Effect of a natural plant ingredient — Pulsatilla saponin B4 on clinical mastitis and serum inflammatory indices in dairy cows

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Research article

Keywords: Clinical mastitis, inflammatory factors, Pulsatilla saponin B4, somatic cell count

Posted Date: August 12th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-48799/v1

Abstract

Background

To investigate the effect of *Pulsatilla* saponin B4, a purified extract form *Pulsatilla Chinensis*, on the treatment of clinical mastitis in dairy cows, 12 healthy cows were used as the control group (group A, no treatment), and 36 cows with mastitis were divided into 3 groups based on the quaque die (QD) intramuscular injection (IM) dose used in the as follows, group B (15 mL), group C (30 mL), group D (60 mL). Identified the microorganisms in milk during the experiment,samples in test groups were cultured, isolated, and verified by quantitative real-time polymerase chain reaction (qPCR). Somatic cell count (SCC) and serum inflammatory indices in cows were measured with a 1-day interval in group C and compared with group A.

Results

Results showed that bacterial detection rates were 100% (group B), 83.33% (group C) and 100% (group D). After the treatment bacterial detection rates dropped to 50.00% (group B), 50.00% (group C), and 41.67% (group D). The medicine was effective in all test groups and showed a dose-effect relationship. Serum haptoglobin (HP), prostaglandin E2 (PGE2), interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-2 (IL-2), interleukin-8 (IL-8), interleukin-10 (IL-10) and tumour necrosis factor alpha (TNF-α), interleukin-4 (IL-4), leukotriene B4 (LTB4) also the SCC were significantly higher than healthy cows ($P < 0.05$) on the day (d) 1 in group C but had no significant difference with group A after treatment.

Conclusion

Treatment with *Pulsatilla* saponin B4 relieved mastitis, leading to relieve of bacterial infection, SCC and levels of serum inflammatory factors effectively in cows with mastitis after being administered for 4–6 days QD.

Background

Mastitis in dairy cows is caused mainly by the inflammatory reaction induced by toxic products secreted by infecting bacteria [1]. With the incidence of clinical mastitis, the permeability of the mammary vein is increased, which in turn enhances the chemotaxis of leukocytes and elevates the somatic cell counts in the milk [2]. Meanwhile, inflammatory agents (exempli gratia endotoxin) will lead to the synthesis and secretion of inflammatory factors (including leukotrienes B4 (LTB4), prostaglandin E2 (PGE2), interleukin-1 alpha (IL-1α), interleukin-1 beta (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumour necrosis factor alpha (TNF-α) [3, 4] and induce hepatic secretion of C-reactive protein (CRP), serum amyloid A protein (SAA), and haptoglobin (HP) [5]. In response to this, the levels of anti-inflammatory factors as interleukin-4 (IL-4) and interleukin-10 (IL-10) increased to inhibit the inflammation [6]. Because of antibiotic resistance, an alternative treatment of mastitis with the lower side effects and lower risk of drug resistance is becoming increasingly desirable [7]. *Pulsatilla* saponin B4 (Anemoside B4, Chemical Abstracts Service number: 129741-57-7) is the main active ingredient of *Pulsatilla Chinensis* and it has a therapeutic role in the case of mastitis as an anti-inflammatory, anti-bacterial, and immune regulating agent [8]. Thus, our study focused on the treatment efficiency and the influence of *Pulsatilla* saponin B4 on the serum inflammatory factors in the cows with mastitis. Furthermore, we provide here an effective non-antibiotic therapy for the clinical mastitis in dairy cows.

Results

Effects of *Pulsatilla* saponin B4 on the cure rate and cure time of clinical mastitis in dairy cows

As shown in Figure 1, all three concentrations of drugs are effective in a 12-day treatment regime, and total cure rates were 75.00%, 75.00%, and 91.67%, average cure time were 6.33 d, 6.11 d, and 6.91 d in group B, C, and D, respectively. Among these six cows in group C were cured, and four cows in group B and D were cured; there were no cured cows after treatment in group B, but 1 and 2 cows in group C and D who were cured after initial shots of the drug, respectively. Group D showed the highest cure rate; however, the average cure time was longer. Thus, group C with 30 mL QD proved most efficient treatment protocol.

