



# The Pharmacological Study on Therapeutic Efficacy of the Herbal Preparation of Hypericum

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## ABSTRACT

Dyspepsia is a condition associated with digestive disorders, including diarrhea, weakness, dehydration, and intoxication. The investigation and application of therapeutic and prophylactic agents for managing gastrointestinal diseases in young cattle, including dyspepsia in newborn calves, is concerning. The present study aimed to investigate the biochemical mechanisms of action of Hypericum in the treatment of dyspepsia in newborn calves. Two groups of black and white breed bulls (five animals per group), aged 8-10 days, were assigned to assess the effects of Hypericum on biochemical parameters, including total protein, albumin, glucose, bilirubin, urea, creatinine, aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase. Calves in the first group (n = 5), exhibiting pronounced signs of dyspepsia, were administered the treatment. This treatment consisted of 250 mL of Hypericum, combined with 500-700 mL of warm water, instead of milk, during the following two feedings. Then, milk was added to each serving of this mixture until the total volume reached 1.25-1.50 liters, and it was given to calves until clinical signs of recovery appeared. Calves of the second group (n = 5) were clinically healthy and received boiled water instead of the Hypericum. The current findings demonstrated that the biochemical parameters of the experimental calves improved following the administration of Hypericum, reaching levels comparable to those of clinically healthy calves. After prophylactic administration of Hypericum at a dose of 6.5 mL/kg during the first six feedings, the general condition of the experimental calves was satisfactory. In the experimental group of calves administered Hypericum at a dosage of 6.5 mL/kg mixed with milk over a period of two days, only one out of fifteen calves exhibited signs of dyspepsia, including liquid feces and lethargic behavior within the initial four days, representing a prevalence of 6.7%. Hypericum could prevent the occurrence of dyspepsia by 93%. The utilization of Hypericum had a therapeutic influence and positively impacted the clinical condition, behavior, and appetite of the calves.

**Keywords:** Albumin, Biochemical indicator, Calf, Dyspepsia, Hypericum, Total protein

## INTRODUCTION

In the context of intensive animal husbandry development, acute gastrointestinal diseases in young animals pose a significant problem. The incidence of dyspepsia in young cattle is up to 80-95%, and the mortality rate in severe toxic forms of dyspepsia reached 70% (Klein-Jöbstl et al., 2014; Kurazeeva et al., 2015). The most common causes of diarrhea in newborn calves are rotavirus, coronavirus, and cryptosporidium. Malabsorption is the main mechanism by which these and pathogenic microorganisms cause diarrhea (Naylor, 2009). Dyspepsia is a polyetiological disease accompanied by digestive disorders, diarrhea, weakness, dehydration, and intoxication (Norboyev and Fayziyeva, 2024). As a result, milk production in sick animals decreases by 15-18%, and their fertility is reduced (Zhirkov et al., 2000). Therefore, the prevention and treatment of neonatal gastrointestinal diseases in young animals are key factors for increasing meat and milk production. In recent years, therapeutic and prophylactic agents for treating gastrointestinal diseases in young cattle, including dyspepsia in newborn calves, have been identified (Gerceva et al., 2023). Treatment of calf dyspepsia should be based on the etiopathogenetic principle and should be aimed at normalizing the digestive process, eliminating dysbacteriosis, dehydration, and intoxication (Norboyev and Fayziyeva, 2024). Despite a highly diverse etiology and pathophysiology of gastrointestinal diseases, treatment with antimicrobials is often considered the first-line therapy. Uncontrolled use of antibiotics leads to the development of dysbiosis in newborn calves and the

