Acta Scientific Veterinary Sciences (ISSN: 2582-3183)

Volume 6 Issue 1 January 2024

Research Article

Antidiarrhoeal Efficacy of Homeopathic Preparation Echinacea Angustifolia Against Naturally Occurring Neonatal Calve Diarrhoea

Mondal Debabrata[1]*, Raguvaran Raja[2], Dosar Shivangi[3], Dixit Shivendra Kumar[1], Narula Ritika Hassija[4] and Khurana Anil[5]

[1]Pr. Scientist, Division of Medicine, IVRI, UP, India

[2]Scientist (SS) Division of Medicine, IVRI, UP, India

[3]JRF, Division of Medicine, IVRI, UP, India

[4]Scientist, CCRH, Delhi India

[5]Director, CCRH, Delhi India

***Corresponding Author:** Mondal Debabrata, Scientist (SS) Division of Medicine, IVRI, UP, India.

DOI: 10.31080/ASVS.2024.06.0804

Received: October 31, 2023

Published: December 18, 2023

Abstract

Background and Objective: Need based clinical research with homeopathic medicine as compared to conventional allopathic treatment is the best bet option to overcome sole dependency on allopath.

Method: Present study was envisaged to evaluate therapeutic potential of homeopathic formulation *Echinacea angustifolia* (Ea) in different potencies viz, (θ, 30C and 200C) against naturally occurring neonatal calf diarrhoea to overcome the detrimental effect of indiscriminate use of antibiotics in dairy herds. Randomised controlled trial performed against naturally occurring clinical cases of neonatal diarrhoea. Therapeutic efficacy was assessed based on resolution of clinical signs and improvement in hematobiochemical and oxidative stress indices.

Results: Clinical recovery was earlier in *E. angustifolia* treated group whereas it was delayed in other groups including standard antibiotic treated groups in this study.

Conclusion: *Echinacea angustifolia* 200C may effectively treat neonatal calf diarrhoea under standard managemental condition and may reduce indiscriminate usage of antibiotic in livestock industry.

Keywords: Diarrhoea; *Echinacea Angustifolia*; Homeopathy; Neonatal

Introduction

Livestock production plays an important role in the national economy. Neonatal calf diarrhoea (NCD) is a major cause of calf morbidity and mortality, poses great threat upon the dairy industry lead to adverse effect on countries economy [1]. Economic losses occur not only from mortality but also from treatment costs and time spent on care as well as subsequent chronic ill thrift with impaired growth performance [2]. Stress plays an important role in predisposing calves to most common problems of diarrhoea during their first few weeks of life.

Neonatal calf diarrhoea is a routine occurrence all over the world and it is caused by both infectious and non-infectious agents. Among the infectious agents it is often caused by *E. coli*, *Salmonella* sp, Corona virus, Rota virus and *Cryptosporidium parvum* [3]. Antibiotics are routinely employed for the treatment of NCD through empirical observation despite confirmatory diagnosis. This approach may lead to the unnecessary use and development of antibiotic-resistant bacteria. WHO guidelines (2018) recommend reduced usage of antibiotic in the food industry [4]. Use of antibiotics in the livestock sector has been increasing to such an extent that it

threatens negative consequences for human health, animal wealth and the environment. Antimicrobial resistance poses serious challenge to the physician and they often switch over to the alternate medicine or therapy for better recovery [5].

Homeopathic remedies have significantt benefits since there is no residual effect on animal products. Veterinarians are constantly seeking less expensive, safer, user-friendly therapeutic agents. Livestock owners are increasingly interested in "natural" and "alternative" methods of disease management, like usage of homeopathy in field condition. Use of traditional methodologies among the rural folks is gaining importance because of their therapeutic value, local availability and cost effectiveness. Homeopathy may be an alternate modality to alleviate therapeutic response against unmet problems from its origin. Randomized controlled trial with homeopathy may bring an alternative to antibiotics.

Most of the infectious diarrhoea is associated with intestinal mucosal damage thereby causing oxidative stress [6] resulting from increased production of free radicals and reactive oxygen species (ROS) and/or a decrease in antioxidant defence which leads to damage of biological macromolecules, disruption of normal metabolism and physiology [7]. Homeopathic formulation *Echinacea angustifolia* is used as an antiseptic, antiviral, and immune stimulant [8]. Supplements of *Echinacea angustifolia* boosts the immune system and can reduce symptoms of infections and decreases inflammation due to illness and also exerts antioxidant effects [10].

Primary aim of the present study is to contribute for improving homeopathy research for the benefit of animal. In cattle, there is limited literature on the use of homeopathic medicines. Study design in homeopathy trials is a matter of debate. Clinical trials on various animals have been reported with homeopathy medicine from time to time but not of well randomized controlled trial with scientific reasoning. Lack of knowledge and understanding might be reasons for the limited use of homeopathy in the present livestock sector.

