Vol. 18, No. 1, Spring, 2022, 34-45 *Original Article*
DOI: 10.22055/IVJ.2022.342990.2468
DOR: 20.1001.1.17356873.1401.18.1.4.2
Journal Homepage: http://www.ivj.ir/

Efficacy of Zataria multiflora essential oil for treatment of Staphylococcus aureus detected by polymerase chain reaction in lactating dairy cows with subclinical mastitis

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Received: 18.03.2022 Accepted: 21.06.2022

Abstract

A treatment containing *Zataria multiflora* essential oil was compared with antibiotic therapy for *Staphylococcus aureus* subclinical mastitis in dairy cows in a field trial. Treatment outcomes in response to ointment were monitored using microbiological culture. Intramammary ointment of a *Z. multiflora* essential oil (Group 1; IOZM) was compared with placebo ointment (Group 2; PO), cefquinome intramammary lactating cow ointment (Group 3; CFQ) and a negative control group (Group 4) in 18 dairy cows with *Staphylococcus aureus* subclinical mastitis each group. Effects on bacteriological cure rate in response to ointment treatment were monitored by culture. Results from this field trials demonstrated that IOZM treatment had the potential to be as effective at eliminating *Staphylococcus aureus* subclinical mastitis as treatment. Following a 14-d and 28-d experimental period, bacteriological responses were observed in 9 out of 18 IOZM treated cows compared with 10 out of 18 CFQ treated cows. Also, the California Mastitis Test grade had no significant effect on treatment. The results of this trial suggest that *S. aureus* subclinical mastitis treatment with *Z. multiflora essential oil,* which indicates that this treatment could be used as an alternative treatment.We successfully treated the *S. aureus* subclinical mastitis in bovine with *Z. multiflora* essential oil and can suggest it's used instead of antibiotic use.

Keywords*:* Cefquinome, Intramammary Ointment, *Staphylococcus aureus,* Subclinical mastitis, *Zataria multiflora*

Introduction

Mastitis is the costliest costly disease in dairy cattle as it is associated with decreased milk production, expensive treatment costs, extra labor and an increased rate of culling (Klaas et al., 2004). Antibiotics are the main proven method for treatment of mastitis; however antibiotic treatment of established mammary infection is only moderately efficacious and requires prolonged milk withdrawal due to residues in milk (Daley and Hayes, 1992). Moreover, the biggest challenge facing the modern dairy industry is the pressure to reduce the use of antibiotics in food-producing animals

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(Mukherjee et al., 2010). Besides, some studies suggest that antibiotics may be relatively ineffective for the treatment of pathogens like S. aureus, which can internalize in eukaryotic host cells, evading the immune system (Barkema et al., 2006). An effective, non-antibiotic mastitis treatment could reduce costs associated with antibiotic therapy and would also relieve some of the pressures facing the agricultural and veterinary sectors to limit the use of antibiotics. Several alternative approaches have therefore been used for the treatment of mastitis, including milking the infected quarter several times a day (Roberson et al., 2004), hydrotherapy, intramammary infusions of glucose solutions (Reinhold et al., 1986) and the use of lysostaphin (Oldham and Daley, 1991) and nisin (Cao et al., 2007; Wu et al., 2007). On the other hand, due to the potential increase of resistance of pathogenic bacteria to antibiotics, researchers are seeking new antimicrobial substances of plant origin as an alternative to ineffective antibiotics. The use of herbal therapies is on the rise (Klepser and Klepser, 1999) and more than 80% of people in developing countries are using traditional medicines including herbal medicine (WHO 2014). These medicines can be used safely if they are prescribed by educated health personnel with the appropriate dose and special caution in pregnancy (Simbar et al., 2008). World Health Organization has recommended all member countries to actively promote native medicines of their respective country (Kamboj, 2000). Avishane Shirazi is the Persian name for Zataria multiflora Boiss (Z. multiflora), belonging to the family Labiatae, and it is native to Iran. This plant is used traditionally in food, especially in yogurt flavoring, as a stimulant, condiment, and carminative and for treatment of pre-mature labor pains and rupture (Sharififar et al., 2007). There are also commercial pharmaceuticals with formulae based on Z. multiflora essential oil. This oil has been used commonly in medicine for the treatment of respiratory tract infections as an antiseptic, antitussive and irritable bowel syndrome treatment. Also, the extracts of aerial parts of Z. multiflora showed anti-inflammatory effects against acute and chronic inflammations in mice and rats (Hosseinzadeh et al., 2000). Several studies reported the positive effect of Z. multiflora for treatment of reproductive disorders such as mycotic vaginitis and bacterial vaginosis in human (Bahadoran et al., 2010; Simbar et al. 2008), and endometritis in cows (Hajibemani et al., 2016). Also, we had previously demonstrated that the Z. multiflora essential oil has in vitro antibacterial activity on Staphylococcus aureus isolated from SCM in dairy cows (Sani et al., 2018). We decided to test the efficacy of that in a field trial.