Bacteriology and PCR results

As shown in Figure 2, bacteria were isolated from the samples of all groups on d 1. Bacterial detection rates were 100% (*Escherichia coli, Cocci, Mycoplasma, Staphylococcus*), 83.33% (*Staphylococcus epidermidis, Bacillus, Staphylococcus aureus, Mycoplasma, Cocci, Staphylococcus aureus, Staphylococcus hemolytic* and *Staphylococcus saprophytic*), and 100% (*Mycoplasma, Escherichia coli, Staphylococcus aureus, Staptococcus saprophyticus* and *Cocci*) in group B, C, and D, respectively. After the treatment bacterial detection rates dropped to 50.00% (group B), 50.00% (group C), and 41.67% (group D).

Effects of *Pulsatilla* saponin B4 on the milk SCC in the cows with clinical mastitis

As shown in Figure 3, milk SCC in group C was significantly higher than that of group A and showed a downstream trend with the treatment. Milk SCC of cows with clinical mastitis dropped significantly from d 1 to d 5 ($P < 0.05$), also from d 7 to d 9 ($P < 0.05$) and showed no significant difference compared to group A on d 11 ($P > 0.05$).

Effects of *Pulsatilla* saponin B4 on serum inflammatory indices in the cows with clinical mastitis

As shown in Table 1, cows with clinical mastitis had very significantly higher serum SAA, HP, PGE2, IL-1α, IL-2, IL-6, IL-8, IL-10, and TNF-α than healthy cows ($P < 0.01$); as well as significantly higher serum LTB4, CRP, IL-1β, and IL-4 than that of healthy cows. This showed a downstream trend with the advancement in treatment except for serum PGE2, in which the concentration was still significantly higher than healthy cows ($P < 0.05$), and the other indices showed no significant difference with the healthy cows ($P > 0.05$). Serum HP, IL-2, and IL-10 concentrations in cows with mastitis after d 3 was significantly lower than d 1 ($P < 0.05$). Serum CRP, SAA, HP, PGE2, IL-1α, IL-2, and TNF-α concentrations in cows with mastitis after d 5 were significantly lower than d 1 ($P < 0.05$). Moreover, after d 7, serum IL-6, IL-4 concentrations in cows with mastitis also became significantly lower than d 1 ($P < 0.05$).

Effects of *Pulsatilla* saponin B4 on the milk bacteria in the cows with clinical mastitis

As shown in Table 2, treatment of *Pulsatilla* saponin B4 eliminated or decreased the infection of *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Klebsiella species, Escherichia coli*, and *Mycoplasma bovis*. The antibiotic resistance of the milk was also decreased in.

Discussion

- **Relationship between the different dose of** Pulsatilla **saponin B4 and the cure rate and SCC of cows with clinical mastitis**

