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Efficacy of *Garcinia mangostana* Linn. and *Achyranthes aspera* Linn. Combined Extracts in the Prevention of Endometritis in Cattle

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ABSTRACT

Endometritis is an important factor in cattle fertility. Pathogenicity and the development of numerous reproductive diseases are directly related to bacterial imbalance in the genital tract. A commercial antibiotic can be relatively costly and can disrupt the animal's usual gut microflora; instead of plant medicals. The aim of this study was to develop an effective artificial insemination (AI) gel from traditional Thai herbs that exhibit bacterial inhibition. Twenty-four female Thai native cattle were divided into two groups: endometritis and healthy. Uterine swabs were isolated, identified, and tested for bacterial biofilm formation *in vitro*. *Brucella ovis*, *Campylobacter fetus*, *Helicobacter trogontum*, and *Arcobacter cryaerophilus* were found in female genitalia with endometritis based on weak biofilm information. *Garcinia mangostana* Linn. and *Achyranthes aspera* Linn. extracts were tested for antibacterial activity using agar dilution assay. A 10 µg/mL concentration of both extracts in combination was effective against the mixed bacterial isolation. The specific AI gel with those extracts was then developed (so-called GA-Gel) *in vivo*. The combined extracts inhibited the endometritis bacteria that expressed antimicrobial activity *in vivo*. Their hematological profiles indicated that the total white blood cells, neutrophils, and lymphocytes counts decreased ($p \leq 0.05$). Compared to healthy cattle, the treated cattle had no significant difference in the levels of aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine. Both *in vivo* and *in vitro* indicated that the GA-Gel was effective for the prevention of an increase of bacteria and can be potentially developed to be an efficient AI gel.

***Keywords:** artificial insemination gel; cattle endometritis; Garcinia mangostana Linn.; Achyranthes aspera Linn*

INTRODUCTION

Metritis and endometritis play important roles in causing infertility, lower performance, early culling, and genetic losses, which can result in dairy cows being unable to reproduce efficiently (Shafique *et al.*, 2021). Bacterial intrauterine contamination by aerobic and anaerobic Gram-positive and Gram-negative microorganisms has been associated with the disease (Mileva *et al.*, 2022). Bacterial contamination in the uterine lumen after artificial insemination is common in dairy cattle. During and after the unhygienic artificial insemination of dairy cows, a wide range of microorganisms arise from the environment, invade the birth canal, and colonize the uterus (Adnane & Chapwanya, 2022; Messman *et al.*, 2020). Most healthy cows will naturally eliminate this contamination within the next 2-4 weeks. The persistence of pathogenic organisms, however, plays a role in developing clinical metritis or clinical endometritis (Sheldon *et al.*, 2020; Drillich & Wagener, 2018). *Arcanobacterium pyogenes*, *Prevotella spp.*, *Fusobacterium necrophorum*, and *Escherichia coli* are the most common pathogenic bacteria isolated from endometritis cases. Several studies have shown that *Staphylococcus* spp., *Streptococcus* spp., and *E. coli* have widely accepted these organisms as the dominant bacteria in the uterus (Adnane & Chapwanya, 2022; Becker *et al.*, 2023). Knowledge of endometritis in dairy cows, its bacterial detection, and antimicrobial susceptibility is sparse and not up to date in Thailand.

Endometritis is usually treated with antibiotics. A range of antimicrobial drugs is often administered by intrauterine infusion or parenteral injection to treat uterine infections (Mileva *et al.*, 2020; Singh & Sethi, 2022). It is crucial to understand the sensitivity of the pathogen to antibiotics before selecting an effective antimicrobial drug for endometritis treatment. As antimicrobial resistance in pathogenic bacteria has become a widespread problem, information on antibiotic susceptibility should be available for the treatment of endometritis. Endometritis is susceptible to several antibiotics, which can be fairly costly and can disturb the animal's normal gut microflora. However, the overuse and indiscriminate use of antibiotics to treat bacterial infections of the

uterus has led to the emergence of antibiotic-resistant bacteria strains. In fact, the overuse of antibiotics has led to multiple drug resistance and increased cattle mortality rates (Diep & Ngu, 2023).

