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Evaluation of ethno veterinary herbal formulation (*Cuminum cyminum* + *Raphanus sativus*) in managing bovine endometritis

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Abstract
The present study was undertaken to evaluate the clinical efficacy of *Raphanus sativus* and *Cuminum cyminum* in treating endometritis under field conditions. The animals aged between two to five calving brought for insemination were randomly subjected to white side test to diagnose the presence of subclinical/clinical endometritis. The animals tested positive were treated with Ethnoveterinary medicine (EVM) therapy, herbal formulation comprising (*Cuminum cyminum* + *Raphanus sativus)* for 5 days. Post-treatment, the recovery percentage against subclinical endometritis with one time EVM therapy was 85.71%. And with the second time EVM therapy, the recovery percentage was 100%. Meanwhile, there is no response in clinical endometritis condition with one time EVM therapy. However, after the second time EVM therapy recovery percentage was 33.33%. Hence, it may be assumed that the EVM therapy is highly effective against subclinical endometritis condition and it is less effective in high degree of clinical endometritis.

Keywords: Endometritis, *Raphanus sativus*, *Cuminum cyminum*

Introduction
Endometritis is one of the most common uterine disorders in dairy cows, causing decreased fertility and high economic losses [1]. After parturition, bacteria from the animal's environment contaminate the uterine lumen of most cattle. Infection persists in the uterus of many animals for more than three weeks, with about 15% of dairy cattle having signs of clinical endometritis [2]. Endometritis is an expensive condition for veterinarians and farmers to manage, rivalling the cost of other endemic diseases such as mastitis. These infections often involve a mixture of pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus, Klebsiella spp., Arcanobacterium pyogenes,* and *anaerobic bacteria* such as *Fusobacterium necrophorum* and *Prevotella* species [3].

The use of ethno veterinary medicine may provide a cheaper and sustainable alternative to synthetic drugs. Of late there is enormous data pertaining to documentation of ethno veterinary practices followed in different parts of the country for various ailments of livestock which include endometritis. However there is a wide gap in accepting these practices due to lack of scientific validation [4].

Raphanus sativus is an Indian traditional plant, family Brassicaceae. The root of *R. sativus* contain 'raphanin' which has already been reported for its antibacterial and antifungal properties. The antibacterial principle 'raphanin' has been found to be strongly active on *Escherichia coli, Pseudomonas pyocyaneus, Salmonella typhi, Bacillus subtilis, Staphylococcus aureus, streptococci* and *Pneumococci*. It is also active against many food borne pathogenic and food spoiling bacteria such as *Listeria, Micrococcus, Enterococcus, Lactobacillus* and *Pedicoccus* species [5].

Cumin (*Cuminum cyminum*) is a flowering plant in the family Apiaceae. Cumin seed is used as a spice for its distinctive flavour and aroma [6]. Despite of its flavouring property, cumin has been found to possess various pharmacological activities such as antimicrobial, antidaibetic, antiepileptic antifertility, anticancer, antioxidant and Immunomodulatory. The reason behind the cumin's medicinal property is mainly due to the presence of various chemical constituents 2.5 to 4.5% volatile oil, 10% fixed oil and proteins, volatile oil mainly consists of 30 to 50% cuminaldehyde, small quantities of a-pinene, b-pinene, phellandrene, cuminic alcohol,

hydrated cuminaldehyde and hydro cuminine which make it suitable for medicinal purpose [7]. The volatile oil of *Cuminum cyminum* was active against *Staphylococcus epidermidis, S. aureus, Corynebacterium diphtheriae, Escherichia coli, Salmonella typhi, Klebsiella pneumonia* [8].

The EVM formulation (*Raphanus sativus* + *Cuminum cyminum*) developed in the EVM centre of TANUVAS, Thanjavur for managing endometritis was found to be highly effective and acknowledged by the practicing veterinarians from various parts of the country [9]. Hence, the present study was undertaken to assess the clinical efficacy of EVM formulation (*Raphanus sativus* + *Cyminum cyminum*) in treating endometritis of dairy cattle and to explore the active principles in *Raphanus sativus* and *Cuminum cyminum* used in the treatment of endometritis.

2. Materials and Methods

The present study was taken to evaluate the clinical efficacy of *R. sativus* and *C. cyminum* in treating under field conditions at Madras Veterinary College Teaching Hospital during the period from March 2017 to June 2017. The animals aged between two to five calving brought for insemination were randomly subjected for white side test to diagnose the presence of subclinical/clinical endometritis. Cervical mucus was aspirated from the os or mid cervix and then transferred to a sterilized test tube. Vaginal swabs were collected aseptically for microbiological examination. Swabs were inoculated into nutrient broth and incubated for overnight at 37°C. The overnight culture was further streaked on nutrient agar, blood agar and Mac Conkey agar, incubated at 37°C for 48 hours. Growth characteristics of the isolates were characterized and recorded [10]. The present study revealed that *E. Coli, Klebsiella spp., Staphylococcus aureus, Strptococcus spp., Bacillus spp.,* are the predominant causative organisms in causing endometritis.