SCC is the count of somatic cells in a millilitre of milk, and contains 98–99% of leukocytes and 1–2% of epilithic cells [9]. Mammary tissues will release inflammatory factors like IL-1β, IL-6, and TNF-α when damaged or infected. This will promote vascular permeability and the sloughing of epilithic cells, also, stimulate the secretion of chemokines, attracting leukocytes to the infected area, and causing the increase of somatic cell count in milk [10]. Thus, SCC reflects the severity of mastitis, and can be used in early diagnosis and treatment evaluation of mastitis in dairy cows [11]. *Pulsatilla* saponin B4 is the main active ingredient of the *Pulsatilla Chinensis*, which has anti-bacterial, anti-inflammatory, vasodilatory, and immunoregulatory properties, and could regulate the expression of related inflammatory cytokines via the nuclear factor kappa-B signalling pathway, relieving the inflammatory response with high efficiency [12, 13]. We showed that all three concentrations of drugs are effective in 12-day treatment but with different cure rates. The total cure rate was 75.00%, 75.00%, and 91.67% respectively for group B, C, and D. In which, group C had the highest cure rate (6 cows), and had the shortest cure time (6.11 d). Bacteria, fungus, viruses and mycoplasma are the main pathogens causing mastitis in dairy cows. Group B, C and D were all infected with *Escherichia coli, Cocci, Mycoplasma* and *Staphylococcus* at different degrees, and the detection of bacteria rata were was 100%. After administration, the bacterial detection rate of each group of cows was reduced to 50.00%, 50.00%, 41.67%, respectively, which indicated that *Pulsatilla* saponins B4 can effectively reduce the degree of bacterial infection in dairy cows with clinical mastitis. Therefore group C is regarded as the best group for clinical treatment of mastitis.

Furthermore, the milk SCC in group C was significantly higher than that of healthy group (group A), and it is consistent with the research of Das D et al. [14]. This indicated that the immune cells were accumulated abundantly in the damaged tissues by the non-specific and fluid immune response. Milk SCC in cows with clinical mastitis started to decrease during the treatment and still showed a downstream trend after treatment, no significant differences with the healthy cows ($P < 0.05$), indicating that *Pulsatilla* saponin B4 may relieve the auto-immune damage by decreasing the secretion of pro-inflammatory factors. In addition, group C and group D had 1 or 2 cows that were cured after few initial shots of drug indicating the *Pulsatilla* saponin B4 may eliminate the pathogenic bacterial and have immune regulation activity in promoting the recovery of mammary tissue. But the mechanism is still needed to be discovered.

- **Effects of** Pulsatilla **saponin B4 on the serum inflammatory factors in cows with clinical mastitis**

The infection-induced inflammatory response is the main cause of clinical mastitis in dairy cows, and it will stimulate the cellular secretion of LTB4, PGE2, and histamine to enhance the immune response, promoting the production of inflammatory factors (interleukins, bradykinin and allergic toxins), and elevating the levels of acute-phase protein (CRP, SAA, HP) [5, 15]. Those acute phase proteins reflect the severity of inflammatory damage in the mammary tissue of dairy cows directly and have a significant correlation with the incidence of mastitis [16]. Inflammatory cytokines can be divided into pro-inflammatory factors (PGE2, LTB4, IL-1, IL-2, IL-6, IL-8, and TNF-α), and anti-inflammatory factors (IL-4 and IL-10). These factors interact and weave a complex regulatory network, regulate vasodilation, vascular permeability, chemotaxis reaction; also inflammatory hyperaemia and exudation, have a strong influence on the incidence, development, and outcome of inflammatory diseases. During the course of clinical mastitis, serum concentrations of IL-1, IL-2, IL-6, IL-8, and TNF-α are significantly elevated, then stimulate the secretion of PGE2 and LTB4, and intensify the tissue damage. Serum CRP, SAA, and HP concentrations also reflect the severity of inflammatory reaction with high sensitivity. Thus, the elevation of those acute phase proteins can also be a criteria of mastitis [17–19]. Our study showed that cows with clinical mastitis had a significantly higher levels of pro-inflammatory factors (IL-1α, IL-1β, IL-2, IL-6, IL-8, TNF-α, PGE2, and LTB4), which may be due to the incidence of mastitis which stimulated body cells to the synthesize and release inflammatory mediators, and induce the inflammatory stress in the dairy cows. Macrophages secreted CRP, SAA, and HP rapidly under the effects of these factors, and increased the severity of inflammation. Bochniarz et al. found that the concentrations of serum anti-inflammatory factors (IL-4 and IL-10) in cows with sub-clinical mastitis were significantly lower than healthy cows [6], but our study showed that the serum concentrations of IL-4 and IL-10 were significantly and very significantly higher than healthy cows respectively. This may be because of the inflammatory symptoms are more obvious in cows with clinical mastitis, and the elevation of acute pro-inflammatory factors damaged the mammary tissues and even the entire body. As a result, the serum concentration of IL-4 and IL-10 increased rapidly to antagonize the inflammation. Current research on *Pulsatilla Chinensis* found that it will inhibit the expression of IL-1, IL-6, IL-8, and TNF-α, down-regulate the levels of acute-phase proteins, elevate the concentration of IL-10 and is considerably efficient in the treatment of inflammatory diseases [20]. Our study showed that serum LTB4 concentration showed no significant difference during the treatment. However, all the other indices showed a downstream trend except for serum PGE2, which the concentration was still significantly higher than the healthy group ($P < 0.05$). All indices had no significant difference with the healthy group on d 7 ($P > 0.05$), which is consistent with current researches and indicated that *Pulsatilla* saponin B4 could down-regulate the serum concentrations of pro-inflammatory factors in the cows with mastitis. We suspect that *Pulsatilla* saponin B4 might inhibit the production and secretion of pro-inflammatory cytokines in epilithic cells and leukocytes to perform an anti-endotoxin function, thus, relief the inflammatory damage of the epilithic cells.