In the present study, Z. multiflora Boiss intramammary ointment was assessed for efficacy in the treatment of bovine SCM due to S. aureus and compares it with antibiotic treatment and PO in naturally infected animals.

Material and Methods

Plant material

Z. multiflora Boiss. (Lamiaceae) tops at the full flowering stage (June and July) were collected from plants growing wild in the Yazd province. The taxonomic identification of plant materials was confirmed by a senior plant taxonomist. Voucher specimens were deposited in the Herbarium of Tehran Faculty of Pharmacy, Tehran, Iran.

Preparation of the methanol extracts

Tops of the plant were dried in the shade, ground in a grinder with a 2 mm in diameter mesh, and about 200 g of dry powdered extracted with 85% methanol using percolation method for 48 h. Solvent removal carried out under vacuum afforded a semisolid mass with a yield of 13%. The resulting extract was fractionated with water and chloroform to give polar and non-polar subfractions.

Isolation of the essential oil

The air-dried and ground herbal parts of the plant collected were submitted for 4 h to water-distillation using a British-type Clevenger apparatus (yield 2.8% v/w). The obtained essential oil was dried over anhydrous sodium sulfate, then stored at 4°C, until tested and analyzed.

Gas chromatography/mass spectrometry analysis

The essential oil was analyzed using a Shimadzu QP 5000 gas chromatograph equipped with an FID detector and HP-5 MS capillary column (30 m · 0.25 mm, film thickness 0.25 lm). Injector and detector temperatures were set at 220 and 290°C, respectively. The Oven temperature was kept at 50°C for 3 min, then gradually raised to 160℃ at 3℃ /min, held for 10 min and finally raised to 240°C at 3°C /min. Helium was the carrier gas, at a flow rate of 1 ml/min. Diluted sample (1/100 in acetone, v/v) of 1.0 ll was injected manually and in the split less mode. Quantitative data were obtained electronically from FID area percent data without the use of correction factors.

Gas chromatography analysis

GC–MS analysis of the essential oil was performed under the same conditions with GC (column, oven temperature, the flow rate of the carrier gas) using a Shimadzu QP 5000 gas chromatograph equipped with a Shimadzu QP 5050 mass selective detector in the electron impact mode (70 eV). Injector and MS transfer line temperatures were set at 220 and 290℃, respectively. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, Wiley 2001 library data of the GC–MS system and literature data (Adams 2001). Alkanes were used as reference points in the calculation of relative retention indices (RRI). GC and GC/MS analysis results are given in Table 1.

Table 1. Chemical composition of Zataria multiflora essential oil (diluted 1/100 in acetone v/v)[a]

Components	% composition	KI[b]
α-thujene	0.08	927
α-pinene	2.2	937
Camphene	0.1	950
3-octanone	0.18	968
β-pinene	0.18	978
Mycerene	0.8	984
ρ-cymene	7.9	1017
β-terpineol	0.9	1026
Y-terpinen	2.5	1055
Linalool	1.2	1090
ρ-menth-1-en-4-ol	1.05	1168
ρ-menth-1-en-8-ol	1.05	1181
Carvacrol methyl ether	1.6	1227
Thymol	14.7	1268
Carvacrol	50.53	1288
Thymyl acetate	0.69	1329
Carvacryl acetate	3.85	1350
Trans-caryophyllene	3.4	1431
Eudema-3,7-dien	0.1	1448
Aromadendrene	2.07	1452
α-humulene	0.2	1467
Cyclosativene	0.1	1472
Ledene	1.06	15.04
Spathulenol	1.02	1578
Caryophylene oxide	1.1	1586
Total	98.59	

