

Investigation of the efficacy of *Tarantula cubensis* extract (Theranekron D6) in the treatment of subclinical and clinical mastitis in dairy cows

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Abstract: The aim of this study was to investigate the effects of the use of Theranekron alone or in combination with intramammary antibiotic therapy for the treatment of subclinical and clinical mastitis on the recovery rate from mastitis. The study material was composed of 177 mammary lobes of 63 Holstein cows with subclinical (groups S1 and S2) and clinical mastitis (groups C1, C2, C3, and C4). In all of the treatment groups, excluding group S2, the California mastitis test scores were determined to have decreased after treatment ($P < 0.05$). In groups C1, C2, C3, and C4, which were treated for clinical mastitis, the somatic cell count (SCC) values were observed to have decreased. The decrease in SCC values was statistically significant except in group C2 ($P < 0.01$). While the SCC values were observed to have decreased in group S1 ($P > 0.05$), which was treated with Theranekron alone, the same values were found to have increased in group S2, which was maintained for control purposes ($P < 0.05$). This study demonstrated that Theranekron D6 could be used as an alternative therapeutic for the treatment of clinical and subclinical mastitis, as it is authorized for the use in cattle with a withdrawal period of zero hours for meat and milk.

Key words: California mastitis test, cow, mastitis, somatic cell count, Theranekron D6

1. Introduction

Defined as inflammation of the mammary gland, mastitis is the most common disease of dairy cattle, and it causes major economic losses (1). The disease reduces milk yield, alters milk composition, and shortens the productive life of affected dairy cattle. Seventy to eighty percent of milk losses caused by mastitis arise from subclinical cases (2). The changes observed in the composition of milk in subclinical mastitis include the presence of plasma proteins, alterations in ion concentrations, the breakdown of local cells, a reduction in the synthetic capacity of the mammary epithelium, and, most importantly, an increase in shedding of somatic cells (3). Although the tests used to determine the number of somatic cells in milk enable diagnosis of mastitis, they do not provide information on its causative agents. Despite the lengthy time they require, the isolation and identification of microorganisms by the culturing of milk samples and the application of antibiograms contribute greatly to the diagnosis and treatment of mastitis as well as to prophylaxis (4).

Nevertheless, mastitis control programs highlight the need for the rapid and timely treatment of cases on the basis of the somatic cell count (SCC) values determined by use of easily applied rapid techniques, irrespective of whether they are caused by environmental or contagious pathogens (5).

Although mastitis develops as a result of polymicrobial infection, the most frequently isolated agents are *Staphylococcus* spp., *Streptococcus* spp., and of gram-negative bacteria, *E. coli* (6,7). Other microorganisms involved in the development of mastitis are *Actinomyces pyogenes*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Mycoplasma* spp., and *Pasteurella multocida* (8).

Depending on the type and virulence of the microorganisms involved, the clinical course of mastitis in dairy cattle varies from mild to fatal. The treatment protocol applied also varies with the severity of mastitis. Generally, therapeutic agents are administered by the intramammary route. When compared to systemic

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administration, the major advantage of intramammary application is the lower dose of active substance required to maintain an adequate concentration of the therapeutic agent at the site of infection. Despite this major advantage, the rate of bacteriological recovery achieved with intramammary therapy is generally low. This results in clinically recovered mammary lobes remaining a source of bacterial infection for the dairy herd. Systemic antibiotics aid in eliminating infection sources, in particular by means of their efficacy in the treatment of chronic infections of the deeper layers of the mammary tissue. Therefore, it is advised that intramammary and systemic antibiotics be used in combination for the treatment of mastitis (9).

Due to the antibiotics used in mastitis treatment protocols leaving residues in milk, the milk of treated animals has to be withdrawn and discarded (10). Furthermore, the frequent and irresponsible use of antibiotics has brought about an increase in the development of antibacterial resistance and a decrease in the efficiency of antibacterial treatment (10). This has created an increased demand for alternative methods in the treatment of mastitis. In recent years, caprylic acid and monocaprylin (11), lysostaphin (12), oxytocin (13), several mastitis vaccines (14), and the immunostimulant levamisole (15) have been used in mastitis treatment.