Furthermore, the concentrations of acute-phase proteins decreased with the decrease in the levels of pro-inflammatory factors, showing that inflammation was resolving rapidly. This indicated that *Pulsatilla* saponin B4 might have a high anti-inflammatory activity which attenuated the acute inflammation and minimized the damage to mammary tissues and body. Differ from current research, we found that serum concentrations of anti-inflammatory factors IL-4 and IL-10 were significantly higher than healthy cows ($P < 0.05$), and showed a downstream trend during the treatment, it may be due to the fact that the secretion of pro-inflammatory factors was more related to the cellular secretion and immune response. In the acute phase of mastitis, levels of pro-inflammatory factors elevated because of the severe inflammation, and the concentrations of anti-inflammatory factors also increased as a consequence of the body's defense mechanism. When the inflammation resolved, the pro-inflammatory factors and anti-inflammatory factors decreased. However, the change in the levels of anti-inflammatory factors was the result of drug administration or as the result of the immune function of the dairy cow still needs to be revealed.

Conclusion

Treatment of *Pulsatilla* saponin B4 can significantly decrease the SCC, eliminate pathogenic bacteria, and down-regulate serum inflammatory indices of cows with clinical mastitis. 30 mL/QD injection in brachiocephalicus muscle for 4–6 days showed the best therapeutic results for clinical mastitis.

Methods

Experiment animals and treatment

The Chinese Holstein cows were obtained commercially from Sichuan Ninggang Animal Husbandry Co., Ltd. Twelve healthy cows and 36 cows with clinical mastitis having similar date of parturition and milk production were selected form 50 healthy and 50 cows with mastitis with weight 612 ± 47 kg, 3-4 years of age, and 2-3 parity in a semi-closed unified dairy farm. Twelve healthy cows were placed into control group (group A), the other 36 cows with clinical mastitis were divided into 3 groups (group B, C, and D) randomly by simple rando mizaton. Intramuscular injection of 15, 30, and 60 mL of *Pulsatilla* saponin B4 QD in the brachiocephalicus muscle of the cows in group B, C and D, respectively was administered, and recorded the first day of the experiment as day 1, continued 4-6 days treatment until the recovery of cows. No administration for the control group. All the groups were under observation for 12 days.All experimental cows had the same feeding and management procedure, and no other diseases occurred, nor antibiotic treatment administration during the experimental period. We selected a small sample size because the *Pulsatilla* saponin B4 was evaluated for the first time in the present study, and therefore, the initial intention was to gather basic evidence regarding the use of this drug in more complex experimental designs.

