



In vitro and In vivo Effects of *Zataria multiflora* Hydro-alcoholic Extract on Bovine *Escherichia coli*

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CALF diarrhea is one of the most important diseases of dairy farms and causes significant economic damage to farmers. Antibiotic-resistant bacterial infection has become a serious concern as antibiotic use has increased, leading to increased use of medicinal herbs. This study was performed to evaluate in vitro and in vivo antimicrobial effect of *Zataria multiflora* on bovine *Escherichia coli*. Sixty calves with *Escherichia coli* diarrhea were selected through microbial culture results. In the first experiment, the antibacterial activities of hydro-alcoholic extract of different levels of *Zataria multiflora* were compared with sulfadimidine to screening against fecal *coli* using a standard protocol of Disc Diffusion Method (DDM). In the second experiment, the calves were randomly treated with hydro-alcoholic extract of *Zataria multiflora* or sulfadimidine. Clinical measurements including rectal temperature, fecal scores, and appetite were assessed. The results of this investigation revealed that a 20 µl of hydro-alcoholic extract of *Zataria multiflora* had antibacterial activity comparable to sulfadimidine. Our clinical results showed that hydro-alcoholic extract of *Zataria multiflora* had superior effects on rectal temperature and stool consistency than sulfadimidine. Also, the use of hydroalcoholic extract of this plant could improve the process of these parameters in calves. The findings show that a hydro-alcoholic extract of *Zataria multiflora* can be utilized to treat diarrhea caused by *E. coli*.

Keywords: Diarrhea, *E.coli*, Holstein calves, Medicinal plants, *Zataria multiflora*.

Introduction

In newborn calves, diarrhea is the leading cause of morbidity and death [1]. The disease is assumed to be involved in 30% of producer losses [2]. Fever, lack of appetite, depression, diarrhea, dehydration, and eventually death are some of the clinical symptoms [3]. *Escherichia coli* (*E. coli*) appears to be a common cause of bacterial diarrhea in calves, resulting in severe scours. [1]. Supportive care, such as fluid therapy and nursing care, is one of the most essential treatments for calf diarrhea, but it is not always effective [4].

Antibiotics have also been used to treat diarrhea. However, increasing antibiotic use has raised serious concerns about antibiotic resistance [5]. As a result, antibiotics has progressively being replaced with medicinal plant in recent years.

Zataria multiflora (*Z. multiflora*) is a thyme-like plant belonging to the Lamiaceae family. This plant is high in carvacrol and thymol, both of which have antibacterial properties against gram-positive and gram-negative bacteria [6]. Antioxidant, anti-inflammatory, antispasmodic, and immune-stimulating effects are also found

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in this plant [7-9]. Studies have shown that the use of *Z. multiflora* in rats and guinea pigs has been effective in treating diarrhea [10]. However, there is no report on the effect of *Z. multiflora* on diarrhea caused by *E. coli* in calves. Thus, the present study aimed to evaluate the in vitro and in vivo antibacterial potential of hydro-alcoholic extract of *Z. multiflora* against bovine *E. coli*.

Materials and Methods

Antibiotic discs were purchased from HiMedia Laboratories Pvt.Ltd.

Preparation of plant extract

In order to prepare this extract, 200 g/L of dried *Z. multiflora* powder was soaked with 70% ethanol. After 48 h, the extracts were filtered using a percolator through Whatman filter paper. Finally, the final extract was collected in 1:2 ratio of plant to liquid extract with 11.85 % W/V dry matter.

Experimental design

Calves with diarrhea less than one week old were monitored from seven industrial farms in Qom province of Iran. A total of 60 calves with *E. coli* diarrhea were chosen based on microbial culture results.

Experiment 1

The disk diffusion technique was used to test the antibacterial activity of different level of *Z. multiflora* (5, 10, 15 and 20 µl) and the antibiotic sulfadimidine on *E. coli* isolated from the feces of calves with diarrhea. The bacterial suspension was cultured on Muller Hinton Agar by sterile swab and blank disc or antibiotic disc. Then bacterial samples were dissolved in normal saline and turbidity was adjusted to 0.5 McFarland spectrophotometrically (OD₆₀₀=0.6). The inhibitory zone diameters were measured after overnight incubation at 37 °C.

Experiment 2

The second experiment was conducted to compare the clinical effects of *Z. multiflora* and sulfadimidine (as a common treatment) on diarrhea caused by *E. coli*. Sixty calves with *E. coli* diarrhea were randomly divided into two groups:

1. Group 1 calves received hydro-alcoholic extract of *Z. multiflora* at a dose of 10 ml per 50 kg of body weight twice daily for 3-5 days based on pre-test experiments.