Efficacy of the homeopathic medication is often debated in various scientific forums across the globe. For the concrete proof of the therapeutic efficacy in homeopathic medicine, it requires clinical evidence on naturally occurring diseased condition of target animals. With this background, the present work was envisaged to evaluate the anti-diarrhoeal potency of *Echinacea angustifolia* in naturally occurring neonatal calf diarrhoea.

Materials and Methods

Medicine and diagnostic kits

Homeopathic drug: *Echinacea angustifolia* of θ, 30C & 200C potency was selected based on literature with empirical background and purchased from DR.WILLMAR SCHWABE INDIA PVT.LTD. Therapeutic drug for *Echinacea angustifolia* 30C & 200C potency constituted by ad lib soaking of drugs in approximately 2 mm size sugar globules whereas *Echinacea angustifolia* θ by mixing 2-3 drops of mother tincture solution in 2-3 ml distilled water. Antibiotic**:** (Ceftriaxone) @10mg/kg b wt for 5 days taken as standard antibiotic for therapeutic trial in calves. SOS Standard Supportive (*L si o´pus* sit - if it is necessary) with rehydration solution consisted of DNS and or Ringer's Lactate (%dehydration x B Wt) IV for 05 days. Diagnostic kits for serum biochemistry were purchased from Coral Company.

Animals and experimental design

Study was conducted on cattle calves maintained at Cattle and Buffalo Farm Unit of LPM Section, ICAR - IVRI. Present study was of veterinary in nature which aims to improve the health and welfare of animals. Necessary ethical approval from CPCSEA, India, a national standard veterinary authority, was obtained prior to the study. After completion of study, all the diarrhoeic calves under trial were rehabilitated and allowed to be the part of main farm animal stock. Neonatal diarrhoeic calves (0 to 28 days) were selected for therapeutic trial. Neonatal diarrhoeic calves were randomly divided into 7 groups (n = 6) consisted of healthy control (Gr. I), disease control (Gr. II), placebo control (Gr. III), standard (Ceftriaxone) antibiotic (Gr. IV) control and homeopathy formulation *Echinacea angustifolia* of different potency, Ea - θ (Gr. V), Ea - 30C (Gr. VI) and Ea - 200C (Gr. VII). Therapeutic efficacy was assessed based on resolution of clinical signs and improvement in hemato-biochemical and oxidative indices. Details of the therapeutic regime have been presented in the table 1.

Sample collection

Pre and post treatment faecal samples were collected from the affected calves for diagnostic and therapeutic evaluation purpose. Samples were stored in plastic pots and analysed shortly. Blood samples with and without EDTA were collected for haematological and blood biochemical study.

Groups (n = 06)	Treatment detail for 07 days
Gr. I (Healthy control)	Nil
Gr. II (Disease control)	Received allopathic therapy after completion of trial as per CPCEA norms
Gr. III (Standard Antibiotic Control)	Ceftriaxone@10mg/Kg BD IM for 7 days + SOS standard supportive therapy
Gr. IV (Placebo control)	Placebo drug (Homeopath formulation without specific compound for homeopathic remedy) + SOS standardsupportive therapy
Gr. V(*Echinacea Angustifolia* θ)	Mixed 2-3drops of mother tincture in 2-3 ml distilled water and of it 4-5drops PO OD alternate days for 7 days + SOS standard. supportive therapy
Gr. VI(*Echinacea angustifolia*-30C)	4-5 globules PO BD for 7 days + SOS standard supportive therapy
Gr. VII(*Echinacea angustifolia*-200C)	4-5globules PO BD for 7 days + SOS standard supportive therapy

Table 1: Therapeutic regimen detail.

Parameters of study

Clinical Evaluation: Diarrhoeic calves were examined routinely for stool colour, consistency and for dehydration, depression if any. Clinical score was assigned to each group on day 0 and 7 based upon the improvement status. Score card details have been presented in table 2.

Clinical Score	Interpretations				
	Stool Consistency score	Lethargy/Weakness/ depression	Dehydration score	Anorexia/ Inappetence	Visible mucous membrane
1 (+)	Normal (well-formed faeces)	Normal (Alert and responsive).	Normal (bright eye and pliable skin).	NO	Pink and Moist
2 (+)	Pasty faeces	Mild depression, calf suckles but not vigorously.	Mild dehydration, slight loss of skin elasticity, skin tents< 3seconds, eyes do not recess into orbit.	YES	Pale and Moist
3 (+)	Semi-liquid faeces, still with a solid component	Moderate depression, calf able to stand, suckling is weak or disorganized	Moderate dehydration, skin tents > 3second but < 10seconds, eyes slightly recessed into orbit	YES	Pale and Dry
4 (+)	Watery faeces	Severe depression, unable to stand or suckle	Severe dehydration, skin tents > 10 seconds, eye smarkedly recessed into orbit.	YES	Dry

Table 2: Clinical Score Card of the affected calves.

