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Clinical Evaluation of *Syzygium aromaticum* and *Cinnamomum zeylanicum* Gels as an Alternative to Antimicrobial Therapy in Ewes Mastitis

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Abstract | Mastitis is a common infectious disease as well as an economically significant disease that affect dairy ewes worldwide. This inflammatory disease leading to reduced milk quality and production, increased veterinary costs, and potential animal welfare concerns. The aim of this study is to investigate the therapeutic efficacy of topical *Syzygium arozmaticum* and *Cinnamomum zeylanicum* gel in treatment of mastitis in ewes. Biochemical identification of bacteria was done to confirm it. The well diffusion method was done by measuring the zone of inhibition of each one, clove gel showed the largest zone compared with cinnamon gel 15mm, 10 mm respectively. Nine ewes were divided to three groups 3 ewes in each one. They were milked-out from the udder of each group before inducing the infection. Group B and C were infused intra-mammary with 1 ml ($7x10^2$ CFU/ml) of *Staphylococcus auras* suspension while the group A was served as a control. After the appearance of clinical signs, group B and group C were treated respectively with *S. aromaticum* gel and *C. zeylanicum* gel applied gently to the udder for five days. Milk of both halves were sampled twice daily for period during the treatment, for bacterial colonies, Somatic cell count, and milk fat content during the experimental infection and treatment. The primary clinical signs of mastitis that appeared after 48 hours of intra-mammary infusions. After treatment bacterial investigation on the 5th day revealed 80% and 60% cure rate in *S. aromaticum* and *C. zeylanicum* gel treated groups respectively. SCC in *S. arozmaticum* gel treated group was reduced significantly to $35x10^3$cell/ml than C. zeylanicum $48x10^3$cell/ml, with no significant increase in milk fat percentage. The therapeutic efficacy of *S. aromaticum* is more than C. *zeylanicum* gel in reducing the SCC and bacterial count in milk.

Keywords | Mastitis, *Syzygium aromaticum*, *Cinnamomum zeylanicum,* Plant extract, Phytochemical

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INTRODUCTION

Mastitis, a prevalent and economically significant condition in livestock, refers to inflammation of the mammary glands. This condition predominantly affects dairy animals, including cows, goats, and sheep, impacting milk production and the overall health of the herd (Nidhal *et al*., 2017; Agharid *et al*., 2022). The main source of mastitis in animals is bacterial- infection, mostly assisted by environmental influences and reduced milking practices.

Bacteria, such as *Staphylococcus aureus, Pseudomonas aeruginosa* and *Streptococcus species*, can invade the udder through the teat duct, which result in inflammation and a range of symptoms (Yousif *et al*., 2008; Thompson-Crispi *et al*., 2013; Liu *et al*., 2017). Mastitis establishes with swelling, warmth, localized breast pain, and redness, mainly accompanied by the formation of hardened or lumpy areas within the affected breast. Modifications in breast milk consistency, flu-like symptoms, including fatigue, body aches, and the occurrence of a fever and chills are considered as a common indicator. Furthermore, some animals may experience general discomfort, nipple discharge, and a decrease in milk yield. While signs can differ, mastitis typically affects one breast in breast-feeding women, they develop quickly. Seeking prompt medical attention is vital for an exact diagnosis and appropriate treatment, as early intervention mitigates the risk of complications and facilitates a quicker recovery (Jassim and Al-Hadithy, 2014; Cheng and Han, 2020). The treatment of mastitis with antibiotics is a standard and effective approach, especially when the condition is caused by bacterial infection. Commonly prescribed antibiotics for mastitis include dicloxacillin, cephalexin, or amoxicillin, chosen based on the specific bacteria involved and their susceptibility to the medication, Antibiotics work by targeting and eliminating the causative bacteria, reducing inflammation, and facilitating the resolution of mastitis (Boakes *et al*., 2018; Wongsawan *et al*., 2019; Tommasoni *et al*., 2023). Furthermore, to antibiotics, relieving of pain by using over-the counter medicines, warm pads, preserving a regular breast-feeding to guarantee appropriate milk drainage which is considered as an important part of treatment. Suitable rest, keep hydration, and nutritional care participate in overall healing process. Clove oil and cinnamon oil, extracted from their plants are identified for their potential antimicrobial, anti-inflammatory, and analgesic properties. Clove oil contains eugenol, a composite with anti-inflammatory and antimicrobial properties (Ibrahim *et al*., 2023). Whereas cinnamon oil contains a good amount of cinnamaldehyde, which also holds an antimicrobial effect. In the context of mastitis, these oils are occasionally considered for their potential to fight bacterial infections and decrease inflammation in the affected breast tissue (Abd El-Aziz *et al*., 2021). Also, some studies revealed the rapid diagnosis of microorganisms such as viruses or bacteria using modern techniques such as Polymerase Chain Reaction (PCR) has revolutionized for understanding of bacterial or viral infections and their impacts on health (Jumaa *et al*., 2019, 2020; Jumaa, 2024; Jbr and Jumaa 2024; Allmjeed *et al*., 2024). By accurately identifying viral or other microorganism's pathogens, researchers can better tailor treatments, including the use of medicinal plants known for their antibacterial or antiviral properties (Jumaa *et al*., 2021; Ibrahim *et al*., 2023). This connection between advanced diagnostic methods and herbal therapies not only enhances the effectiveness of treatments but also promotes a holistic approach to managing viral or bacterial infections or modulate the immune system and offering a synergistic strategy to improve long-suffering outcomes. This study aimed to determine if the topical gels containing *Syzygium arozmaticum* and *Cinnamomum zeylanicum* are effective in treating of mastitis in ewes.

