

## Research Article



# Biological Activity and Pharmaco-Therapeutic Efficiency of *Calligonum leuocladum* B. Dosage Forms in the Treatment of Endometritis of Cows

ISATAY TUSUPOVICH JAKUPOV, GULZHAN TURSUNOVNA YESZHANOVA, GULNUR KURBANALIEVNA MAMYTBKOVA\*

S. Seifullin Kazakh Agrotechnical Research University, Astana, 010011, Kazakhstan.

**Abstract** | The aim of this study is to obtain dosage forms from plant raw materials *Calligonum leuocladum*, to study their pharmacotherapeutic activity and use for the treatment of endometritis in cows. A total of 270 cows were examined by clinical methods after calving, of which 45 cows showed signs of acute endometritis. Subsequently, the bacterial background of uterine mucus in sick cows was investigated and *St. pyogenes*, *E. faecalis*, and *E. coli* were isolated. For the complex treatment of acute postpartum endometritis, the cows of the experimental groups were treated with the phytopreparation *C. leuocladum*. For comparison, the drug «Biometrosanit» was used in the treatment of cows of the control group. Phytopreparations of *C. leuocladum* were suppositories obtained from extracts for intrauterine administration, with 20% and 40% content of *C. leuocladum*. Previously, phytopreparations were tested for antimicrobial (diffusion in agar by serial double dilutions) and antiradical activity (comparison with Butylhydroxyanisole (BHA), as well as for toxicity and the ability to cause irritation or an allergic reaction from the mucous membranes. In cows that used a suppository with a 40% content of *C. leuocladum*, the earliest recovery periods were revealed ( $6 \pm 0.5$  days), the number of cured animals was 80%. The use of intrauterine suppositories with 20% content of *C. leuocladum* did not provide a high therapeutic effect (53.3%). The cows of the experimental groups showed positive dynamics in the content of metabolic components in the blood. The compared drug «Biometrosanit» caused the recovery of 73.3% of control animals. Medicinal forms of *C. leuocladum* were obtained by drying, grinding, extracting extract from roots, stems in the Soxhlet extractor. The pharmacological and therapeutic efficacy of the ethanol extract of *C. leuocladum* has been studied. A toxicity test was carried out on white mice using 10%, 20%, 40% concentration of *C. leuocladum*, no death of mice was recorded within 30 days. When studying the irritating and allergic effects of *C. leuocladum* on rabbits, it was found that no irritating effect and allergic reaction was observed within 24 hours in animals with 10%, 20%, 40% content of *C. leuocladum*. Studies of the antimicrobial properties of *C. leuocladum* extract with 10%, 20%, 40% concentrations, suppositories containing 20% and 40% of *C. leuocladum* showed antimicrobial activity against *E. coli*, *St. aureus*. *C. leuocladum* shows the highest antimicrobial activity at 40% concentration of  $18 \pm 0.1$ . *C. leuocladum* has a high antiradical activity. The study of the therapeutic efficacy of suppositories containing 20% and 40% of *C. leuocladum* in the treatment of endometritis in cows ( $n=45$ ) provides recovery of 80% of cows. Thus, tests for biological activity, as well as the effect of *C. leuocladum* on mucous membranes, indicate its good antiradical properties and activity against *E. coli*, the absence of toxicity, irritating and allergenic properties. The information obtained by us will expand knowledge in the field of veterinary phytopharmacology and help develop safe and effective measures for the treatment of postpartum diseases of cows.

**Keywords** | Herbal medicine, Uterine diseases, Bacterial culturing, *Calligonum leuocladum*, Endometritis, Cows.

**Received** | April 25, 2023; **Accepted** | May 15, 2023; **Published** | June 10, 2023

\***Correspondence** | Gulnur Kurbanalieva Mamytbekova, S. Seifullin Kazakh Agrotechnical Research University, Astana, 010011, Kazakhstan; **Email:** gulnur4284@mail.ru

**Citation** | Jakupov IT, Yeszhanova GT, Mamytbekova GK (2023). Biological activity and pharmaco-therapeutic efficiency of *Calligonum leuocladum* B. dosage forms in the treatment of endometritis of cows. Adv. Anim. Vet. Sci. 11(7): 1200-1208.

