

## Therapeutic effect of oregano essential oil on subclinical bovine mastitis caused by *Staphylococcus aureus* and *Escherichia coli*

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(Received: November 13, 2015; Revised: December 12, 2015; Accepted: December 24, 2015)

**Abstract :** Oregano (*Origanum vulgare*) is an aromatic herb belonging to the *Lamiaceae* family. This study evaluated the therapeutic effect of oregano essential oil (OEO) on clinical bovine mastitis caused by *Staphylococcus* (*S.*) *aureus* and/or *Escherichia* (*E.*) *coli*. Thirty-two quarters from 18 lactating cows with subclinical mastitis were selected from a dairy farm and were divided into 4 groups (8 quarters/group): negative control (CON), positive control (GEN) and two OEO-treated groups (OEO-1 and OEO-2). Each group was intramammarily treated with saline, 10 g/tube of gentamicin ointment, and single and double doses of 0.9 mL OEO ointment twice a day for three days, respectively. After the treatments, physical udder conditions were greatly improved in GEN and OEO groups. In CON group, somatic cell counts (SCCs), number of white blood cells (WBC) and bacteria were increased. However, in OEO groups, SCCs and number of WBC were significantly decreased and *S. aureus* and *E. coli* were not detected in milk as compared with those before the treatments as well as GEN group. These results suggest that OEO may be a useful alternative to antibiotics for the control of subclinical bovine mastitis caused by *S. aureus* and/or *E. coli*.

**Keywords :** bovine mastitis, *Escherichia coli*, oregano essential oil, *Staphylococcus aureus*, therapeutic effect

### Introduction

Bovine mastitis is an infection of the mammary gland and is the major illness for dairy ruminants that leads to reduce milk production, and is often associated with cattle disorders such as fever and rumen motility [5]. Mastitis-related economic losses are due to reduction in yield, increased treatment costs, discarded milk, increase in culling, pharmacologic costs, and increased labor costs [1]. In the United States, the economic damage to the dairy industry is approximately 2 billion dollars annually, which has a similar impact in Europe. Bovine mastitis also carries public health significance in addition to its economic importance [22].

Bacteria such as *Escherichia* (*E.*) *coli* and *Staphylococcus* (*S.*) *aureus* are the major causes of bovine mastitis in many countries. *E. coli* infections cause clinical bovine mastitis, which is often accompanied by severe clinical symptoms including hot and swollen udders, fever, and loss of appetite. In addition, *S. aureus* is well recognized as a major pathogen

responsible for subclinical mastitis in cattle that is caused by its colonization and internalization in epithelial and endothelial cells of mammary gland [14].

In European countries and United States, 57% of *S. aureus* isolates (811 cases) from bovine mastitis showed to be  $\beta$ -lactamase positive [6]. In Korea, over 66% of *S. aureus* isolates (402 cases) from bovine mastitis were resistant to penicillin, and approximately 2.7% of the isolates were resistant to three or more antibiotics [18]. In recent study, 9 of 27 *E. coli* isolates from bovine mastitis were resistant to antibiotics such as ampicillin, neomycin and trimethoprim, and nearly 15% of the *E. coli* isolates were resistant to two antibiotics, ampicillin and trimethoprim [9].

Recently, the emergence of multidrug resistance in mastitis-causing bacteria has complicated management, prevention and treatment of mastitis [3]. As a result, many researchers have investigated efficient alternatives to antibiotics for the treatment of bovine mastitis. These include probiotics [10], phytobiotics [17], bacteriophages [8] and plant essential oils

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[12, 25].

Essential oils generally regarded as safe show antimicrobial proprieties and antibacterial resistance that has not been reported after prolonged exposure [20]. In previous studies, oregano essential oil (OEO) displayed antimicrobial activity against antibiotic-resistant to *S. species* and multidrug-resistant to *E. coli* in bovine mastitis [22].

Oregano (*Origanum vulgare*) is an aromatic herb belonging to the *Lamiaceae* family, and is commonly found throughout Asia, Europe, and Northern Africa [26]. In natural remedies, Oregano is used to treat respiratory and gastrointestinal disorders, dyspepsia, painful menstruation, rheumatoid arthritis, cholesterolemia and urinary tract disorders [26]. OEO contains several ingredients, most of which possess important antioxidant and antimicrobial properties [2]. Carvacrol and thymol which constitute approximately 78–85% of OEO, are principally responsible for the antibacterial activity of the oil, and other minor components such as the monoterpene hydrocarbons  $\gamma$ -terpinene and *p*-cymene also contribute to its antimicrobial activity [4].