Sample collection

10 mL tail venous blood of experimental cows were collected before morning feeding at 8 am. The sampling was done on the days 1, 3, 5, and 7 in group B, C, and D, also on same day during the experiment in group A. Collected venous blood was placed in a centrifuge tube without anticoagulant and centrifuged at 3 000 rpm for 10 min to separate serum after 1 hour rest at room temperature (20-25 ℃), and upper serum was transferred to EP tube, stored at -70 ℃. Washed the udder by warm water, and sterilized by 75% ethanol. Discarded the first three streaks of milk, then measured the SCC of the milk in the fourth streak on the days 1, 3, 5, 7, 9, and 11 in group A and group D. And averaged the SCC of group A during the experiment. All sampling process were administrated before dosing.

Animals treatment after experimentation

Breeding management of cows in group A and cured cows in group B, C, and D returned to normal after the study, and the rest were treated by other medicine until cured. All cows will be fed until death.

Equipment and reagents

The equipment shown below: Centrifuge (Sigma, Germany); Ultra-low temperature freezer (Haier, China); Clean bench (Sujing Co., Ltd, China); Full wavelength microplate reader (Thermo Scientific, U.S.A), UV-visible spectrophotometer (Lambda 45, U.S.A), Microsampler (Thermo Scientific, U.S.A); Electronic thermostatic water bath (Zhongxing Instrument, China), Stratagene Mx3005P (Agilent Technologies, U.S.A).

The reagents shown below: *Pulsatilla* saponin B4 (100 mL/bottle, concentration 66%, ethanol solution), donated by Sichuan Innovate Medical Technology Co., Ltd, Chengdu, China, identified by the National Engineering Research Center of Traditional Chinese Medicine Solid Preparation Manufacturing Technology of Jiangxi University of Traditional Chinese Medicine; ELISA kit of IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, LTB4, PGE2, SAA, and HP, all were purchased from Shanghai Enzyme Biotechnology Co., Ltd, Shanghai, China. Milk pathogenic bacteria DNA extraction kit, bioeasy Co., Ltd, Shenzhen, China. Eight combined bovine mastitis pathogen nucleic acid detection reagents kit, bioinfee Biotechnology Co., Ltd, Shenzhen, China.

Criteria of clinical mastitis

All cows with clinical mastitis were diagnosed by the same branch veterinarian of the dairy farm. Detailed criteria included red and swollen mammary gland with sensitive tenderness, higher surface temperature of the incidence quarter, significantly decreased milk production, milk with yellow or red color, SCC higher than 500 000/mL, and other abnormal traits [21].

Clinical cure and bacteriological cure

A cow was regarded as clinically cured if both milk and mammary gland had a normal appearance in the clinical examination performed approximately 24 hours after the last infusion.

A cow was considered bacteriologically cured if a microorganism was identifiedin the milk sample collected on d 1, and the same species was not isolated in any of milk samples collected posttreatment (d 1 or 11). If the same pathogen isolated on d 1 was identified in either of the posttreatment samples, the quarter was considered noncured. Only cows with culture results for posttreatment milk samples were included in this evaluation. In addition, samples with negative culture (no growth) on d 1 were not included in the analysis of bacteriological cure.

PCR tests and indices measurement

Bacterial incubation and isolation were operated on the milk sample of d1 and d 11 of all test groups, then use qPCR (40 loops, threshold fluorescence 500 dR) to verify the bacteria (*Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Klebsiella species*, *Escherichia coli, and Staphylococcus aureus*) and β-lactam resistance.

Serum concentrations of IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, LTB4, PGE2, SAA, and HP in experimental cows were measured by the ELISA kit.

SCC measurement and cure rate calculation

Detected the SCC of milk by De Laval milk somatic cell detector and observed the clinical symptoms of the cows with mastitis to judge the treatment condition every day during the experiment, and then calculated the cure time, effective rate, and cure rate.

Data Analysis

Used SPSS 19.0 to verify the data distribution, distinguished the differences and correlations between groups by independent sample t-test and Person relation analysis, respectively. All data was performed by `X ± SD, compared data with P-value lower than 0.05 was considered to be significant, lower than 0.01 was considered to be very significant.