**DOI** | <https://doi.org/10.17582/journal.aavs/2023/11.7.1200.1208>

**ISSN (Online)** | 2307-8316



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Uterine diseases in cattle occur at all stages of the reproduction cycle but the majority of cases is found in the postpartum period. The inflammation of the uterus is generally defined as metritis or endometritis, with several gradations, e.g. puerperal metritis, clinical metritis, clinical or subclinical endometritis (Madoz et al., 2014). Whether uterine diseases have a negligible, moderate or detrimental effect on fertility is still under discussion and depends on definitions and classification. After parturition, a broad diversity of bacteria with >200 different species has been found in postpartum period (Helfrich et al., 2020). The microbiota of the uterus can indicate not only infection, but also the presence of inflammation.

Treatment of endometritis with antimicrobial agents occur with varying degrees of success, inconsistent rates of recovery, high treatment costs, milk utilization, microbial resistance, and decrease of phagocytic activity of polymorphonuclear leukocytes (Williams et al., 2005, Otto et al., 2015, Sheldon et al., 2017, Drillich et al., 2018, Helfrich et al., 2020).

7 days after the treatment intrauterine infusion of ceftiofur did not affect the prevalence of subclinical endometritis and positive uterine culture. However, it reduced detection rate of positive uterine microflora in cows with clinical endometritis (29.0 vs 51.4%) and reduced the overall prevalence of *Arcanobacterium pyogenes* (1, 0 versus 7.6%). Observation showed that cows with clinical endometritis had an increased prevalence of *A. pyogenes* (10.3 vs. 1.5%), *E. coli* (5.9 vs. 0.75%), and an overall positive uterine culture (41.2 vs. 22.4%); however, only increased prevalence of *A. pyogenes* (10.2 vs 1.5%) was observed in cows with subclinical endometritis (Galvão et al., 2009).

Effectiveness of intrauterine therapy with cefapirin in the purulent form of endometritis was defined in the work (Tison et al., 2017).

Currently, the development of effective, safe and rational drugs and forms from plant raw materials for the treatment and correction of animal health is relevant.

It was determined that hydroxytyrosol, a natural phenolic alcohol with strong antiradical activity, may increase the role of endogenous antiradical systems and is effective in the treatment and prevention of uterine pathologies in dairy cows (Gugliandolo et al., 2020).

In this respect *Calligonum leucocladum* belongs to advanced study plants. *C. leucocladum* (calligonin, white-skinned dzhuzgun, Polygonaceae family) is a shrub, 50-120 cm

high. About 30 species of *C. leucocladum* are found in desert and semi-desert steppes in Kazakhstan (Azat et al., 2021). Information on the systematization and study of the areas of distribution of various species of *Calligonum* in work (Zhang et al., 2020) provided. Like all desert plants, calligonin contains tannins, as well as catechins, dehydrocatechins, essential oil, flavonoids. The plant extract has anti-inflammatory, antipyretic, hypoglycemic and gastro-protective properties. In addition, dosage forms of *C. leucocladum* have a pronounced antiradical, immunomodulatory activity.

The study of biologically active ingredients of the roots of *Calligonum polygonoides* determined thirty-one compounds, the main components of which were pyrogallol and palmitic acid, which have antimicrobial and allelopathic activity (Abd-El Gawad et al., 2020).

In article (Kiani et al., 2019) studied the effect of *Calligonum comosum* extract on the development of endometrial lesions in mice. It was found out that *C. comosum* inhibits the growth and formation of cyst that develops endometriotic lesions in mice, which may be useful in the treatment of endometriosis.

Authors (Abdo et al., 2015) discuss the hepatoprotective and chemoprotective effects of *C. comosum* containing polyphenolic antioxidants. The chemopreventive effect of *C. comosum* extract was shown on a rat animal model. It significantly suppressed the increase in serum liver enzymes, including aspartate aminotransferase, alanine aminotransferase, and  $\gamma$ -glutamyltransferase.

The aim of the research is to obtain dosage forms from the roots and stems of *Calligonum leucocladum*, as well as to study their pharmacotherapeutic activity and use for the treatment of endometritis in cows.