[a] Relative percentages of the compounds were obtained electronically from FID area percent data.
[b] Kovats index on non-polar DB-5 ms column in reference to n-alkanes

Intramammary ointment preparation

White Beeswax (2 mg), Hard Paraffin (3mg), Cetostearyl Alcohol (5mg), White Soft Paraffin or Yellow Soft Paraffin (90 mg) were melted together and stirred. Then the source of heat was removed and the stirring was continued until the mass reached room temperature (placebo ointment). After that, the Simple Ointment

was melted and incorporated gradually into the extract of *Zataria Multiflora* (0.5%, 50 mg/ointment) according to the previous *in vitro* study (Sani et al., 2018). The Whole content was finally stirred until congeals (Sawant and Tajane, 2016).

Selection of animals and experimental protocol

Cows were selected from one herd consisting of dairy Holstein cows. Following the selection of suitable quarters of each cow as an *S. aureus* subclinical mastitis case, a trial was undertaken in June 2017. All cows enrolled in the study were routinely milked three times a day where pre-milking udder preparation consisted of washing with water, forest ripping, post-milking teat dipping and drying teats with service with paper towels. Seventy-two *S. aureus* subclinical mastitis lactating cows (15–50 days postpartum) were used to evaluate the treatment. These cows were maintained in the animal shed of the institute under identical environmental conditions and were divided into four equal groups. Selection of quarters was made using a combination of CMT and bacteriological culture from quarter milk samples for *S. aureus* SCM detection. And finally, *S. aureus* infection was confirmed using PCR method (Trung et al., 2015). Quarters were blocked according to parity (1, 2, or ≥ 3) and milk production and assigned to 4 groups. Group I consisted of 18 cows with *S. aureus* SCM that were treated with an intramammary ointment of a *Zataria multiflora essential oil* (IOZM) for three days with 12h interval. Group II consisted of 18 cows with *S. aureus* SCM that were treated with placebo ointment (PO). Group III consisted of 18 cows with *S. aureus* SCM that were treated with cefquinome (Cefquinome sulfate, Cobactan LC; Intervet) according to manufacture recommendation (CFQ). Group IV consisted of 18 cows with *S. aureus* SCM that were treated as a control group.

Milk sampling protocol

Milk from each cow was collected in sterile vials after cleaning the teat orifice with 70% ethyl alcohol and after discarding a few streams of milk. The milk was obtained for bacteriological and CMT analyses on two occasions, 7 days apart before treatment for detection of *S. aureus* SCM and on day 14 and 28 post-treatment for evaluation of *S. aureus* SCM treatment according to CMT and bacteriological analyses, again.

S. aureus SCM quarters were enrolled in the study based on positive CMT results (scores T, 1, 2 or 3) at the time of first pre-treatment sampling and isolation of *S. aureus* in the two samples obtained 7 days apart. *S. aureus* SCM cure was defined as a treated infected quarter that was bacteriologically negative for the *S. aureus* isolated at 14 and 28 days after the last treatments, and a negative CMT.

California Mastitis Test

The CMT was performed on-site by one expert person using the method described by Schalm *et al.* In brief, after discarding first three streams, milk was milked from aseptic quarters to CMT plate and mixed with equal reagent and agitated for 15 seconds. According to the reactions obtained, the results were classified as follows: negative, traces, 1, 2, and 3, recorded as −, ±, +, ++, and +++, respectively. Samples with CMT score trace, 1, 2, or 3 were considered positive.

Bacteriological analyses

CMT-positive samples were transported to the laboratory on ice. All samples were incubated at 37ºC for 18 hours and then inoculated on selective Chapman (Merck, Germany) agar and 5% sheep blood agar plates. Hemolysis, morphology, and pigmentation were scored after 24 hours incubation at 37ºC. Growth on Chapman agar was scored after a 48 hours incubation at 37ºC. Colonies yielding Gram-positive cocci were subjected to biochemical tests

for catalase, oxidase (DrySlide Oxidase Difco, USA), acetoin production (VP), and anaerobic mannitol fermentation (Difco, USA). Coagulase test was performed as follows. Two drops of cultures in TSB (Trypticase Soy Broth, Merck, Germany) were added to tubes containing 0.5 ml of citrate rabbit plasma (Bacto Coagulase Plasma, Difco, USA) and clot formation was observed every two hours for a 24 hour period. *S. aureus* strain ATCC 25923 was used as a positive control. Production of DNAse has tested in agar DNAse, using HCl 1.5 M to visualize halo formation around the colonies. (Vieira-da-Motta et al., 2001).