For each cow, three different investigators were involved as follows: a first investigator administered the treatment and collected sample based on the randomization table. This investigator was the only person aware of the treatment group allocation. A second investigator was responsible for testing the cure rate, bacterial infection rate, somatic cell count, inflammatory factors, and qPCR. Finally, a third investigator (also unaware of treatment) analyzed the above test results.

Abbreviations

QD: quaque die; IM:intramuscular injection; qPCR:quantitative real-time polymerase chain reaction; SCC:somatic cell count; d:day; LTB4:leukotrienes B4; PGE2:prostaglandin E2; IL-1α:interleukin-1 alpha; IL-1β:interleukin-1 beta; Il-2:interleukin-2; IL-4:interleukin-4; IL-6:interleukin-6; IL-8:interleukin-8; IL-10:interleukin-10; TNF-α:tumour necrosis factor alpha; CRP:C-reactive protein ; SAA:serum amyloid A protein; HP:haptoglobin

Declarations

Acknowledgements

The authors are grateful to the farmers for allowing the collection of samples.

Authors' contributions

LHS and YLF provided the concept on this study. LHS, BLQ and SKL designed the study. LCY, XLZ, JBX, YZ and YS acquired the samples. SMY, SZC, BLQ, LCY, and YZ interpreted the data. ZTS, SLY, YLF and KD developed and provided the experimental *Pulsatilla* saponin B4. BLQ and LHS drafted the work. LHS revised the manuscript. All authors read and approved the manuscript.

Funding

This study has been supported by the National Key R&D Program of China (2018YFD0500900) and the Sichuan Department of Science and Technology Support Project (2019YJ0650).The funders had no role in the design of the study, collection, analysis and interpretation of data, and in writing the manuscript.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All procedures were approved by Insti-tutional Animal Care and Use Committee of Sichuan Agricultural University (approval number: DYY-13309).

Consent for publication

All authors to have approved the submitted version (and any substantially modified version that involves the author's contribution to the study).

Competing interests

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Tables

Table 1. The effect of *Pulsatilla* saponin B4 on the inflammatory factor index of clinical mastitis in dairy cows

Index	Goup A (*n*=12)	Group C (*n*=12)			
		1st day	3rd day	5th day	7th day
CRP (mg/L)	6.55±1.68^{b}	8.25±2.50^{a}	7.02±2.08^{ab}	6.66±1.05^{b}	6.47±1.93^{b}
SAA (μg/mL)	6.95±1.01^{C}	10.86±3.20^{A}	9.40±2.63^{Ab}	8.85±2.31^{bc}	8.71±2.60^{bc}
HP (ng/mL)	167.37±36.40^{B}	236.34±55.99^{A}	178.46±45.14^{B}	168.68±35.05^{B}	160.01±52.80^{B}
LTB4 (ng/mL)	11.15±2.69^{b}	14.71±3.82^{a}	14.49±4.77^{a}	14.27±4.68^{a}	14.13±4.78^{a}
PGE2 (pg/mL)	253.70±52.08^{C}	351.80±56.39^{A}	331.47±82.63^{AB}	307.83±66.35^{b}	299.62±51.36^{b}
IL-1α (pg/mL)	175.90±47.49b^{C}	228.47±69.62^{A}	210.98±63.04^{Ab}	181.65±60.07^{bC}	163.23±47.55^{C}
IL-1β (pg/mL)	276.71±81.61^{b}	358.78±113.73^{a}	331.88±99.60^{ab}	325.92±98.89^{ab}	295.64±98.06^{ab}
IL-2 (pg/mL)	449.65±95.09^{B}	606.38±130.78^{A}	500.38±151.23^{b}	470.21±145.64^{b}	415.76±121.10^{B}
IL-6 (pg/mL)	97.67±22.63^{C}	155.00±39.96^{A}	134.52±38.21^{AB}	124.65±42.53^{b}	117.07±36.88^{bC}
IL-8 (pg/mL)	108.61±38.62^{B}	165.45±53.22^{A}	153.94±50.83^{a}	152.60±54.40^{a}	139.76±47.86^{AB}
TNF-α (pg/mL)	122.65±33.50^{C}	174.65±49.07^{A}	151.22±41.09^{Ab}	143.77±35.69^{bc}	133.20±46.61^{BC}
IL-4 (pg/mL)	13.42±4.36^{b}	16.56±3.71^{a}	14.28±4.87^{ab}	13.78±4.98^{ab}	12.48±4.60^{b}
IL-10 (pg/mL)	109.41±24.04^{B}	146.54±31.43^{A}	122.99±26.85^{b}	119.47±26.07^{b}	119.84±30.35^{b}

