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Therapeutic Efficacy of Garlic (*Allium sativum*), Turmeric (*Curcuma longa*) and Eucalyptus (*Eucalyptus globules*) Extracts in the Treatment of Bovine Endometritis

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ABSTRACT

Background: Endometritis is one of the major causes of subfertility in bovines. The present study was conducted to evaluate the therapeutic efficacy of ethanolic extracts of garlic, turmeric and eucalyptus in the treatment of bovine endometritic as an alternative to antibiotics.

Methods: Forty crossbred cows affected with endometritis were equally divided into 4 groups. Group I (control) cows were treated intrauterinely (i.u) with streptopenicillin 2.5 g + 30 ml normal saline for 3 days and group II, III and IV cows were treated (i.u) with 30-40 ml of ethanolic extract of garlic, turmeric and eucalyptus for 3 consecutive days. The animals were inseminated artificially on subsequent estrus after the treatment.

Result: Bacterial count in uterine flushing declined significantly after treatment in all the four groups. The percentage of reduction in bacterial count was higher in garlic treated cows than in control and other treatment groups. A significant increase in percentage of PMNs was found after treatment in all the groups. The overall conception rate was 40% in control group, 50% in group II and 30% in group III and IV. It was concluded that ethanolic extract of garlic for the treatment of endometritis resulted in better conception rate and significantly higher percentage reduction of bacterial load than other treatment and control groups.

Key words: Bovine, Endometritis, Eucalyptus, Garlic, Turmeric.

INTRODUCTION

The productivity of the cattle is highly influenced by genetics and environment. The reproductive efficiency and productivity of animals are interlinked and are needed to be synchronized for the expression of complete production potential of cattle. The biggest concern is to ensure and maintain the reproductive challenges of cattle and ensure one calf per year which needed to be optimized to maintain the efficient production cycle. Repeat breeding due to endometritis in cattle and buffaloes covers the major percentages of reproductive issues in cattle (Selvaraju *et al.,* 2005) which hinder the optimized reproduction cycle in cattle of which bacterial endometritis constitutes a major cause.

The innate defence mechanism in the uterus prevents invading bacteria from colonizing in the uterus but when this mechanism gets impaired, bacteria may colonize in the uterus and results in endometritis. Clinical endometritis refers to the local inflammation of the endometrium, characterized by the presence of purulent or mucopurulent material originating from the uterus, not accompanied by systemic illness (Sheldon *et al*. 2006) and the degree of clinical endometritis depended on the nature of the cervical discharge *i.e.* from mild to severe-mucus with flakes to purulent (Gautam *et al.,* 2009). Treatment of clinical endometritis in cattle and buffalo is challenging and the treatment option should be mandated according to the identified intrinsic and extrinsic risk factors (Kumar *et al.,* 2020). Several treatment schedules and drugs were used for the treatment of endometritis in cattle like immuno-modulators and lugol's iodine (Palanisamy *et al.* 2015), plant extracts (garlic, neem, turmeric, ashwagandha *etc.*), honey (Abdul-Hafeez *et al*. 2019), streptopenicillin with adjuvants like EDTA - tris (Selvaraju *et al.* 2003), aminoseletone and primalact (Shabunin *et al.,* 2020) and obtained varying results. Although treatment of endometritis with antibiotics, antibacterial agents, immunomodulators and hormones have met with varying degree of success, the inconsistent recovery rate, high cost of treatment, milk disposal after

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antibiotic treatment, immergence of microbial resistance of antibacterial drugs and reduced phagocytic activity of polymorphonuclear leukocytes, the need for alternative strategies is inevitable.

Use of herbal medicines has long been used to reduce the side effects and toxicity of allopathic medicines. The extracts of garlic, turmeric, neem, eucalyptus, ashwagantha, *etc.*, had been shown to possess some of antimicrobial, anti-inflammatory and immunomodulatory effects due to their active principles and other alkaloids. These extracts had been used since long years for the treatment of various diseases and disorders in human medicine and in recent years in the field of veterinary medicine. Extract of garlic (Kumar, 2013 and Singh, 2016) and hydro-alcoholic extract of turmeric (Kumar, 2016) were found to be an effective broad spectrum antibacterial and had been suggested as alternative therapy for repeat breeding cattle and buffaloes. Eucalyptus plant extract has been reported to possess antimicrobial property against *Bacillus subtilis, Salmonella typhi, Staphylococcus aureus, Klebsiella pneumonia, Staphylococcus epidermidis, Enterococcus faecalis* and *Proteus mirabilis* (Hossam, 2008). Considering the above points, the present study was designed with the following objectives (1) to study the therapeutic efficacy of herbal extracts on the cytology of the endometrium in bovine endometritis and (2) to study the influence of herbal treatments on the conception rate of the cows with clinical endometritis.

