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## Combination therapy with *Echinacea purpurea* extract and Quercetin is effective in hematological alterations in neonatal calf diarrhoea

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### Abstract

Hematological changes associated with diarrhea in calves were intended for this study. The experiment started from September 2019 to Feb 2020. The study was conducted on 163 calves, ranging between 1-2 months of age. The samples were collected from 24 calves with *E.coli* diarrhea and 6 healthy calves for comparison after the treatment of all the groups with Quercetin which is a known antioxidant and *Echinacea purpurea* for 7 days. Blood samples were analysed for blood components and biochemical parameters in serum. The study showed a significant increase ( $p < 0.05$ ) in white blood cells but no significant change was noted hemoglobin and TEC indicating an alteration in the immune system of calves to respond to the existing infection.

**Keywords:** Neonatal diarrhea, Echinacea, antioxidant, immunomodulator, hematology etc.

### Introduction

Neonatal calf diarrhoea is an economically important disease reported in calves throughout the world [1]. Being multifactorial disease it involves a complex interaction between host, pathogen and the environment [2]. Proper diagnosis and early treatment are crucial in preventing calf mortality associated with this disease. The unavailability of vaccines against different pathogens involved in neonatal calf diarrhoea is also a matter of concern [3]. Under the present scenario, boosting the host immune response to improve the overall animal health is one of the strategies to combat neonatal calf diarrhoea. Immune function is excessively dependent on the state of oxidant and anti-oxidant balance in the body [4]. Oxidative stress has been implicated to play a major role in the pathology of neonatal calf diarrhoea as evinced by different studies [5, 6].

*Echinacea purpurea* and quercetin are potent antioxidants. *Echinacea purpurea* is known to display anti-inflammatory, anti-viral and anti-fungal activities [7]. In addition, *Echinacea purpurea* extract has the ability to modulate host immune response in calves [8]. Quercetin also has demonstrated to exhibit protective roles in experimentally induced-diarrhoea in some studies [9, 10]. From the above findings, we can generalise that both *Echinacea purpurea* and quercetin have a possible protective effect against various forms of diarrhoea. The haematological and biochemical parameters of the diarrhoeic calves serve as a prognostic marker and often help the clinician to adopt a better therapeutic protocol [4]. Hence, this study is designed to evaluate the potential of *Echinacea purpurea* plant extract, quercetin and its combination in reversing the hematological alterations in naturally infected neonatal diarrhoeic calves.

### Material and method

#### Care and use of animals

The animal experimentation in this study was carried out as per the guidelines of Institute Animal Ethics Committee and the protocol (CPCSEA-DADF), approved by the Committee for the Purpose of Control and Supervision of Experiments on animals (CPCSEA), India.

#### Trial animals

Newly born calves within range of 0-8 weeks with an approximate weight of 20-40kg were enrolled for the study. The cattle calves were weaned immediately after birth and reared in calf sheds. The calves that were born on the farm during September 2019 to February 2020 were the participants of the study.

In this study, diarrhea cases in calves were selected on the basis of fecal consistency score (FCS) and clinical dehydration with or without elevation of rectal temperature (Walker *et. al.*, 1998). A total of 163 diarrhoeic calves (0 to 4 weeks) had been included in the study period. For the diagnosis of *E.coli* about 1.0 gm of sterile faecal material (Directly from rectum) was collected from each diarrhoeic calf.

### Experimental protocol

30 calves will be taken for the study, out of which 6 will be healthy ones and 24 diarrheic calves. The calves of group I will be taken as healthy controls. Group II will be treated alone with the standard supportive therapy that is fluids (Ringer's lactate, intalyte- intravenously, bid) and antimicrobial drugs (*viz.* Sulphonamide+Trimethoprim @1ml per 20kg bwt. bid, Metronidazole @500 mg, intravenously). Group III calves will be given Quercetin @10mg/kg body weight orally, for 7 days alongwith standard treatment. Group IV will be treated with herbal *Echinacea purpurea* @ 250 mg/kg body weight orally, for 7 days alongwith standard treatment. While group V will be given combination of Quercetin and *Echinacea purpurea* at given dose rates along with the standard supportive therapy.

