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Effect of Administration of Garlic Extract and $PGF_2\alpha$ on Hormonal Changes and Recovery in Endometritis Cows*

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ABSTRACT : The efficacy of garlic extract and $PGF_2\alpha$ in the treatment of endometritis in cows was evaluated. A total of 26 parous cows affected with endometritis were randomly allocated into three groups. Group I (n = 10) animals were infused (IU) with 10 ml garlic extract mixed with 90 ml normal saline, three times at 12 h interval starting from the day of estrum, whereas the animals of Group II (n = 10) were treated with a single injection of $PGF_2\alpha$ (25 mg Lutalyse) on the 10^{th} to 12^{th} day after estrus, and group III (n = 6) remained as control. Cervico-vaginal mucus (CVM) was collected from each animal at pre- and post-treatment estrus and subjected to white side test, pH determination and total bacterial load. The clinical recovery of cows was assessed by negative white side test reaction, pH value and total bacterial count of CVM at subsequent estrus. The recovered animals were inseminated with frozen-thawed semen twice at 12 h intervals and pregnancy was confirmed at 45-60 days following insemination. A significant decline ($p<0.05$) in pH of CVM was observed in both the treatment groups at subsequent estrus. After treatment there was a significant ($p<0.05$) reduction in bacterial load, whereas, it was increased in control group. A total number of 65 isolates were identified in CVM samples comprising mostly of facultative anaerobic bacteria. Plasma T_4 and T_3 concentrations were increased in all the treated animals, whereas, a decline was observed in cortisol levels following treatment. The overall conception rate was 50% in treated groups as compared to nil pregnancy in the control. (**Key Words :** Cows, Endometritis, Garlic Extract, $PGF_2\alpha$, Bacterial Load, Thyroid Hormones, Conception Rate)

INTRODUCTION

Among the periparturient diseases of dairy cattle endometritis alone is reported to have greatest impact on fertility (Borsberry and Dobson, 1989). Under normal circumstances, the uterine defence mechanism (UDM) prevents invading bacteria from colonizing in the uterus (Hussain and Daniel, 1991) but when this mechanism gets impaired or weakened, bacteria may colonize in the uterus and lead to the endometritis (Vandeplassche and Bouters, 1983). Although treatment of endometritis with antibacterial agents and antibiotics have met with varying degree of success, the inconsistent recovery rate, high cost of treatment, milk disposal after antibiotic treatment, immergence of microbial resistance of antibacterial drugs and reduced phagocytic activity of polymorphonuclear leukocytes (PMN Cells) are obvious disadvantages of their use.

This situation have posed a great challenge in the management of endometritis. Hence, there is an urgent need to find out an alternative therapy for treatment of uterine infections by using natural substances as a means of activation of natural defence mechanism in the uterus. Use of certain plant products as a therapeutic agent has become a subject of recent scientific investigations. Garlic (Allium Sativum), one of such agent is being used since ancient times for its cytotoxic, antitumor, antifungal, antibacterial, antiviral, antiprotozoal properties (Ledezma and Apitz, 1998). In Sushruta sanhita- the ancient Indian Literature of medicine, garlic has been recommended remedy for hemorrhoids, rheumatis, dermatitis, abdominal pain, cough, leprosy etc. Significant growth inhibition of fungi and bacteria was also demonstrated using aqueous extract of garlic by Elnima et al. (1983). Similarly, prostaglandin $F_2\alpha$ is known for uterotonic effect and stimulation of phagocytosis by uterine leukocytes (Steefan et al., 1984).

Furthermore, the circulatory levels of thyroxine (T_4) and tri-iodothyronine (T_3) have been found to play an important role in correlating the persistant infection as well as subclinical condition of infection (David et al., 1998). A decrease in T_4 and T_3 concentrations occurred due to constant caloric deprivation or to enhanced endogenous cortisol production (Peterson and Ferguson, 1989).

