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Alternative therapeutic approach of ovine subclinical mastitis using the ethanolic roots extract of *Capparis spinosa*

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Abstract

Background: Over the past decades, *Capparis spinosa* has been considered a traditional therapy for relieving different illnesses. Mastitis causes a decrease in milk production and is usually treated with injectable and intra-mammary antibiotics.

Aim: Investigating the therapeutic effects of *C. spinosa* root extract on subclinically mastitic ewes.

Methods: Totally, 164 lactating ewes were selected randomly from the flocks that existed in some areas belonging to Al-Najaf City (Najaf, Iraq) from September to December (2022). Each study animal was subjected to direct sampling of milk before and once each week for 6 weeks (42 days) post treatment to be tested directly by the California mastitis test (CMT).

Results: Concerning phytochemical testing of ethanolic root extract, the findings revealed a significant increase in the concentration of alkaloids, flavonoids, polyphenols, and tannins when compared to other components such as coumarins, saponin, glycosides, amino acids, and steroids. In this study, there were 44.51% infected ewes with subclinical mastitis, involving 25.61%, 13.41%, and 5.49% for scores 1, 2, and 3, respectively. In comparison with pre-treatment week, insignificant alteration was seen in the values of all scores in therapeutic week 1. However, significant differences were initiated in values of score 0 in week 2; score 0 and score 2 in week 3; score 0, score 1, and score 2 in week 4; and values of all scores in weeks 5 and 6.

Conclusion: This represents the first Iraqi study aimed at the treatment of subclinical mastitis in sheep using the root extract of *C. spinosa*. Phytochemical testing of ethanolic extract revealed the presence of variable amounts of chemical compounds that reflect their effects on treated animals by decreasing the number of infected ewes with the disease. Moreover, studies are greatly important to estimate the therapeutic effects of other parts of *C. spinosa* such as leaves and seeds, on the disease and other animal diseases.

Keywords: Caper, Ewes, Herbal therapy, California mastitis test, Somatic cell count.

Introduction

Capparis spinosa is a perennial shrub of the *Capparaceae* Family under the *Brassicales* Order in the *Magnoliopsida* Class, which featured features morphologically a high degree of heterogeneity and plesiomorphic properties (Rahnavard and Razavi, 2017; Shilla *et al*., 2019). This plant grows naturally in many countries in Asia, America, North Africa, and Europe, is important economically, and has considerable trade at an international level, especially in the United States, India, and China (Kdimy *et al*., 2022). Biochemical studies of leaf, flowers, fruits, seeds, and root extracts exhibited that *C. spinosa* has variable contents of phenolic compounds and flavonoids based on multiple environmental, geographical, and processing factors (Stefanucci *et al*., 2018; Grimalt *et al*., 2022). Several studies on different parts of this plant revealed numerous pharmacological activities such as antimicrobial, antifungal, antiparasitic, antibacterial, anti-inflammatory, antioxidant, anthelmintic, antidiabetic, liver protective, antihyperlipidemia, analgesic, sedative, and anticonvulsant (Rizvi and Ali, 2016; Beshah *et al*., 2020; Nadaf *et al*., 2023).

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Mastitis is one of the more common health problems affecting animals which occur due to inflammation of the parenchyma of the mammary gland, resulting in physical and chemical alteration in milk with pathological changes in glandular tissues (Saleem *et al*., 2021). Many etiologies and risk factors have been incriminated in the occurrence of disease in addition to bacterial invasion, which represents the main cause of mastitis in field animals such as camels, cattle, goats, and sheep (Constable *et al*., 2017). Mastitis has particular importance in field animals since the disease decreases the quantity and quality of the yielded milk, reduces the weight gain in newborns fed on milk of affected animals, and affects animal welfare (Poławska *et al*., 2012).