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emergence of resistant bacterial strains to these medications (Dobrzanska et al., 2020; Du et al., 2023). Pursuing novel pharmaceuticals and investigating the biochemical mechanisms of their influence in the prevention and treatment of dyspepsia in newborn calves is an urgent priority issue. Medicinal plants offer potential as alternative or complementary treatments. Medicinal plants, due to their multifaceted effects resulting from plant-specific, multi-component compositions, can potentially function as multi-target medicines. Based on European ethnoveterinary studies, a total of 223 medicinal plant species have been identified for treating gastrointestinal and respiratory diseases. Based on these studies, it was found that the most promising candidates for gastrointestinal diseases are *Allium sativum* L., *Mentha x piperita* L., and *Salvia officinalis* L. (Ayrle et al., 2016). Additionally, a complex phytomineral preparation was created from opoka from the Taskala deposit and a decoction of camelthorn (*Alhagi maurorum*), and investigated for the treatment of diarrhea in calves. The findings suggested a high therapeutic effect of this preparation (Montayeva et al., 2024). The present study aimed to identify potential medicinal plant species for preventing and treating gastrointestinal and respiratory diseases in calves and piglets, and to investigate the biochemical mechanisms of the herbal drug, Hypericum, in treating dyspepsia in newborn calves.

## MATERIALS AND METHODS

### Ethical approval

The Animal Ethics Committee of the Federal Center for Toxicological, Radiation and Biological Safety, Kazan, Russian Federation, approved all animal handling protocols used in the current study (no 1 dated 12.02.2023).

### Study design

The herbal preparation of Hypericum, based on St. John's Wort, was used for the present study. 300 g of St. John's Wort herb was placed in gauze, poured with 6-7 liters of boiling water, and left until the temperature dropped to 70°C. Then, 250 g of glucose, 50 g of sodium chloride, 10 g of calcium lactate, 5 g of ascorbic acid, and 220 g of sodium carboxymethylcellulose (Blanose) were added to the infusion. After dissolving the added components, enough water at 70°C was added to bring the total volume to ten liters. The resulting solution was left for 12-15 hours at room temperature, and periodically stirred several times. Then, the gauze with the remnants of St. John's Wort was squeezed and discarded. Before use, the preparation was heated to 37-38°C and thoroughly shaken into a homogeneous mass (Rakhmatullin and Sklyarov, 2020). The present study was designed to investigate the dynamics of biochemical blood parameters in calves with dyspepsia before and after the use of Hypericum and to determine the prophylactic effectiveness of Hypericum. The current study was conducted at the Federal State Budgetary Institution, Federal Center for Toxicological, Radiation, and Biological Safety, Kazan, Russia.

To examine the impacts of the pharmaceutical compound of Hypericum on the biochemical parameters of blood, two groups of black-and-white calves, aged 8 to 10 days, were assembled. The first group, consisting of five calves exhibiting pronounced signs of diarrhea, was administered Hypericum orally. The administration of Hypericum continued for five days. For therapeutic purposes, sick calves received a mixture of 250 ml of Hypericum with 500-700 ml of warm water instead of milk during the subsequent two drinking sessions. Then, this mixture was added to each subsequent portion of milk, increasing the total portion volume to 1.25-1.5 liters. The treatment was given orally for five days until the animal had clinically recovered. The calves of the second group, consisting of five calves, were clinically healthy and received 750-950 mL of boiled water instead of the treatment. The calves in the experiment underwent a clinical examination, and their physiological development, response to external influences, reception of cow's milk, and digestive and excretory systems were determined. The frequency of defecation, the consistency, and the color of feces were noted. Blood (5-6 mL) was collected from the jugular vein of calves, both before treatment administration and on the fifth day after the last treatment. The content of total protein and albumin, bilirubin, creatinine, urea, glucose, the activity of aspartate (AST) and alanine aminotransferases (ALT) as indicators of liver damage syndrome, lactate dehydrogenase, and amylase enzymes were studied before treatment administration and on the fifth day after the last administration of the treatment.