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Table 1. Bacterial load (X±SE) in cervico-vaginal mucus of cows treated with different non-specific immunomodulators

Groups	Bacterial load ($\times 10^6$/ml)		
	Pre-treatment estrus	Post-treatment estrus	% Reduction
I (Garlic extract, n = 10)	144.20 ± 55.33^{A}	0.86 ± 0.45^{Ba}	98.12 ± 0.96^{a}
II (Prostaglandin $F_2\alpha$, n = 10)	73.57 ± 25.22^{A}	0.91 ± 0.24^{Ba}	92.8 ± 4.10^{a}
II (Control)	30.65 ± 16.03^{A}	41.32 ± 16.62^{Ab}	$-(36.62\pm25.26^{b})$

Values within same column (a, b) and within same row (A, B) having different superscripts differ significantly ($p<0.05$).

Similarly in stressful animals, an increase in plasma cortisol concentration causes an alteration of neutrophil function and an increased susceptibility to bacterial infection (Roth and Kaeberle, 1982).

Hence, the present experiment was conducted to test the therapeutic effects of garlic extract and prostaglandin F_2 alpha on the recovery and hormonal changes in endometritic cows.

MATERIALS AND METHODS

This study was carried out on twenty six crossbred cows (Freisian×Sahiwal), maintained at Military Dairy Farm, Bareilly, U.P. India. All the animals were kept under the similar feeding and managemental conditions during the period of experiment. All the cows were in age group of 4-8 years and in their 2nd to 4th lactation and were considered for experimentation on the basis of presence of white flakes in cervicovaginal mucous (CVM), pH and color reaction of CVM to white side test (Popov, 1969). The CVM and blood samples were collected from all the animals at pre and post treatment estrus. The plasma was separated and stored at-20°C till hormone assay.

The garlic cloves were crushed and mixed with 70% alcohol. The mixture was placed in a water bath for 4-6 hr at 65-70°C and allowed overnight incubation at room temperature (25-30°C). It was filtered and the tincture (filtrate) was mixed with saline at 10% concentration and used as described by Esparza et al. (1996).

Twenty six endometritic cows were randomly divided into 3 groups; two treatment groups of 10 animals each and a control group which included 6 animals. The group I cows (n = 10) were given 10 ml garlic extract mixed with 90ml normal saline by intrauterine (IU) route thrice at 12 h interval, starting on the day of estrum. Whereas, the animals of group II (n = 10),were treated with single injection of 25mg of dinoprost tromethamine (Lutalyse,Novartis India Ltd.) between 10^{th} to 12^{th} day after estrus.The animals of group III (n = 6) served as control and received single IU infusion of 100 ml sterilized normal saline at estrum.

Prior to bacteriological examination the CVM was mucolysed by mixing with sterile distilled water in a vortex shaker for 10-15 minute and divided into two halves. One half was used for bacterial count and other half for identificaton of organisms after direct inoculation on nutrient, blood and Mackonkey agar plates. The bacterial load was determined by calculating colony forming units on the agar plates after 24 h and species identification was done by observing morphology of organisms after Gram's staining. The genus of each isolates was identified by carrying out primary identification tests (Shape, growth in air, presence of spore, motility, catalase, oxidation/ fermentation tests and acid production in glucose as described by Cowan and Steel (1974). Identification of species of each genera was done by secondary biochemical tests (Indole, MR, V.P.Citrate, acid production in sugars, growth on specific media, coagulase etc.) as per recommendations of Cowan and Steel, 1974.

The plasma thyroxine (T_4) and tri-iodothyronine (T_3) levels of endometritic as well as non-endometritic cows were estimated using solid phase ^{125}I RIA kit (Izotop, Institute of Isotopes Co. Ltd., H-1535, Budapest). Similarly, plasma cortisol levels were measured by solid phase ^{125}I RIA kit (ICN Bio medicals, Inc. Diagnostics Division).

Following administration of garlic extract and $PGF_2\alpha$ the recovered animals (based on white side negative reaction test) were considered as non-endometritic or normal cows at subsequent estrus. The peripheral concentrations of plasma T_4, T_3 and cortisol in endometritis (non-recovered cows) were compared to those of non-endometritis/normal (recovered cows) irrespective of group of treatment.

The clinical recovery of cows was assessed by monitoring the changes in pH, nature and white side test reaction of estrual mucus and total bacterial count of CVM at subsequent estrus. The cows during subsequent standing estrus were inseminated twice at 12 h interval with frozen/thawed semen from fertile crossbred bulls. Pregnancy was confirmed by rectal palpation at 45-60 days following insemination.