## MATERIALS AND METHODS

### TECHNOLOGY FOR OBTAINING DOSAGE FORMS FROM RAW MATERIALS OF *C. LEUCOCLADUM*.

Scientific research was conducted in the laboratory of the Department of «Veterinary Medicine» at S. Seifullin Kazakh Agro Technical Research University, as well as in the agricultural units of the Akmola region and in the laboratory of the Institute of Applied Chemistry (Astana, Kazakhstan).

Memmert 500» vacuum drying cabinet was used for drying the raw materials to remove moisture by evaporation and removal of the formed vapors at 150°C for 1 hour. Grinding of *C. leucocladum* raw materials was carried out in a ball mill. The size of the crushed final product is 200–150 mg.

Soxhlet extractor - special standard equipment was used to extract the extract from the roots and stems of *C. leuocladum*.

Suppositories (Figure 1) for intrauterine insertion with 20% and 40% content of the active substance was obtained from the extract of *C. leuocladum*.



Figure 1: Suppositories

Dosage forms obtained from roots and stems of *C. leuocladum*, were studied at 10%, 20%, 40% concentrations for irritating and allergic properties, toxicity, antimicrobial properties (Table 1).

Table 1: Suppository composition (m=5,0)

Nº	Dosage forms	Dosage form composition	The content of active ingredients in the dosage form
1	Suppositories containing 20% <i>C. leuocladum</i>	<i>C. leuocladum</i> extract	1,0
		<i>Adrenalini hydrochloridum</i>	0,1
		<i>Lanolinum anhydricum</i>	0,2
		<i>Oleum Cacao</i>	3,7
2	Suppositories containing 40% <i>C. leuocladum</i>	<i>C. leuocladum</i>	2,0
		<i>Adrenalini hydrochloridum</i>	0,1
		<i>Lanolinum anhydricum</i>	0,2
		<i>Oleum Cacao</i>	2,7

Tests for the toxicity of the *C. leuocladum* extract were carried out on white mice - group 1 experimental, group 2 control, (m = 18-19 g), (n = 8). The extract of *C. leuocladum* in the tested concentrations, at a dose of 0.1 mg/kg, was given every 6 hours, for one day (Chinedu et al., 2013).

The *C. leuocladum* extract was tested for irritating and allergic properties on 3 groups of rabbits (n = 9), selected

according to the principle of analogues. The first (n = 3) and second (n = 3) groups were experimental, the third group (n = 3) - control. Rabbits of the first group received subcutaneously injection with 100 mg (0.1 ml) of a 10% liquid extract of *C. leuocladum* once. Animals of the second group were injected with a 20% liquid extract of *C. leuocladum* in a similar dose, then the drug was injected intramuscularly, at the same dose twice a day. Animals of the control group were given water for injection. After 14 days, 2 drops of *C. leuocladum* extract, 10 and 20% concentration, respectively, were injected into the conjunctival sac of experimental animals, and 2 drops of double-distilled water were injected into the control animals. The reaction was checked after 15 minutes (rapid reaction) and after 24 hours (delayed type hypersensitivity). Evaluation was made according to the following scale: no reaction - 0; mild redness of the lacrimal duct - 1; redness of the lacrimal duct and sclera towards the cornea - 2; redness of the entire conjunctiva and sclera, with itching, scratching, possible development of purulent ophthalmitis - 3 (Gritsyuk et al., 2021).

During the testing the antimicrobial activity of *C. leuocladum*, extract and suppositories of *C. leuocladum* were taken in different concentrations and with different concentration of active substance (Balouiri et al., 2016).

The antimicrobial activity of *C. leuocladum* was evaluated by the method of diffusion in agar by the method of serial double dilutions, and the minimum suppressive concentration (MSC) was also evaluated.

The extract and suppositories of *C. leuocladum* were taken in various concentrations containing the active substance for testing the antimicrobial and antiradical activity of *C. leuocladum* to observe the antimicrobial activity.

The antimicrobial activity was evaluated by agar diffusion method, by serial two-fold dilution method, and the assessment of the minimum inhibitory concentration (MIC) was also carried out.

The microbial composition of uterine mucus in cows with endometritis was preliminarily determined.

Antiradical activity of ethanol extracts of *C. leuocladum* was determined by the method of inhibition of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), which was added to the test solutions. Then, the optical density of the solutions was measured, and the antiradical activity of the observed objects was calculated using the formula. The concentration-dependent, optical density of the observed solutions were measured using Cary 60 UV-Vis spectrophotometer at a wavelength of 520 nm (Sroka, 2006).