Plant materials are used as natural medical systems for antibacterial, anti-inflammatory, and anti-oxidative purposes as they have few side effects compared to conventional antibiotics and are also pathogen tolerant (Michalak, 2022; Parham *et al.*, 2020). *Garcinia mangostana* Linn. (mangosteen) is a tropical fruit commonly used as a health food and dietary supplement in Southeast Asia. Many studies have shown that the mangosteen fruit has antibacterial, antioxidant, anti-proliferative, anti-carcinogenic, and anti-inflammatory properties. Xanthones are chemicals substances contained in the pericarp that include garcinone B, garcinone E, and alpha-mangostins. The anti-inflammatory effect of α-mangostin has been shown to have an inhibitory effect against various bacteria, including *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* (Tatiya-Aphiradee *et al.,* 2019; So-In & Sunthamala, 2022). *Achyranthes aspera* Linn. is also a medicinal plant native to Asia, South America, and Africa. *A. aspera* Linn. has been found to have antibacterial, antifungal, thyroid-stimulating, antioxidant, anti-inflammatory, anti-arthritic, immunomodulatory, wound healing, anti-obesity, antispasmodic, anticancer, and hepatoprotective effects. This plant has been shown to contain a variety of phytochemicals, including saponins, phenolic compounds, flavonoids, alkaloids, steroids, and terpenoids (Sinan *et al.,* 2020; Sunthamala *et al.*, 2021). Previous studies demonstrated the efficacy of *Garcinia mangostana* Linn. and *Achyranthes aspera* Linn. against various bacteria, suppression of the release of proinflammatory cytokines, and neutralization of reactive oxygen species (ROS) by increasing antioxidant enzyme activity (So-In & Sunthamala, 2022; Sunthamala *et al.*, 2021).

However, there is a synergistic effect of combining two natural extracts, which increases the efficacy of preventing bacterial infections and reduces inflammation caused by artificial insemination in cattle. Thus, a key need is to develop innovative and effective artificial insemination (AI) gel in order to reduce the spread and effectively control the disease. None of the AI gel products produced in Thailand are non-toxic, particularly those extracted from traditional Thai herbs. Thus, this study focused on identifying the causative bacteria that cause endometritis in cattle and then developing an effective artificial insemination (AI) gel from traditional Thai herbs to inhibit the progression of bacterial dispersion.

MATERIALS AND METHODS

Experimental Design

Two to three years of female native Thai cattle from Kalasin province were from traditionally managed farms. After 30 days of general observation (no treatment), we started the experiment by selecting 24 of them and dividing them into four groups (six cattle per group): (1) the group that was diagnosed with endometritis and was then treated with the GA-Gel; (2) cattle with endometritis, and then treated with a commercial AI gel; (3) the group with healthy cattle that were subsequently treated with the GA-Gel; and (4) the group with healthy cattle that were subsequently treated with a commercial AI gel.

The cattle with subclinical endometritis were categorized into groups 1 and 2. Ultrasonography was employed as a diagnostic tool, relying on detecting intrauterine fluid and assessing uterine diameter. The presence of a limited quantity of fluid in the uterine cavity and/or increased thickness of the uterine walls can indicate the presence of endometrial inflammation (Osawa, 2021).

All cattle were kept semi-intensive, i.e., from 06:00 PM to 6:59 AM in the barn and from 07:00 AM to 05:59 PM on the floor. The diet consisted mainly of grass and hay, without control of additional grain and vitamins for individual animals. The climatic conditions for the study were from January 2022 to July 2022, i.e., during the Thai winter season. The study was conducted under the supervision of a veterinarian and in accordance with the proposal (IACUC-MSU-27/2022) approved by the Committee on Ethics and Standards for the Rearing and Use of Animals for Scientific Purposes of Mahasarakham University

Sample Collection

Samples of uterine swabs of all cattle were taken from both endometritis and healthy cattle to isolate and identify bacteria. Sampling was performed as follows: (1) The vulva area was wiped with a tissue and then disinfected with 70% alcohol, (2) A sterilized colposcope was inserted into the cow's vagina, (3) A sterilized swab was pushed through these instruments to collect the uterine sample. The swabs were kept in a test tube with Brain Heart Infusion (BHI) broth, and later, the samples were refrigerated at 2 °C to 8 °C until the bacteria were isolated and identified (Bicalho *et al.,* 2017). To determine the hematological profiles of all cattle, blood samples were collected and subjected to triplicate analysis. The blood sample was collected from the jugular vein and placed in the tubes containing the anticoagulant agent, ethylenediaminetetraacetic acid (EDTA). Plasma was extracted from blood by centrifugation at 400 g for 10 minutes at 4 °C and stored at -20 °C for subsequent analysis at the Hematology Laboratory, Faculty of Veterinary Medicine, Khon Kaen University, Thailand (So-In & Sunthamala, 2023).

Hematological and Biochemical Analyses

Plasma was analyzed using an automatic chemical analyzer (Konelab 20i, Thermo Fisher Scientific) at the Veterinary Laboratory diagnostic service, Faculty of Veterinary Medicine, Khon Kaen University, Thailand. The hematological parameters include red blood cells (RBC), hemoglobin, platelets, compressed red blood cells (PCV), and white blood cells (WBC) such as mono-

cytes, eosinophils, neutrophils, and lymphocytes. The biochemical parameters include Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Blood urea nitrogen (BUN), and creatinine (Kongchian *et al.*, 2020).