MATERIALS AND METHODS

Microorganism

The *Staphylococcus aureus* strain LOC-M2020 obtained from Physiology, Biochemistry and pharmacology Department College of Veterinary Medicine, University of Baghdad.

Re-identification of *Staphylococcus aureus*

The bacteria were inoculated on nutrient agar plate using a sterile technique. The plate was incubated aerobically at 37°C for 24 hours.

Biochemical tests

Catalase test was prepared by transferring a small portion of bacterial colony on a clean glass slide, then a drop of 3% of hydrogen peroxide (H_2O_2) was added to investigate the presence of bacteria. While, coagulase test was done by placing a drop of normal saline on a glass slide, and a colony was emulsified in it, then a drop of plasma was added to investigate the presence of bacteria.

Plants collection

Fifty grams of dried *(Syzygium aromaticum)* (clove buds) and *(Cinnamon zeylanicum)* (barks) were bought from local market in Baghdad during October 2023.

Clove and cinnamon barks extraction

By using a grinder, the dried clove buds and cinnamon barks samples were ground. The Soxhlet apparatus was filled up with 50 grams from each sample separately. Ethanol 90% was put in a rounded flask with the steaming chip granules. At 70-80 °C, the extraction procedure went on for seven to eight hours. The mixture was then filtered, and the crude extract was obtained by evaporating the solvent using a rotary vacuum evaporator. The extract was weighed and stored for further test (Ibrahim *et al*., 2023).

Phytochemical analysis

Through color reactions with different reagents, the test was able to detect the biochemical components present in distinct plant extracts of *S. aromaticum* and *C. zeylanicum*. Using a standard approach, flavonoids, phenolic, alkaloids, steroids, saponins, glycosides, tannins, diterpine, carbs, and protein were measured from each plant (Ibrahim *et al*., 2023).

Preparation of gel

According to Osanloo *et al.* (2022), gel formulation is made using the physiologically and pharmaceutically inert and non-sensitizing Carbapol polymer, which is used as a stabilizer and suspender for many pharmaceutical products. A magnetic stirrer set to 700 rpm and 30°C for 30 to 60 minutes was used to continuously stir 1.0 gram of carbopol powder in 80 ml of deionized water to create carbopol 1%. A deionized water was then added to bring the volume up to 100 milliliters. A few drops of triethanolamine (TEA) were added to bring the pH to approximately 7. To prepare a carbapol gel containing plant extract at concentration of 0.05%, the amount of *S.aromaticum* and *C. zeylanicum* extract needed were 50 mg, respectively which were added to 100 gram of carbapol gel to obtain the final desired concentration.