Antiradical activity (ARA) was determined in the ethanol extract of *C. leuocladum* in the concentration range of 0.1, 0.25, 0.5, 0.75 and 1 mg/ml using the following formula:

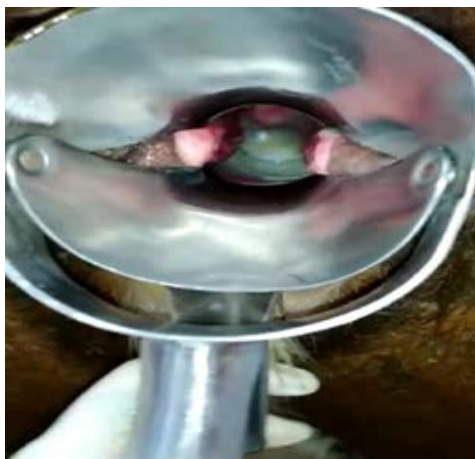
$$ARA (\%) = (A_0 - A_t) / (A_0 * 100) \quad (1)$$

where:  $A_0$  – optical density of the control sample;  
 $A_t$  – optical density of the working sample.

The therapeutic efficacy of *C. leuocladum* in the treatment of endometritis in cows was tested using suppositories containing 20 and 40% of *C. leuocladum*, by clinical, hematological and biochemical methods.

Clinical observations of animals were carried out, including external research methods - examination, palpation, as well as internal - vaginal, rectal.

A total of 270 cows were examined after calving, belonging to the farms of the Akmola region of Kazakhstan. To make a diagnosis of endometritis, clinical studies of animals were carried out, including external examination, palpation, as well as internal vaginal, rectal examination methods (Figure 2).



**Figure 2:** Vaginal examination

When conducting external and vaginal examination the following factors were considered: the integrity, color of the skin and mucous membranes, their swelling and hyperemia, the condition of the vaginal part of the cervix, the degree of opening of the cervical canal, the volume and nature of the cervical-vaginal mucus (Jakupov et al., 2021). During rectal examination the following parameters have been defined: the consistency and diameter of the cervix, the size and consistency of the uterine horns, the thickness of their walls, the rigidity of the uterus, the size, shape, mobility and consistency of the ovaries, and the presence of follicles, cysts or corpus luteum on their surface (Jakupov et al., 2016). Clinical methods have established signs of acute endometritis in 45 cows.

**STATISTICAL DATA**

The obtained data was processed using the Microsoft Excel 2019 program, as well as using regression analysis and Spearman's correlation coefficient.

**RESULTS**

Toxicity of *C. leuocladum* extract was tested on white mice. Within 24 hours, behavioral reactions, coordination of movements and position in space of the animals of the experimental group were normal, reflex sensitivity, frequency of respiratory movements, consistency of fecal masses, frequency of diuresis remained unchanged. When studying the chronic toxicity of the ethanol extract of *C. leuocladum* (at a dose of 0.1 mg/kg, peros, for 30 days), no death of experimental mice was registered. In addition, positive dynamics of changes in live weight of experimental animals was observed compared to that in the control group of animals. Long-term injection of *C. leuocladum* extract caused an effect on the biomass of mice. After two weeks of injection of 10% extract of *C. leuocladum* we observe increase of the live weight of white mice by 16.7%. And with 20% concentration of the dosage form of *C. leuocladum* it reached 23.2%, compared with the original, while the live weight of mice in the control group increased by 11.4% in two weeks.

Tests for irritant and allergenic properties of *C. leuocladum* extract were carried out on rabbits. After instillation of the extract on the conjunctiva of the eyes in animals of the experimental and control groups, the reaction was evaluated after 15, 30 minutes and after 1; 2; 3; 4; 5; 6 and 24 hours. The evaluation of the reaction in animals was made according to the following scale: the first group - 0, there is no irritating effect of the drug. The reaction in animals of the second group - 1, slight reddiness the lacrimal duct and mucous membrane. Allergic reactions in the form of tears and outflows, eyelid edema and vascular hyperemia were not observed in animals of both experimental groups. The results of the study of uterine mucus of cows for the presence of microorganisms are shown in Table 2.