Polymerase chain reaction for *S. aureus* confirmation

Molecular typing of the target gene from 16S rRNA was performed by the method of Trung et al. (2015), with some modification. Cells from an overnight culture in TSB were immersed into 300 µl universal lysis solution (200 mM NaOH, 1% SDS) and heated for 5 minutes at 95°C. An equal volume of 1 M Tris-HCl was added to neutralize the pH to 7.5. This aqueous solution was subjected to standard phenol/ chloroform/isoamyl alcohol extraction. Briefly, 600 µl of the aqueous solution was transferred into a 1.5 ml Eppendorf tube, an equal volume of phenol/chloroform/isoamyl (24/25/1) was added and vortexed for 5 minutes and centrifuged at 13,000 g. The upper aqueous phase was then transferred to an Eppendorf tube and an equal volume of isopropanol was added. After thorough mixing, the solution was centrifuged at 16,000 g for 30 minutes to pellet the DNA. Precipitated DNA was washed twice with 70% ethanol and reconstituted in 150 µl TE (25 mM Tris-base pH8.0, 1 mM EDTA) and used as a template for PCR in 25 µl reactions containing 10 mM Tris-HCl pH 8.3/50 mM KCl/1.5 mM Mg^{2+}, 250 µM of each deoxynucleotide triphosphate and1mM of specific primers. For amplification the following specific primers were used: 5'-AAGGGCGAAATAGAAGTGCCGG-3' (forward) and 5´-ATGGTCGGTTCCTTAGAAAACAAAC TTG-3' (Reverse) were used. Thermal cycling comprised initial denaturation at 95°C for 4 minutes, 35 cycles of 94°C for 25 seconds, 58°C for 45 seconds and 72°C for one minute. PCR products were separated by horizontal electrophoresis on 1.4% agarose gels (Sigma, USA) in TAE running buffer (1 M Trizma base, 3 M Sodium Acetate, 0.5 M EDTA). DNA fragments were stained with Ethidium Bromide (25 µg ml-1 Sigma, USA) for 15 minutes and visualized in a UV transilluminator (Sigma, USA). The sizes of the amplification products were estimated by comparison with a 100bp DNA step ladder.

Statistical Analyses

Data were organized in excel worksheets and then the data statistically were analyzed by SAS package software (SAS Inst. Inc., Cary NC ver. 9.1-2005). Analysis was performed using Non-parametric Chi square test in NPAR1WAY procedure where significant level was 0.05.

Results

PCR evaluation

The PCR product, a single DNA band of approximately 515 bp, was detected and confirmed in all *S. aureus* strains isolated from milk samples and *S. aureus* reference strain (Figure 1).