Pharmacodynamics analysis

Assessment of antibacterial activity

The agar well diffusion technique was done for evaluation the antibacterial action of the plant extract, five ml of standardized bacterial stock suspensions (1.5×10^8 cfu/ml) of *Staphylococcus aureus* was add to 200 ml of sterile Mueller Hinton agar. Twenty-five ml of the agar was dispersed into Petri dishes, after solidification, making 4 wells (six mm) in diameter were made, after that wells were loaded with 0.1 ml of each extract concentration (100, 200, 300, 400, and 500) mg/ml. The plates were then put in the incubator in upright position at 37 Co for 24 hours. Three plates were made for each extract concentration and the strength of these extracts was dictated by measuring the inhibition zone diameter around every well against the tested bacteria. Standard error and mean were calculated (Ibrahim *et al.*, 2023).

Induction of experimental mastitis

Nine ewes in the middle of their first lactation were randomly divided into three groups. An overnight inoculated trypticase soy agar with *S. aureus* strain LOCM2020 was utilized to prepare 7×10^2 cfu/ml bacterial suspensions in phosphate buffer saline; Six ewes were served as a model for experimental mastitis. After the appearance of clinical signs, and California Mastitis test of both mastitis and control milk.

Treatment protocol

Clinical mastitis detected and spread among three groups, each group containing three animals: Group A: consider as a control group which was not given any treatment. Group B: Treated with application of gel containing clove extract 0.05%. Group C: Treated with application of gel containing cinnamon extract 0.05%. Both formulations were applied gently to the ewe's udders two times a day for five consecutive days after every milking.

Parameters

Clinical sing, milk samples were gathered before infection, after infection, and after treatment for California Mastitis Test (CMT), Somatic cell count, milk yield, milk fat content % and microbiological investigation (Tommasoni *et al.*, 2023).

RESULTS

Bacterial identification

Culture isolation

The cultured colonies exhibited a smooth, opaque and golden-yellow pigmentation on nutrient agar (Figure 1).

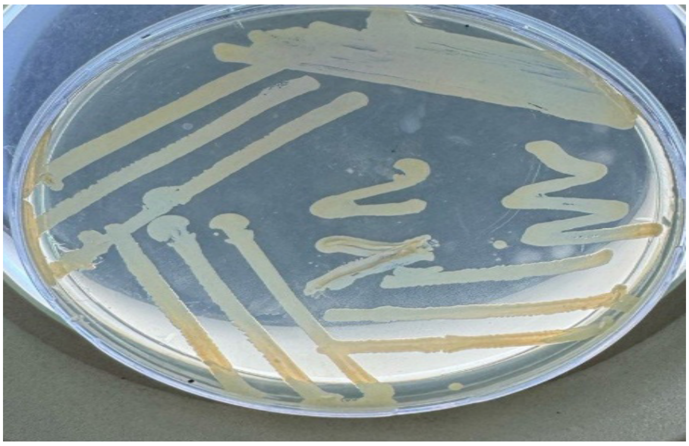

Figure 1: The appearance of *Staphylococcus aureus* on nutrient agar.

Catalase and coagulase tests

The result revealed the appearance of immediate bubbles which indicate a positive result of catalase. A slide method test showed the appearance of clumping within 10 seconds which indicate the positive result of coagulase test which confirming the presence of *Staphylococcus aureus* (Figures 2, 3).

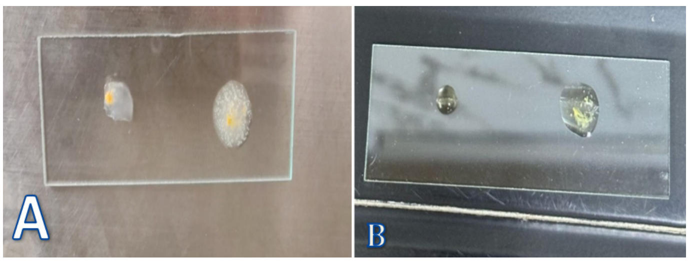

Figure 2: (A) Positive catalase test showing immediate bubble formation and (B) positive slide coagulase test showing visible clumping within 10 seconds, confirming the presence of *Staphylococcus aureus.*

Extraction of plant

Extraction of clove bud and cinnamon barks with 90% ethanol gave a pale yellow and deep brown color, respectively with yield percentage of 25 % and 32%, respectively.

