



## ORIGINAL ARTICLE

# Evaluation of Histopathologic and Clinical improvement effects of *Zataria multiflora* Extract on Necrotic Vaginitis in postpartum Dairy cows

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### ABSTRACT

*Zataria multiflora* Boiss. (ZM) is a thyme-like plant belonging to the Lamiaceae family that grows wild only in Iran, Pakistan and Afghanistan. This plant with the vernacular name of Avishan-e-Shirazi (Shirazi thyme) in Iran is a valuable medicinal and condimental plant. It has several traditional uses as an antiseptic, carminative, stimulant, diaphoretic, diuretic, anesthetic, anti-spasmodic and analgesic. The aim of present study was to evaluate of histopathologic and clinical improvement effects of *Zataria Multiflora* extract on necrotic vaginitis in postpartum dairy cows. 50 Animals were divided into the 2 control and treatment groups. Control group received common method to wit betadine 10% three times on days 1,3 and 5 as well as penicillin for 3 consequence days. In treatment group, 40-50CC thyme extract 0.5% was administrated vaginally. Histopathologic findings showed healing process in both groups. Our data showed that both treatment methods are effective in treatment of vaginal necrosis (Diagram 1). In comparison, data showed that thyme extract is more effective than penicillin + betadine. Our study in accompany with above mentioned documents prove the antimicrobial activity of thyme extract. At the end, we hope to expand this protocol on human society with different concentrations and carriers in treatment of necrotic vaginitis.

**Keywords:** *ZatariaMultiflora*, vaginitis, necrosis, dairy cow.

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### INTRODUCTION

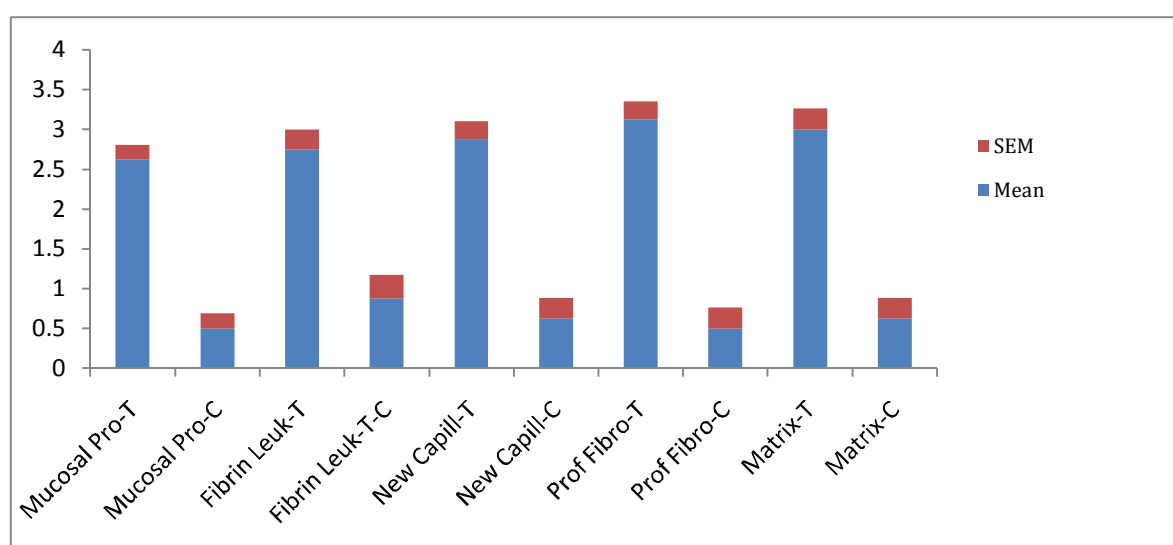
Nowadays most industrial dairy herds' economy is considered by reducing the cost of animal husbandry. The most common factor that had a negative impact on herd reproductive indicators and subsequently significantly reduces herds' economic benefit is genital infections in the postpartum period. Necrotic vaginitis is more common after dystocia due to fetomaternal disproportion in dairy cows in which the vaginal wall gets torn and crush due to being too heavy-handed manipulation. The condition is one of the most common causes of infertility in dairy cows and in addition to threaten animal health may make uterus to infection and yields to decrease in milk production and weakness probably death. There was a different ways of treatment including general antibiotics and local administration of antiseptics but they had low success however impair uterus mechanisms of defense. On the other hand, due to the increasing resistance of pathogenic bacteria to antibiotics, researchers seeking new antimicrobial substances of plant origin as an alternative to ineffective antibiotics. *ZatariaMultiflora* is from family of Lamiaceae which is native of Shiraz [10]. Since 16th century, thyme has been introduced officially as a medicinal plant pharmacopoeia and all valid registered [3,4]. Thyme is of the mint family and has anti-inflammatory, antioxidant and antiseptic activities. The most important compound of thyme isthymol and carvacrol which have phenolic structures. Based on *ZatariaMultiflora* anti-inflammatory and antiseptic activities, the aim of present study was to evaluate of histopathologic and clinical improvement effects of *ZatariaMultiflora* extract on necrotic vaginitis in postpartum dairy cows.