The data were analysed as per standard procedure (Snedecor and Cochran, 1994). Student's "t" test and Duncan's multiple range test (d.m.r.t.) were used to compare the means of pH, bacterial load within groups and between the groups, respectively. The recovery rate data of cows was also tested by d.m.r.t. The 5 percent level of significance ($p<0.05$) was accepted as indicating statistically significant differences.

RESULTS

The CVM of endometric cows was either purulent (50-60%) or mucopurulent (40 to 50%) at the time of treatment

Table 2. Proportionate population of micro-organisms isolated from cervico-vaginal mucus of endometritis cows

Type of bacteria	No. of isolates	Percent total
Escherichia coli	16	24.6
Staphylococcus aureus	14	21.5
Pseudomonas aeruginosa	10	15.4
Bacillus sp.	6	9.2
Streptococcus sp. (Group D)	6	9.2
Proteus sp.	5	7.7
Actinomyces pyogenes	4	6.2
Klebsiella sp.	4	6.2

and showed either moderate or slight positive reaction to white side test. At subsequent estrus, however, the estrual CVM turned clear in 70% animals treated with garlic extract and 80% by $PGF_2\alpha$, as compared to 33.3% of control animals.

The recovery rate of cows from endometritis assessed in terms of response to white side test (negative) was higher in garlic extract (50%) and $PGF_2\alpha$ (60%) treated animals as compared to control (16.7%).

The mean pH at pretreatment estrus varied from 7.75±0.11 to 8.05±0.11 which declined significantly ($p<0.05$) at subsequent estrus in treated animals. However, in control group the pH of CVM did not differ significantly between pre and post treatment estrus (7.75±0.11 vs. 7.83±0.21).

The bacterial load in estrual CVM at pre-treatment estrus was statistically similar in all the groups and declined significantly ($p<0.05$) at subsequent estrus in treatment groups (Table 1). However, the bacterial load in CVM remained similar in control group at pre and post-treatment estrus. The percent reduction of bacterial load was greater in treatment groups than control animals which had, 36.62+25.26 percent increase at subsequent estrus (Table 1).

The bacteriological investigation of CVM revealed the presence of a number of bacterial species (Table 2). All animals (Treated and Control) sampled had positive bacteriology. Among them, *E. coli* (24.6%) was the most frequently isolated followed by Staphylococcus aureus (21.5%), Ps. aeruginosa. (15.4%). Other than these, *Bacillus sp*. and *Streptococcus sp*. (9.2% each), Proteus sp. (7.7%), *A. pyogenes* (6.2%) and *Klebsiella sp*. (6.2%) were also recorded. These were mostly facultative anaerobes. The study revealed that the overall conception rate increased by 50% following administration of garlic extract and dinoprost tromethamine against nil pregnancy in control group (Table 3).

The mean concentration of thyroxine (T_4), triodothyronine (T_3) and cortisol estimated at pre and post treatment estrus are given in Table 4. The concentration of

Table 3. Bacterial isolates of CVM in treated and control cows

Group I	Pre-treatment	Post-treatment	Pregnancy results
1	*Ps.aeruginosa, E coli*	*Ps. aenuginosa, E. coli*	-
2	*Staph. Aureus, Streptococcus sp.* (Group D)	*Streptococcus sp.* (Group D)	+
3	*Bacillus sp.*	*Bacillus sp.*	+
4	*Bacillus sp, E. coli, Staph. aureus*	*Bacillus sp., E. coli, Staph. aureus*	-
5	*Proteus sp., E. coli*	Not identified	-
6	*Proteus. sp., A. pyogenes*	*Proteus sp.*	-
7	*Ps. aeruginosa*	*Ps. aeruginosa*	-
8	*Ps. aeruginosa, E. coli, Streptococcus sp.* (Group D)	*E. coli, Ps. aeruginosa*	+
9	*E. coli*	*E. coli*	+
10	*Klebsiella sp. E. coli*	*E. coli, Klebsiella sp*	+
Group II			
1	*A. Pyogenes, E. coli*	*E. coli*	+
2	*Bacillus sp., Staph. aureus, Klebsiella sp.*	*Staph. aureus*	-
3	*Streptococcus sp. (Group D)*	*Streptococcus sp.* (Group D)	+
4	*Staph. aureus, E. coli.*	*Staph. aureus*	+
5	*Ps. aeruginosa*	*Not identified*	-
6	*E. coli, Ps. aeruginosa*	*E. coli, Ps. aeruginosa*	-
7	*Staph. Sureus, Streptococcus sp.* (Group D)	*Streptococcus sp.* (Group D)	-
8	*E. coli, A. pyogenes*	*E. coli*	+
9	*Staph. aureus, E. coli, Proteus sp.*	*E. coli*	-
10	*Ps. aeruginosa*	Not identified	+
Group III (Control)			
1	*Ps. aeruginosa*	*Ps. aeruginosa*	-
2	*Staph. aureus*	*Staph. aureus*	-
3	*E. coli, Staph. aureus*	*E. coli, Staph. aureus*	-
4	*Ps. aeruginosa*	*Ps. aeruginosa*	-
5	*Staph. aureus. E. coli*	*Staph. aureus,E. coli*	-
6	*Ps. aeruginosa, Proteus sp.*	*Ps. aeruginosa, Proteus sp.*	-