2. Group 2 calves received oral sulfadimidine (as the common treatment for diarrhea) at a dose of 30 ml per 50 kg body weight on the first day and the second to fifth day, they received a dose of 15 ml per 10 kg of body weight as prescribed by a veterinarian as a control group. Each ml of sulfadimidine solution contained 333 mg of sodium sulfadimidine.

Parameters such as rectal temperature, appetite status, stool consistency, number of stools, and difference in stool consistency score were examined. During the study period, the calves' stool consistency and appetite status were assessed using the following scoring system:

Consistency of stool score: 0-Natural stool, 1-Pasty stool, 2-Semi liquid stool, 3-Liquid stool.

Appetite status score: 3-Natural appetite, 2-Poor appetite, 1-Loss of appetite.

Statistical analysis

Results were analyzed by using SPSS software. The result of disk diffusion was compared using the Duncan's test. The means of in vivo data were compared using the paired T- test and independent T test.

Results

The results of the analysis of *Z. multiflora* hydro-alcoholic extract are shown in Table 1. The findings revealed that the obtained hydro-alcoholic extract of *Z. multiflora* contained 0.19 % thymol and 0.43 % carvacrol. The results of disk diffusion showed that the antibacterial activity of *Z. multiflora* has a dose-dependent impact on *E. coli* compared to sulfadimidine (Figure 1). Although sulfadimidine had a higher inhibitory effect on *E. coli* than the *Z. multiflora* groups, the difference was not significant as compared to the 20 µl *Z. multiflora* level.

The reported measurement uncertainty estimated regarding K=2 and confidence level of approximately 95%.

Table 2 summarizes the effects of *Z. multiflora* and sulfadimidine in newborn calves with diarrhea on rectal temperature, appetite, and stool consistency in vivo. The rectal temperature in the *Z. multiflora* group decreased by 0.97°C after

TABLE 1. The compounds and properties of hydro-alcoholic extract of *Zataria multiflora*.

Row	Test	Method of Analysis	Result
1	Description & Appearance	Visually & organoleptic	Dark brown colored, Clear solution, Strongly odor of Thyme
2	Specific Gravity (g/ml)	USP40<841>	0.989
3	Refractive Index (nD)	ISIRI2274-6	1.3711
4	pH	ISIRI22083	6.50
5	Dry residue (%w/w)	BP2017	11.85
6	Thymol (%w/w)	In House	0.19
7	Carvacrol (%w/w)	In House	0.43

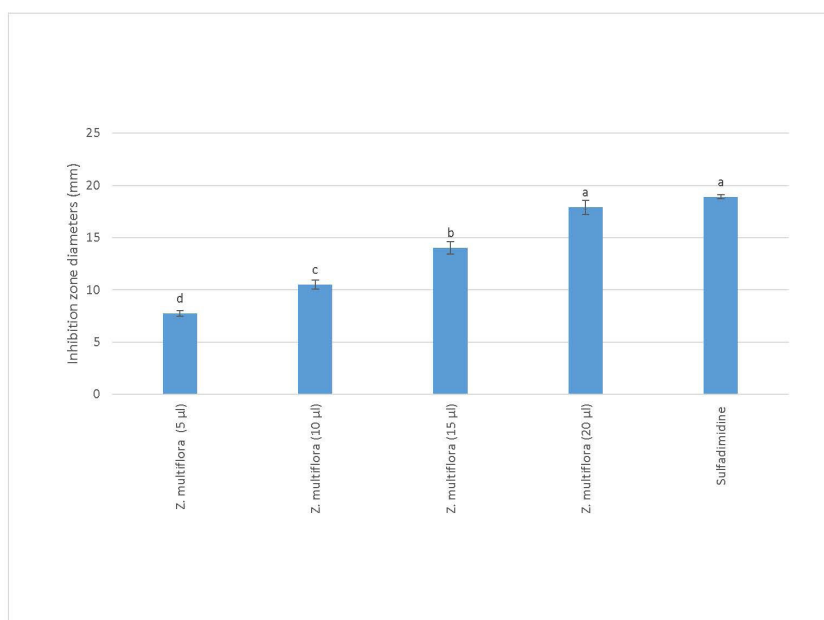


Fig. 1. The inhibition zone diameters (mm) of hydro-alcoholic extract of *Zataria multiflora* and sulfadimidine against bovine *Escherichia coli* by disc diffusion assay.

a, b, c, d Different letters indicate a significant difference ($P < 0.01$).

treatment compared to before treatment, which was significantly different from the control group ($P < 0.001$). There was no statistically significant difference in appetite status improvement between the two groups during the trial. The fecal consistency of the treatment group decreased significantly from 2.55 (loose stool) to 0.21 (rigid and almost typical), while the fecal consistency of the control group declined from 2.66 to 0.50 ($P < 0.001$; Table 2). The *Z. multiflora* and sulfadimidine (control) groups had 2.44 ± 0.19 and 2.48 ± 0.23 days of effectiveness, respectively

(Table 2). The difference in effectiveness duration between the two groups was not statistically significant.