**Table 2:** The results of the study of the bacterial background of the uterine mucus of cows with acute endometritis.

Group number	Bioprobe	Selected types of microorganisms
1	cows, experimental group	<i>St. pyogenes</i> 10 <sup>8</sup> CFU/ml, <i>E. faecalis</i> 3*10 <sup>3</sup> CFU/ml
2	cows, experimental group	<i>St. pyogenes</i> 10 <sup>7</sup> CFU/ml, <i>E. coli</i> 10 <sup>5</sup> CFU/ml
3	cows, control group	<i>St. pyogenes</i> 10 <sup>7</sup> CFU/ml, <i>E. coli</i> 10 <sup>6</sup> CFU/ml

The data in Table 2 show that microorganisms *S. pyogenes*

10<sup>8</sup> CFU/ml, *E. coli* 10<sup>6</sup> CFU/ml were detected in various concentrations in the uterine mucus taken from cows for bacteriological examination.

The results of tests for the sensitivity of the isolated microflora to *C. leuocladum* are shown in Table 3.

Table 3 shows that *C. leuocladum* extract at concentrations of 10%, 20%, 40% and suppositories with 20 and 40% content of *C. leuocladum* show moderate antibacterial activity against the gram-negative test strain *E. coli* ATCC 25922. At the same time, *C. leuocladum* medications show the highest antibacterial activity at a concentration of 40% (18±0.1) with a minimum inhibitory concentration (MIC) of 12.5 µg/ml, while the MIC of the extract at concentrations of 10% , 20% and 40% *C. leuocladum* suppository was 25 µg/ml.

**Table 3: Antimicrobial activity of dosage forms of *C. leuocladum***

Dosage forms	Unit of analysis	Activity
<i>C. leuocladum</i> - ethanol extract, 0,5%, 1%, 2%, 5%,10%, 20%, 40%	<i>E. coli</i>	+ active
	<i>St. aureus</i>	+ active
	<i>Ps. aeruginosa</i>	- inactive
<i>C. leuocladum</i> - suppositories 20%, 40%	<i>E. coli</i>	+ active
	<i>St. aureus</i>	+ active
	<i>Ps. aeruginosa</i>	- inactive

*C. leuocladum* medications had no activity against *Ps. Aeruginosa* at all concentrations studied.

Butylhydroxyanisole (BHA) was taken as a control to determine the antiradical activity (formula 1) of *C. leuocladum* (Table 4).

The results of the study of antiradical activity showed that *C. leuocladum* has a high activity compared to Butylhydroxyanisole at all concentrations studied.

For the purpose of studying the therapeutic efficacy of the suppository containing 20% and 40% of *C. Leuocladum* the research was conducted on lactating cows of the Holstein-Friesian breed (n = 45), aged 3-5 years with clinical signs of acute endometritis.

Cows with acute postpartum endometritis were divided into 3 groups, according to the principle of analogues. The first (n=15) and second (n=15) groups were experimental, the third group (n=15) served as control. *C. leuocladum* phytopreparation was included in the treatment of acute postpartum endometritis in cows of the first and second groups. The treatment of cows of the third group included the medication "Biometrosanit". Treatment was carried out according to the following scheme (Table 5).

According to Table 6, each group included a complex treatment regimen - this is intrauterine suppositories with a different composition, and the use of additional medications. For uterine contraction a 0.5% solution of propranolol was injected intramuscularly, a rectal massage of the uterus was performed to remove exudate from the uterine cavity. Then the cows were injected with 2 suppositories intrauterine, once every 24 hours. All animals, intramuscularly, with an interval of 48 hours, were injected with Trivitamin P. The duration of treatment in all experimental groups averaged 8-10 days. The results of treatment of postpartum endometritis are shown in Table 6.

The data in Table 6 show that in herbal treatment of acute catarrhal endometritis with a 20% content of *C. leuocladum* resulted in 53.3% of the animals recovered. The therapeutic effect reached 80% in cows of the second group, where a suppository with a 40% content of *C. leuocladum* was used. The recovery result was 73.3% in the third group, where the "Biometrosanit" was used. The earliest recovery time was observed in the second group (6±0.5 days), the latest in cows of the first group.

In the study on the effectiveness of *C. leuocladum* suppositories in animals, hematological blood parameters were investigated (Table 7).