In the clinical form of the disease, there was swelling, heat, pain, and edema in the mammary gland; however, a large proportion of mastitic glands are not readily detectable by manual palpation or by visual examination of the udder and milk, known as "subclinical infection" (Peters *et al*., 2015; Pavlenko *et al*., 2018). In the field, several subclinical cases cannot be detected until they are indirectly diagnosed to identify the somatic cell concentration in the milk of the affected animal (Kamal *et al*., 2014; Saleem *et al*., 2021; Tanni *et al*., 2021). The California mastitis test (CMT) can qualitatively estimate amounts of DNA in milk secretions, which is useful because the concentrations of DNA and white blood cells are directly correlated (Windria *et al*., 2021).

Worldwide, various parts of some medicinal plants were used for the control of mastitis in domestic animals, mostly cattle (Gomes *et al*., 2019; Lopes *et al*., 2020; Srichok *et al*., 2022). Hence, this represents the first Iraqi study aimed at detecting the therapeutic effect of the ethanolic root extract of *C. spinosa* in controlling ovine subclinical mastitis.

Materials and Methods

Animals

In total, 164 lactating ewes were selected from flocks found in some areas belonging to Al-Najaf City (Najaf, Iraq) during September–December 2022. Each study animal was subjected to the direct collection of approximately 50 ml of milk samples from both udder quarters under aseptic conditions into a labeled disposable plastic container that was transported to the laboratory for testing by CMT.

Preparation of C. spinosa

Initially, roots of *C. spinosa* were collected from different regions of Al-Najaf province, washed, dried, and crushed to obtain the root's powder using the grinder. Then, a total of 100 g of dried powder and 70% of ethanol alcohol were put in a thimble piece and set in the extraction Soxhlet apparatus (Gerhardt, Germany) at a temperature of 45°C. Post filtration, vacuum drying, and concentration to semi-solid mass, percentage yield was detected as the following [Percentage yield (%) = Weight of extract (gm)/Weight of crushed plant material 100] (Banso and Adeyemo, 2006).

The extract was collected and stored in aseptic glass vials covered with aluminum foil, and kept frozen at 4ºC until used (Fig. 1). The solution was freshly prepared immediately before use and the dose was individually adjusted according to animal body weight (Rawri *et al*., 2013).

Phytochemical screening

As described by other studies, coumarins, proteins and amino acids, steroids, carbohydrates and glycosides, saponins, tannins, polyphenols, flavonoids, and alkaloids were estimated as detailed by other studies (Morsy, 2014; Anza *et al*., 2015; Gbadamosi and Kalejaye, 2017; Singh *et al*., 2018).

CMT

According to the manufacturer's instructions (Weizur, India), milk from each udder's quarter was tested to detect the score of each positive case as follows: score (0) for grey non-thickening mixture, score (1) for grey/light purple slightly thickening mixture, score (2) for purple distinct thickening mixture, and score (3) for elevated dark purple gel surface. The CMT was performed on positively infected animals and repeated weekly after therapy for 28 days.

Therapeutic approach by roots extract of C. spinosa

The suspension of required concentrations was prepared through the dissolving of concentrated extract in distilled water to obtain 3 different doses as follows; 200, 250, and 300 mg/kg (Rawri *et al*., 2013).

The positively infected ewes with subclinical mastitis were divided according to the severity of infection into score 1, score 2, and score 3. Finally, the positively infected animals were treated daily for 28 days according to their scores (1, 2, and 3) with 200, 250, and 300 mg/kg B.W., respectively, to detect the effect of the root extract of *C. spinosa* in curing the infected animals.

Statistical analysis

The *t-test* and One-Way ANOVA in the GraphPad Prism Software version 6.0.1 (GraphPad Inc., USA) were used to identify significance throughout study values at $p < 0.05$ (Gharban *et al*., 2022).

Ethical approval

The current study was licensed by the Scientific Committee of the Faculty of Veterinary Medicine at the University of Kufa (Najaf, Iraq) as well as the College of Medicine and College of Veterinary Medicine at the University of Wasit (Wasit, Iraq).