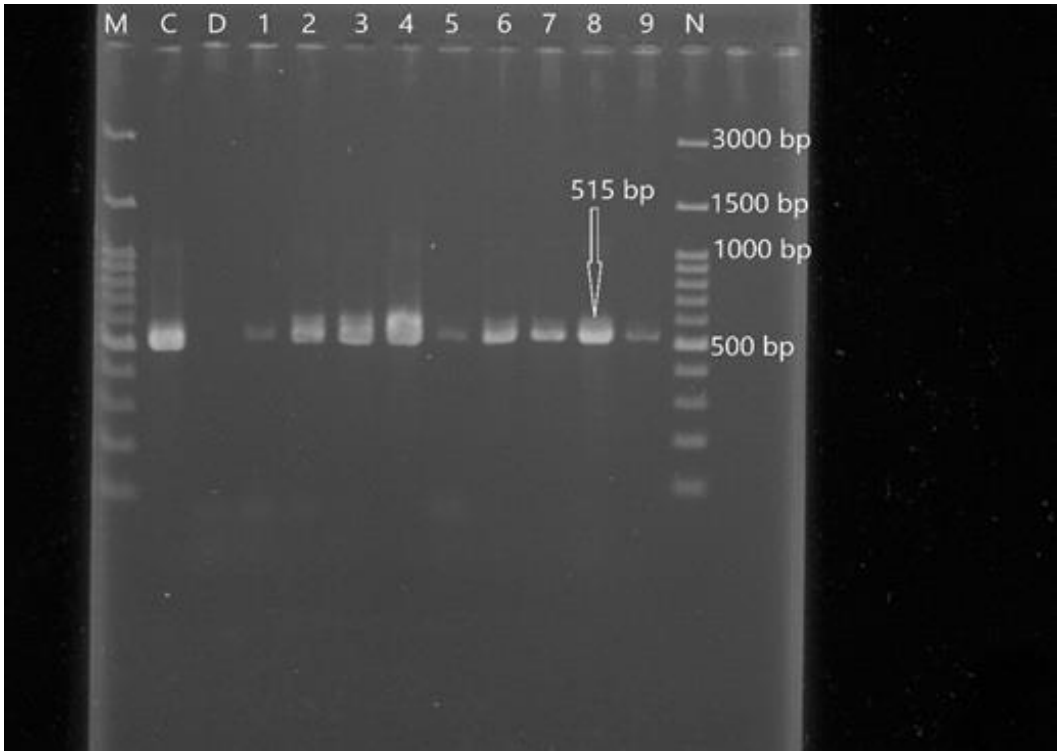

Figure 1. Agarose gel electrophoresis of PCR amplification products from *S. aureus* 16S rRNAs recovered from subclinical mastitic milks– lanes 1-9: mastitic milk samples –lane C: reference strain *S. aureus* ATCC 25923 as a positive control- lane D: negative control (Distilled water)- lanes M and N: DNA molecular weight markers.

Chemical composition of the essential oil

Air-dried herbal parts of *Z. multiflora* were subjected to hydrodistillation using a Clevenger apparatus and the pale yellow-colored essential oil was obtained (yield 2.8% v/w). The results obtained by GC–MS analysis of the essential oil of the plant are presented in Table 1.

Cure rate

Fifty-four *S. aureus* SCM quarters were treated with IOZM, PO, and cefquinome intramammary antibiotic and 18 quarters were used as a control group (non-treated). The parity distribution of the cows is shown in Table 2. Percentage of cure in IOZM and CEQ groups were also significantly lower in ≥ 3 parity compared with other parity groups (p=0.04 and P=0.02, respectively).

Table 2. parity distrobution in control and three treatment groups

Group	Parity	No	Cure rate (%)
IOZM	1	6	83.33[a]
	2	6	50[a]
	≥ 3	6	16.66[b]
P value			0.04
PO	1	6	16.66[a]
	2	6	0[a]
	≥ 3	6	0[a]
P value			0.31
CFQ	1	6	83.33[a]
	2	6	66.66[a]
	≥ 3	6	16.66[b]
P value			0.02
Control	1	6	16.66[a]
	2	6	0[a]
	≥ 3	6	0[a]
P value			0.31

Different lowercase letters at each column are significantly different.
SCM: subclinical mastitis, IOZM: intramammary ointment of a *Zataria multiflora essential oil*, PO: placebo ointment, CFQ: cefquinome intramammry lactating cow ointment.

S. aureus isolation frequency in post-treatment samples was significantly lower than in pretreatment samples, in both IOZM and CEQ groups isolated (P=0.004, P=0.002, respectively, Table 3). However, no difference was observed in S. aureus isolation frequency between pre and posttreatment samples in PO and control groups (P>0.05, Table 3). CMT positive regardless of CMT score in post treatment samples was significantly lower than in pretreatment samples, in both IOZM and CEQ groups (p=0.02, Table 4). However, no difference was observed in CMT positive samples in PO and control groups regardless of CMT score (p=0.46, p>0.05, respectively, Table 4). The Cure rate was significantly similar in day 14 and 28 post-treatment samples in all groups (P>0.05, Table 5).