The results of blood tests indicated an increase in the erythrocyte sedimentation rate (ESR) in cows with endometritis and an increase in the leukocytes level.

After treatment procedures these changes were subtle in animals of the control group, compared with those in second experimental group.

Thus, after treatment the level of hemoglobin in the blood of experimental animals increased by 25, 34% and 27.83%, respectively, while in control group this figure was 19.9%.

The leukocytes level in the blood of animals of all groups slightly exceeded the standard values due to the presence of an inflammatory process in the body. However, after treatment, the white blood cells level decreased with the physiological parameters reaching 32.75% in the second and 35.6% in the third group. A downward trend in leukocytes level in the blood was also observed in control group and amounted to 13.25%.

Also a significant decrease in ESR in all groups was observed. However, the achievement of the standard value was observed in cows of the third group.

**Table 4:** Antiradical activity (%) of *C. leuocladum* at various concentrations of ethanol extract

№	Test substances	Extract concentration (mg/ml)				
		0,1	0,25	0,5	0,75	1,0
1	Butylhydroxyanisole (BHA)	80,82	81,23	80,30	83,08	83,88
2	Ethanol extract of <i>C. leuocladum</i>	85,22	85,44	85,89	85,14	85,31

**Table 5:** Treatment scheme for cows with acute endometritis

Group	Treatment scheme	Dosing type, dosage, dosage frequency
1 – experimental group (n = 15)	0,5% propranolol solution	Intramuscularly, at a dose of 15 ml per injection, for 5 days;
	<i>C. leuocladum</i> suppositories, 20%	Intramuscularly, 2 suppositories, once a day, for 5 days
	Trivitamin P	Intramuscularly, 10 ml, twice, with an interval of 48 hours.
2 - experimental group (n = 15)	0,5% propranolol solution	Intramuscularly, 15 ml, once a day, for 5 days
	<i>C. leuocladum</i> suppositories, 40%	Intramuscularly, 2 suppositories, once a day, for 5 days
	Trivitamin P	Intramuscularly, 10 ml, twice, with an interval of 48 hours.
3 – control group (n = 15)	0,5 propranolol solution	15 ml intramuscularly once a day for 5 days
	Biometrosanit	Intrauterine, 2 tablets, once a day, for 5 days
	Trivitamin P	Intramuscularly, 10 ml, twice, with an interval of 48 hours.

**Table 6:** Therapeutic effectiveness of the treatment of acute catarrhal endometritis in cows

Animal groups	Number of animal treated	Recovery time, days	Number of recovered animals, heads	Recovery %
1 – experimental group	15	8±0,5	8	53,3
2 - experimental group	15	6±0,5	12	80,0
3 – control group	15	7±0,5	11	73,3

**Table 7:** Dynamics of hematological blood parameters of cows in the second experimental group before and after treatment

Ser. №	Parameters	Standard values min... max	Control I M ± m		Experimental II M ± m		Experimental III M ± m	
			Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
1	Hemoglobin, g/l	80-150	86,3±0,32	107,8±0,95*	86,9±0,48	116,4±0,129	87,4±0,96	121,1±0,23*
2	Red blood cells, 10 <sup>6</sup> /µl	5-10	4,41±1,2	4,2±0,78*	4,1±0,57	4,79±0,08*	4,34±0,05	5,03±0,119
3	White blood cells, 10 <sup>3</sup> /µl	4-12	14,18±0,58	11,3±1,23*	13,89±1,7	9,34±0,68*	13,82±0,98	8,9±0,62*
4	Lymphocytes%	45-75	62,0±0,96	59,6±1,33*	65,6±1,46	57,5±0,56*	61,9±0,76	57,6±0,89*
5	Monocytes%	2-7	6,0±0,34	2,2±0,82*	6,4±0,26	2,8±0,38*	5,9±0,09	2,4±0,023*
6	Granulocytes%	26-51	37,0±0,8	39,2±0,29*	38,12±1,2	40,0± 0,18*	36,6±0,76	41,0±0,1*
7	Erythrocyte sedimentation rate (ESR), mm/h	0,5-1,5	6±0,033	3±0,045	7±0,032	2±0,066	6±0,034	1,5±0,08

Notes: \* - P ≤ 0,001

The obtained results of biochemical analysis of blood indicated a decrease in the level of glucose, total protein, calcium, phosphorus in cows with endometritis (Table 8).

