recorded as 66.7% in inseminated cows. Subsequently, pregnancy rate was recorded as 55.6% on per-rectal palpation as against nil pregnancy in control group. It was observed that similar treatment was employed earlier (Rahi *et al.,* 2014), who reported 75% recovery rate and 62.5% conception rate in endometritis cows treated with Garlic extract IU plus Ashwagandha powder orally. However, they found higher recovery (87.5%) and conception (75.0%) rate when both Garlic extract and Ashwagandha were administered through intrauterine route.

Table 1: Mean pH and PMN cell count before and after treatment in infectious repeat breeder cows.

Group	Before treatment		After treatment			
			Recovered		Non-recovered	
	pH	PMN, %	pH	PMN, %	pH	PMN, %
Garlic Extract	8.3±0.1[a]	9.4±0.5[c]	7.3±0.1[b]	2.9±0.3[d]	8.4±0.0	8.0±0.0
Garlic + Ashwagandha	8.5±.01[a]	10.0±0.3[c]	7.4±0.1[b]	4.7±0.3[d]	8.2±0.0	8.5±0.0
Control	7.8±0.2	9.9±0.4	-	-	8.4±0.1	9.7±0.5

[a,b,c,d]$p<0.05$

The mean pH value of CVM reduced from 8.5±0.1 to 7.4±0.1 in recovered cows whereas, remained same (8.2±0.0) in non-recovered cows after treatments. WST became negative in all recovered cases while remained positive in non-recovered cases on subsequent estrus after treatment. Average PMN cell count was 10±0.3% before treatment in this group, which reduced to 4.7±0.3% ($p<0.05$) in recovered cases, whereas, reduced (8.5±0.0%; $p>0.05$) in non-recovered cows. The mean immunoglobulin level (mg/ml) in group-II increased from 6.8±0.4 to 12.3±0.9 after treatment. No report citing immunoglobuline flocculation test results could be traced from the literature for comparison of the current findings in infectious repeat breeding.

In conclusion, the study showed that although bare Garlic extract yielded higher recovery rate (83.3%), however the final pregnancy rate on treated basis was similar for both treatment protocols irrespective of chronic or fresh repeat breeding. Statistically, recovery (83.3 *vs.* 75.0%), conception (60.0 *vs.* 66.7%) and pregnancy rate (50.0 *vs.* 55.6%) in Garlic extract therapy *vs.* combination therapy differed ($p>0.05$) in endometritis cows.

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