To assess the prophylactic effect of Hypericum, two groups of black-and-white calves aged 8-10 days were designed. For the prevention of gastrointestinal diseases in calves of the first group (15 heads), within two days after birth, all calves were given 6.5 mL/kg of the Hypericum medicinal solution once with colostrum of the first six feedings. According to the results of preliminary studies and the findings of Garmaev and Garmaev (2019), it has been established that the most optimal condition is a six-fold preventive administration of the treatment. Considering the composition of the treatment, it had a beneficial influence on the immune system of calves. Calves in the second group (15 heads) were given boiled water within two days after birth and were fed using a nipple drinker. The rates of morbidity and mortality among the calves were used to determine the prophylactic effects.

### Biochemical examination

During the experimental studies, the total protein was assessed using the biuret reaction (Lubran, 1978; Tietz et al., 1995; Kondrakhin et al., 2004). The glucose content was determined by the unified glucose oxidase method of orthotolidine oxidation (McMillin, 1990; Tietz, 1995). Activities of ALT and AST were determined using the unified dinitrophenylhydrazine method (Lala et al., 2024). Serum lactate dehydrogenase (LDH) activity was measured by the 2,4-dinitrophenylhydrazine reaction. L-Lactate, in the presence of serum enzyme and NAD, was oxidized to pyruvate, which was then detected through a color reaction with 2,4-dinitrophenylhydrazine (Li et al., 1980; Kondrakhin et al., 2004; Farhana et al., 2023).

### Statistical analysis

The results of the experimental studies were processed using the method of variation statistics. therefore, the STATISTICA 13 application software was used. Statistical comparisons between different groups were conducted using one-way analysis of variance (ANOVA). The Least Significant Difference (LSD) test was used to compare variances among means at a significance level of  $p < 0.05$ . Mean data were expressed with standard deviation.

## RESULTS AND DISCUSSION

The occurrence of acute digestive disorders and liver pathology in young animals in the early postnatal period of development indicates the presence of severe metabolic disorders in the breeding stock (Hussain et al., 2014; Urbutis et al., 2023). Additionally, the metabolism of growing animals is characterized by elevated intensity, making them highly sensitive to changes in maintenance and feeding conditions (Batrakov et al., 2021).

The mechanism of action of the medicine was related to its complex mode of action. The treatment contains St. John's wort, which, according to Klemow et al. (2011), exhibited anti-inflammatory, antimicrobial, antiviral, and antidepressant effects.

At the beginning of the experiments, clinical signs such as general weakness, decreased movement, diarrhea, and a decreased sucking reflex were observed in the calves with dyspepsia in the experimental group. After administering Hypericum water mix, the severity of clinical signs, such as diarrhea and depression, in the calves of the experimental group decreased within 24 hours. Additionally, Hypericum had a noticeable therapeutic effect on the clinical condition, behavior, and appetite of calves, and no adverse reactions were detected. On the third day of treatment administration, the calves in the experimental groups were active, with a pronounced appetite, natural reflexes, and accelerated sucking reflexes. Clinical signs of diarrhea in the treatment group entirely resolved by the fourth day of treatment administration. Corresponding biochemical blood parameters, measured before treatment and five days after the last treatment administration, are presented in Table 1.

**Table 1.** Dynamics of biochemical parameters of blood in black and white breed bulls aged 8-10 days

Measurement results Indicators	Before treatment		After treatment	
	Experimental group	Control group	Experimental group	Control group
Total protein (g/l)	45.2 ± 3.14*	58.7 ± 3.96	62.6 ± 3.42	62.5 ± 4.21
Albumin (g/l)	24.05 ± 1.9*	35.32 ± 2.8	32.9 ± 4.9	32.1 ± 5.7
Glucose (mmol/l)	1.27 ± 0.21*	2.92 ± 0.47	5.2 ± 0.78	4.3 ± 0.69
Bilirubin (μmol/l)	2.63 ± 0.44	1.92 ± 0.54	2.01 ± 0.75	1.43 ± 0.15
Urea (mmol/l)	5.69 ± 0.64*	2.92 ± 0.38	5.78 ± 2.38*	2.02 ± 0.4
Creatinine (mmol/l)	62.7 ± 7.93	70.3 ± 3.54	84.2 ± 16.5	68.5 ± 2.63
Alanine aminotransferases (U/l)	19.0 ± 2.86	14.1 ± 1.18	20.3 ± 3.91	11.8 ± 1.64
Aspartate aminotransferases (U/l)	32.3 ± 4.56*	56.8 ± 2.69	38.8 ± 5.4	47.2 ± 3.1
AST/ALT ratio	1.99 ± 0.47	2.92 ± 0.33	2.63 ± 0.76	3.82 ± 0.59
Lactate dehydrogenase (U/l)	1623.2 ± 31.96	1735.8 ± 48.1	1629.8 ± 262.7	1812.1 ± 29.1