Faecal examination

Initially, faecal samples were subjected to centrifugal-flotation technique to detect the presence of parasitic ova/oocyst in faeces. Faecal samples were further subjected to bacteriological examinations by isolation and identification. Samples were inoculated in buffered peptone water (BPW) and incubated at 37°C overnight. A loopful of bacterial growth was streaked on MacConkey's (Hi-Media, Pvt. Ltd., India) agar and incubated for 24h at 37°C aerobically. Antimicrobial susceptibility testing of isolated *E. coli* was performed using Kirby- Bauer disc diffusion method on Mueller-Hinton agar (HiMedia, Pvt. Ltd., India) as per guidelines of the Clinical and Laboratory Standards Institute. Briefly, McFarland 0.5 standardized suspension of the bacteria in nutrient broth was prepared and incubated for 6-8h and using sterile cotton swab streaked over the entire surface of Mueller-Hinton agar. Discs containing known concentrations of each antimicrobial drug was then placed onto the inoculums surface of MHA plates and incubated at 37°C aerobically for 16-18h. Clear zones of bacterial growth inhibition were measured in mm using a measuring calliper. The antimicrobials and their concentrations used for the susceptibility test were Ampicillin (AMP 10µg), Enrofloxacin (EX10µg), Amikacin (AK30µg), Amoxyclav (AMC 20/10µg), Nalidixic acid (NA 30µg), Cefotaxime (CTX30µg), Ceftriaxone (CTR 30µg), Tetracycline (TE 30µg), Cefotaxime/Clavulanic acid (CEC 20µg), Trimethorpim sulpha (COT 15 µg)

Haematology and serum biochemistry

Complete blood count (CBC) was performed as per standard procedures and the serum biochemical parameters viz. blood urea nitrogen (BUN), creatinine, alanine amino transaminase (ALT), as-

partate amino transferase (AST) and total protein were analysed using standard diagnostic kits by UV-Spectrophotometric method.

Oxidative stress indices estimation

Oxidative Stress indices in the affected calves were measured by total antioxidant assay (DPPH and FRAP assay).

- **DPPH (1, 1 diphenyl 2, picrylhydrazl) Assay:** The free radical scavenging activity of serum was measured by DPPH (1, 1 diphenyl 2, picrylhydrazl) assay with slight modification [7]. It measures the free radical scavenging activity in terms of hydrogen donating ability or radical scavenging property of serum. Serum sample (100 µl) was mixed with 2 ml of DPPH Solution (0.2 mM) prepared in methanol. The mixture was allowed to incubate at room temperature for 30 min. After completion of incubation period, 1 ml of chloroform was added and centrifuged at 3000 rpm for 5 min. The absorbance of clear solution was measured at 517 nm. A 100 mM of DPPH solution prepared in methanol was used as a control. Percent inhibition of DPPH free radical (scavenged %) was calculated based on the following equation

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

- **FRAP (Ferric Reducing Antioxidant Power) Assay:** To determine the total antioxidant capacity of serum, a modified FRAP assay was used with little modification [10]. The fresh working FRAP reagent was prepared by mixing 10 volumes of 300mmol/L acetate buffer (3.1 g of CH_3COONa and 16 mL of CH_3COOH), pH 3.6, with 1 volume of 10 mmol/L TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mmol/L HCl and 1 volume of 20 mmol/L $FeCl_3$. Serum sample (50µl) was mixed with 1.5 mL of FRAP reagent and kept at dark for 10 min. The resulting intense blue colouration (Ferrous tripyridyltriazine complex) was subsequently measured at 593 nm. Aqueous solution of $FeSO_4 \cdot 7H_2O$ (100-1000 M) was used as standard. The data was expressed as FRAP value (µM/mL Fe (II).
- **Therapeutic Evaluation:** Efficacy of the therapy was assessed based on the onset of drug action, improvement in clinical score, haematology and serum biochemistry.
- **Statistical Analysis:** JMP 9.0 software was used for analysis of the data. Independent "t" test was employed to determine the statistical significance of blood parameters. The statistical analysis was considered significant at $p < 0.05$.