### Blood samples

Blood samples were collected on day 0, 3 and 7 of the treatment. All samples were collected aseptically in sterile containers, and kept in EDTA and serum vials for further use. The concentration of haemoglobin (gm/dL) in the whole blood was measured by modified sahli's haemoglobinometer or acid haematin method [11].

The total erythrocyte count (TEC) and total leukocyte count (TLC) were done manually on Neubauer's chamber as per the standard protocol. Hemoglobin concentration (g/dl) in the whole blood was estimated by the cyanomethemoglobin method [12].

The differential leukocyte count (DLC) in whole blood was carried out by method described by [12]. Neutrophils, lymphocytes, monocytes, eosinophils and basophils were counted up to 100 cells and expressed as percentage.

### Results

The mean values of Hb at the commencement of the treatment on day 0 were (12.300±.469, 10.666±.855, 11.233±.149, 11.766±.540, 11.600±.500); in CONT, ST, ST+QUE, ST+EP, ST+QUE+EP, respectively. No significant difference in Hb (Table1) was observed in healthy control group and the animals treated with ST, ST+QUE, ST+EP, ST+QUE+EP on

day 3 and day 7 of the therapy.

There was a non- significant ( $p= .984$ ) interaction found between the treatment means and the Hb concentration.

TEC (Mean± SE) values were 6.43±.24, 6.08±.29, 6.01±.20, 6.31±.26, 6.050±.28 on day 0 in healthy control group, standard treatment group, animals treated with Quercetin, Echinacea, combination of Quercetin and Echinacea groups, respectively. There was no significant difference observed between healthy and treatment groups of diarrhea positive groups throughout the therapeutic period. Non significant ( $p=>.05$ ) interaction was noted between treatments and the TEC mean concentration.

Statistically a significant ( $p=.004$ ) interaction was found between treatments and TLC values. The mean values of TLC on day 0 (before treatment) were 10733.33±452.66, 7725±690.943, 8531.66±186.83, 10183.33±406.95, 8425±386.38 in healthy control group, animals treated with Quercetin, Echinacea, combination of Quercetin and Echinacea groups, respectively. The mean TLC values were significantly lower ( $p< .05$ ) in diarrheic calves (animals treated with Quercetin, Echinacea, combination of Quercetin and Echinacea groups) in comparison to healthy group on day 0. The mean TLC concentration ranged from 10733.33±452.66 to 11056.66±699.09 in healthy group of animals and there was no significant difference observed over the days when received only placebo therapy. (Table 1).

There was a significant increase in TLC values from day 0 to day 3, while no significant change observed from 3 to 7<sup>th</sup> day of treatment. Also, there was significant change between TLC in healthy and other treatment groups on day 3 and day 7 of treatment except in treatment group which showed non-significant values as compared to healthy control over the days (0 to 7) of treatment. Further there is significant elevation of TLC in group treated with combination of Quercetin+ Echinacea on day 7.

In DLC, the mean values of absolute Neutrophil, lymphocyte, Monocyte, Eosinophil and basophil counts ranged from 32.83±1.87 to 36.85±2.07, 58.500±1.176 to 52.666±1.498, 8.666±.421 to 10.500±.670 in healthy calves.

There was no significant difference in DLC value from day 0 to day 7 in healthy calves that received only placebo treatment while day 7 there was a significant decrease in lymphocyte values, there is no significant difference in monocyte mean value throughout 7 days period of treatment. In all the four treatment groups, no significant ( $p=.67$ ) difference found between the treatment as compared to the healthy control animals in monocytes on any day of treatment (before and after).