Results

Concerning phytochemical testing of ethanolic root extract of *C. spinosa*, the findings revealed a significant increase ($p < 0.0122$) in concentration of alkaloids (19.05%), flavonoids (19.05%), polyphenols (14.29%), and tannins (14.29%) when compared to other components such as coumarins (9.52%), saponin

Fig. 1. Collected roots and extracts of *C. spinosa* for the current study.

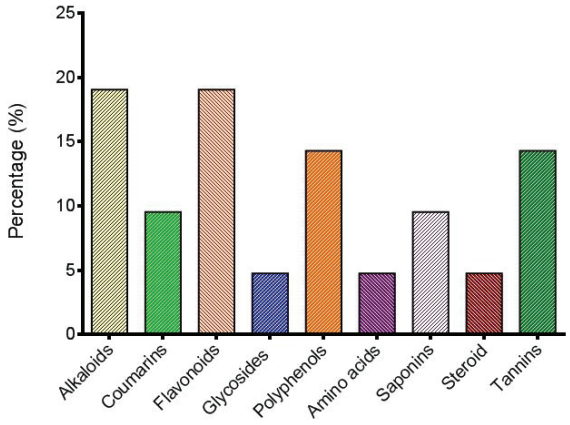

Fig. 2. Concentrations of chemicals existed in the ethanolic roots extract of *C. spinosa.*

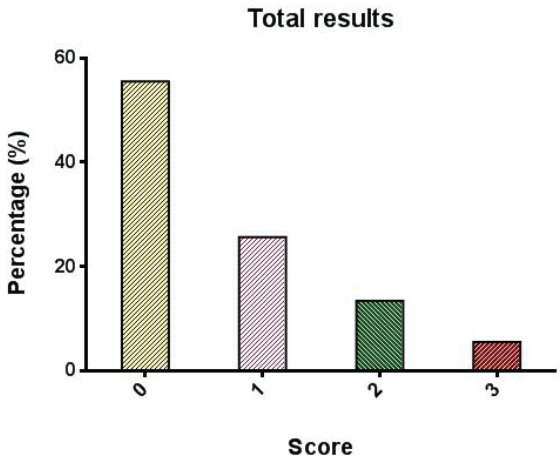

Fig. 3. Scores of study ewes examined by the CMT.

(9.52%), glycosides (4.76%), amino acid (4.76%), and steroid (4.76%) (Fig. 2).

In total, 164 milk samples tested by the CMT, there were 73 (44.51%) positive lactating ewes infected with subclinical mastitis, which involved 42 (25.61%), 22 (13.41%), and 9 (5.49%) positive animals for scores 1, 2, and 3, respectively ($p < 0.0137$) (Fig. 3).

During the therapeutic weeks of the study, root extract has a positive effect on infected animals (Tables 1–7). In comparison with pre-treatment week (0 week), insignificant alteration ($p > 0.05$) was seen among all scores of therapeutic week 1. However, significant alteration ($p < 0.05$) was initiated at score 0 in week 2; score 0 and score 2 in week 3; score 0, score 1, and score 2 in week 4; and values of all scores in weeks 5 and 6.

Discussion

Several studies have confirmed that, in comparison with untreated quarters, the treatment of subclinical mastitis can potentially reduce forward transmission of infection to other healthy ones and the incidence of clinical mastitis and increase bacteriological cure rates by reducing somatic cell count (Steele and McDougall, 2014; McDougall *et al*., 2022). The higher rate of subclinical mastitis among study ewes demonstrated that this disease remains a major problem in fields. In sheep, although etiologies and incidence rate of disease are variable in different countries due to different factors, it was 9.23% in Iran (Beheshti *et al*., 2010), 15% in Greece (Fragkou *et al*., 2014), 28.14% in Ethiopia (Gebrewahid *et al*., 2012), and 32.2% in Portugal (Queiroga, 2017).