Result

Fecal samples collected from all the diarrheic calves were found negative for endoparasitic infection by microscopic examination of parasitic ova/oocyst. On bacteriological examination, pink colored *E. coli* colonies were identified in MacConkey's agar and were sub-cultured into nutrient agar to get a pure colony, followed by sub-culture in eosin methylene blue (EMB) agar. Green metallic sheen colonies were further confirmed by gram staining (Figure 1) as well as biochemical reaction test to identify enterobacteriaceae family by HiMedia IMViC (Indole, Methyl Red, Voges Proskauer and Citrate) test for final confirmation as *E. coli* (Figure 2). Isolates confirmed as *E. coli* were again processed for conformational identification by PCR amplification of *E. coli* specific *uidA* gene after boiling and snap chilled extracted DNA as template. PCR was carried out using *uidA* primers by the program comprised of 94°C for 5 min followed by 35 cycles of annealing at 55.2 °C for 10 s, extension at 72 °C for 1 min, denaturation at 94 °C for 10 s and a final extension of 72°C for 10 min in the thermal cycler (Eppendorf, Germany). The amplified DNA (5 µL per lane) was run on a 2% agarose gel at constant 70 V. Gel was visualized under UV light (Figure 3). *E. coli* were found resistant to all tested antibiotics except amikacin and cefotaxime (Figure 4).

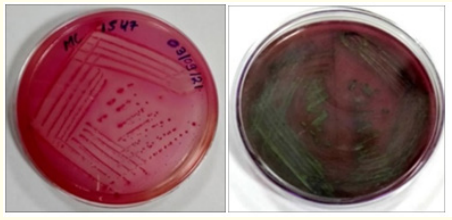

Figure 1: *E. coli* isolates from diarrheic calf stool.

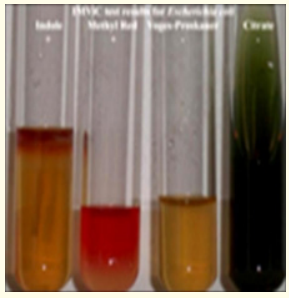

Figure 2: Biochemical Tests of *E. coli* isolates from diarrheic calf stool.

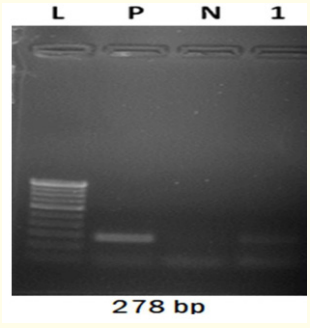

Figure 3: Amplification of *E. coli* specific uidA gene.

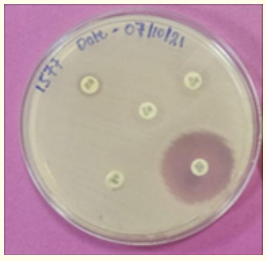

Figure 4: ABST of isolated *E. coli.*

Grs.	Anorexia/Inappetance		Visible Mucous Membrane		Lethargy/Weakness/ depression		Stool consistency		Dehydration	
	0 Day	7 Day	0 Day	7 Day	0 Day	7 Day	0 Day	7 Day	0 Day	7 Day
Gr.I	1 (+)	2 (+)	2 (+)	3 (+)	1 (+)	2 (+)	1 (+)	2.6 (+)	3 (+)	3.8 (+)
(Healthy	(A)	(A)	(AB)	(A)	(A)	(A)	(A)	(A)	(A)	(A)
Control)										
Gr.II	1 (+)	2 (+)	1.3 (+)	2.8 (+)	1 (+)	2 (+)	1 (+)	2.6 (+)	2.3 (+)	3.8 (+)
(Disease	(A)	(A)	(A)	(A)	(A)	(A)	(A)	(A)	(A)	(A)
Control)										
Gr. III	1.3 (+)	1.6 (+)	2.1 (+)	2.1 (+)	1.5 (+)	1.6 (+)	1.1 (+)	2.8 (+)	2.1 (+)	3.3 (+)
(Standard	(A)	(A)	(A)	(A)	(A)	(A)	(A)	(A)	(A)	(A)
Antibiotic)										
Gr.IV	1.3	1.6 (+)	2.1 (+)	2.6 (+)	1.6 (+)	1.8 (+)	1.3 (+)	2.3 (+)	2.5 (+)	3.5 (+)
(Placebo	(+) (A)	(A)	(AB)	(A)	(A)	(A)	(A)	(AB)	(A)	(A)
Control)										
Gr.V	1.1 (+)	1.6 (+)	1.8 (+)	2.6 (+)	1.3 (+)	1.8 (+)	1.3 (+)	2.6 (+)	2.5 (+)	3.5 (+)
(Ea-θ)	(A)	(A)	(AB)	(A)	(A)	(A)	(A)	(A)	(A)	(A)
Gr.VI	1.1 (+)	1.8 (+)	2 (+)	2.8 (+)	1.1 (+)	1.6 (+)	1 (+)	2.5 (+)	2.6 (+)	3.6 (+)
(Ea-30C)	(A)	(A)	(AB)	(A)	(A)	(A)	(A)	(A)	(A)	(A)
Gr.VII	1.3 (+)	1.8 (+)	1.8 (+)	2.6 (+)	1.1 (+)	1.8 (+)	1.3 (+)	2.6 (+)	2.5 (+)	3.8 (+)
(Ea-200C)	(A)	(A)	(AB)	(A)	(A)	(A)	(A)	(A)	(A)	(A)

Table 3: Comparative clinical score of calves' at pre and post treatment period.