Phytochemical analysis

Active components detection of clove bud and cinnamon barks extract in 90% ethanol were recorded in Table 1. The extract screening revealed the presence of the different chemicals; alkaloids, phenol, saponins, flavonoids, tannins and glycosides, steroids.

Table 1: Phytochemical analysis of plant extract.

Phytochemical	Clove bud	Cinnamon barks
Flavonoids	+++	++
Phenolic	+++	++
Alkaloids	+	+++
Steroids	+	+
Saponins	+	+
Glycosides	+	+
Tannins	+	+
Carbohydrates	-	+
Protein	+	-

+ = positive , ++ = good presence, +++= strong presence, – = not detected

Sensitivity test

Using different concentrations of the plant extract in agar well diffusion methods, resulting in different sizes of inhibition zone against *staphylococcus aureus*. The size of inhibition zones was different according to concentration of the plant extracts. Results indicated that *S. aureus* were more sensitive significantly (P<0.05) to *S. aromaticum* extract than *C. zeylanicum* in a concentration dependent Figure 3.

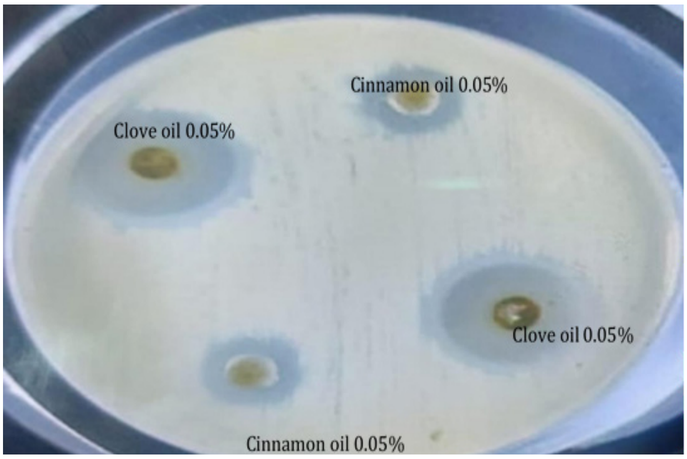

Figure 3: A zone of inhibition of clove oil and cinnamon extract against *S. aureus*.

Induction of mastitis

The progression of experimental induction of mastitis was illustrated in the (Tables 2 and 3). The primary clinical signs of mastitis that appeared after 48 hours of intra-mammary infusions of ≈ 7 x 10^2 CFU/ml of *S. aureus* in ewes were hard udder by palpation, pain during milking of the infused half and localized warmth of the infused udder in comparison to the control udder, after 48 hours later, body temperature raised by 0.1-1.5 °C in comparison to control (38.5 °C), restricted movement, congested mucous membranes, engorgement of capillary blood vessels and watery milk appearance (Figures 4A, 5 and 6).

Table 2: Clinical singe of mastitis by *S. aureus* in ewes.

No. infused CFU of bacteria	7 x 10^2 CFU/ml (two infusions during 24 hrs)
Prevalence of Infection	90%
Lag time of infection	72 hours
Primary clinical signs	Hard udder by palpation localized temperature pain during milking
Progressed clinical signs	Rectal temperature 40 – 41 °C. Engorged blood capillary vessels. Congested mucous membranes. Restricted movement. Watery milk appearance.

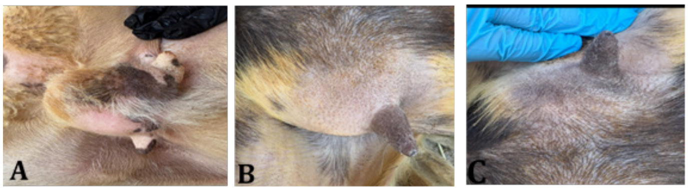

Figure 4: (A) Clinical singe of Mastitis by *S. aureus* in ewes, (B) treated ewes with cinnamon gel 0.05% (C) treated ewes with clove gel 0.05%.

Figure 5: California mastitis test.

Table 3: Somatic cell count, milk yield and milk fat content % before and after treatment.