Bacterial Isolation and Identification by Polymerase Chain Reaction

In the first step after collection, bacteria on the swab samples were propagated on ice in BHI broth for 2 hours. The bacteria were cultured at 37 °C for 6 hours before the bacterial cells were collected for DNA extraction. The mixed bacteria were preserved in a 15% glycerol broth and stored at -20 °C for further experiments. The total DNA was extracted from mixed bacterial cultures. Bacteria strains were subsequently detected by polymerase chain reaction (PCR). The specific primers used to amplify and identify bacteria strains include *Brucella ovis*, ORFs AO503 (Xavier *et al.*, 2010), *Campylobacter fetus*, ctdA gene (Asakura *et al.*, 2008), *Helicobacter trogontum* (Mendes *et al.*, 1996), and *Arcobacter cryaerophilus*, 23S rRNA (Houf *et al.*, 2000), which are listed in Table 1. The PCR products were electrophoresed on a 1.5% agarose gel at 110 V, 400 mA for 30 minutes. The images were finally visualized under the UV light.

Antimicrobial Susceptibility Test

The agar dilution assay was used to study the minimum inhibitory concentration (MIC) of antimicrobial agents and crude extracts on Mueller-Hinton agar according to Clinical and Laboratory Standards Institute (CLSI) recommendations (Shang *et al.*, 2019; Malinowski & Kłossowska, 2010). Antibacterial agents include gentamicin, penicillin, streptomycin, and polymyxin B at a concentration of 1-64 μg/mL. The extracts of *G. mangostana* Linn. and *A. aspera* Linn. were used for antibacterial susceptibility at a concentration of 1.25-10 μg/mL. The result of inhibition was not evident in any colony at any site. The experiment was performed in triplicate, with the experiments being triplicate dependent.

Biofilm Formation Assay

Biofilm formation assay was determined using a quantitative spectrophotometric microtiter plate assay. 200 μL of mixed bacteria cultures at a concentration of 0.5 McFarland standard was cultured in Trypticase soy broth (TSB) in a 96-well plate for 24 hours. Each sample was cultured in triplicate, with the experiments being triplicate dependent. After 24 hours, the culture media was discarded. The bacterial biofilm was stained with 200 μL of 0.1% crystal violet in absolute ethanol for 10 min and then washed 3 times with distilled water. The stained biofilm was dissolved with 200 μL of absolute ethanol and then transferred to a new 96-well plate. The absorbance was examined at 570 nm using a plate reader. The arithmetic mean of absorbance (Abs) was compared to that of the negative control (*Escherichia coli* ATCC 25922). Biofilm formation was classified into 4 categories, i.e., no biofilm formation (Abs sample/Abs control), weak biofilm formation (Abs control < Abs sample / 2 × Abs control), moderate biofilm formation (2 × Abs control < Abs sample / 4 × Abs control), and strong biofilm formation (4 × Abs control < Abs sample) (Bochniarz *et al.*, 2018).

Preparation of *Garcinia mangostana* Linn. Combined with *Achyranthes aspera* Linn. Artificial Insemination Gel

One kilogram of air-dried and grated herbs was extracted by maceration with 70% ethanol for seven days. A Rotary Evaporator R-II (Buchi Company, Flawil, Switzerland) was used to evaporate the crude extract under reduced pressure. Weighing the dried residue of extracted herbs enabled the calculation of the percentage yield. The biologically active chemicals, including phenols and flavonoids were examined according to So-In & Sunthamala (2022). The total phenolic and total flavonoid content of the extracts were compared with gallic acid and quercetin, respectively. The GA-Gel was prepared by using the extract of *G. mangostana* Linn. and *A. aspera* Linn. at the rate of 10 μg/mL as well as thickening agent, humectants, preservative, pH adjusting agent, and sterile distilled water to obtain a slippery hydrogel (Figure 1). The GA-Gel underwent comprehensive testing to evaluate its microbiological, physical, and chemical characteristics, thereby guaranteeing the product's safety and quality.

In Vivo Evaluation of Effectiveness of *Garcinia mangostana* Linn. Combined with *Achyranthes aspera* Linn. Extract gel

The GA-Gel and commercial artificial insemination (AI) gel were used for treatment *in vivo*. In two groups,

Table 1. Specific primers for detection of endometritis-associated pathogenic bacteria

Gene	Primer sequence	Product size (bp)	References
Brucella ovis, ORFs AO503	Forward: 5′-GCCTACGCTGAAACTTGCTTTTG-3′	228	
	Reverse: 5′-ATCCCCCCATCACCATAACCGAAG-3′		Xavier *et al.*, 2010
Campylobacter fetus, ctdA gene	Forward: 5′-AACGACAAATGTAAGCACTC-3′	397	
	Reverse: 5′-TATTTATGCAAGTCGTGCGA-3′		Asakura *et al.*, 2008
Helicobacter trogontum	Forward: 5′-CATAGGTAACATGCCCCA-3	888	
	Reverse: 5′-CTGTTTCAAGCTCCCC-3′		Mendes *et al.*, 1996
Arcobacter cryaerophilus 23S rRNA	Forward: 5′-TGCTGGAGCGGATAGAAGTA-3′	257	
	Reverse: 5′-AACAACCTACGTCCTTCGAC-3′		Houf *et al.*, 2000