Cure rate was significantly higher in IOZM and CEQ groups than PO and control groups in day 14 and 28 post-treatment (P<0.001, P=0.003, respectively, Table 5), but there were not any significant differences between IOZM and CEQ groups and between PO and control in day 14 and 28 post-treatment (P>0.05, Table 5).

Table 3. *Staphylococcus aureus* isolation in SCM samples

	Staphylococcus aureus isolation in SCM samples			P value
	Pretreatment	Post treatment		
	Day 0 and 7	Day 14	Day 28	
Quarters	n/n (%)	n/n (%)	n/n (%)	
IOZM group	18/18 (100)[a]	9/18 (50)[b]	9/18 (50)[b]	0.004
PO group	18/18 (100) [a]	18/18 (100) [a]	17/18 (94.44) [a]	P>0.05
CFQ group	18/18 (100) [a]	8/18 (44.44) [b]	8/18 (44.44) [b]	0.002
Control group	18/18 (100) [a]	18/18 (100) [a]	17/18 (94.44) [a]	P>0.05

Different lowercase letters at each row are significantly different.
SCM: subclinical mastitis, IOZM: intramammary ointment of a *Zataria multiflora essential oil*, PO: placebo ointment, CFQ: cefquinome intramammry lactating cow ointment.

Table 4. Effect of CMT on SCM treatment in treatment and control groups

SCM samples with CMT T & +				SCM samples with CMT ++ & +++			
	Pretreatment	Post treatment		Pretreatment	Post treatment		P value
	Day 0 and 7	Day 14	Day 28	Day 0 and 7	Day 14	Day 28	
Quarters	n/n (%)	n/n (%)	n/n (%)	n/n (%)	n/n (%)	n/n (%)	
IOZM group	10/10(100) [a]	5/10 (50)[b]	5/10 (50)[b]	8/8 (100)[a]	4/8 (50)[b]	4/8 (50)[b]	0.02
PO group	9/9(100) [a]	9/9 (100) [a]	9/9 (100) [a]	9/9 (100) [a]	9/9 (100) [a]	8/9(88.88) [a]	0.46
CFQ group	9/9(100) [a]	4/9(44.44)[b]	4/9(44.44)[b]	9/9 (100) [a]	4/9(44.44)[b]	4/9 (44.44) [b]	0.02
Control group	10/10(100)[a]	10/10(100)[a]	9/10 (90) [a]	8/8 (100) [a]	8/8 (100) [a]	8/8 (100) [a]	P>0.05

Different lowercase letters at each row are significantly different.
CMT: California Mastitis Test, subclinical mastitis, IOZM: intramammary ointment of a *Zataria multiflora essential oil*, PO: placebo ointment, CFQ: cefquinome intramammry lactating cow ointment.

Table 5. Comparison of cure rate in treatment and control groups between days 14 and 28 post treatment

Cure rate in SCM samples			
	Day 14	Day 28	P value
Quarters	n/n (%)	n/n (%)	
IOZM group	9/18 (50)^{Aa}	9/18 (50)^{Aa}	0.987
PO group	0/18 (0)^{bb}	1/18 (5.55)^{Bb}	0.785
CFQ group	10/18 (55.55)^{Aa}	10/18 (55.55)^{Aa}	0.987
Control group	0/18 (0)^{bB}	1/18 (5.55)^{bB}	0.785
P value	<0.001	0.003	

^{a,b} Different letters at each column and ^{AB} different letters in a row showed significantly differences. SCM: subclinical mastitis, IOZM: intramammary ointment of a *Zataria multiflora essential oil*, PO: placebo ointment, CFQ: cefquinome intramamamry lactating cow ointment.

Discussion

Mastitis is a major production limiting disease of dairy animals all over the world. It not only adversely affects animal health but also deteriorate the quality as well as quantity of milk. Generally, the control and treatment of mastitis are based on antibiotic therapy, vaccination and improving managemental practices. Antimicrobial resistance has become a common problem, especially in the areas of bacterial chemotherapy (Nandivada and Amyes, 1990). The present study aimed to evaluate the effectiveness of Z. multiflora essential oil for the treatment of naturally occurring SCM caused by mastitis with *S. aureus*. Z. multiflora is a thyme-like plant that grows wild in central and southern Iran, Afghanistan, and Pakistan. Thyme is an herbal remedy with antibacterial properties including gram-positive and negative bacteria. Its extract has been used to treat gastrointestinal disturbances, respiratory disorders, and coughs due to colds, bronchitis and pertussis, laryngitis and tonsilitis, minor wounds, the common cold and as an antibacterial agent in oral hygiene.