Table 2. the qPCR of the pathogenic bacteria and gene in the milk of cows with clinical mastitis

target genes	*Pseudomonas aeruginosa*		*Streptococcus agalactiae*		*β-lactam resistance*		*Streptococcus dysgalactiae*		*Mycoplasma bovis*		*Klebsiella Species*		*Escherichia coli*	
time/ cows	before	after	before	after	before	after	before	after	before	after	before	after	before	after
1	-	-	29.98++	-	38.14	-	-	-	-	-	-	-	-	-
2 (not cured)	-	-	-	39.44	-	32.22+	-	-	-	-	-	-	34.62?	-
3	-	-	-	-	29.62++	-	-	38.55	-	-	-	-	-	-
4	-	-	26.66++	-	-	38.55	38.7	-	-	-	-	-	31.83+	-
5	-	-	-	-	-	-	-	-	25.46++	-	25.46++	-	33.01+	38.92
6 (relieved)	-	-	-	-	-	38.58	-	38.8	29.18++	32.69+	29.18++	-	36?	-
7	-	-	31.12+	-	-	36.47	-	-	-	-	-	-	39.14	-
8	-	-	31.78+	-	-	36.98	-	-	-	-	-	-	-	-
9	-	-	35.02?	37.72	-	35.58	-	-	-	-	-	-	30.5+	-
10(not cured)	-	-	26.52++	30.49+	-	39.52	36.73?	-	-	-	-	-	32.49+	-
11	-	-	-	-	-	38.34	29.23++	38.42	-	-	-	39.71	35.35?	-
12	-	-	-	-	-	37.8	-	-			-	-	32+	39.63

Note?unmber in the table means loops until reached the threshold value (500 dR), ++ after numbers means positive, + means slightly positive, and ? means suspective, - means not reached the threshold value after 40 loops.

Figures

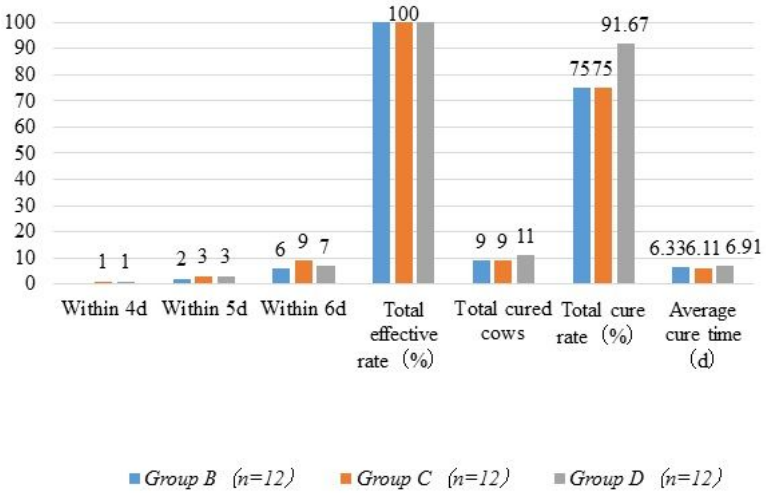
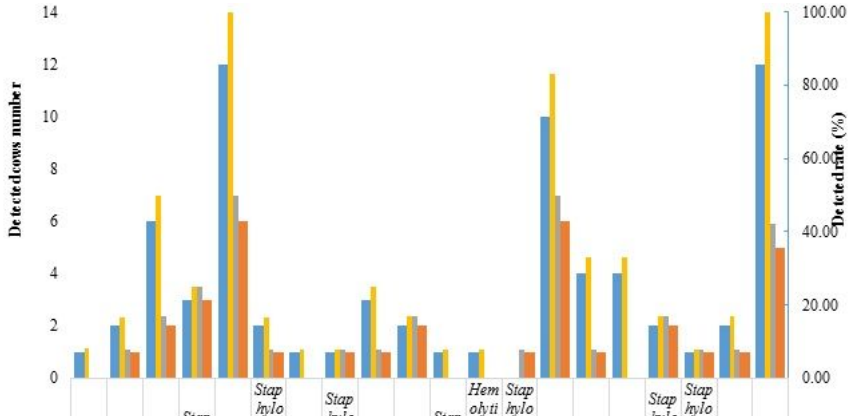