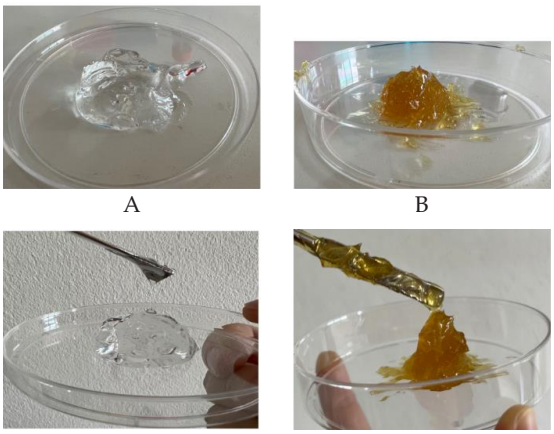

Figure 1. Characteristics of insemination gels. A and C are commercial insemination gels, and B and D are the insemination gels in this study containing *Garcinia mangostana* Linn. and *Achyranthes aspera* Linn. extracts (GA-Gel).

each treatment gel was applied to two separated and isolated areas of endometritis lesions, vagina, urethra, and vulva. The GA-Gel was prepared aseptically in the laboratory at Mahasarakam University, Thailand, and applied liberally with a gel bottle. Following Frye *et al.* (2019), this GA-Gel concentration was chosen to meet commercial safety standards. For seven days, each drug was applied once daily to the same affected area. On days 1 and 7, photographs were taken in natural light using a Canon EOS Rebel T5 digital SLR camera and a regular EF-S 18e55 mm f/3.5-5.6 IS II lens. A scoring scale was developed based on published criteria (Frye *et al.*, 2019) for physical anatomy identification and in consultation with a veterinarian. The severity of each endometritis was assessed prior to drug administration (day 1) and again 24 hours later (day 7) by a professional veterinarian blinded to treatment type. The bacteria density in the endometritis lesions and in the uterus of the cattle was determined by the bacterial count on day 1 and day 7 after treatment with the GA-Gel.

Statistical Analysis

The prevalence of the pathogenic bacteria groups was determined by calculating the ratio of cattle that tested positive for the bacteria to the total number of cattle. Statistical differences in bacteria prevalence were tested using the Chi-square and Fisher's exact tests in Minitab (version 16.0) software. The experimental results of hematological profiles were examined using one-way ANOVA and Tukey's multiple comparison tests (GraphPad Soft Inc. La Jolla, CA, USA, Prism version 10). The $p \leq 0.05$ level was deemed statistically significant.

RESULTS

Phytochemical Screening of Ethanol Crude Extracts

Table 2 shows the phytochemical screening for the presence of phenolics and flavonoids in crude extracts of *G. mangostana* Linn. and *A. aspera* Linn. The total phenolic and flavonoid contents of *G. mangostana* Linn. extract were 100.91 ± 2.64 g GAE/100 g crude extract and 111.76 ± 1.21 g QE/100 g crude extract, respectively. In the *A. aspera* Linn. extract, the total phenolic and total flavonoid contents were 7.79 ± 3.59 g GAE/100 g crude extract and 66.27 ± 3.06 g QE/100 g crude extract, respectively.

Identification and Biofilm Formation of Bacteria from the Metritis and Healthy Cattle Groups

Genomic DNA was extracted from the mixed bacteria of six clinical and healthy cattle. The bacteria were identified using gene-specific primers for *B. ovis*, *C. fetus*, *H. trogontum*, and *A. cryaerophilus*. Amplification was confirmed by agarose gel electrophoresis of the PCR results. Table 3 shows the bacteria detected in cattle with endometritis, such as Brucella ovis and Arcobacter cryaerophilus, which had prevalences that were statistically significantly higher than in the healthy group. *C. fetus* was found more frequently in the healthy group than in the endometritis group. *H. trogontum* was not found in either group of cattle. A study on the characteristics of biofilm formation in the isolates of bacteria showed a weak biofilm of bacteria in the endometritis group,

Table 2. Phytochemical screening for the presence of phenolics and flavonoids in crude extracts of *Garcinia mangostana* Linn. and *Achyranthes aspera* Linn.