To the authors' knowledge, no literature report is available on the use of Theranekron in mastitis treatment, despite its wide range of use in domestic animals. Theranekron is a homeopathic remedy containing extract of the spider *Tarantula cubensis*. This substance, which is of a peptide structure, enables the amelioration of necrotic structures, and thus the healing of wounds by accelerating epithelialization (16,17). Demonstrated to induce antiphlogistic, demarcating, and necrotizing effects, Theranekron (18) is used in the supportive treatment of mammary tumors in dogs (19), oral lesions associated with bluetongue disease in cattle (20), foot and mouth disease in cattle (21), and retentio secundinarum in cows, and has been indicated to shorten the involution period of the uterus (22).

The present study was aimed at investigating the use of Theranekron D6 in the treatment of subclinical and clinical mastitis, as an alternative to conventional antibiotic therapy.

2. Materials and methods

2.1. Farm and herd management

This study was conducted at a large-scale modern holding belonging to Saray Carpet, Agriculture and Stock Farming Inc., located in the Develi district of Kayseri Province in Turkey. The study material comprised 177 mammary lobes of 63 Holstein cows that were suspected to have mastitis on the basis of the electrical conductivity of the milk measured at milking.

The electrical conductivity of the milk was measured by an automatic milking system at each milking. Animals that presented with high conductivity and reduced milk yield were selected for clinical examination, as they were suspected to have mastitis.

The animals were subjected to uniform management and nutrition conditions throughout the study and were provided with water ad libitum.

2.2. Assessment of milk samples

The milk samples were first assessed by the California mastitis test (CMT). The CMT results were scored as described by McDougall (23).

SCCs were performed using a somatic cell counter (DeLaval, Sweden) within a short time. The number of somatic cells in the milk samples was determined by counting the cells stained with a DNA-specific fluorescent probe and propidium iodide. Milk samples, 60 µL in volume, were drawn into cassettes and the loaded cassettes were placed into the DeLaval cell counter for the determination of the number of somatic cells per microliter of milk.

2.3. Bacteriological examination

For this purpose, firstly, the teats were cleansed with a warm and nonirritant antiseptic solution. After discarding the first milk, 10-mL milk samples were collected and transferred to the laboratory in cooled thermos flasks on the day of collection.

The samples were transferred to the laboratory under cooled storage and, once transferred to the laboratory, were homogenized. The samples were inoculated onto blood agar containing 7% defibrinated sheep blood (Merck, 110328) and MacConkey agar (Merck, 105465) for bacteriological analyses. The agar plates were incubated at 37 °C under aerobic conditions for 24–48 h. Sabouraud 4% dextrose agar (Merck, 105438) was used for mycological analyses. The cultures were incubated at 24 °C for 5–7 days. The bacteria and fungi grown on the media were identified by the use of conventional methods (8,24).

2.4. Study protocol

The CMT was applied to milk samples from the dairy cows, which were classified according to their milk yield and milk conductivity scores. The animals were later allocated to 2 groups, on the basis of their CMT scores, namely the subclinical mastitis group (group S) and the clinical mastitis group (group C). These 2 groups were further divided into subgroups, on a random basis (groups S1, S2, C1, C2, C3, and C4). Group S1 received 10 mL of Theranekron D6 (Richter Pharma AG, Austria) by subcutaneous (SC) route, while group S2, which was maintained for control purposes, was administered 10 mL of physiological saline by SC route. Of the groups treated for clinical mastitis, group C1 received Theranekron alone, group C2 was given an intramammary antibiotic

alone, group C3 received a combination of Theranekron and an intramammary antibiotic, and group C4 received a double dose of Theranekron (2 times 10 mL in 2 days). The intramammary antibiotic therapy involved the administration of 2 doses of Ubrolixin (200 mg Cephalexin, 100,000 IU Kanamycin, Boehringer Ingelheim, Germany).

For bacteriological examination, milk samples were taken prior to treatment. Furthermore, the ear tag (unique individual identification) number, age, daily milk yield, lactation period, body condition score (BCS), and locomotive score of each animal were recorded.

2.5. Statistical analysis

For the determination of the statistical significance of the efficacy of the treatment in each group, the CMT scores determined before and after treatment were compared using Wilcoxon's test, and the SCC values were compared by the paired-samples t-test. Statistical analyses were performed using the NCSS 2009 (Version 9.0.5) software package (25).

This study was approved by the Local Ethics Committee for Animal Experiments of Erciyes University in Kayseri, Turkey (ERÜ-HADYEK:14/84).

3. Results

Information on the age, lactation period, body condition score, daily milk yield, and locomotive score of the animals included in the study is presented in Table 1.

The distribution of the SCC values according to the CMT scores before and after treatment is given in Table 2. It was observed that the SCC values increased in parallel with increased CMT scores (Figure 1).