Although previous studies examined the antimicrobial activity of OEO against pathogenic bacteria originating from bovine mastitis [20], few studies investigated the medicinal effect of OEO on bovine mastitis caused by pathogenic bacteria. The present study evaluated the therapeutic potential of OEO on clinical bovine mastitis caused by *S. aureus* and/or *E. coli*.

## Materials and Methods

### Experimental animals

This study was conducted in a dairy farm located in the Changwon area of Gyeongsang province, Korea. Thirty-two quarters from 18 lactating cows with clinical mastitis that had not been responding to treatment for over one weeks, were selected for this study. Clinical mastitis was defined as having somatic cell counts (SCCs) greater than  $2.0 \times 10^5$  cells/mL [21]; and the detection of *S. aureus* and/or *E. coli* in milk. The 32 quarters were divided into negative control (CON; 8 quarters), positive control (GEN; 8 quarters) and two OEO-treated groups [OEO-1 (8 quarters) and OEO-2 (8 quarters)], and the somatic cells and bacteria in the milk were counted.

### Treatment

CON was treated with saline twice a day for 3 days. GEN was treated with 10 g/tube of gentamicin ointment (Daesung Gentamicin Cream; Daesung Microbiological Labs, Korea), twice a day for 3 days. OEO-1 and OEO-2 had single and double doses of OEO ointment containing 0.9 mL of OEO (Eco-Mast 10 mL/tube; Daehan New Pharm, Korea) infused into the inflamed quarters twice a day for 3 days, respectively.

### Observation of udder condition

At the end of the treatment, physical conditions of the udders were evaluated by visual observation and palpation.

According to the previous study [19], physical changes in the udders including swelling, redness, pain and heat, were classified by using a 3-point severity score at the time of enrolment (0 = normal, 1 = mild, 2 = moderate, and 3 = severe).

### SCCs in milk

Approximately 10 mL of milk samples were daily collected from each quarter under aseptic conditions into sterilized conical tubes before and after the intramammary treatment of each drug for 3 days. SCCs were determined by using an automatic cell counter (Somacount; Bentley Instruments, USA).

### Enumeration of *S. aureus* and *E. coli* in milk

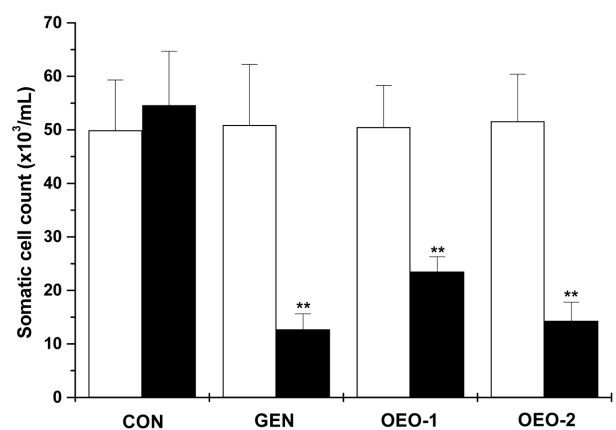
After collection of milk samples from each quarter, milk samples were diluted 20 times with sterilized distilled water. Each 0.1 mL of the diluted solution was spread onto CHROMagar Staph aureus (CHROMagar, France) in duplicate to count *S. aureus* and CHROMagar *E. coli* (CHROMagar) in duplicate to count *E. coli*. After inoculation, selective media were incubated at 37°C for 24 h and the typical color colonies (*S. aureus*, pink; *E. coli*, blue) were counted and represented as colony forming units (CFU)/mL.

### Analysis of total white blood cells

On 24 h after the last injection of OEO ointment, 5 mL of blood samples were collected from the tail vein of the dairy cows. Total white blood cell (WBC) was analyzed using an automatic blood cell analyzer (Advia 120 hematology analyzer; Bayer, USA).

### Statistical analysis

All results were represented as the mean  $\pm$  SD. Statistical analyses were performed using SPSS software (ver. 18.0;



**Fig. 1.** Change of somatic cell count before (□) and after (■) intramammary treatment with oregano essential oil (OEO) in cattle with mastitis. Negative control (CON) treated with saline; positive control (GEN) treated with 10 g/tube of gentamicin ointment twice a day for 3 days; single (OEO-1) and double (OEO-2) doses of 0.9 mL OEO ointment twice a day for three days. Asterisks (\*\*) indicate  $p < 0.01$  compared to before treatment.