Crude extracts	Phytochemicals	
	Total phenolics (g GAE/100 g crude extract)	Total flavonoids (g QE/100 g crude extract)
Garcinia mangostana Linn.	100.91 ± 2.64	111.76 ± 1.21
Achyranthes aspera Linn.	7.79 ± 3.59	66.27 ± 3.06

Table 3. Identification and biofilm formation of bacteria from the endometritis and healthy cattle groups

Pathogens	Healthy group (n=6)	Endometritis group (n=6)	Fisher's exact test ($p \leq 0.05$)
Brucella ovis	0 (0%)	3 (50%)	0.000
Campylobacter fetus	5 (83%)	1 (17%)	0.000
Helicobacter trogontum	0 (0%)	0 (0%)	1
Arcobacter cryaerophilus	1 (17%)	3 (50%)	0.000

which was statistically significantly more frequently formed than in healthy cattle (Table 4).

The Antimicrobial Effect of *Garcinia mangostana* Linn. Combined with *Achyranthes aspera* Linn.

The study focused on the antimicrobial activity of materials on bacteria isolated from cattle. The result showed that the combined extract inhibited bacteria isolated from endometritis cattle at a concentration of 10 µg/mL, as shown in Figure 2. Antibiotics such as gentamycin, penicillin, and streptomycin, as well as polymyxin B inhibited bacteria at a concentration of 8-32 µg/mL. The bacteria in the endometritis group were more strongly inhibited after treatment with gentamycin, penicillin, and streptomycin than in the control group. The group treated with polymyxin B showed the same inhibition pattern in both endometritis and healthy groups.

Hematological Profiles in Cattle

Hematological profiles were measured in the cattle blood from the jugular vein to confirm the effect of combined herbal extract gel on hematology. The amounts of erythrocytes, hemoglobin, platelets, PCV, monocytes, and eosinophils were not statistically different in all groups (Figure 3A, 3C, 3D-F, and 3H-I, respectively). When using GA-Gel in the endometritis group, the total number of WBC, neutrophils, and lymphocytes

Table 4. Biofilm formation characteristics of bacteria in endometritis and healthy groups

Biofilm formation characteristics	Healthy group (n=6)	Endometritis group (n=6)	Fisher's exact test (p≤0.05)
Non-adherant	61%	44%	0.023
Weak	39%	56%	