While no clinical signs were observed in the mammary glands of animals with clinical and subclinical mastitis for which the SCC of the milk was determined to be above 7,000,000, in some other cases in which the SCC value was 1,000,000 the mammary lobe was observed to display slight hyperemia. Therefore, when establishing the groups for clinical and subclinical mastitis, a CMT score of +1 and a SCC value of 1,000,000 and above were set as the limit values.

Table 1. Some descriptive parameters for cows used in this study.

Cattle traits	N	Min	Max	Mean ± std. deviation
Age (year)	63	2.00	9.02	3.96 ± 1.33
Daily milk yield (L)	63	3.00	45.30	22.32 ± 8.87
Lactation day (day)	63	9.00	308.00	92.10 ± 74.95
Body condition score	63	2	4	3.10 ± 0.52
Locomotive score	63	1	4	1.56 ± 0.74

N: The number of cows.

Table 2. CMT scores related to SCC (×1000) values.

	CMT score	N	Mean ± std. deviation
Before treatment	0	51	240.7 ± 194.4
	1	56	982.9 ± 662.2
	2	29	2446.5 ± 1603.9
	3	41	5168.8 ± 1749.0
After treatment	0	81	259.6 ± 281.8
	1	54	1062.2 ± 563.0
	2	24	2393.9 ± 1183.7
	3	18	4889.8 ± 1935.6

N: The number of mammary lobes.

The comparison of the CMT scores of the treatment groups calculated for the pretreatment and posttreatment periods demonstrated that the decrease observed in the CMT scores posttreatment in all of the treated groups was statistically significant (Table 3). While the decrease observed in the CMT scores of the treatment group with subclinical mastitis (group S1) upon therapy was significant ($P < 0.05$), no alteration was observed in the scores of the control group (group S2) ($P > 0.05$).

The statistical assessment of the pretreatment and posttreatment SCC values of the groups with clinical mastitis demonstrated a decrease in all groups. Excluding group C2, the decrease in the SCC values of all groups was significant ($P < 0.01$). Of the groups with subclinical mastitis, group S1, which received treatment, displayed a decrease in the SCC values, albeit not statistically significant ($P > 0.05$), while group S2, which was maintained for control purposes, presented an increase ($P < 0.05$) (Table 4) (Figure 2).

The microbiological analysis of the milk samples collected before treatment yielded the isolation of *S. aureus* and *E. coli* at high rates (Table 5).

In all of the groups (groups C and S), neither body condition scores nor locomotive scores had an effect on treatment.

4. Discussion

In cows, multiple environmental and individual factors affect the risk of contracting subclinical mastitis. As these include, among others, predisposing factors such as adverse management and feeding conditions, poor hygiene, milking method, climate, seasonal changes, anatomical and physiological disorders, immune deficiency, and secondary diseases, the treatment procedures that may be applied fall within a rather wide range. Apart from the CMT and SCC, which are based on the increase in somatic cells in milk, microbiological tests are needed in

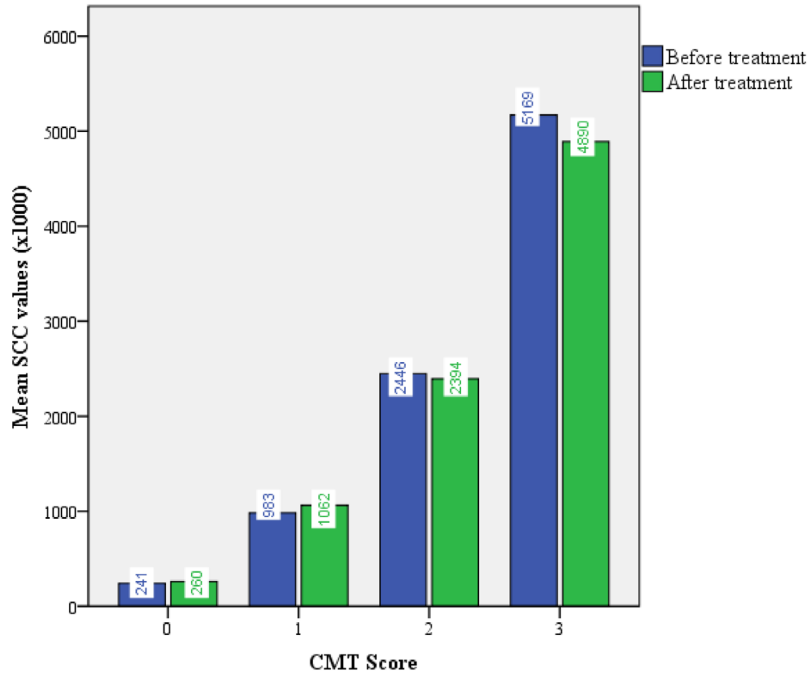


Figure 1. The relationship between CMT and SCC values in this study.

the diagnosis of subclinical mastitis in order to increase the number of treatment options that may be employed.