(+) denotes pregnant and (-) denotes non-pregnant.

Table 4. Effect of treatment of different non-specific immunomodulators on thyroxine (T_4), trilodothyronine (T_3) and cortisol level in endometritis cows

Groups	Hormonal profile (Means±SE)					
	T_4 (nmol/lit.)		T_3 (nmol/lit.)		Cortisol (ng/ml)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Group I (Garlic extract)	35.79±5.56^{A}	38.86±3.34	2.03±0.24^{a}	2.44±0.27^{b}	4.58±1.69	1.80±0.82
Group II ($PGF_2\alpha$)	21.17±1.95^{aB}	41.63±2.60^{b}	2.11±0.21	2.68±0.40	3.27±0.81^{a}	1.25±0.33^{b}
Group II (Control)	29.86±2.89^{A}	31.97±5.38	1.57±0.19^{a}	2.09±0.22^{b}	2.04±0.63	3.45±1.32

Values within same row (a, b) and within same column (A, B) having different superscripts differ significantly ($p<0.05$).

Table 5. Peripheral concentration of plasma T_4, T_3 and cortisol in endometritis and normal cows

Groups	T_4 (nmol/lit)	T_3 (nmol/lit)	Cortisol (ng/ml)
Endometritis cows	28.33±2.47^{a}	1.80±0.11	4.40±0.69
Normal cows	42.73±4.66^{b}	2.25±0.29	2.89±0.92

Values within same column having different superscripts (a, b) differ significantly ($p<0.05$).

both thyroid hormones tended to increase at post treatment estrus. However T_4 level was significantly higher in $PGF_2\alpha$ -treated animals (41.63±2.60 vs. 21.17±1.95, $p<0.05$), whereas T_3 concentration remained significantly greater (2.44±0.27 vs. 2.03±0.24) only in Garlic-extract treated animals. The mean cortisol concentration at pre treatment estrus ranged in between 2.04±0.63 to 6.77±1.38 ng/ml (Table 4), which got reduced at post treatment estrus. However, this reduction in cortisol levels was found significant ($p<0.05$) in $PGF_2\alpha$-treated animals (1.25±0.33 vs. 3.27±0.81). In contrast, the cortisol levels tended increase at post treatment estrus (3.45±1.32 vs. 2.04±0.63 ng/ml) in control animals.

Furthermore, the concentration of T_4 was significantly greater (42.73±4.66, $p<0.05$, Table 5) in normal than endometritic cows (28.33±2.47). Similarly, an increased levels of T_3 (2.25±0.29) was observed in normal cows as compared to those affected with endometritis (1.80±0.11 n mol/lit). Alternatively, these endometritis cows had higher (4.40±0.69 ng/ml) levels of plasma cortisol than the normal cows (2.89±0.92 ng/ml), though their means were statistically insignificant.

DISCUSSION

The colour reaction of CVM to white side test became negative (colourless) in greater proportion of animals following treatment with non-specific immunomodulators. This could be explained on the basis of number of leukocytes present in the uterine discharge. The normal uterine discharge contained too low population of leukocytes to cause any change of colour, whereas, the discharge from endometritis animals has been found to possess more number of leukocytes causing colour reaction to white side test (Popov, 1969).