The results of the study of the blood biochemical components after treatment showed an increase of most stud-

ied parameters within the standard values, while in the blood of the animals of the experimental group there was a tendency to reduce the enzymatic activity of ALT (34.9 µkat/l before treatment, 21.1±0.45 µkat/l after treatment, P ≤ 0.05).

**Table 8:** Dynamics of biochemical values of blood in cows before and after treatment

Ser. №	Parameters	Standard value min... max	Control group(n-15)		Experimental first group(n-15)		Experimental second group(n-15)	
			(M ± m)		(M ± m)		(M ± m)	
			Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
1	Total protein, g/l	60-120	79,5±0,63	78,3±0,43*	74,4±0,19	85,0±0,53*	71,05±0,56	83,15±0,74*
2	Glucose, mmol/l	2,53-2,97	1,6±0,4	2,2±0,52*	1,55±0,04	2,46±0,15*	1,48±0,87	2,55±0,23*
3	Calcium, mmol/l	1,62-3,37	1,65±0,22	2,40±0,01	1,83±0,7	2,26±0,24*	1,7±0,11	2,8±0,48*
4	Phosphorus, mmol/l	0,81-2,72	0,93±0,37	1,86±0,14*	1,03±0,03	1,57±0,74*	0,76±0,13	1,77±0,32*
5	ALT, mkkat/l	6,9-35	28,4±0,96	27,1±0,98*	31,1±2,65	26,2±1,77*	34,9±1,11	21,1±0,45*
6	AST, mkkat/l	45-110	96,60±0,7	93,2±1,23*	99,6±2,51	92,0±2,05*	104,45±0,8	84,15±0,63

Note: \*- P ≤ 0,05

As can be seen from the Table 9, there was a slight increase in total protein level; after treatment, there was a significant decrease in total protein in the blood of experimental cows by 7.9%, and by 15.5% in control cows (P ≤ 0.05), with alignment with normative values. The glucose, calcium and phosphorus level in the blood of cows in post partum period was significantly lower than the physiological values in experimental and control animals. However, after the treatment we observed adjusting and stimulating within the physiological parameters.

## DISCUSSION

In providing the country's population with food, dairy cattle breeding is of paramount importance, the necessary condition for the intensive management of which is to ensure the health of the breeding stock. The solution of this problem is hindered by the widespread endometritis in cows. Endometritis in cows causes significant economic damage due to reducing milk yield, premature culling of highly productive cows, morbidity in newborn calves, and the cost of treating sick animals.

Despite the available scientific achievements, the problem of endometritis continues to be one of the urgent tasks for veterinary medicine. In the fight against endometritis, it is almost impossible to do without the use of antimicrobial drugs that can cause many side effects.

Naturally, there is a need to create new environmentally safe, effective drugs. The preparations made on the basis of plant raw materials deserve attention.

The advantage of herbal preparations is that they contain biologically active substances that act on the human and animal body in a complex way. In addition, phytopreparations are characterized by bioavailability and the absence of side effects.

The authors (Otto et al., 2015) compile the results on bacterial complications of uterine involution and early diagnosis, treatment and prevention of postpartum uterine diseases and their impact on milk production and reproductive function. They believe that medications that are successfully used in clinical diseases do not always reduce the incidence of cytological endometritis. In addition, treatment of dairy cows with acute postpartum metritis, prostaglandins, nonsteroidal anti-inflammatory drugs in combination with antibiotics leads to an increase in infertility days in cows (Jeremejeva et al., 2012).

Postpartum uterine disease is associated with the release of *E. coli*, *T. pyogenes*, and anaerobic pathogenic bacteria. Perception of bacteria or their toxins, such as lipopolysaccharide, by the innate immune system triggers inflammatory responses (Sheldon et al., 2017).

A comparative analysis of uterine bacteria of cows with different indicators of vaginal discharge (VDS) showed the predominance of *T. pyogenes*, *E. coli* and *Staphylococcus spp.* in the intrauterine flora of all groups, while the relative number of bacteria differed between VDS groups and these data confirm studies that showed a huge variety of bacteria belonging to 202 different species, with *T. pyogenes* (13.2%), *E. coli* (11.2%), *S. xylosus* (5.4%), *B. pumilus* (5.2%) and *S. uberis* (4.9%) predominating (Drillich et al., 2018).