\*  $p < 0.05$  in comparison with the results of the control group

According to the present results, it should be noted that in unhealthy calves before the use of Hypericum, the amount of total protein, albumin, glucose, and AST was significantly lower ( $p < 0.05$ ) than in clinically healthy animals in the control group, by 23%, 27%, 56.5% and 43.12%, respectively. Albumin is the primary protein component of blood, primarily produced in the liver, and it plays a crucial role in transporting water and metabolites from tissues. Hepatic

albumin synthesis is not a high priority, and it occurs when the body is adequately nourished. A poor nutritional state, inflammation, exposure to hepatotoxins, and exposure to high colloid osmotic pressure inhibit synthesis (Moman *et al.*, 2022). A decrease in albumin levels is a clear indicator of a liver detoxification system disorder in calves with dyspepsia. Decreased production of albumin is a rare cause of hypoalbuminemia. Significant and severe chronic hepatic impairment is necessary before a noticeable decrease in plasma albumin levels is observed. Most commonly, inadequate synthesis of albumin in the presence of increased catabolism due to significant systemic illness contributes to an overall hypoalbuminemia (Gounden *et al.*, 2021).

The total urea amount in the experimental group before treatment was significantly higher ( $p < 0.05$ ) by 51.3% compared to the control group measurements. Protein degradation was elevated in the bodies of unhealthy calves; consequently, the urea concentration in the blood of all experimental calves was significantly higher than in the control group ( $p < 0.05$ ). The amount of bilirubin in the experimental group before treatment was insignificantly higher ( $p > 0.05$ ) by 37%. The current results indicated that the occurrence of metabolic and functional disorders in the body systems caused an endogenous intoxication. The predominance of catabolic processes over anabolic processes resulted in the decompensation of the body's regulatory systems and the accumulation of proteolytic enzymes, kinins, and other biologically active products of protein degradation, as well as inflammatory mediators, in toxic concentrations. The accumulation of toxic substances in the body subsequently causes liver dysfunction (Velikanov, 2017).

Calves with dyspepsia exhibited significantly lower concentrations of total protein ( $p < 0.05$ ). A reduction in total protein, albumin, and glucose levels signified the dominance of catabolic processes in the bodies of calves suffering from diarrhea.