Table 1. Effect of *C. spinosa* on scores of infected ewes in the 1st therapeutic week.

Pre-treatment		Post treatment/score [no. (%)]			
Score	**Total no.**	**0**	**1**	**2**	**3**
1	42	3 (7.14%)	39 (92.86%)	0 (0%)	0 (0%)
2	22	0 (0%)	1 (4.55%)	21 (95.45%)	0 (0%)
3	9	0 (0%)	0 (0%)	0 (0%)	9 (100%)
Total	73	3	40	21	9

Table 2. Effect of *C. spinosa* on scores of infected ewes in the 2nd therapeutic week.

Pre-treatment		Post treatment/score [No. (%)]			
Score	**Total no.**	**0**	**1**	**2**	**3**
1	40	8 (20%)	32 (80%)	0 (0%)	0 (0%)
2	21	0 (0%)	4 (19.05%)	17 (80.95%)	0 (0%)
3	9	0 (0%)	0 (0%)	0 (0%)	9 (100%)
Total	70	8	36	17	9

Table 3. Effect of *C. spinosa* on scores of infected ewes in the 3rd therapeutic week.

Pre-treatment		Post treatment/score [No. (%)]			
Score	**Total no.**	**0**	**1**	**2**	**3**
1	36	5 (%)	31 (%)	0 (0%)	0 (0%)
2	17	2 (%)	6 (%)	9 (%)	0 (0%)
3	9	0 (0%)	1 (11.11%)	1 (11.11%)	7 (77.78%)
Total	52	7	38	10	7

Table 4. Effect of *C. spinosa* on scores of infected ewes in the 4th therapeutic week.

Pre-treatment		Post treatment/score [no. (%)]			
Score	**Total no.**	**0**	**1**	**2**	**3**
1	38	17 (44.74%)	21 (55.26%)	0 (0%)	0 (0%)
2	10	2 (20%)	3 (30%)	5 (50%)	0 (0%)
3	7	1 (14.29%)	1 (14.29%)	3 (42.86%)	2 (28.57%)
Total	55	20	25	8	2

Table 5. Effect of *C. spinosa* on scores of infected ewes in the 5th therapeutic week.

Pre-treatment		Post treatment/score [no. (%)]			
Score	**Total no.**	**0**	**1**	**2**	**3**
1	25	6 (24%)	19 (%)	0 (0%)	0 (0%)
2	8	1 (12.5%)	3 (37.5%)	4 (50%)	0 (0%)
3	2	0 (0%)	1 (50%)	1 (50%)	0 (0%)
Total	35	7	23	5	0

Table 6. Effect of *C. spinosa* on scores of infected ewes in the 6th therapeutic week.

Pre-treatment		Post treatment/score [no. (%)]			
Score	**Total No.**	**0**	**1**	**2**	**3**
1	23	16 (69.57%)	7 (30.43%)	0 (0%)	0 (0%)
2	5	1 (20%)	3 (60%)	1 (20%)	0 (0%)
3	0	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total	28	17	10	1	0

Table 7. Total results for the effect of *C. spinosa* on scores of infected animals among different study weeks.

Total no.	Week	Score (%)			
		0	**1**	**2**	**3**
164	0	91 (55.49%)	42(25.61%)	22 (13.41%)	9 (5.49%)
	1	94 (57.32%)	40 (24.39%)	21 (12.81%)	9 (5.49%)
	2	102 (62.2%)	36 (21.95%)	17 (10.37%)	9 (5.49%)
	3	109 (66.46%)	38 (23.17%)	10 (6.1%)	7 (4.27%)
	4	129 (78.66%)	25 (15.24%)	8 (4.88%)	2 (1.22%)
	5	136 (82.93%)	23 (14.02%)	5 (3.05%)	0 (0%)
	6	153 (93.29%)	10 (6.1%)	1 (0.61%)	0 (0%)
p value		0.0156	0.0219	0.0313	0.0474