The main constituents of the EO of *Z. multiflora* (Table 1) are phenolic compounds such as carvacrol and thymol. However, the compositions of the EOs of herbs and spices can vary greatly depending upon geographical region, plant variety, and age, and the method of drying and extraction of the oil (Bagamboula et al., 2004; Valero and Salmeron, 2003). In this regard, whereas Azizkhani et al. (2013) found that the major components of *Z. multiflora* Boiss. obtained from the Fars Province of Iran were the phenolic compounds carvacrol (71.12%) and no thymol in the present study the major phenolic component of *Z. multiflora* Boiss. obtained from the Yazd province of Iran were carvacrol (50.53%) and thymol (14.7%), similar to the findings of Shaffee and Javidnia, (1997).

In the present study, there were no signs of toxicity in either clinical trial. A significant difference in the cure rate occurred between the control (5.55%), PO (5.55%) groups and IOZM (50%), CEQ (55.55%) groups. In the latter study, *Z. multiflora* EO showed antibacterial activity against clinical isolates of methicillin-resistant and methicillin-sensitive strains of *S. aureus (*Mahboubi and Bidgoli 2010*)* and *S. aureus* ATCC 29213 (Azizkhani et al. 2013). Our *in vitro* previous study showed that *Z. multiflora* possesses antibacterial activity on *S. aureus* isolated from subclinical mastitis Sani et al. (2018). The current study showed that *Z. multiflora* has *in vivo* antibacterial effect against *S. aureus* in bovine SCM.

In the past, other alternative treatments were used for SCM and have the potential

for treatment. Intramammary infusion of a live culture of Lactococcus lactis had similar treatment effect with amoxicillin-clavulanic acid in chronic subclinical or clinical S. aureus mastitis (Klostermann et al., 2008). Owens et al. (1997) previously found that using a combination of penicillin and novobiocin, bacteriological cure rates for clinical mastitis caused by *S. aureus* could be as high as 70% if treatment was initiated at the early stage of infection (<28 d) and could be as low as 35% for animals with chronic infections (Klostermann et al., 2008). Also, *Nigella sativa* extract was useful for the treatment of naturally occurring SCM in lactating dairy cows caused by *S. aureus* (60%) *(*Ghodrati Azadi and Farzane, 2010). Another new approach in SCM treatment is the use of antimicrobial peptides, especially the intramammary infusion of nisin (Roy et al., 2016). In addition to herbal therapy for SCM treatment during lactation, dry cow therapy with herbal ointment also useful such as herbal IMM product (Phyto-Mast; Bovinity Health LLC, Narvon, PA) that cure rate appeared to be similar among this and penicillin-dihydrostreptomycin (Mullen et al., 2014).

Parity affected the cure rate in both IZOM and CFQ group. Parity probably impacts treatment in several ways. First, the bacteriological cure rate decreases with age. Second, the magnitude in SCC reduction that can be achieved, the following cure, is less in older cows because their pretreatment SCC was noted to be lower (Deluyker et al., 2001). Deluyker et al. (2005) reported that Bacteriological cure rate was significantly higher for lower parity, in subclinical *S. aureus* mastitis. And also Sandgren et al. (2008) find that factors such as age, and previous history of mastitis can also affect recoveries as it has been shown that mastitis in older cows, or cows with a previous history of mastitis, is more difficult to treat than in younger animals.

Previously, bacteriological cure rates for *S. aureus*-induced SCM was found to be 43% for amoxicillin and cephapirin (Wilson et al., 1999). Klostermann et al. 2008 reported that 38% of naturally occurring chronic subclinical *S. aureus* mastitis cases treated with amoxicillin-clavulanic acid. Kasravi et al. (2011) showed that bacteriological cure rates of persistent sub-clinical *S. aureus* mastitis were 66.66% with cefquinome standard therapy, and also cure rate in cows with subclinical *S. aureus* mastitis with a combination of nafcillin with sodium penicillin and dihydro streptomycin sulfate was reported 38.9% (Khoramian Tousi et al., 2016). Thus the cure rate with an antibiotic in our study appeared to be similar to cure rates of some antibiotics.