Rectal temperature changes had a decreasing trend in both treatments during the four days of the experiment (Fig. 2).

Also, the appetite status trend is shown in Figure 3, which was rising in both treatments. The consistency of stool was most severe on the last day of the experiment, which decreased in the following days in both treatments (Fig. 4).

TABLE 2. Rectal average temperature, appetite status and stool consistency of newborn calves with *E. coli*-induced diarrhea in hydro-alcoholic extract of *Zataria multiflora* (treatment) and sulfadimidine (control) groups (Mean \pm SE).

Item	Treatment (n= 30)			Control (n= 30)			Difference		P value ²
	before	after	P value ¹	before	after	P value ¹	treatment	Control	
Rectal temp.	39.75 \pm 0.48	38.78 \pm 0.32	<0.001	39.27 \pm 0.50	38.86 \pm 0.39	<0.001	0.97 \pm 0.15	0.41 \pm 0.17	<0.001
Appetite status	1.90 \pm 0.31	2.97 \pm 0.19	<0.001	1.81 \pm 0.69	2.88 \pm 0.33	<0.001	1.07 \pm 0.69	1.07 \pm 0.12	N.S
Stool consistency	2.55 \pm 0.10	0.21 \pm 0.11	<0.001	2.66 \pm 0.08	0.50 \pm 0.13	<0.001	2.34 \pm 0.12	2.16 \pm 0.23	<0.001
Duration of effectiveness	2.44 \pm 0.19			2.48 \pm 0.23			----	----	0.23

P¹: paired T-test P²: independent T-test N.S= not significant

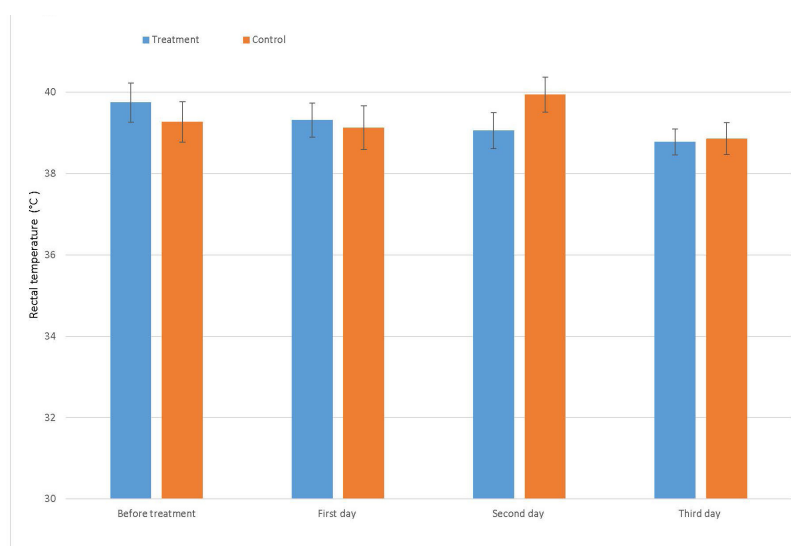


Fig. 2. The trend of rectal temperature changes of newborn calves with *E. coli*-induced diarrhea in hydro-alcoholic extract of *Zataria multiflora* (treatment) and sulfadimidine (control) groups.