Clinical score was assigned to each group on the basis of therapeutic improvement in the parameters like anorexia/inappetance, status of mucous membrane, lethargy, weakness/depression and stool consistency, dehydration status before and after therapy. Score was assigned in a scale of 1^+ to 4^+ according to the condition of animal and 1+ was considered as no improvement and 4+ was considered as improvement. Details of score card have been presented in table 3. Calves received Ea - 200c recorded earlier recovery even by 2^{nd} to 3^{rd} day of therapy by resolution of clinical signs with the normal level of blood biochemical parameters. All calves received Echinacea (θ and 200c) recovered and some even earlier than antibiotic therapy which was reconfirmed by stool culture examination.

Examination of complete blood count revealed (Table 4) no significant difference in the HB, TLC, TEC and PCV level on day 0 and day 7 in all treatment groups. No significant difference was noticed in the AST level on pre and post treatment in all treatment groups. With respect to ALP level, there was significant ($p < 0.05$) difference noticed on day 7 in Gr. V and VI but within the reference values (Table 5). Total protein level showed no significant difference on pre and post treatment in all groups except the Gr. III wherein

Grs.	HB (g/dl)		TLC (× 10^3/µl)		TEC (× 10^6/µl)		PCV (%)	
	0 Day	7 Day	0 Day	7 Day	0 Day	7 Day	0 Day	7 Day
Gr.I	10.3 ± 1	10.5 ± 1	9516 ± 388	10323 ± 32	12.6 ± 2.1	13.4 ± 2.	3.1 ± 3.0	31.7 ± 3.
(Healthy	(A)	(A)	6(D)	30(BCD)	(ABC)	7	(A)	5(A)
Control)						(ABC)		
Gr.II	7.7 ± 1.3	7.7 ± 0.9	13483 ± 67	13615 ± 67	9.3 ± 3.9	11.2 ± 2.	23.1 ± 3.9	23.1 ± 2.
(Disease	(CDE)	(CDE)	93(ABC)	93(AB)	(C)	7	(CD)	7 (CD)
Control)						(BC)		
Gr.III	8.4 ± 2.4	8.5 ± 2	12.4 ± 4.3	12.0 ± 4.3	11.1 ± 4.2	10.2 ± 2.	25.3 ± 6.7	25.2 ± 5.
(Standard	(DE)	(E)	(E)	(E)	(BC)	2(BC)	(BCD)	0
Antibioti								(BCD)
c)								
Gr.IV	7.3 ± 0.8	7.0 ± 0.4	11941 ± 53	10702 ± 52	11.8 ± 2.4	12.3 ± 4.	22.2 ± 2.6	21.1 ± 1.
(Placebo	(BCDE)	(BCDE)	58	639	(BC)	4	(D)	8(D)
Control)			(ABCD)	(BCD)		(ABC)		
GroupV	8.2 ± 0.8	8.6 ± 1.0	9685 ± 218	16.3 ± 2.9	16.3 ± 2.9	13.9 ± 2.	24.8 ± 2.5	26.3 ± 3.
Grs. (Ea-0)	(BCDE)	(BCD)	3(BCD)	(CD)	(AB)	8	(BCD)	0
						(ABC)		(BCD)
Gr.VI	9.4 ± 1.2	9.3 ± 1.4	15508 ± 18	12163 ± 15	12.8 ± 4.3	12.8 ± 4.	28.4 ± 4.0	29 ± 3.8
(Ea-30C)	(AB)	(AB)	36(A)	81	(ABC)	3	(AB)	(A)
				(ABCD)		(ABC)		
Gr.VII	11.35 ± 2.	11.9 ± 3.8	20.3 ± 8.52	14 ± 5.30	9.63 ± 2.0	9.3 ± 2.3	34.15 ± 8.5	36.2 ± 1
(Ea-200C)	80(A)	8(A)	(B)	(B)	(B)	(B)	3(B)	1.73
								(B)

Table 4: Haematological changes of calves on pre and post treatment period.