**Table 1.** Udder conditions before and after oregano essential oil (OEO) treatments

Groups	Conditions	Treatment							
		Before				After*			
		0 <sup>†</sup>	1	2	3	0	1	2	3
CON	Swelling	4	3	1	0	3	3	2	0
	Redness	6	1	1	0	4	2	2	0
	Pain	6	2	0	0	5	2	1	0
	Heat	6	1	1	0	4	2	2	0
GEN	Swelling	5	2	1	0	5	3	0	0
	Redness	6	1	1	0	8	0	0	0
	Pain	7	1	0	0	8	0	0	0
	Heat	7	1	0	0	8	0	0	0
OEO-1	Swelling	5	2	1	0	4	4	0	0
	Redness	7	1	0	0	8	0	0	0
	Pain	7	1	0	0	8	0	0	0
	Heat	7	1	0	0	8	0	0	0
OEO-2	Swelling	4	3	1	0	6	2	0	0
	Redness	6	1	1	0	8	0	0	0
	Pain	7	1	0	0	8	0	0	0
	Heat	6	1	1	0	8	0	0	0

\*Severity on 24 h after the last treatment of drug. <sup>†</sup>0, normal; 1, mild; 2, moderate; 3, severe.

**Table 2.** Change in counts of *Staphylococcus (S.) aureus* and *Escherichia (E.) coli* in milk

Groups	Bacteria	Treatment	
		Before	After*
CON	<i>S. aureus</i> (CFU/mL)	496 ± 57	540 ± 72
	<i>E. coli</i> (×10 <sup>2</sup> CFU/mL)	42.2 ± 3.8	45.1 ± 4.2
GEN	<i>S. aureus</i> (CFU/mL)	517 ± 56	ND
	<i>E. coli</i> (×10 <sup>2</sup> CFU/mL)	45.2 ± 5.1	ND
OEO-1	<i>S. aureus</i> (CFU/mL)	513 ± 48	ND
	<i>E. coli</i> (×10 <sup>2</sup> CFU/mL)	49.4 ± 4.4	ND
OEO-2	<i>S. aureus</i> (CFU/mL)	527 ± 62	ND
	<i>E. coli</i> (×10 <sup>2</sup> CFU/mL)	51.3 ± 4.9	ND

Values are expressed as mean ± SD from three independent experiments. \*24-h after the last treatment of drug. ND, not detected.

SPSS, USA). Data were analyzed using one-way analysis of variance (ANOVA), followed by Student's two tailed *t*-test. The value of  $p < 0.05$  or  $p < 0.01$  was used as the criterion for statistical significance.

## Results

### Udder conditions

Changes in physical conditions of the udders are presented in Table 1. After saline treatment, moderate swelling, redness, pain and heat of udders were observed in 2, 2, 1 and 2 quarters of CON group, respectively. However, only mild swelling of udders was observed in 3 quarters of GEN group, 4 quarters of OEO-1 group, and 2 quarters of OEO-2 group.

### Change of SCCs in milk

The changes of SCCs in milk are shown in Fig. 1. In CON, SCCs after treatment were slightly higher than those before treatment. However, these differences were not significant. In GEN, OEO-1 and OEO-2, SCCs were significantly decreased compared with those before treatment ( $p < 0.01$ ). There were no significant differences among GEN and OEO groups.

### Changes of bacterial numbers in milk

The numbers of *S. aureus* and *E. coli* in milk are presented in Table 2. Although in CON group, the numbers of *S. aureus* and *E. coli* in milk were increased on 24 h after saline treatment for 3 days, there were no significant differences between before and after the treatment. In GEN, OEO-1 and

**Table 3.** Change of total white blood cells in blood samples

Groups	Treatment	
	Before	After*
CON	9.64 ± 0.47	9.71 ± 0.45
GEN	9.72 ± 0.53	7.71 ± 0.39 <sup>†</sup>
OEO-1	9.57 ± 0.50	8.07 ± 0.36 <sup>†</sup>
OEO-2	9.68 ± 0.57	7.78 ± 0.41 <sup>†</sup>

Values are expressed as mean ± SD from three independent experiments. \*24-h after treatment. <sup>†</sup>vs. before treatment at  $p < 0.05$ .

OEO-2 groups, *S. aureus* and *E. coli* were not detected in the milk on the fourth day after the treatment for 3 days.