The present study revealed parallel results for the CMT and SCC in terms of mastitis treatment (Table 1). However, while the animals with subclinical mastitis, of which the SCC values were below 1,000,000 presented with clinical signs in the mammary gland (hyperemia, swelling, pain, etc.), 9 mammary lobes (9/93 - 9.67%) pertaining to animals with clinical mastitis, which had SCC values of 7,000,000 and above and a CMT score of +3, did not display any sign of inflammation. Therefore, it was concluded that subclinical and clinical mastitis could not be differentiated on the basis of CMT scores, SCC values,

and clinical signs alone, and it was decided to use a SCC value of 1,000,000 as a limit value.

In a previous study, the SCC values of animals with CMT scores of +1, +2, and +3 were determined as 150,000, 2,140,000, and 8,740,000, respectively (26), whereas, in the present study, the SCC values of the animals with CMT scores of 0, +1, +2, and +3 were 240,700, 982,900, 2,446,500, and 5,168,800, respectively. Moreover, the increase in the CMT values was consistent with the increase in the SCC in our study (Table 2).

In research conducted in Konya Province in Turkey (27), the microorganisms isolated and identified from milk samples, taken from 125 mammary glands reacting

Table 3. CMT scores in the respective groups before and after treatment.

Treatment groups	N	CMT scores (median and 25%-75% percentile)		Statistical significance (Wilcoxon signed ranks test)
		Before treatment	After treatment	
Theranekron D6 (C1)	29	2 (1-3)	1 (0-1)	P < 0.001
Intramammary antibiotic (C2)	19	2 (1-3)	1 (0-2.25)	P < 0.05
Clinical mastitis				
Theranekron D6 + intramammary antibiotic (C3)	20	3 (2.25-3)	2 (1-2.75)	P < 0.01
Theranekron D6 double dose (C4)	25	2 (2-3)	1 (1-2)	P < 0.01
Subclinical mastitis				
Theranekron D6 (S1)	50	1 (0-1)	0 (0-1)	P < 0.05
Control (S2)	34	0 (0-0)	0 (0-0)	P > 0.05

N: The number of mammary lobes.

Table 4. SCC values in the respective groups before and after treatment.

Treatment groups	N	SCC values (×1000) (Mean ± SEM)		Paired differences	Statistical significance (paired sample t test)
		Before treatment	After treatment		
Theranekron D6 (C1)	29	2976.00 ± 402.44	1375.86 ± 248.65	1600.14	P < 0.001
Intramammary antibiotic (C2)	19	3167.58 ± 523.92	2079.42 ± 647.02	1088.16	P > 0.05
Theranekron D6 + intramammary antibiotic (C3)	20	4347.55 ± 437.65	2571.80 ± 374.80	1775.75	P < 0.001
Theranekron D6 double dose (C4)	25	3450.96 ± 422.68	2225.32 ± 324.75	1225.64	P < 0.01
Subclinical mastitis Theranekron D6 (S1)	50	449.86 ± 41.72	393.96 ± 71.74	55.90	P > 0.05
Control (S2)	34	234.68 ± 37.04	519.88 ± 135.62	-285.21	P < 0.05

N: The number of mammary lobes.

positively for CMT, were *S. aureus* (47.3%), *C. pyogenes* (16.3%), *E. coli* (8.2%), *C. albicans* (6.5%), *S. agalactiae* (6%), and other microorganisms (15.7%). On the other hand, in the present study, the microorganisms isolated were *S. aureus* (41.17%), *E. coli* (47.05%), *A. pyogenes* (19.6%), *B. subtilis* (15.68%), *P. aeruginosa* (13.72%), coagulase-negative *Staphylococcus* (CNS) (13.63%), *P. vulgaris* (7.84%), *E. faecalis* (3.92%), and *C. albicans* (1.96%) (Table 5).

Animals suffering from mastitis can be diagnosed rapidly and reliably on the basis of SCC values. Although bacteriological examination has been reported to be important for the diagnosis as well as treatment and prophylaxis of mastitis (4), the feasibility of this procedure

needs to be discussed in terms of labor, costs, and duration. For this reason, the significance of the determination of the treatment protocol to be applied in view of the SCC value and the timely application of the protocol has been highlighted (5).