Our data on the presence of antimicrobial properties of *C. leuocladum* against gram-positive and gram-negative microorganisms are consistent with those of (Yahia et al., 2020).

The results of our study of the antiradical activity of *C. leuocladum* are consistent with the results of (Khan et al., 2015) and (Badria et al., 2007), who studied the antiradical and antifungal activity of the methanol extract of *C. polygonoides*. The obtained results indicate that *C. polygonoides*



causes inhibition of the growth of *A. niger*, and also causes a significant antiradical effect.

Our studies have not revealed the antifungal activity of *C. leucocladum* against *C. albicans*, therefore we consider it necessary to continue research on the fungicidal properties of *C. leucocladum*.

Studies of the chronic toxicity of *C. leucocladum* are consistent with the results of (Sakuov et al., 2017), who established the safety of long-term daily intragastric injection of *C. leucocladum* to white mice. Thus, when determining the amitoxic, irritating and allergenic properties of *C. leucocladum*, allergic and toxic manifestations in rabbits were not revealed.

Due to high content of tannins (up to 10-12%), citric acid (up to 5%), alkaloids (up to 1.3%), flavonoids (0.31-0.61%), polysaccharides, coumarins, and leucoanthocyanidins with anti-inflammatory and antitumor properties, and more reserves of raw materials in nature *Calligonum* (Suleimen et al., 2022) may be considered as possible sources of medicinal raw materials, on the basis of which it is possible to develop the medication production that provide a good therapeutic effect.

## CONCLUSION

When studying the therapeutic efficacy of suppositories with a 20% content of *C. leucocladum* used in the complex treatment of acute endometritis in cows, 53.3% of animals recovered, while a drug with a 40% content of *C. leucocladum* provided recovery in 80% of cows, and, in a shorter time -  $6 \pm 0.5$  days. The use of medicinal forms of *C. leucocladum* for treatment determined positive dynamics in the content of total protein, glucose, calcium and phosphorus in blood, and also led to corrections in hematological parameters in sick cows during recovery process.

Thus, the use of phytopreparations of *C. leucocladum* in a complex of therapeutic manipulations, which has antimicrobial and high antiradical activity, is able to suppress the formation of free radicals that aggravate the pathogenesis of the disease, leads to an improvement in the course of endometritis in cows, a reduction in recovery time, correction of metabolic parameters in the blood within physiological normative values.

Therefore, we believe that the creation and use of preparations based on *C. leucocladum* raw materials in veterinary medicine will expand the arsenal of modern herbal preparations with a wide range of pharmacological activity that are safe for animals.

## RECOMMENDATION

Our studies have shown that extract of *C. leucocladum* has antimicrobial and high antiradical activity, therefore it is necessary

- further research on the fungal properties of *C. leucocladum*.
- further development of safe and effective measures for the treatment of postpartum diseases of cows.

## ACKNOWLEDGMENTS

The authors are grateful to the Director of the Institute of Applied Chemistry (Astana, Kazakhstan) candidate of chemical sciences, PhD Suleimen Ye.M. The work was financially supported by the Science Committee, Ministry of Education and Science, Republic of Kazakhstan, Program-Targeted Financing (Grant No. BR06429242 «Scientific support of veterinary wealth and food safety»).

The results of the study of the blood biochemical components after treatment showed an increase of most studied parameters within the standard values, while in the blood of the animals of the experimental group there was a tendency to reduce the enzymatic activity of ALT (34.9  $\mu$ kat/l before treatment,  $21.1 \pm 0.45$   $\mu$ kat/l after treatment,  $P \leq 0.05$ ).

## NOVELTY STATEMENT

The authors developed dosage forms (suppositories) based on extract of *C. leucocladum*, studied their pharmacotherapeutic activity for the treatment of cows's endometritis.

## AUTHOR'S CONTRIBUTION

All authors have contributed in this manuscript from planning, writing, comprehensive discussion until decision to publish

## ETHICAL APPROVAL

All events involving animals were held in accordance with the model Law on responsible Treatment of Animals of the Interparliamentary Assembly of the Member States of the Commonwealth of Independent States (March 27, 2017)

## CONFLICT OF INTEREST

The author have declared no conflict of interest.



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