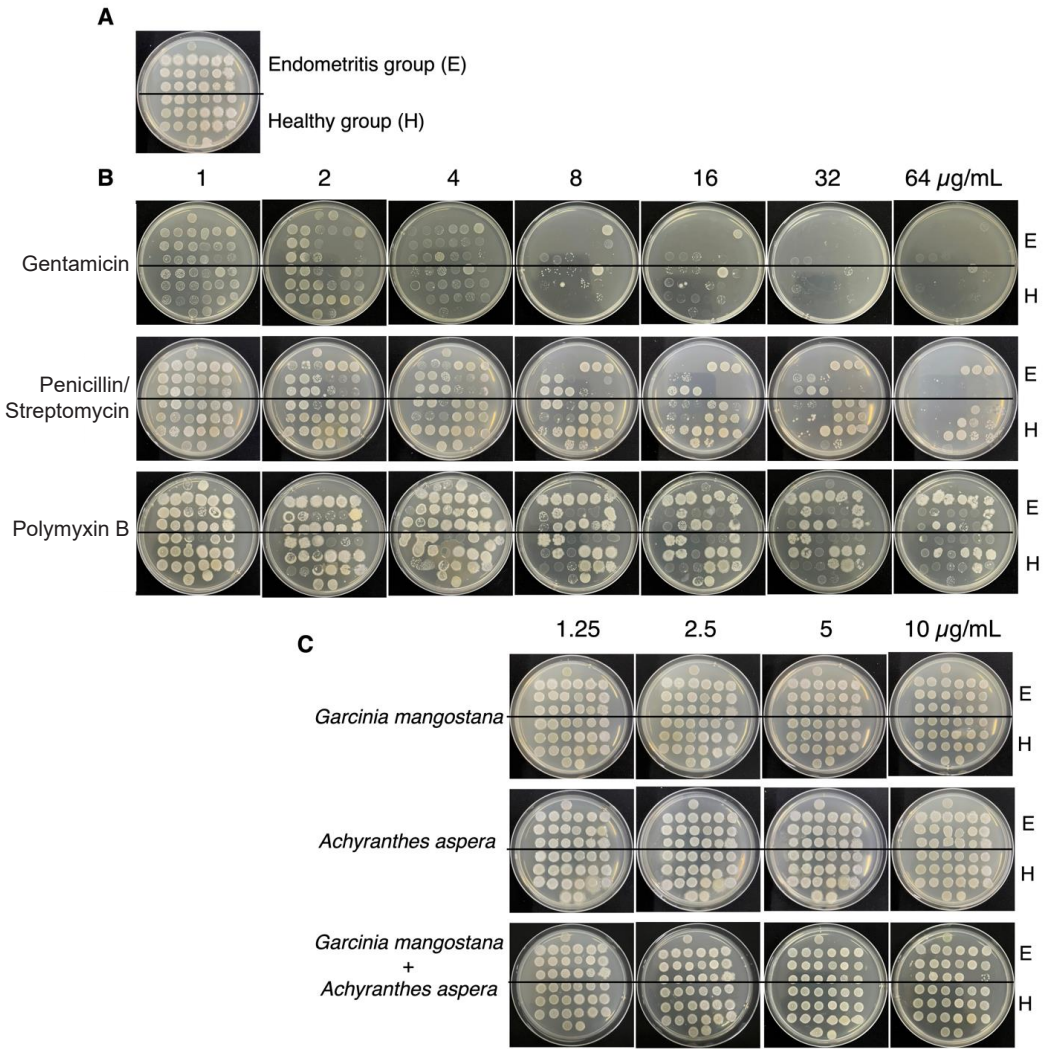

Figure 2. Antimicrobial activity of the herbal extracts. (A) Total bacteria in healthy and endometritis groups without antibiotics, (B) Total bacteria in healthy and endometritis groups with antibiotics such as gentamycin, penicillin and streptomycin, and polymyxin B at vary concentrations of antibiotics (1-64 µg/mL), and (C) Total bacteria in healthy and endometritis groups with *Garcinia mangostana* Linn., *Achyranthes aspera* Linn., and the combined extracts at vary concentration of extracts (1.25-10 µg/mL). E= endometritis group; H= healthy group.

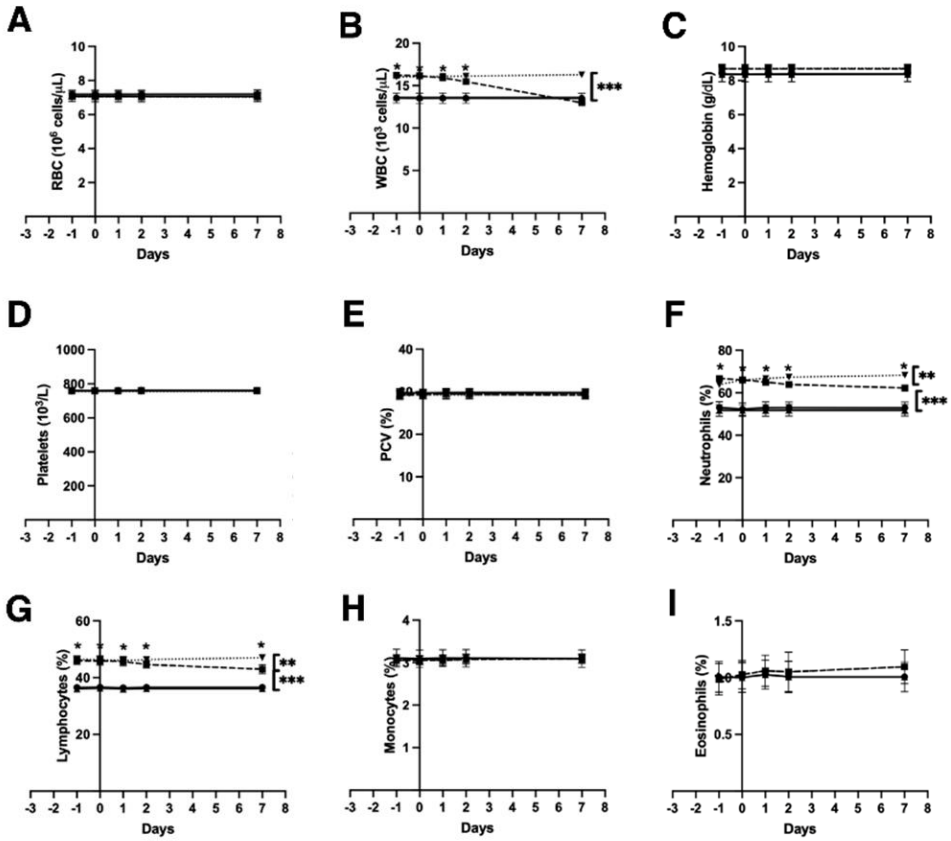

Figure 3. The hematology profiles of cattle with the GA-Gel compared to the commercial gel in healthy and endometritis groups. (A) red blood cells (RBC), (B) white blood cells (WBC), (C) hemoglobin, (D) platelets, (E) compressed red blood cells (PCV), (F) neutrophils, (G) lymphocytes, (H) monocytes, and (I) eosinophils. The data are presented as the mean ± SD based on 6 cattle per group with triplicates in each. Different symbols indicate a significant difference among different levels (*p≤0.05, ***p≤0.01, ***p≤0.001). ▲= Healthy commercial; ▼= endometritis commercial; •= healthy GA-Gel; ■= endometritis GA-Gel..

significantly decreased ($p<0.05$) (Figures 3B, 3F, and 3G, respectively).