Administration of Hypericum resulted in the resolution of clinical signs, such as liquid feces and depressed behavior, in three days. Administering treatment to the calves for five days did not cause toxicosis in the experimental animals. Complete recovery in overall health in calves occurred on the tenth day. There was no significant difference in the biochemical parameters of the blood of calves after treatment in the experimental group compared to healthy calves ( $p > 0.05$ ), except for the urea level. The present results indicated that administering Hypericum led to improvements in the biochemical parameters of the experimental calves, which improved to the level of clinically healthy calves. The current findings demonstrated that Hypericum quickly entered the regulatory system of calves after administration and exhibited therapeutic effects. According to Fayzieva *et al.* (2024), in the calves of the experimental group treated with the probiotic MAXLAC/DW, blood glucose at the beginning of treatment averaged  $2.64 \pm 0.28$  mmol/L and decreased significantly by the fifth day of treatment to  $2.48 \pm 0.18$  mmol/L. Total protein increased significantly from  $54.8 \pm 1.70$  to  $67.4 \pm 1.82$  g/l by the fifth day of treatment. It was observed that the total bilirubin level in the blood of the experimental group, which was injected with Vetom 1.2 probiotic (*Bacillus subtilis* VKPM I-10641), averaged  $4.2 \pm 0.05$  and  $4.4 \pm 0.06$   $\mu$ mol/l. The activities of AST and ALT enzymes decreased to  $0.58 \pm 0.06$  ( $9.67 \pm 1.00$ ) and  $0.42 \pm 0.06$  ( $7.00 \pm 1.00$ ) mmol/s/l (U/l), respectively, by the end of the experiments (Norboyev and Fayziyeva, 2024). Montayeva *et al.* (2024) reported that the created Kazakh complex phytomineral preparation for treating diarrhoea in newborn calves reduced the activity of ALT by 22.4% and AST by 28.7%. Additionally, the total protein level in the calves of the experimental group reached its maximum value, significantly surpassing the comparable metrics of the calves in the control group by 6.1%.

The prophylactic administration of Hypericum at a dose of 6.5 mL/kg during the first six feedings improved the overall health of the experimental calves, with no signs of toxicosis (Table 2). It should be noted that in the calves of the experimental group, which were given Hypericum at a dose of 6.5 mL/kg added to milk for two days, only one calf out of 15 fell ill within the first four days, representing a 6.7% incidence. The Hypericum prevented dyspepsia by 93%. Over a 30-day observation period, no diarrhea was observed in the calves of the experimental group. In contrast, all calves in the control group developed dyspepsia after calving, resulting in a 100% incidence (Shcherbakov *et al.*, 2022).

In the control group, four calves died, representing 26.7% of the total. Explaining the mechanism of the preventive action of Hypericum, it is worth noting that the primary cause of dyspepsia in calves is metabolic disorders in cows during pregnancy, resulting from an inadequate diet and physical inactivity. Metabolic disorders in cows during pregnancy have adverse effects on the embryonic development of calves, resulting in calves born weaker, with reduced natural resistance and susceptibility to diseases (Mischenko *et al.*, 2021).

**Table 2.** Preventive action of Hypericum in black and white breed bulls aged 8-10 days for five days

Group	Number of calves	Percentage of dyspepsia morbidity	Percentage of cattle deaths
Experimental	15	6.3	0
Control	15	100	26.7

## CONCLUSION

The oral administration of Hypericum at a dosage of 6.5 mL/kg for five days effectively restored impaired protein and carbohydrate metabolism, as well as liver function, in calves suffering from dyspepsia, bringing these parameters to levels comparable to those of healthy calves. The administration of Hypericum at a dosage of 6.5 mL/kg demonstrated both therapeutic and prophylactic efficacy in managing calf dyspepsia. Further studies are required to elucidate the impact of Hypericum on the immune system of calves.

## DECLARATIONS

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### Authors' contributions

Emil Rakhmatullin, Ilnur Kadikov, and Ruslan Rakhmatullov developed the study design and substantiated its methodology. Ekaterina Mayorova organized and conducted experimental studies, and Ekaterina Kurshakova and Danil Sagdeev took part in the selection of blood from calves. Andrey Korchemkin conducted the statistical analysis of the results. Emil Rakhmatullin, Ilnur Kadikov, and Ruslan Rakhmatullov analyzed the obtained data and provided a draft of the manuscript. Alsu Makaeva edited and reviewed the manuscript. All authors participated in discussing the results, reviewing the analyzed data, writing the article, and agreeing on the final edition of the manuscript for submission.

### Competing interests

The authors declared no conflict of interest.

### Ethical considerations

The study was originally conducted, and all the analyzed data were prepared based on the experiment results.

### Availability of data and materials

All data of this study are available upon reasonable requests from the authors.

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