Grs.	AST (IU/L)		ALP (IU/L)		TP (g/dl)		BUN (mg/dl)		CRE (mg %)	
	0 Day	7 Day	0 Day	7 Day	0 Day	7 Day	0 Day	7 Day	0 Day	7 Day
Group I (Healthy Control)	14.3 ± 5.2 (EF)	15.3 ± 7.1 (EF)	5.4 ± 2.7 (DE)	6.4 ± 2.0 (DE)	6.5 ± 0.85 (A)	6.8 ± 1.3 (A)	18.7 ± 8.4 (CDE)	21.1 ± 15.2 (BCD)	1.4 ± 0.2 (A)	1.3 ± 0.2 (AB)
Group II (Disease Control)	9.3 ± 4.2 (FG)	9.3 ± 5.3 (FG)	5.3 ± 2 (DE)	5.0 ± 2.6 (E)	7.5 ± 1.3 (A)	7.5 ± 1.6 (A)	11.7 ± 4.7 (EF)	10.5 ± 4.7 (F)	0.8 ± 0.2 (AB)	0.8 ± 0.3 (B)
Group III (Standard Antibiotic Control)	33.3 ± 6.5 (AB)	34.6 ± 7.1 (A)	21 ± 9.3 (A)	4.7 ± 1.9 (E)	6.7 ± 1.1 (A)	3.2 ± 1.2 (C)	18.7 ± 4.1 (CDE)	19.0 ± 5.5 (CDE)	1.0 ± 0.4 (AB)	1.0 ± 0. (AB)
Group IV (Placebo Control)	5.7 ± 5.1 (G)	5.5 ± 3.1 (G)	4.6 ± 1.6 (E)	20.0 ± 9.4 (AB)	3.6 ± 1.2 (BC)	5.6 ± 0.9 (ABC)	11.9 ± 6 (EF)	10.7 ± 4.4 (F)	1 ± 0.25 (AB)	1.1 ± 0.3 (AB)
Group V (Ea-0)	16.6 ± 3.2 (E)	19.3 ± 3.0 (DE)	13.8 ± 5.4 (C)	14.9 ± 5.2 (E)	6.3 ± 1.1 (A)	6.1 ± 0.8 (ABC)	14.9 ± 3 (DEF)	16.2 ± 2.5 (CDEF)	0.7 ± 0.29 (B)	0.7 ± 0.1 (B)
Group VI (Ea-30 C)	24 ± 10 (CD)	26.1 ± 11.1 (BC)	14.2 ± 2 (C)	16.4 ± 2.7 (BC)	7.2 ± 2.6 (A)	7.8 ± 1.8 (A)	20.1 ± 4.7 (CD)	23.1 ± 5.9 (ABC)	1.0 ± 0.3 (AB)	1.1 ± 0.2 (AB)
Group VII (Ea-200C)	23.83 ± 5.77 (A)	24.83 ± 6.04 (AB)	18.8 ± 6.67 (A)	23.8 ± 10.5 (A)	9.3 ± 2.06 (A)	9.5 ± 2.0 (A)	18.3 ± 8.11 (B)	15.5 ± 6.68 (B)	1.26 ± 0.10 (A)	1.4 ± 0.25 (A)

Table 5: Serum biochemical changes of calves on pre and post treatment period.

Grs.	FRAP (µM/mL)		DPPH (%)	
	0	7	0	7
Gr.I (Healthy Control)	1229 ± 106 (G)	1331.2 ± 96 (F)	48.1 (CD)	50.1 (BC)
Gr.II (DiseaseControl)	1307 ± 241 (FG)	1363.3 ± 329 (EF)	52.5 (ABC)	48.6 (CD)
Gr. IIII (StandardAntibioticControl)	820.6 ± 112 (DE)	959.4 ± 74 (CD)	48.1 (A)	56 (A)
Gr.IV (PlaceboControl)	1450 ± 124 (I)	1546.4 ± 124 (H)	58.3 (CD)	58.3 (AB)
Ea-0	1619 ± 48 (ABC)	1732 (A)	40.1 (EF)	36.5 (F)
Ea- 30 C	1596.3 ± 129 (BC)	1672.3 ± 142 (AB)	46.3 (CD)	43 (DE)
Ea- 200 C	1287.5 ± 250.11 (B)	1456.33 ± 347.01 (C)	65.33 ± 21.43 (C)	57.66 ± 22.39 (D)

Table 6: Oxidative stress changes in calves on pre and post treatment period.

significantly ($p < 0.05$) reduced total protein was recorded on day 7. BUN and creatinine levels were not significant in all groups on pre and post treatment days.

With respect to FRAP assay (Table 6), significantly ($p < 0.05$) increased total antioxidant level noticed on Day 7 in Group V as compared to other groups. DPPH level was not significant on pre and post treatment in all groups except in Group VII. Calves received Ea - θ recovered by 05 to 09 days whereas 03 calves received Ea -30cdid not recover and these animals were subjected to allopathic therapy till recovery.