The overall pH of CVM in endometritis cows was towards alkaline side as also has been reported by others (Bindrawan et al., 2002; Deori et al., 2004). Which can occur due to more metabolites of bacteria and the inflammatory exudates (Salphale et al., 1993). After the administration of non-specific immunomodulators, the pH of CVM in treated cows declined at subsequent estrus. This may be attributed that once the infection from the uterus is eliminated, the pH drops towards the neutral side.

In our study, most commonly isolated bacteria were E. coli, Staphylococcus aureus and Pseudomonas aeruginosa. These observations are in agreement with several reports (Arora and others, 2000; Bindrawan et al., 2002; Deori et al., 2004) in endometritic animals. These bacteria were facultative anaerobes and normal inhabitants of reproductive tract. They may acquire a pathogenic role when the defense mechanism of uterus become weaken due to stress factors such as extreme changes in weather, poor sanitation, etc.

The fifty percent that recovered from endometritis and became pregnant in garlic extract treated group, is similar with the findings of Esparza and others (1996). This could be due to the significant reduction of bacterial load in garlic extract-treated animals, as garlic extract is well known for its antimicrobial property against Gram positive and Gram negative bacteria (Yoshida et al., 1998; Chung et al., 2003). The treatment with that garlic extract has been shown to stimulate the release of cytokines such as IL-2, IFN-α, IFN-γ and increase the natural killer activity and enhances phagocytic activity of peritoneal macrophages (Kyo and others, 1998). The therapeutic efficacy of garlic for its cytotoxic, antitumor, antifungal, antibacterial, antiviral, antiprotozoal properties for which it is being used traditionally since long time is thought to be caused by an organic sulphur compound 'Ajoene' (4,5,9 -trithiadodeca 1,6, 11-triene-9-oxide) (Ledezma and Apitz, 1998). Further, garlic is also reported to contain selenium, vit. A, B, C and E (Williamson, 2002).

The observation that treatment with $PGF_2\alpha$ caused a significant reduction of bacterial load in CVM and

conception (50%) is in agreement with the findings of several others (Kaneko et al., 1996; Sood et al., 2003). Rao and others (2001), however, observed a higher (77%) conception rate in endometritis cows treated with $PGF_2\alpha$. There are several reports (Roy and others, 1990; Steefan and others, 1994; Sood and others, 2003) indicating that endometritic cows recovered earlier after administration of $PGF_2\alpha$, and tended to have better reproductive performance. In fact, the exogenous $PGF_2\alpha$ therapy removes the suppressive effect of progesterone on the uterine defense mechanism or, alternatively stimulate it through estrogen. The $PGF_2\alpha$ induced myometrial contractions (Lindell and Kindahl, 1983), helps in expelling debrises and micro-organisms from the uterine lumen. Additionally, $PGF_2\alpha$ may have stimulatory effect on the phagocytic activity of uterine PMNs (Paisley and others, 1996), Hence, the luteolytic action of $PGF_2\alpha$ has become therapy of choice for endometritis in cows where a functional corpus luteum is present.

The lower levels of T_4 and T_3 observed in endometritis cows of present study are in agreement with the earlier in cows with uterine infection (Abede and Eley, 1992; Elecko et al., 1990) and repeat breeding buffaloes (Mahendran et al., 2001). The lower levels of thyroid hormones in endometritis cows might be due to the physiologic, pharmacologic or stress of the animals, as also observed by David et al. (1998). The administration of our treatment have resulted in increased levels of T_4 and T_3 which might be due to the elimination of bacterial infection and stress in these animals.

The increased levels of cortisol at pre-treatment estrus is in accordance with observations of Bindrawan et al. (2002) and that of Shalaby (1997) who also recorded an increase in cortisol concentrations in endometritic cows compared to healthy. It has been reported that the cortisol levels fluctuate around 2 ng/ml in non-stressful cows and a multifold increase (up to 27 ng/ml) in its concentration may occur under stress (Kaufmann and Thun, 1998). The decline in cortisol levels during post-treatment estrus can thus be considered an indication of elimination of stress.

This study indicates therapeutic values of garlic extract and $PGF_2\alpha$ in endometritic cows.

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