MATERIALS AND METHODS

The cows brought in estrum to the Large Animal Gynaecology ward, Department of Clinics, Veterinary College and Research Institute, Namakkal during over a period of one year (2019 to 2020) with history of repeat breeding were subjected to thorough gynaecological examinations. The cervical mucus from the mid cervix was collected in these animals aseptically using a sterile sheath and transferred to a sterile test tube for white side test as per the method described by Palanisamy *et al.* (2015). Forty animals which tested positive for white side test were confirmed for endometritis and selected for the study. These animals were divided into four equal groups (groups I, II, III and IV). The cervical mucus was collected from these animals to study the nature of cervical mucus, pH, white side test and bacterial load on the day of estrum (day 0) just prior to treatment. The uterine flushings were collected by infusing sterile normal saline just prior to the treatment in all the animals (*i.e.* day 0) and on day 3 after treatment for endometrial cytology and for the analysis of percentage of polymorphonuclear cells. Polymorphonuclear (PMNs %) cell count in the uterine flushing was assessed by preparing smears from the cell suspension received by centrifugation of uterine flushing (3000 rpm for five minutes) on clean grease free glass slides and stained by Giemsa stain.

The group I, II, III and IV cows were treated with 2.5 gm of streptopenicillin (diluted in 30 ml of normal saline) and 30 ml of 10% ethanolic extract of garlic, turmeric and eucalyptus, respectively through intrauterine route for three days. The cervical mucus was collected on the subsequent estrus in all the animals to study the nature, pH, white side test and bacterial count. All the animals which tested negative for white side test on the subsequent estrus were inseminated and those animals which returned to heat were inseminated on the second subsequent estrum after the treatment. The pregnancy was verified per-rectally on day 45 by ultrasonography. The nature of the cervical mucus was classified as mucopurulent, cloudy and clear and the pH of the cervical mucus was assessed using the pH paper. The bacterial count was evaluated by the number of colonies on the plate count agar by streaking the cervical mucus. Briefly, 3% plate count agar was prepared aseptically in laminar airflow in 100 mm petridish and allowed for solidification. The cervical mucus samples collected were streaked on the plate count agar aseptically using a sterile loop under laminar airflow and incubated for 24 hours and the bacterial colonies were counted. The number of bacterial colonies and the percentage of reduction of bacterial colonies were studied in the cervical mucus before and after treatment. The data obtained were analysed statistically by the analysis of variance (ANOVA) using SPSS 20.0 software package.

RESULTS AND DISCUSSION

Nature of cervical mucus

The observations on the nature of the cervical mucus and pH of the cervical mucus in the present study before and after treatment in the treatment and control groups are shown in the Table 1. The nature of cervical mucus in control and treatment groups was muco-purulent and cloudy in all the animals before treatment. On subsequent estrus after treatment the percentage of animals that had clear cervical mucus were 80.00, 90.00, 90.00 and 80.00 in groups I, II, III and IV, respectively.

pH of cervical mucus

The pH of the cervical mucus before treatment in all the groups ranged from 7 to 9 with an average of 8.35. The normal pH of the cervical mucus in cows ranged from 6.5 to 7.4 and the deviation of the pH towards alkalinity might be due to the uterine infections. The pH of the cervical mucus after treatment in all the groups ranged from 6 to 8 with average of 6.8. The statistical analysis of the pH of CVM before and after treatment revealed a highly significant ($P<0.01$) difference within the groups however the difference was not statistically significant between the groups.

White side test

The results of the white side test performed before and after treatment in all groups are depicted in the Table 1. The percentage of the animals that tested negative for white side test in all the groups after treatment were 80.00, 90.00, 90.00 and 80.00 in groups I, II, III and IV, respectively. However

the difference was not statistically significant which might be due to the equal efficacy of the herbal extracts. However, the ethanolic extract of garlic and turmeric are highly effective on the nature of cervical mucus compared with the other treatment and control groups. The result of the present study was in accordance with the results of Singh (2016) and Bhardwaz *et al.* (2018) following treatment with garlic extract in crossbred cows.

Bacterial load

Bacterial load could be a diagnostic indicator to assess health status of uterus and has been used to assess the level of infection (Dhaliwal, 2001). The mean bacterial load (10^6 per ml) at before treatment in all the four groups ranged from 250 to 335 colonies and after treatment ranged from 19 to 25 colonies and the results are depicted in Table 2. In all the treatment and control groups a highly significant decline ($P<0.01$) in bacterial colony count was observed in post-treatment estrus cervical mucus samples. The percent reduction (Mean±SE) in bacterial load in animals treated with streptopenicillin, ethanolic extract of garlic, ethanolic extract of turmeric and ethanolic extract of eucalyptus were 92.69±0.79, 93.37±0.49, 90.42±0.22 and 89.82±1.09, respectively. The percentage of reduction in the bacterial load between the groups I and IV and II and III differed significantly ($P<0.05$), however the difference was highly significant between groups II and IV ($P<0.01$). The per cent reduction in the bacterial load between the groups I and II did not differ significantly which indicated that the control and garlic groups were equally effective in the reduction of bacterial load in the uterus.