## MATERIALS AND METHODS

This study was carried out in 3rd and 5th stations of Moghan livestock and husbandry complex on 50 cases with necrotic vaginitis in which they had lacerations, bruises and crush the vagina and vestibule. Animals were divided into the 2 control and treatment groups. Control group received common method to wit betadine 10% three times on days 1,3 and 5 as well as penicillin for 3 consequence days. In treatment group, 40-50CC thyme extract 0.5% was administrated vaginally. Treatment group did not receive any other treatment. Sampling from vaginal tissue was executed before (Figures 1 and 2) and after study on day 10. Tissue specimens collected from vagina, were fixed in 10% buffered formalin, embedded in paraffin and 5  $\mu$ m thick microscopic sections were prepared through hematoxylin-eosin staining method. Statistical analysis on the incidence of lesions was performed using Fisher's exact probability test or Chi-square test and the data including body weight were compared by ANOVA test and Tukey post-hoc test. The results were considered statistically significant if the P value was 0.05 or less.

## RESULTS

Histopathologic findings showed healing process in both groups. Our data showed that both treatment methods are effective in treatment of vaginal necrosis (Diagram 1).

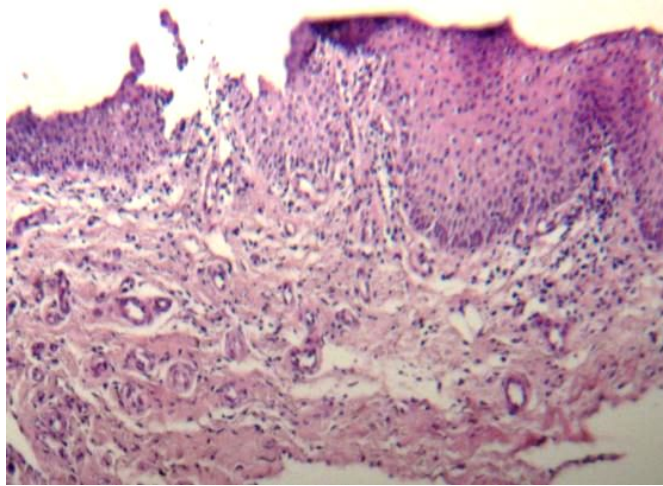


**Diagram 1:** mean of changes in vaginal mucosa in both groups. Data presented as mean $\pm$ SD.

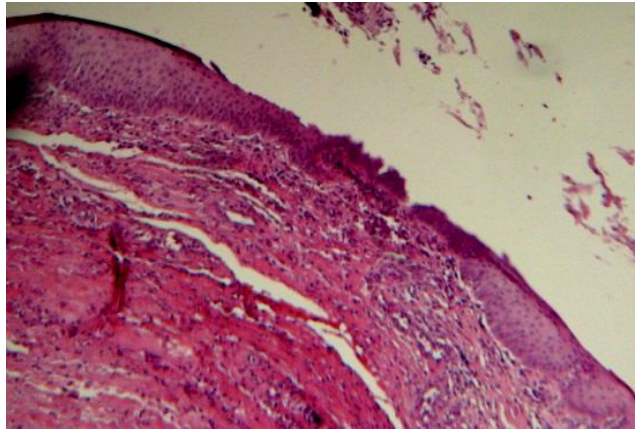