Liver and Kidney Function in Cattle

Liver and kidney functions were measured to confirm the effectiveness of GA-Gel on liver enzymes, such as ALT, AST, BUN, and creatinine. There was no statistical difference between groups ($p<0.05$), as shown in Figure 4A-4D.

Female Genital Tissue in Cattle

In this study, the effects of GA-Gel on female genital tissue in cattle were compared with those of commercial AI gel. There were no effects on female genital tissue, whether pain, swelling, redness, or inflammation, in all groups after 7 days (Figure 5A-5D). The bacteria density at the lesions of GA-Gel administration before and after 7 days were not significantly different ($p>0.05$). The total aerobic bacteria density of the healthy group before and after 7 days of administration of GA-Gel were 36.5±3.3 and 34.5±3.2 colonies ($p=0.95$), respectively, while in the endometritis group before and after 7 days of administration were 50.5±15.8 and 43±10.2 colonies ($p=0.52$), respectively.

DISCUSSION

This study compares the efficacy to inhibit the growth of pathogenic bacteria of a combined herbal extract gel of *G. mangostana* Linn. and *A. aspera* Linn. (so-called GA-Gel) with the commercially AI gel in artificial insemination of cattle. There have been no studies of this form of a gel for artificial insemination. First, this study reported the identification of *B. ovis*, *C. fetus*, and *A. cryaerophilus* bacteria in the female genital organs of cattle with endometritis. Although there have been no reports on the isolation of bacteria from endometritis in cattle in Thailand, the studies by Campos-Múzquiz *et al.* (2019); Caruso *et al.* (2019) and Khurana *et al.* (2021) show the differences in bacterial isolation between healthy and endometritis cattle, especially *B. ovis* and *A. cryaerophilus*, suggesting that the disease may be caused by different pathogens.

The study also revealed a significantly higher prevalence of *C. fetus* in the healthy group compared to the endometritis group. Cows affected by genital campylobacteriosis do not have systemic abnormalities and can spread the disease through contaminated equipment or by artificial insemination with contaminated semen (Campos-Múzquiz *et al.,* 2019). Campylobacteriosis often has limited clinical impact

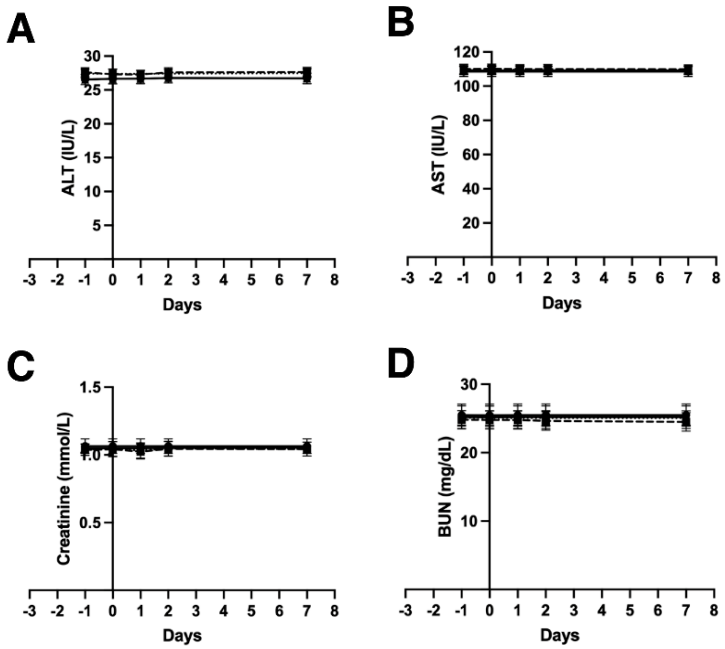

Figure 4. The liver and kidney function of cattle with the GA-Gel when compared to the commercial gel in healthy and endometritis groups. (A) alanine transaminase (ALT), (B) aspartate aminotransferase (AST), (C) blood urea nitrogen (BUN), and (D) creatinine. The data are presented as the mean ± SD based on 6 cattle per group with triplicates in each. Different symbols indicate a significant difference among different levels (*p≤0.05, ***p≤0.01, ***p≤0.001). ▲= Healthy commercial; ▼= endometritis commercial; •= healthy GA-Gel; ■= endometritis GA-Gel.