Groups	SCC		Milk yield ml/day		Milk fat content %	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Group A	6.5x10^4	7.4x10^4	228±17	216±11	8.17±0.21	8.25±0.19
Group B	6.8x10^4	3.5x10^4	312± 15	542± 12	8.65±0.15	8.87±0.41
Group C	7.2.x 10^4	4.8.x 10^4	298±13	470±18	8.42±0.20	8.53±0.25

Figure 6: Grayish watery appearance

Table 4: Shedding of *Staph. aureus* (CFU/ml) in milk during and after gel application.

Time day	Control infected untreated	Treated with clove gel	Treated with Cinnamon gel
0	$72x\ 10^3$	$87x\ 10^3$	$82x\ 10^3$
1	$84x\ 10^3$	$54x\ 10^2$	$61x\ 10^2$
2	$69x\ 10^3$	$33x\ 10^2$	$42x\ 10^2$
3	$75x\ 10^3$	$12x\ 10^2$	$15x\ 10^2$
4	$64x\ 10^3$	N.D	N.D
5	$81x\ 10^3$	N.D	N.D

ND: Not detected.

Table 4 showed the result of microbial plate counting of *S. aureus* colonies the bacterial count a remarkable decline ($P \leq 0.05$) in the number of colonies after gel containing extract application of the 2nd day; this drop had been remained throughout the 2nd day of treatment in comparison to control group, while at the 4th day the plates that streaked with treated milk showed no bacterial growth in comparison to control group; that absence remained along the time of bacterial growth tracking with significant observed udder health restoration after complementary therapy and post-care after therapy (Figure 4B, C).

DISCUSSION

The main problem related to mastitis incidence persists despite the fact that various control strategies for the disease have been in place for a long time. Although the primary treatment strategy still involves the use of antibiotics, the increasing usage of herbal therapies can be attributed to their limited effectiveness (Cheng and Han, 2020). *In vitro* research has indicated promise for the use of plant medications in the management of ewe mastitis (Baskaran *et al*., 2009; Choi *et al*., 2012; Fratini *et al*., 2014; Budri *et al*., 2015; Piotr *et al*., 2018). Furthermore, there is paucity of research that examine the effectiveness of natural formulations in vivo for treating mastitis in ewes (McPhee *et al*., 2011; Alekish *et al*., 2017). Additionally, only a small number of them concentrated on using plant products to treat mastitis in ewes (Hase *et al*., 2011; Mullen *et al*., 2014; Sheehan *et al*., 2022). The goal of this study was to create a pharmacological formulation in the form of gel that could be applied to the affected udder. The suggested gel's antibacterial capability and phytochemical composition were previously examined in vitro as plant gel components are usually acknowledged to be harmless (Kovačević *et al*., 2021a, b; Saadoon, 2022; Tomanić *et al*., 2022). The development of pharmacological formulations has been aided by the encouraging findings of these investigations (Tomanić *et al*., 2022). This study revealed that clove buds and cinnamon barks extract contain several active phytochemical components like flavonoids, phenol, alkaloid, steroid, saponin, glycoside, and tannin. These ingredients working alone or in combination may cause a broad-spectrum antimicrobial activity against both bacteria and fungi. Clove oils produce antibacterial activity against *S. aureus*, at different dilutions of the extracts, these outcomes agree with the result of other workers (Adarsh *et al*., 2020; Al-Dujaily and Mahmood, 2021; Lone *et al*., 2022) who revealed the presence of these phytochemicals using different solvent extraction. Numerous studies testified the presence of bioactive phytochemicals like alkaloids; carbohydrates, phenolics, proteins, tannins, glycosides, saponins and fibers in this plant are responsible for their antimicrobial potential (Jain and Argal, 2014). Mastitis caused by *staphylococcus aureus* affected by the action of clove oil which may attributed to the lipophilic nature of clove oil that facilitates its interaction with the phospholipid in the membrane of bacteria, thereby increased the permeability and cause damage to the membrane (Radünz *et al*., 2019). Cinnamon oil contains a good amount of phenolics which has ability to cross through the lipid layers of bacterial cell membrane and interact with proteins to stop them from doing their functions normally, the modification in the permeability of membrane and the loss of functional proteins transporting molecules and ions imbalance the microbial cell. This consequently head to denaturation of several enzymes and proteins, cytoplasmic coagulation, and the loss of ions and metabolites (Vasconcelos *et al*., 2018). The combination of extracts from *Syzygium aromaticum, Cinnamomum bark* displayed significant antibiofilm and antibacterial activities against clinical isolates and a reduction in the virulence factors (Mustafa and Wasman, 2020; Ranjani *et al*., 2022). The purpose of high reliance on medicinal herbs might be due to high cost of traditional drugs, poorsocioeconomic conditions of the farmer and their inaccessibility to the modern veterinary practices that are usually in city areas and fewer resources for treating large population of animals (Mussarat *et al*., 2014). The experimental induction of *Staphylococcus aureus* intramammary was induced by dipping teat with newly grown suspension of bacteria in the middle of their first lactation. Although variation in the challenging procedures and physical status of studied