In comparison, data showed that thyme extract is more effective than penicillin + betadine. In term of histopathology, in treatment group, proliferation of vaginal mucosa was more significant than control group. These changes were  $2.625\pm 0.18$  and  $0.5\pm 0.18$  in treatment and control groups, respectively ( $P<0.001$ ; Figures 3 and 4). Also in treatment group, aggregation of leucocytes plus fibrin in submucosa area was more significant than control group. These changes were  $2.750\pm 0.25$  and  $0.8750\pm 0.29$  in treatment and control groups, respectively ( $P<0.001$ ; Figures 3 and 4). Proliferation of new vessels in submucosa in treatment group was more significant than control group indicates rapid healing process in this group. These changes were  $2.875\pm 0.22$  and  $0.625\pm 0.26$  in treatment and control groups, respectively ( $P<0.001$ ; Figures 3, 4).

Proliferation of fibroblasts in submucosa in treatment group was more significant than control group indicates rapid healing process in this group. These changes were  $3.125\pm 0.22$  and  $0.500\pm 0.26$  in treatment and control groups, respectively ( $P<0.001$ ; Figures 3, 4).

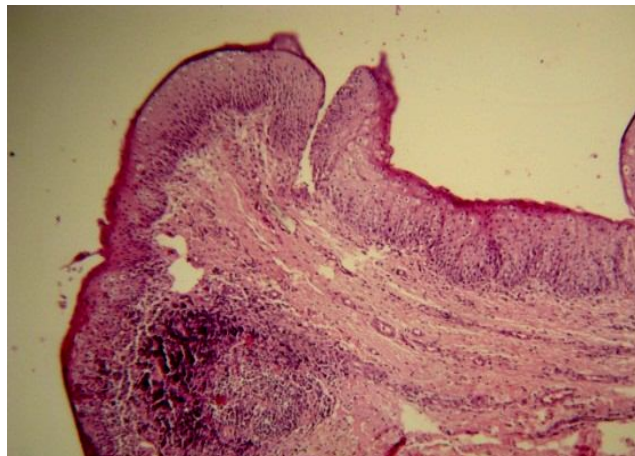
Expanded substitution of connective tissue in submucosa in treatment group was more significant than control group indicates rapid healing process in this group. These changes were  $3.00\pm 0.26$  and  $0.625\pm 0.26$  in treatment and control groups, respectively ( $P<0.001$ ; Figures 3, 4).



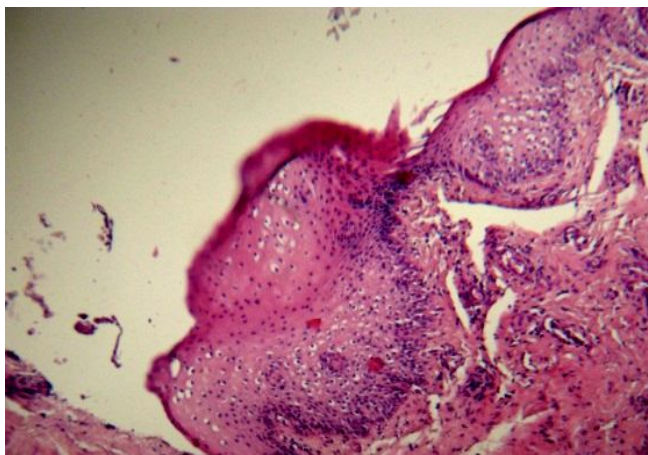
**Figure 1:** microscopic view of necrotic vaginitis from pretreatment cows (treatment group) in which epithelial necrosis with infiltration of inflammatory cells and onset of healing process with angiogenesis are obvious (H&E, 40x).