### Changes of total WBC numbers

The number of total WBC in the blood samples is shown in Table 3. In CON group, WBC numbers after saline treatment for 3 days were slightly increased compared with those before the treatment. However, WBC numbers in GEN, OEO-1 and OEO-2 groups after each treatment for 3 days, were significantly decreased compared with those before the treatment ( $p < 0.05$ ).

## Discussion

At the first time, this study carried out the application of OEO known as antibacterial activity to treat bacterial bovine mastitis and identified OEO as a potential candidate for the control of bovine mastitis.

The antibacterial mechanism of OEO activity against bovine mastitis caused by bacteria was to induce an increase in bacterial membrane permeability, which further affects pH homeostasis and the equilibrium of inorganic ions [16]. Eventually, the phenolic compounds of OEO inhibit the growth of bacteria by altering the bacterial cell surface, which may inhibit the adhesion of bacteria to mammary epithelial cells [24].

SCCs have been used as an indicator of bovine mastitis in dairy cows in order to estimate the therapeutic effects for the mastitis [29]. Quarter SCCs below  $2 \times 10^5$  cells/mL are considered as healthy udder for most practical purposes [21]. In the previous study, SCCs of quarters with mastitis were reduced to near normal levels (ca.  $2 \times 10^5$  cells/mL) after the intramammary treatment with *Linum usitatissimum* fixed oil (2.5 mL/udder) once a day for 7 days, but total SCCs in all treated-quarters were slightly higher than normal levels [12]. After intramammary infusion of nisin (18,000 IU/mg) at a dose of 2,500,000 IU once daily for 3 days, the number of mammary quarters with SCCs greater than  $5.0 \times 10^5$  cells/mL was significantly decreased, but SCCs in all quarters treated with nisin were above normal levels [28]. On the other hand, SCCs in quarters inoculated once with  $10^6$  CFU/mL of *Lactobacillus perolens* CRL 1724 decreased to the control value ( $2 \times 10^5$  cells/mL) at day 5 post-treatment [10]. When considering the dosage and infusion times, in the

present study, SCCs in OEO-1 group treated with single dose of 0.9 mL OEO twice a day for 3 days were dropped to normal levels on 24 h after the last treatment, indicating that OEO has a therapeutic effect greater than those used in the previous studies.

Nowadays, conventional treatments against bovine mastitis still use antibiotic therapy, although alternatives including herbal and homeopathic approaches are becoming more popular [11]. After treatment with 125 mg of ceftiofur hydrochloride once a day for 8 days, bacteriological cure after 21 days was 47.4% (9/19) for *S. aureus* [27]. Additionally, bacteriological cure rates for bovine mastitis intramammarily administered with 75 mg of cefquinome three times at 16-h intervals were 66.7% (2/3) for *S. aureus* and 77.4% (5/7) for coliforms [13]. In contrast, bacteria were eliminated from the *E. coli* challenged- quarters of cows treated with enrofloxacin (5 mg/kg first intravenously 12-h post-challenge, then twice subcutaneously 36- and 60-h post-challenge) within 3.8 days, and from cows treated with 1.5 g of lactoferrin within 5.8 days [15]. In the present study, bacteriological cures in OEO-1 on 24 h post-treatment were 100% for both *S. aureus* and *E. coli*, which suggests that OEO can be used as an alternative treatment for clinical bovine mastitis caused by *S. aureus* and/or *E. coli* because OEO has higher bacteriologic cure rates than those antibiotics used in the previous studies.

Somatic cells are mainly milk-secreting epithelial cells that have been shed from the lining of the gland and WBC has entered the mammary gland in response to injury or infection [23]. Mastitis is generally accompanied by a huge influx of leukocytes into the infected udder with secretion of inflammatory mediators into the milk compartment. Following infection of the udder with pathogenic organisms, WBC increases in the blood of the cow, and SCCs in the milk also increase [7]. In the present study, after infection with pathogenic bacteria in the udder, both WBC in blood and SCCs in milk increased compared with those before infection, which coincides with similar findings in previous studies [7, 22]. However, in OEO groups, SCCs and number of WBC were significantly decreased as compared with those before the treatments as well as GEN group.

In conclusion, results from this study suggest that OEO may be a useful alternative to antibiotics for the control of bovine mastitis caused by *S. aureus* and/or *E. coli*.

## Acknowledgments

This research was supported by Daehan New Pharm Co. Ltd. in Seoul, Korea.

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