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animals and these outcomes is complying with (Enger *et al*., 2018) who conveyed that intra-mammary infusion of *Staphylococcus aureus* caused recognized intra-mammary infection in eighteen of the nineteen infused quarters with increasing in the somatic cell count. The existence of inflammatory alterations in mammary gland can be determined by increasing in somatic cell count, which might lead to changes in the constituents of milk such as fat, protein and lactose, etc. and reduction in milk yield (Ahmed and Yousaf, 2021; Kamal, 2022). The reduction in milk yield was seen when somatic cell count raised and these results were in accordance with prior research (Bansal *et al*., 2005; Neamah *et al*., 2020). Though, it was found that the milk fat rate was raised. In this study, the use of alternative therapy such as clove oil and cinnamon oil to treat mastitis and avoid antibiotic resistant because the consistent use of antibiotics, sometimes misused or overused, builds a selective pressure on both pathogenic bacteria and commensal bacteria, such as gut or skin microbiota, that can result in the prevalence of antibiotic-resistant bacteria, and the spread of resistant bacteria or genes (Mohanad *et al*., 2018; Caneschi *et al*., 2023). These plant extract oils have an antibacterial effect by causing a leakage of cellular components and loss of ions due to the increased membrane permeability, disruption of protein pumps, and the depletion of ATP leading to cell lysis and death (Saad *et al*., 2013; Karm, 2019; Mahbula *et al*., 2022). In a study comparing the effects of clove, oregano, and a control group that did not receive any treatment, it was discovered that cattle fed these essential oils during the treatment period produced 4.3% more milk than the non-supplemented cows. They also showed improved feed efficiency, increased milk production, increased milk protein, and decreased counts of somatic and bacterial cells (Merkhan, 2019; Al-Suwaiegh *et al*., 2020). As dairy sheep were fed a mixture of EOs, their milk output increased and their somatic cell count significantly decreased as compared to the infected group (Giannenas *et al*., 2011).

CONCLUSIONS

Ewes' mastitis has a very significant global impact because of the decreased yield and low milk quality. The dairy industry suffers significant financial losses due to ewe's mastitis, a severe illness that can be safely treated and prevented. Plant-derived EOs and certain of their constituents hold a great deal of promise in this regard. One of the most global concerns facing the food and veterinary industries is the presence of antimicrobial residues in milk, as it can disrupt the manufacture of dairy products and lead to the development of resistance. Further in vivo research on the withdrawal duration of essential oil-based pharmaceutical formulations in the treatment of bovine mastitis is necessary to determine the efficacy of EOs since the costs associated with rejecting milk due to the withdrawal phase may exceed the costs of the therapy.

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NOVELTY STATEMENT

This study provides the first comparative evaluation of *Syzygium aromaticum* and *Cinnamomum zeylanicum* gels as alternative topical therapies for mastitis in ewes in Iraq, demonstrating the superior efficacy of *S. aromaticum* in reducing bacterial load and somatic cell count without affecting milk quality.

AUTHOR'S CONTRIBUTION

RSJ and OMSI: Designed the experiment. RSJ and BMA: Interpreted the data and drafted the manuscript. BMA and RSJ: Carried out the experiment, investigation, and resources, OMSI, BMA, RSJ. RSJ, and OMSIK: Performed the statistical analysis and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Ethical approval

All procedures in this study followed the guidelines of local Ethics Committee at the College of Veterinary Medicine, University of Baghdad and approved under no. P.G./1210 on 24 June 2024.

Generative AI and AI-assisted technology statement

We did not use AI technology for the current research, except Grammarly for Grammar and spell check.

Conflict of interest

The authors have declared no conflict of interest.

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