Histopathological changes

During the trial period, one diarrhoeic calf of Gr. V (Ea - θ) died which was subjected to histopathological changes if any due to *E. coli* diarrhoea and the same calf was excluded from the therapeutic trial. Tissue samples subjected to hematoxylin and eosin (H.E) stain for histopathological changes and revealed mild to moderate fatty changes in the hepatocytes of periportal mid zonal and central area of liver. Mild infiltration of neutrophils with few monocytes was depicted in the hepatic sinusoids. Mild fibro cellular infection was evident in the portal triad area. In spleen moderate macrophage reaction in sub capsular splenic pulp and in red pulp area was present. Highly engorged and hemorrhagic red pulp with moderate presence of plasma cells and splenomegaly with macrophage cell proliferation was evident (Figure 5).

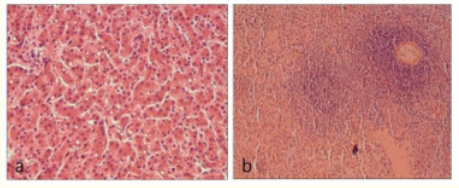

Figure 5: Histopathological changes in Liver (A) and Spleen of diarrhoeic calf (B).

Discussion

Indiscriminate use of antibiotics in the livestock sector threatens negative consequences due to its residual effect on human health, animal wealth and the environment. Undifferentiated diarrhoea in young calves (neonatal calf diarrhoea) is a routine occurrence all over the world and even India. Homeopathic remedies have significant benefits since there is no residual effect on animal products, nor does it generates resistant microorganisms. Primary aim of the study was to contribute for improving homeopathy research for the benefit of animal. In cattle, there is limited literature on the use of homeopathy. Study design in homeopath trials is a matter of debate. Clinical trials on various animals have been reported with homeopath medicine from time to time but not of well randomized controlled trial with scientific reasoning.

Fundamental clinical research and a battery of on field testing are the basis to establish level of confidence. Efforts have been given to develop therapeutic regimen on the basis of prospective observational data and therapeutic consequences. Study was focused on strategic combination of observation based effective methodologies to achieve sustainable therapeutic effect.

In the present study, all fecal samples were found negative for protozoan and helminth infections and were found positive for *E. coli* by gold standard bacteriological diagnostic tests. Treatment of diarrhoeic calves with antimicrobial agents based on bacteriological culture and sensitivity tests is rarely practised routinely even in organised dairy farm. This practice led to emergence of antimicrobial resistance (AMR) in the animal population. *E. coli* isolates obtained from calves exhibited 75% [9], 83% [11] and 87% [12] resistance for ampicillin and for tetracycline (54.3%) and meropenem (2.5%). Similar kind of observation was noticed in the present

study where *E. coli* were found resistant to all tested antibiotics except amikacin and cefotaxime/clavulanic acid. In addition, multidrug resistant *E. coli* isolates 139 (49.6%) were also reported [13]. Antimicrobial resistance was higher in organized dairy farm as compared to unorganised dairy sector. AMR is an emerging problem across the globe leading to huge economic losses in livestock enterprises. Multidrug resistant *E. coli* is an emerging concern in animal husbandry which makes the existing conventional antimicrobial therapy ineffective. Emergence of antimicrobial population and possible transmission of the same into human food chain warrant cheap and effective alternate medicine to treat neonatal calf diarrhea. Study reported that homeopathic medications might have protected the intestinal epithelium, and consequently prevented diarrhea in children [14]. Also *E. angustifolia* was 50% effective in controlling neonatal calf diarrhea and reduces (60%) use of antibiotics [14]. In addition, *E. angustifolia* reduces the number of pathogenic bacteria in fecal samples which contributes to the improvement of intestinal health favoring nutrient absorption.

Clinical recovery was earlier in *E. angustifolia* treated group whereas it was delayed in other groups including standard antibiotic treated groups of the present study. Oxidative stress measured by FRAP assay revealed significantly ($p < 0.05$) increased total antioxidant level on day 7 in Group (Ea - θ) as compared to other groups. Calves received Ea - 200c recorded best recovery even by 2^{nd} to 3^{rd} day of therapy by clinical symptom, and oxidative stress reduction. Calves received *Echinacea angustifolia* (**θ** and 200c) recovered earlier than antibiotic therapy which was reconfirmed by stool culture examination.