**Figure 2:** microscopic view of necrotic vaginitis from pretreatment cows (control group) in which epithelial necrosis with infiltration of inflammatory cells and onset of healing process with angiogenesis are obvious (H&E, 40x).



**Figure 3:** microscopic view of necrotic vaginitis from posttreatment cows (treatment group) in which healing of epithelial necrosis with mild degenerative changes with infiltration of inflammatory cells with angiogenesis are obvious (H&E, 40x).



**Figure 4:** microscopic view of necrotic vaginitis from pretreatment cows (control group) in which epithelial necrosis with infiltration of inflammatory cells and onset of healing process with angiogenesis are obvious (H&E, 40x).

#### DISCUSSION AND CONCLUSION

Causes of Inefficiency are different in reproduction cows and differentiate between these causes is much harder. Postpartum infections are one of the most common diseases in the postpartum period may be involved in dairy cattle and reduce reproductive performance. Plants are always either traditional medicines from public sources or pure products so can be replaced with some drugs. In recent decades, numerous studies have been taken to choose, extract and different mechanism of action of plant compounds against pathogens. In present study the healing action of thyme extract was compared with penicillin + betadine and our data showed that thyme extract is more effective than penicillin + betadine. Although there was no document in veterinary medicine to determine its effects, but in medical science, there are many cases of vaginal necrosis treated with thyme extract. In a study by Barati et al., (2003) reported that administration of thyme extract cause make changes in uterine surface like antiseptics including betadine, oxytetracycline and etc. [2]. The results of this study are also in line with this research, because most of the infections agent in vaginitis is also *Arcanobacterium pyogenes*. Thyme is also used in extract-treated group is accelerated significantly in the development of vaginal mucosal tissue compared with controls. Antibacterial activity of thyme extract is because of thymol and carvacrol which make changes in the cell membrane permeability to hydrogen and potassium ions, thus, yields to increase in intracellular PH and accelerate production of ATP and bacterial death. Considering that, thymol and carvacrol are phenolic compounds in the thyme which have antiseptic activity. These two compounds are the same with exception of the hydroxyl position. thymol and carvacrol are most common in human [6]. Antimicrobial activity of these are due to increase the permeability of the cell membrane in which they can cathodize with cations and impair vital activities [7,8,9]. In a study by Zahraei et al., (2005) on contiguous bacteria inducing mastitis, treatment of mastitis due to *Staphylococcus aureus* and *Streptococcus agalactiae* reported significant [5]. It seems that in compare with common methods like betadine, thyme extract is more effective and nature origin with no effects on vaginal surface and drug residue. Also, bacteriological studies have showed that MIC for this extract on *Arcanobacterium pyogenes* and *Fusobacterium necrophorum* in treatment of vaginitis is effective. Singh and Singh, [11] showed that ethanolic extract of thyme has significant activity against *E. coli* enterohemorrhagic. Data obtained from present study is compatible with that study. In a study by Gholamhosseinian et al., [8] it has been shown that thyme extract inhibits the growth of *E. coli*. Also, Sharififar et al., [10] showed the efficacy of thyme extract against *S. aureus* and *E. coli*. In another study the activity of thyme extract was compared with antibiotics and has shown that effect of thyme extract on *Salmonella typhimurium* in more than tetracycline but is less than chloramphenicol and trimethoprim + sulfamethoxazole. Based on data obtained from their study and restriction use of antimicrobial agents they suggest to compare other medicinal plants to be replace [1]. Our study in accompany with above mentioned documents prove the antimicrobial activity of thyme extract. At the end, we hope to expand this protocol on human society with different concentrations and carriers in treatment of necrotic vaginitis.

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