The intramammary administration of antibiotics with the aim to treat mastitis, irrespective of antibiograms, limits the success of the treatment applied. Furthermore, considering that the majority of the antibiotics used for the treatment of mastitis leave residues in milk, which ultimately leads to milk losses due to milk withdrawal and discarding, alternative treatment options are desirable (10). With the administration of Theranekron D6 a decrease in values in the SCC could be observed in all groups (C1, C3,

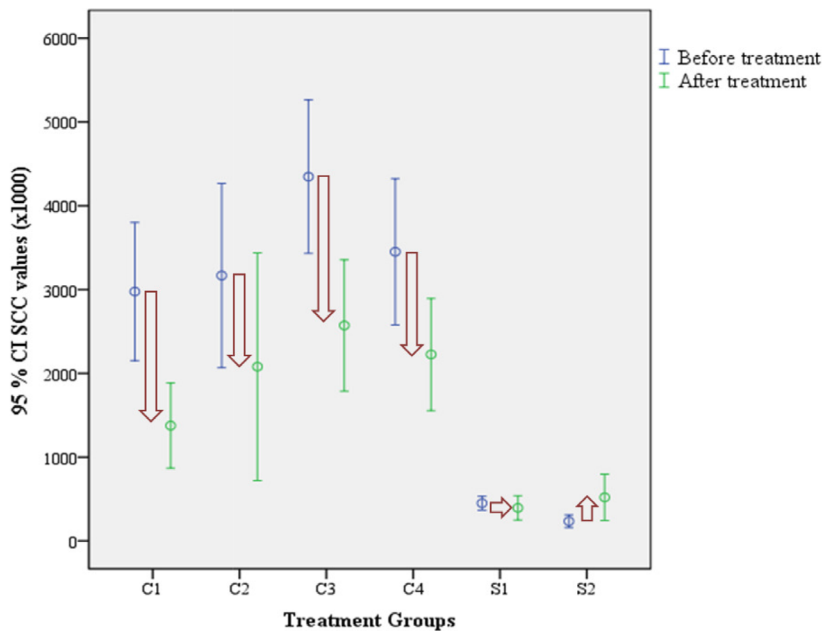


Figure 2. SCC values in the respective groups before and after treatment.

Table 5. Microorganisms isolated from milk samples in the study groups.

Microorganisms	Isolation rate (%)
<i>E. coli</i>	47%
<i>S. aureus</i>	41.1%
<i>A. pyogenes</i>	19.6%
<i>B. subtilis</i>	15.6%
<i>P. aeruginosa</i>	13.7%
CNS	13.6%
<i>P. vulgaris</i>	7.8%
<i>E. faecalis</i>	3.9%
<i>C. albicans</i>	1.9%

C4). However, a marked decrease in the number of SCC in group C2 was observed.

A statistically significant decrease was observed in the posttreatment SCC values of all groups except C2 (intramammary antibiotic), compared to the pretreatment values. The lack of statistical significance of the decrease in group C2, which received antibiotic therapy, pointed to the need for the selection of the most appropriate antibiotic and/or antibiotic combination based on antibiogram results.

The observation of a decrease in the SCC values of group S1 and the occurrence of a statistically significant increase in the SCC values of group S2, which was maintained for control purposes, demonstrated the efficacy of Theranekron in cases of subclinical mastitis.

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In each of the dairy cows included in the study, more than one of the mammary lobes were determined to have clinical and/or subclinical mastitis. It was concluded that, in the treatment of animals with inflammation in more than one mammary lobe, the use of a single dose of Theranekron D6 would effectively reduce SCC values. Due to its authorized withdrawal period of zero hours, it provides the advantage that no milk has to be withdrawn and discarded, which ultimately leads to a reduction in the treatment cost.

In conclusion, in the present study, it was demonstrated that the use of Theranekron D6 for the treatment of clinical and subclinical mastitis was effective in the reduction of the SCC values, and when combined with the appropriate intramammary antibiotic the success of the treatment protocol increased. It was also ascertained that, due to the withdrawal period of zero hours, the method emerged as an alternative treatment option offering less economic loss, particularly for the treatment of subclinical mastitis. The results of the present study, in which Theranekron D6 was used for the first time in the treatment of clinical and subclinical mastitis, are considered to constitute reference information for future research to be conducted on this subject.

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