Cured quarters, however, are defined as not only being pathogen-free but also having negative CMT. As CMT that considered as qualitative index of milk somatic cell number was decreased on day 14 and 28 post-treatment in cured samples from IOZM group, and in contrast, in post-treatment samples, in the present study, there was no evidence of a return to high CMT levels of the pretreatment samples and also CMT level in post-treatment samples has no any significant difference between control and PO groups, therefore, the present results suggest that IOZM can be considered as beneficial ointment for treatment of subclinical *S. aureus* mastitis during lactation. . Our result showed that the severity of SCM had not significant difference between cefquinome and IOZM, and also between control and PO groups. Therefore, IOZM can be used effectively in all subclinical *S. aureus* mastitis with any severity. Deluyker et al. (2005) and Deluyker et al. (2001) also reported that the geometric mean somatic cell count did not differ in d 21 and 28 post- treatment between control and Pirlimycin treatment in SCM during the lactation period.

In conclusion, IOZM can be used as an alternative treatment where the use of antibiotic treatments is limited or even prohibited in *S. aureus*-induced SCM.

Acknowledgments

We are grateful to Mr. Arab Prefecture Dairy Farming by whom the bulk milk samples were taken.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

The study was funded by Semnan University, Semnan, Iran

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Received: 18.03.2022
Accepted: 21.06.2022

DOI: 10.22055/IVJ.2022.342990.2468
DOR: 20.1001.1.17356873.1401.18.1.4.2

اثربخشی اسانس آویشن شیرازی در درمان استافیلوکوکوس اورئوس تعیین شده با PCR در گاوهای شیری مبتلا به ورم پستان تحت بالینی

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تاریخ دریافت: ۱۴۰۱/۲/۲۸ تاریخ پذیرش: ۱۴۰۱/۳/۳۱

چکیده

درمان ورم پستان تحت بالینی ناشی از استافیلوکوکوس اورئوس در گاوهای شیری با اسانس آویشن شیرازی و آنتی‌بیوتیک در یک کارآزمایی بالینی مقایسه شد. موفقیت درمان به پماد با استفاده از کشت میکروبیولوژیکی مونیتور شد. پماد داخل پستانی اسانس آویشن شیرازی (گروه ۱؛ IOZM) با پماد پلاسیبو (گروه ۲؛ PO)، پماد داخل پستانی سفکینوم (گروه ۳؛ CFQ) و با گروه کنترل منفی در ۱۸ گاو شیری مبتلا به ورم پستان تحت بالینی ناشی از استافیلوکوکوس اورئوس در هر گروه مقایسه شد. اثرات میزان بهبود باکتریولوژیکی در پاسخ به درمان پماد توسط روش کشت بررسی شد. نتایج حاصل از این کارآزمایی بالینی نشان داد که درمان IOZM پتانسیل از بین بردن ورم پستان تحت بالینی استافیلوکوکوس اورئوس را دارد. پس از یک دوره آزمایشی ۱۴ روزه و ۲۸ روزه، پاسخ‌های باکتریولوژیک در ۹ گاو از ۱۸ گاو تیمار شده با IOZM در مقایسه با ۱۰ گاو از ۱۸ گاو تیمار شده با CFQ مشاهده شد. همچنین عدد CMT تأثیر معنی‌داری بر درمان نداشت. نتایج این کارآزمایی نشان داد که درمان ورم پستان تحت بالینی استافیلوکوکوس اورئوس با اسانس Z. multiflora می‌تواند به عنوان یک درمان جایگزین مورد استفاده قرار گیرد. ما با موفقیت ورم پستان تحت بالینی استافیلوکوکوس اورئوس را در گاو با اسانس Z. multiflora درمان کردیم و می‌تواند پیشنهاد کند که به جای استفاده از آنتی‌بیوتیک استفاده شود.

کلمات کلیدی: سفاکینون، پماد داخل پستانی، استاف ارئوس، ورم پستان تحت بالینی، زاتاریا مولتی فورا

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