For conducting White Side Test (WST), 1 mL of estrual cervical mucus was heated with equal volume of 5 to 10% sodium hydroxide up to boiling point and after cooling the intensity of colour changes was studied [11]. Based on the results, 10 animals which were positive for WST were selected further investigation.

The selected experimented animals were firstly treated with 10 gm of wet grounded *Cuminum cyminum* and smeared on the tongue of the animal followed by one piece of *Raphanus sativus* (150-200 g/day) given orally daily once for 5 days. The recipe was prepared afresh for every day administration.

The owners were advised to report for subsequent oestrus to study the effect of treatment. Based on the WST, the animals which recovered (as indicated by no colour) were inseminated and the animals which were still positive for WST were subjected for the same treatment in the same cycle. The animals were followed for next two oestrus cycles. Based on recovery the observations were recorded.

The components of EVM formulation for endometritis (*R. Sativus* and *C. Cyminum*) was subjected to phytochemical screening [12].

2.1 Statistical Analysis

The data obtained in the study were subjected to statistical analysis as described by Snedecor and Cochran [13] for calculating percentage of recovery.

3. Results

3.1 Incidence of subclinical / clinical endometritis

A total of 40 animals were screened, of which 10 animals (25%) were found to be positive for endometritis.

3.2 Bacteriological isolation and identification

The bacteriological isolates were revealed that the presence of *E. Coli, Klebsiella spp., Staphylococcus aureus, Strptococcus spp., Bacillus spp.,*

3.3 Response for EVM formulation

The details of the response to the EVM formulation was presented in Table: 1. Out of 10 numbers of animals, two were exhibited apparently clear discharge, five were exhibited slightly cloudy discharge one was exhibited cloudy discharge and the remaining two were exhibited white purulent discharge during gynaecological examination.

After the EVM therapy, Six numbers of Subclinical endometritis animals were negative for white side test and one number of subclinical endometritis affected animal was positive for the white side test. In the animals with clinical endometritis, all the three were not showed the response to EVM therapy and the cervical discharge from the following heat was still positive; hence these animals had again undergone to the EVM therapy for another five days from the day of discharge. Out of these four two were negative for white side test and it showed the persistence of endometritis after the therapy and these animals repeated another five days for the treatment. Even after the second treatment, two were showed negative for the white side test and two were showed positive for the white side test.

In total, the recovery percentage against subclinical endometritis with first time EVM therapy *(R. sativus + C. cyminum)* is 85.71%. And with second time EVM therapy *(R. sativus + C. cyminum)*, the recovery percentage is 100%. Meanwhile, there is no response in clinical endometritis condition with first time EVM therapy *(R. sativus + C. cyminum)* and after the second time EVM therapy *(R. sativus + C. cyminum)* the recovery percentage is 33.33% (Table: 2).

The EVM formulation for endometritis was analysed for phytochemical molecules. The result showed that *Raphanus sativus* and *Cuminum cyminum* both had alkaloids, saponins, tannins, terpenoids, flavonoids, carbohydrates, aminoacid & protein, volatile oil, ascorbic acid and diterpines. Raphanus sativus alone had steroids, anthocyanins and glycosides; Likewise, *Cuminum cyminum* alone had sterols.

4. Discussion

Endometritis continues to be a major problem in dairy industry causing severe economic loss to the farmers, despite various efforts taken to contain its occurrence. The currently available treatment involves intra uterine or systemic administration of antimicrobials which often lead to emergence of antimicrobial resistance [14].The present study applied the EVM formulation *(R. sativus + C. cyminum)* recommended by EVM centre, Thanjavur in the treatment of bovine endometritis

The animals aged between two to five calving brought for insemination at MVC teaching hospital were randomly subjected to white side test to diagnose the presence of subclinical/clinical endometritis of which 25% of animals tested were found to be positive for uterine infections. The bacteriological studies of vaginal swabs from the affected animals revealed that the presence *E. Coli, Klebsiella spp.,*

Staphylococcus aureus, Strptococcus spp., Bacillus spp., as the predominant organisms which were earlier reported to be the main risk factors associated with the development of endometritis [3, 10].

One time EVM treatment for five days was initiated in the subclinical and clinical endometritis affected animals. Out of the seven animals with Subclinical endometritis, six animals were negative for white side test and one was positive for the white side test after the onetime EVM therapy. While the three animals with clinical endometritis does not respond to the onetime EVM therapy.