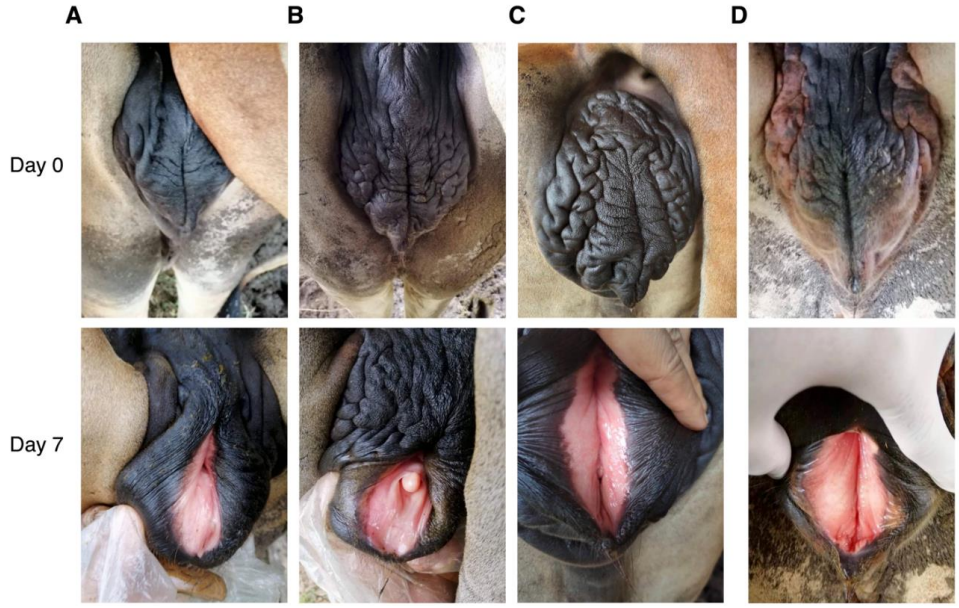

Figure 5. Exhibits the external and internal characteristics of vagina in cattle in days 0 and 7. (A) Healthy group with GA-Gel, (B) Endometritis group with GA-Gel, (C) Healthy group with commercial gel, and (D) Endometritis group with commercial gel.

on a small number of animals in a herd, while a larger number of carriers remain asymptomatic. *C. fetus* is an important sexually transmitted infection in cattle, leading to infertility and abortions (Campos-Múzquiz *et al.,* 2021). However, it does not directly cause of endometritis. The main consequences of the disease include premature embryonic mortality, fetal death, and impaired fertility. After infection, immunity gradually develops, and the majority of cows later achieve conception after two or more repeated mating attempts, even in the presence of a persistent organism in the caudal reproductive system (Michi *et al.,* 2016; Campos-Múzquiz *et al.,* 2021; Knipper *et al.,* 2022).

Furthermore, the antimicrobial activity of combined extracts of *G. mangostana* Linn. and *A. aspera* Linn. on mixed bacteria isolated from cattle was showed in this result. At a concentration of 10 μg/mL of the combined extracts, the effect inhibits the growth of

these bacteria. An important extract constituent of *G. mangostana* Linn. is α-mangostin, which has been shown to ward off bacterial infection by disrupting membranes and allowing the passage of intracellular contents (So-In & Sunthamala, 2022). Not only has its antimicrobial activity been reported, but also its anti-inflammatory and antioxidant effects. Secondary metabolites of *A. aspera* Linn., such as alkaloids, tannins, and polyphenols also have an important function as antibacterial agents and can be used to combat antibiotic resistance (Sunthamala *et al.,* 2021; Ahmad *et al.,* 2022; Mengie *et al.,* 2021). The herbs used to develop the artificial insemination gel *G. mangostana* Linn. and *A. aspera* Linn. with a pH of 6.5, do not contain parabens, dyes, flavor enhancers, and microorganisms.

In this study, GA-Gel was tested for 7 days in both healthy and endometritis groups. The reduction in the total number of WBC, neutrophils, and lymphocytes in the endometritis group was associated with the reduction of pathogens compared to the healthy group. While many studies have confirmed the potential of the herbs, the threshold safety limits of nephrotoxic and hepatotoxic are yet not known. Undesirable side effects of medication are inevitable, especially, the renal system, that is associated with the excretion of toxic wastes from the body and the liver that is associated with the metabolism of drugs, xenobiotics, herbs and their removal, and thus they are susceptible to the adverse effects of these harmful chemicals. This study discusses about the effects of herbs on imposing toxic effects in liver and renal system. In addition, examination of the liver enzyme function markers ALT, AST, and renal function enzyme markers BUN and creatinine confirmed the effects of acute systemic toxicity. The values were normal in all groups, indicating that the GA-Gel has no effect on the liver and kidneys. The study also showed no effect on female genital tissue (inflammation, swelling, redness, and heat).

CONCLUSION

The development of an advanced and efficient gel for artificial insemination (AI) aimed at reducing pathogen transmission and effectively managing the disease in cattle. Pathogens, including *Arcobacter cryaerophilus*, *Brucella ovis*, *Campylobacter fetus*, and *Helicobacter trogontum*, were successfully isolated from both healthy and endometritis-afflicted cattle. The combination of *Achyranthes aspera* Linn. and *Garcinia mangostana* Linn. demonstrated antimicrobial properties without causing toxicity to the liver, kidneys, or genital system. These findings suggest that GA-Gel holds significant antimicrobial potential in veterinary medicine. However, further research is necessary to confirm its safety and efficacy in other species.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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