As infectious agents have developed resistance against various antibiotics; different therapies have served for the treatment of the disease, such as antibiotics (Golder *et al*., 2016), herbal (Rathaur *et al*., 2020), and homeopathic (Ferreira *et al*., 2022) therapies. Among these agents, herbal therapy is currently to cure not only intra-mammary infections but also systemic infections. Many authors confirmed that herbals can prevent udder infections without the concerns of eliminating milk and meat antibiotic residues, discarding milk tainted with antibiotics, and bacterial resistance (Olivares-Pérez *et al*., 2015; Rajala-Schultz *et al*., 2021; Tomanić *et al*., 2023).

Due to the biochemical and bioactive compounds, *C. spinosa* was extensively investigated by various analytical studies (Rahnavard and Razavi, 2017; Zhang and Ma, 2018). However, limited worldwide studies focused on the isolation and detection of the bioactive materials that existed in the roots of this plant. Based on our data, phytochemical screening for the contents of the roots extract showed that this plant had different compounds with various concentrations as seen by Satyanarayana *et al*. (2008) who detected that *C. spinosa* contains many phytochemical constituents from various parts of the plant, such as alkaloids in roots and seeds. In addition to alkaloids, the roots of *C. spinosa* have many bioactive compounds like flavonoid compounds (rutin and quercetin and polyphenol), which are commonly found within the various parts of the plant as stems, leaves, roots, fruits, berries, and flowers (Zhang and Ma, 2018). The phytochemical properties of the solvents employed, particularly their polarity, might be the initial reason for the yield variation. Other reasons include geographical drying time, species origin, extraction process, and ambient circumstances, according to various research. Additional variables, including pH and temperature, may have a direct influence on the yields of extraction (Chedraoui *et al*., 2017).

As reported by different researchers, the extracts of *C. spinosa* parts are considered potential source to restrain the growth of various bacteria that can acquire resistance to antibiotics, including amoxicillin, erythromycin, cephalexin, ciprofloxacin, piperacillin, teicoplanin, tetracycline, and vancomycin (Muraih *et al*., 2020; Saliem and Abdulridha, 2022). Muhaidat *et al*. (2013) detected that the crude fractions obtained from *C. spinosa* have higher degrees of antibacterial potency toward Gram-positive organisms in comparison with Gram-negative bacteria, indicating that the contents of *C. spinosa* are natural antibiotics that are actively and typically related to fractions of phytochemical contents. In different studies, the findings showed that some chemical contents of the *C. spinosa* extracts have potentially possessed antimicrobial activity such as alkaloids, tannins, and flavonoids (Eltawaty, 2018; AlMousa *et al*., 2022). Also, Tagnaout *et al*. (2016) mentioned that the methanol extracts of caper can be

considered a good source of antibiotics and antioxidants for therapeutic and nutraceutical industries. Extracts of *C. spinosa* are also rich in antioxidants that stop the advent of oxidative stress that occurs in mastitis, improve the cure rate, and reduce the severity of the disease (Yang *et al*., 2010; Mansour *et al*., 2016; Jiménez-López *et al*., 2018).

Conclusion

Herbal therapy has gained remarkable care in the scientific community because of its multidirectional and pleiotropic activities. In this regard, the ethanolic root extract of *C. spinosa* revealed great potential since it decreased the SCCs, and promised alternative medicine to cure subclinical mastitis in sheep. Moreover, research is necessary to establish if the long-term consumption *C. spinosa* reduces the occurrence or severity of subclinical and clinical forms of disease among sheep or other domestic animals.

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Conflict of interest

The authors have no conflict of interest to disclose.

Authors' contributions

BAA, MHMM, and ADL: Collection of plant, extraction of extract, and treatment of study animals. HAJG: Clinical examination, CMT testing, and statistical analysis. The authors read and accepted this copy manuscript.

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Data availability

All data supporting the findings of this study are available within the manuscript.

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