Percentage of PMNs

The percentage of PMNs before and after treatment in control and treatment groups is shown in Table 2. The percentage of PMNs in all the groups ranged from 23-28 before treatment and 46 to 50 after treatment. The statistical analysis of the percentage of PMNs differed highly significant within groups before and after treatment, however the difference was not statistically significant between the groups.

Conception rate

The conception rate in the group I, II, III and IV animals were 40%, 50%, 30% and 30%. The difference in the conception rate between the groups was not statistically significant although the conception rate was higher in the group II animals followed by group I, III and IV animals.

According to Parmar (2021), an ideal treatment of endometritis not only eliminates the bacteria in the uterine cavity and sub endometrial layers, but also enhances the uterine defense mechanisms (UDM). Although the treatment

Table 1: Effect of extracts of garlic (*Allium sativum*), turmeric (*Curcuma longa*) and eucalyptus (*Eucalyptus globules*) on Physio-chemical characteristics of cervical mucus in crossbred cows with endometritis.

Parameter	Group I (Streptopenicillin)		Group II (Garlic)		Group III (Turmeric)		Group IV (Eucalyptus)	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
Nature of cervical mucus								
Muco-purulent	70.00 (7)	20.00 (2)	60.00 (6)	0.00 (0)	70.00 (7)	10.00 (1)	70.00 (7)	20.00 (2)
Cloudy	30.00 (3)	0.00 (0)	40.00 (4)	10.00 (1)	30.00 (3)	0.00 (0)	30.00 (3)	0.00 (0)
Clear	0.00 (0)	80.00 (8)	0.00 (0)	90.00 (9)	0.00 (0)	90.00 (9)	0.00 (0)	80.00 (8)
White side test								
Positive	100 (10)	20.00 (2)	100 (10)	10.00 (1)	100 (10)	10.00 (1)	100 (10)	20.00 (2)
Negative	0.00 (0)	80.00 (8)	0.00 (0)	90.00 (9)	0.00 (0)	90.00 (9)	0.00 (0)	80.00 (8)
CVM pH								
pH	8.30±0.21^{a}	6.60±0.16^{b}	8.30±0.21^{A}	7.00±0.21^{B}	8.30±0.21^{p}	6.70±0.21^{q}	8.50±0.17^{x}	6.90±0.18^{y}

Table 2: Effect of extracts of garlic (*Allium sativum*), turmeric (*Curcuma longa*) and eucalyptus (*Eucalyptus globules*) on bacterial count, PMN (%) and conception rate in crossbred cows with endometritis.

Groups	Treatments	Bacterial count (10^6/ml)	Percentage reduction of microbial load	PMN (%)	Conception rate (%)
Control (n=10) (Streptopenicillin)	Pre-treatment	333.10±26.49^{a}	92.69±0.79^{pq}	22.80±1.70^{A}	40.00
	Post-treatment	23.20±1.87^{b}		46.20±1.74^{B}	
Garlic (n=10)	Pre-treatment	295.20±22.03^{a}	93.37±0.49^{q}	25.90±1.33^{P}	50.00
	Post-treatment	19.10±1.36^{b}		48.10±1.89^{Q}	
Turmeric (n=10)	Pre-treatment	251.90±11.89^{a}	90.42±0.22^{pr}	24.20±1.20^{R}	30.00
	Post-treatment	24.20±1.38^{b}		49.20±1.04^{S}	
Eucalyptus (n=10)	Pre-treatment	263.20±13.29^{a}	89.82±1.09^{r}	26.80±1.07^{X}	30.00
	Post-treatment	25.60±1.63^{b}		49.70±1.49^{Y}	

of endometritis in bovine is mainly achieved using intrauterine antibiotics, antiseptics and rarely by hormones, the inconsistent results, high cost of treatment and inhibition of natural uterine defense mechanism warrants an alternate therapy due to multiple drug resistance pathogens (MDR). Antimicrobials of plant origin are not only effective in the treatment of infectious diseases but also reduce the side effects associated with the use of antibiotics or antimicrobials (Cunha, 2001 and Mahour *et al.,* 2021). The treatment of endometritis in cattle in an alternative strategy dated long back in 1990s. The use of a variety of plant extracts and other forms have been used for its treatment. In the present study, the ethanolic extract of garlic and turmeric had promising results on the nature, pH and white side test negativity than eucalyptus and control groups. The improved nature of cervical mucus, pH of cervical mucus and conception rate could be due to the significant reduction of bacterial count.