Diarrhoea caused by enteric infections is a major factor in morbidity and mortality worldwide. Enteric pathogen triggers intestinal inflammation and loss of absorptive surface [15]. Most of the infectious diarrhoea is associated with intestinal mucosal damage thereby causing oxidative stress [6] resulting from increased production of free radicals and reactive oxygen species (ROS) and/or a decrease in antioxidant defence which leads to damage of biological macromolecules, disruption of normal metabolism and physiology [7]. Supplements of *Echinacea angustifolia* boosts the immune system [8] and can reduce symptoms of infections and decreases inflammation due to illness and also exerts antioxidant effects [10].

Considering the fast pace of resistance to antimicrobial agents than the development of novel therapeutic agents, there is possibility of crisis in existing conventional treatments. Such crisis can be easily addressed by using economic and effective homeopathic remedy either alone or in combination with other supportive therapeutic agents.

Conclusion

Similarly, antidiarrheal potency of *Echinacea angustifolia* 200C was evident against naturally occurring neonatal calf diarrhoea in the present study denoting alternate therapeutic remedy against nonspecific neonatal calf diarrhoea.

Acknowledgement

Authors acknowledge financial support of DG, CCRH, Min of AYUSH, GOI to accomplish the present study and also Director, ICAR - IVRI for extending requisite facilities.

Conflict of Interest

Nil.

Bibliography

1. Bartels CJ., *et al*. "Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves". *Preventive Veterinary Medicine* 93.2-3 (2010): 162-169.

2. Windeyer MC., *et al*. "Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age". *Preventive Veterinary Medicine* 113.2 (2014): 231-240.

3. Meganck Vanessa., *et al*. "Evaluation of a protocol to reduce the incidence of neonatal calf diarrhoea on dairy herds". *Preventive Veterinary Medicine* 118.1 (2015): 64-70.

4. Aidara-Kane Awa., *et al*. "World Health Organization (WHO) guidelines on use of medically important antimicrobials in food-producing animals". *Antimicrobial Resistance and Infection Control* 7 (2018): 1-8.

5. Fortuoso Bruno F., *et al*. "Homeopathic treatment as an alternative prophylactic to minimize bacterial infection and prevent neonatal diarrhea in calves". *Microbial Pathogenesis* 114 (2018): 95-98.

6. Cheng Yan., *et al*. "Changes of intestinal oxidative stress, inflammation, and gene expression in neonatal diarrhoea kids". *Frontiers in Veterinary Science* 8 (2021): 598691.

7. Birben Esra., *et al*. "Oxidative stress and antioxidant defense". *World Allergy Organization Journal* 5 (2012): 9-19.

8. Woelkart K., *et al*. "Bioavailability and pharmacokinetics of Echinacea purpurea preparations and their interaction with the immune system". *International Journal of Clinical Pharmacology and Therapeutics* 44.9 (2006).

9. JK Aronson "(in Meyler's Side Effects of Drugs, 2016). *Echinacea Angustifolia*: Text book of natural medicine (5th Edition) (2020).

10. Yamada Koji., *et al*. "A comparison of the immunostimulatory effects of the medicinal herbs Echinacea, Ashwagandha and Brahmi". *Journal of Ethnopharmacology* 137.1 (2011): 231-235.

11. Wani SA., *et al*. "Diarrhoeagenic *Escherichia coli* and salmonellae in calves and lambs in Kashmir: absence, prevalence and antibiogram". *Revue Scientifique et Technique* 32.3 (2013): 833-840.

12. Galland John C., *et al*. "Prevalence, antibiotic susceptibility, and diversity of *Escherichia coli* O157: H7 isolates from a longitudinal study of beef cattle feedlots". *Applied and Environmental Microbiology* 67.4 (2001): 1619-1627.

13. Khawaskar Damini P., *et al*. "Pathotyping and antimicrobial susceptibility testing of Escherichia coli isolates from neonatal calves". *Veterinary Research Communications* 46.2 (2022): 353-362.

14. Jacobs Jennifer., *et al*. "Treatment of acute childhood diarrhea with homeopathic medicine: a randomized clinical trial in Nicaragua". *Pediatrics* 93.5 (1994): 719-725.

15. Hodges Kim and Ravinder Gill. "Infectious diarrhea: Cellular and molecular mechanisms". *Gut Microbes* 1.1 (2010): 4-21.

16. Bokelmann JM. "Echinacea (Echinacea purpurea, Echinacea angustifolia)". *Medicinal Herbs in Primary Care. Elsevier* (2021): 317-322.

17. Brand-Williams., *et al*. "Use of a free radical method to evaluate antioxidant activity". *LWT-Food Science and Technology* 28.1 (1995): 25-30.

18. Benzie Iris FF and John J Strain. "The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay". *Analytical Biochemistry* 239.1 (1996): 70-76.

19. Godambe., *et al*. "Species specific PCR based detection of Escherichia coli from Indian foods". *3 Biotech* 7 (2017): 1-5.