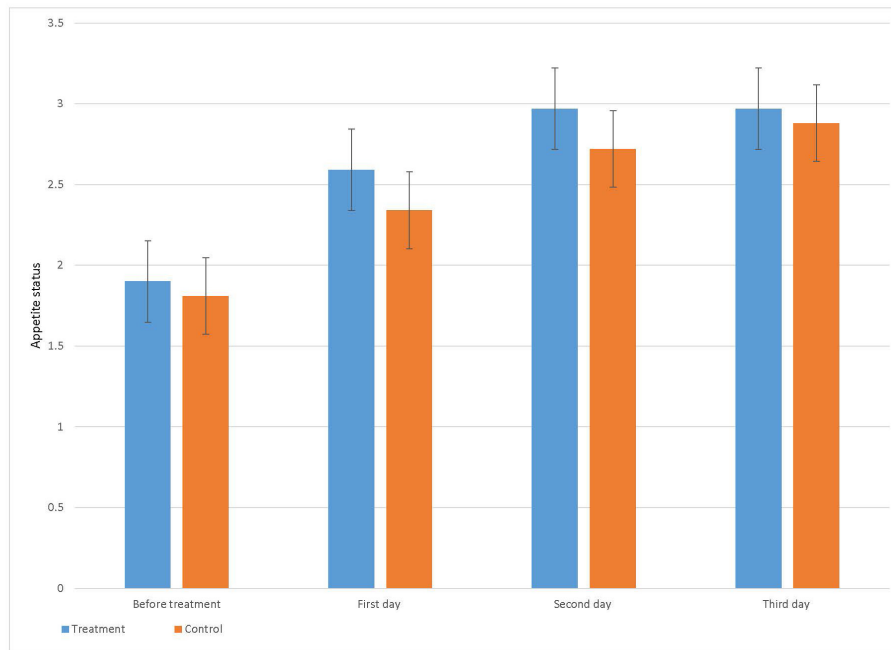


Fig. 3. The trend of appetite status changes hydro-alcoholic extract of *Zataria multiflora* (treatment) and sulfadimidine (control) groups .

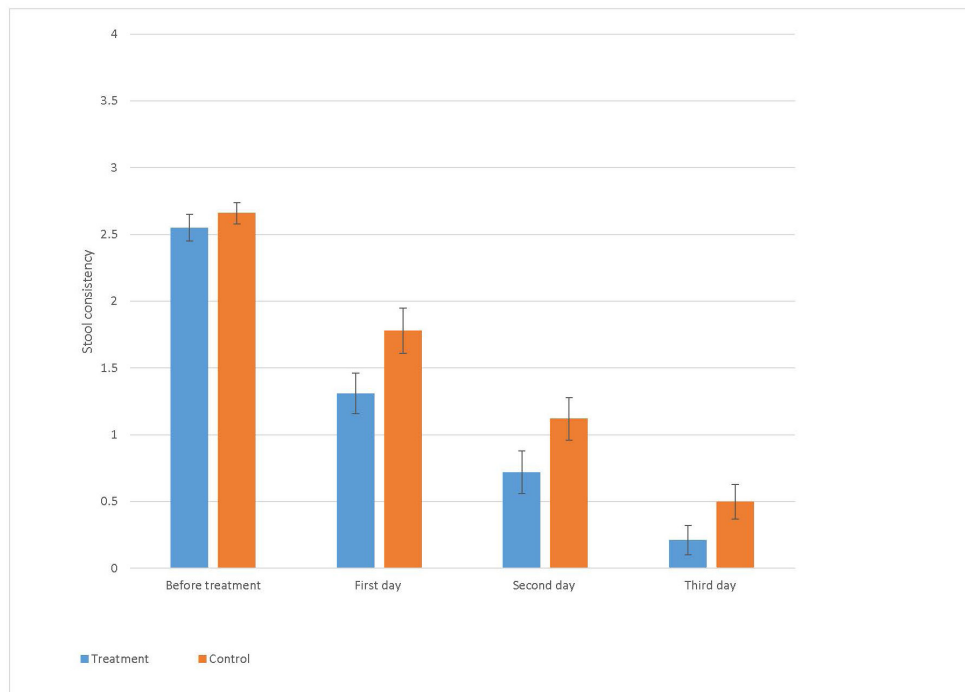


Fig. 4. The trend of stool consistency changes of newborn calves with *E. coli* S-induced diarrhea in hydro-alcoholic extract of *Zataria multiflora* (treatment) and sulfadimidine (control) groups.

Discussion

In vitro and in vitro results of *Z. multiflora* hydro-alcoholic extract showed that it has significant antibacterial properties on bovine *E. coli*. *Z. multiflora* had a superior effect in improving the rectum temperature and stool consistency of calves with diarrhea compared to sulfadimidine. It has been documented that the bacterial flora of the small intestine change in calves with diarrhea regardless of age of the calf and source of diarrhea [11]. Probably part of the beneficial effects of *Z. multiflora* is that it prevents the survival of *E. coli* as well as modulates intestinal bacterial flora. These results were consistent with the results of the strong antimicrobial effects of *Z. multiflora* on different types of microorganisms [12]. In this regard, Fazel et al. reported that the hydro-alcoholic extract of *Z. multiflora* showed antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Shigella flexneri*, and *Salmonella Typhi* [13]. *Z. multiflora* has proven to have anti-inflammatory effects in the treatment of both chronic and acute inflammation in mice and rats [14]. It has been hypothesized that carvacrol and thymol activities, which are the key components of *Z. multiflora*, can disrupt the outer and inner membranes and interact with membrane proteins and intracellular targets [15], as well as periplasmic enzymes [16]. Thus, the antidiarrheal activity of *Zataria multiflora* extract in calf might be attributed to the major components of this plant such as thymol and carvacrol, as well as the synergistic actions of compounds.

Conclusion

As a result, the hydro-alcoholic extract of *Zataria multiflora* can be utilized as an alternative treatment for diarrhea caused by *E. coli* in newborn calves.

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Conflict of interest

The authors declare no conflict of interest.

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