In the present study increase in the PMNs in the animal treated with garlic extract was comparable with all the other treatment and control groups. However, Singh (2016) reported an increased total cell count (TCC) after treatment with garlic. Kyo *et al.* (1998) reported that the treatment with garlic extract had shown to stimulate the release of cytokines such as IL-2, IFN-α, IFNγ which increased the natural killer activity thus enhancing the phagocytic activity of macrophages. The per cent reduction (Mean±SE) in bacterial load in animals treated with ethanolic extract of garlic was 93.37±0.49 which was highly significant with eucalyptus and significant with turmeric group. Our results were in accordance with the results of Sarkar *et al.* (2006) who evaluated the therapeutic efficacy of garlic extract and $PGF_2\alpha$ in the treatment of endometritis in cows and suggested that after treatment with garlic extract, there was a significant reduction in bacterial load and percentage of reduction in bacterial count was 98.12±0.96 which was higher than the control and $PGF_2\alpha$ treated groups.

The improved percentage of reduction of bacterial count in garlic might be due to the good antibacterial properties (Meriga *et al.*, 2012 and Viswanathan *et al.*, 2014) against both Gram positive and Gram negative bacteria (Chung *et al.* 2003). The antibiotic activity of 1 mg of biologically active principle allicin in garlic is equated to that of 15 IU of penicillin (Sadanandan *et al.,* 2014). The conception rate in garlic treated animals was 50% and our results are in accordance with Alagar *et al.* (2018) who reported 50% conception rate in endometritis affected buffaloes treated with garlic extract and suggested that the treatment with garlic showed regeneration of submucosal endometrial gland and hyperplasia of the glandular cells which resulted in fully grown surface epithelium in buffaloes. Improved recovery rate and conception rate were obtained by Kumar *et al.* (2009) and Kumar *et al.* (2013) in buffaloes treated with garlic extract. However, Selvaraju *et al.* (2003) observed a conception rate of 50.00% in endometritic cows treated EDTA-tris-streptopenicillin and suggested that regeneration of lining epithelium of endometrium with scattered mononuclear cell infiltration in sub-epithelial area was seen in EDTA-tris-streptopenicillin treated cows.

The nature of the cervical mucus and response to white side test in group III (turmeric) animals were comparable with the group II animals, however, the percentage of reduction of bacterial count and conception rate in these animals were lower than the garlic treated animals. The possible reasons could be the biologically active ingredient curcumin in turmeric which possess more potent immunomodulatory and anti-inflammatory properties rather than antimicrobial properties which was indicated by the percentage of PMNs (24.20±1.20 vs 49.20±1.04) before and after treatment. The findings in the study were also supported by Jagetia and Aggarwal, (2007) who reported that the expression of multiple pro inflammatory cytokines such as TNF, 1L-1, 1L-2, 1L-6, 1L-8, 1L-12 and chemokine's through inactivation of NF- Kappa B, the transcription factor was regulated by the turmeric in their study. These results were in contrast with the findings of Kumar (2016) who explained that hydroalcoholic extract of turmeric shown maximum zone of inhibition among ashwagandha and garlic herbal extracts and suggested that turmeric was found to be a broad spectrum antibacterial.

In control and group IV animals the nature of the cervical mucus and response to white side test were comparable but the percentage of reduction in bacterial load and conception rate were higher in control group than in group IV. The use of eucalyptus is limited in the treatment of endometritis and the results are not comparable with the garlic and turmeric group. Hayat *et al.* (2015) reported that the antimicrobial property of the eucalyptus was active against few Gram positive bacteria and inactive against *Escherichia coli* and *Pseudomonas aeruginosa* and Fratini *et al.* (2017) also did not observe inhibition zone diameter (IZD) results using eucalyptus essential oil against *S. aureus* and *E. coli*. Hayat *et al.* (2015) also suggested that eucalyptus has more potent anti-oxidant and anti-inflammatory activity compared to antibacterial property. The reduced results in eucalyptus group animals of this study might be due to the limited and selective antimicrobial property against only specific bacteria.

CONCLUSION

It is concluded that the ethanolic extract of garlic for the treatment of endometritis resulted in better conception rate and significant percent reduction of bacterial count than control and other treatment groups. Further studies are warranted for standardization of dose based on the concentration of biologically active compounds in the garlic extracts. The combination of herbal extracts in the treatment of endometritis in cattle and buffaloes could be a better future alternative.

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