Figure 1

The effect of different dosage Pulsatilla saponin B4 on cure time and cure rate of clinical mastitis in dairy cows



Cows detected on d1	1	2	6	3	12	2	1	1	3	2	1	1	0	10	4	4	2	1	2	12
Cows detected on d11	0	1	2	3	6	1	0	1	1	2	0	0	1	6	1	0	2	1	1	5
Detection rate on d1 (%)	8.33	16.67	50.00	25.00	100.0	16.67	8.00	8.00	25.00	17.00	8.00	8.00	0.00	83.00	33.00	33.00	17.00	8.00	17.00	100.0
Detection rate on d11 (%)	0.00	8.00	17.00	25.00	50.00	8.00	0.00	8.00	8.00	17.00	0.00	0.00	8.00	50.00	8.00	0.00	17.00	8.00	8.00	42.00

Figure 2

The detection of bacteria in milk of various test groups

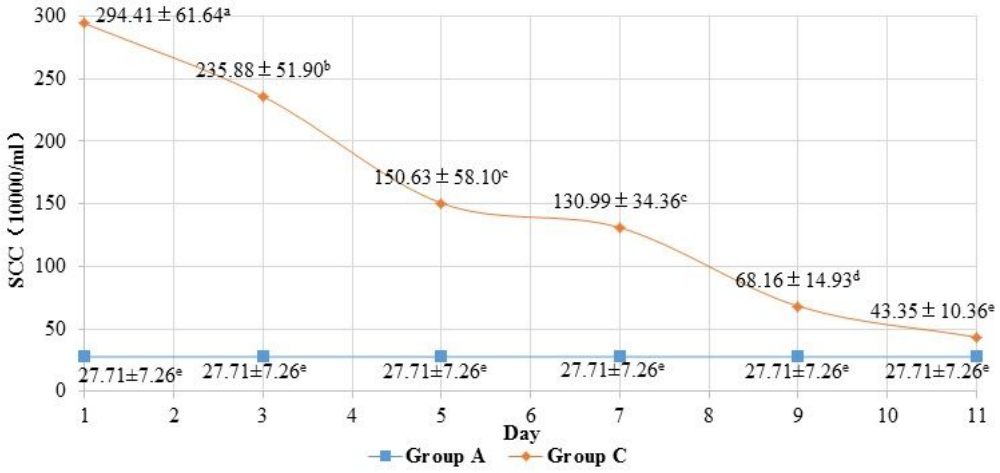


Figure 3

The effect of Pulsatilla saponin B4 on somatic cell counts of clinical mastitis in the milk of dairy cows Note: the same letters show that there is no significant difference (P > 0.05), and the different letters show that there is a significant difference (P < 0.05), independent of case, only capital letters indicate a significant difference (P 0.01), the same as below.

Supplementary Files

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