In the present study, the recovery from subclinical endometritis with one time EVM therapy was 85.71% and with second time EVM therapy was 100%. Meanwhile, there was no response in one time EVM therapy in clinical endometritis where as during second time EVM therapy the recovery percentage was 33.33%.

In India, major endometritis causing organisms are *S. Aureus, Streptococci, E. coli, Klebsiella spp.,* Hence, treatment is primarily aimed at reducing the microbial load of the endometritis condition. In this study *R. sativus* root and *C. cyminum* seed were used which were reported to possess activity against both gram positive and gram negative organisms. The results were in accordance with the findings of Sharma *et al*. [16].

The different extracts contained alkaloids, carbohydrates, phenolic compounds, flavonoids, amino acids and volatile oil. The aqueous, methanolic and hydrophobic radish extracts or specific phytochemicals that are present in radishes including Glucosinolate and iosthiocyanate. These results were in agreement with the findings of Aruna *et al.* [15]. The antimicrobial activity of the *R. Sativus* is contributed by raphanin. Our bacteriological examination revealed *E.coli, Klebsiella spp., Staph. Aureus, Streptococci spp., and Bacillus spp.,* Raphanin is strongly active on inhibit the growth of *Staphylococcus aureus, Bacillus spp., streptococci, Pneumococci.* gainst many food born pathogenic and food spoilage bacteria such as *Listeria, Micrococcus, Enterococcus, Lactobacillus* and *Pedicoccus sp* [16].

Similarly the phytochemical constituents present in the alcoholic extract and volatile oils of cumin inhibited the growth of *Klebsiella pneumonia* and its clinical isolates by improvement of cell morphology, capsule expression and decreasing urease activity [7].

The antimicrobial activities and biofilm-formation preventive properties of *Cuminum cyminum* essential oils and chlorhexidine were assessed against *Streptococcus mutans* and *Streptococcus pyogenes* is mainly because of terpenoids in the cumin and thus their synergistic effect would be the reason for success rate in endometritis treatment under field conditions [17]. Above reports supports the study, the components of the EVM formulation having antimicrobial activity which is responsible for the clearance of subclinical endometritis.

Table 1: Results of the animals treated for endometritis by using EVM formulation *(R. Sativus* and *C. cyminum*)

Animal No.	Discharge colour	Response to white side test	Treatment (EVM therapy for 5 days)	Discharge colour in the following heat	Response to white side test	Artificial Inseminatin	Discharge in the following heat	Discharge colour	Response to white side test	Artificial Insemination	Discharge in the following heat
1	Slightly cloudy	Positive	Yes	Clear	Negative	Done	No	-	-	-	-
2	Cloudy	Positive	Yes	Clear	Positive	No *	No	Apparently clear	Positive	No*	Yes
3	Apparently Clear	Positive	Yes	Clear	Negative	Done	No	-	-	-	-
4	White purulent discharge	Positive	Yes	Slightly Cloudy	Positive	No *	Yes	Clear	Negative	Yes	No
5	Slightly cloudy	Positive	Yes	Apparently clear	Positive	No *	Yes	Clear	Negative	Yes	No
6	Apparently clear	Positive	Yes	Clear	Negative	Done	Yes	Apparently clear	Negative	Yes	No
7	Slightly cloudy	Positive		Clear	Negative	Done	No	-	-	-	-
8	Slightly cloudy	Positive	Yes	Clear	Negative	Done	Yes	Apparently clear	Negative	Yes	No
9	Slightly cloudy	Positive	Yes	Clear	Negative	Done	No	-	-	-	-
10	White purulent discharge	Positive	Yes	Cloudy	Positive	No*	Yes	Clear	Positive	No*	Yes

*: EVM therapy repeated for another 5 days

Table 2: The recovery percentage after EVM therapy against bovine endometritis

S. No.	Disease condition	Recovery after the first duration of treatment			Recovery after the Second duration of treatment		
		No. of animals treated	No. of animals recovered	Recovery (%)	No. of animals treated	No. of animals recovered	Recovery (%)
1.	Subclinical endometritis	7	6	85.71	1	1	100
2.	Clinical endometritis	3	-	-	3	1	33.33

5. Conclusion

The findings of the present study placed on record the evidence based evaluation of a successful EVM formulation (*Raphanus sativus* and *Cuminum cyminum*) used against endometritis for its rational pharmacological basis. The information collected from the available literature on the antimicrobial, anti-inflammatory and properties substantiate the rationality of the combination. The phytochemical analysis of the formulation, revealed the presence of compounds possessing these activities which justifies its use in endometritis therapy under field conditions.

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