**Table 1:** Showing the hematological values of all the test groups on day 0, 3 and 7 of the study.

Group	Day 0	Day 3	Day 7	Group Mean
<b>HB</b>				
Gr. I	12.300±.469 <sup>aA</sup>	12.333±.348 <sup>aA</sup>	12.333±.356 <sup>aA</sup>	12.32±.21
Gr. II	10.666±.855 <sup>aA</sup>	10.566±.761 <sup>aA</sup>	10.833±.535 <sup>aA</sup>	10.68±.39
Gr. III	11.233±.149 <sup>aA</sup>	10.533±.356 <sup>aA</sup>	11.533±.574 <sup>aA</sup>	11.1±.23
Gr. IV	11.766±.540 <sup>aA</sup>	11.766±.660 <sup>aA</sup>	12.100±.281 <sup>aA</sup>	11.87±.28
Gr. V	11.600±.500 <sup>aA</sup>	11.966±.366 <sup>aA</sup>	12.300±.223 <sup>aA</sup>	11.95±.21
<b>Total</b>	11.51±.250	11.43±.259	11.82±.2	
<b>TEC</b>				
Gr. I	6.43±.24 <sup>aA</sup>	6.90±.13 <sup>aA</sup>	6.90±.12 <sup>aA</sup>	6.74±.11
Gr. II	6.08±.29 <sup>aA</sup>	5.80±.17 <sup>aB</sup>	6.03±.14 <sup>aA</sup>	6.03±.14
Gr. III	6.01±.20 <sup>aA</sup>	6.20±.21 <sup>aAB</sup>	6.16±.14 <sup>aA</sup>	6.16±.14
Gr. IV	6.31±.26 <sup>aA</sup>	6.23±.34 <sup>aAB</sup>	5.95±.23 <sup>aA</sup>	5.95±.23
Gr. V	6.050±.28 <sup>aA</sup>	6.050±.30 <sup>aAB</sup>	6.35±.39 <sup>aA</sup>	6.15±.18
<b>Total</b>	6.18±.11	6.23±.12	6.28±.11	

TLC				
Gr. I	10733.33±452.66 <sup>aA</sup>	10358.33±559.37 <sup>aA</sup>	11056.66±699.09 <sup>aA</sup>	10719.44±299.51
Gr. II	7725±690.943 <sup>aB</sup>	9608.33±706.33 <sup>bA</sup>	10341.66±902.86 <sup>bA</sup>	9225±376.91
Gr. III	8531.66±186.83 <sup>aB</sup>	10508.33±172.19 <sup>bA</sup>	11908.33±375.14 <sup>bAB</sup>	10316.11±412.65
Gr. IV	10183.33±406.95 <sup>aA</sup>	11179.16±516.65 <sup>aA</sup>	11583.33±435.10 <sup>aAB</sup>	10981.94±285.02
Gr. V	8425±386.38 <sup>aB</sup>	10733.33±565.31 <sup>bA</sup>	12958.33±530.02 <sup>cB</sup>	10705.55±524.41
Mean	9119.66±264.36	10477.5±249.91	11571.66±264.69	

## Discussion

Haematological parameter is one of the imperative tools to assess the level of infection and monitor the therapy in neonatal calves suffering from diarrhoea [13]. In our study, most of the diarrhoeic calves exhibited marked leucopenia, neutrophilia and lymphopenia. Similar results with neutrophils and lymphocyte count were recorded in neonatal diarrhoeic calves by Brar and co-workers [13]. A recent investigation also reported similar haematological findings as our study in neonatal calf diarrhoea with respect to TEC, TLC, neutrophil and lymphocyte count [13]. The therapy with Echinacea, quercetin and their combination was successful in normalising the neutrophil and lymphocyte counts along with the total leukocyte count. Echinacea was able to improve the haematological parameters in broiler chicken infected with *E. coli* [14]. The extract of Echinacea species is known to have hematinic potential in healthy equines [15]. Quercetin was able to alleviate leucocytosis and neutrophilia in experiments with diabetic rats [16].

## Conclusion

The above study elucidates that *Echinacea purpurea* and Quercetin when included in the treatment regimen of diarrhoeic calves can successfully elevates the neutrophils and other leukocytes which is useful in combating the disease and shows that the enhancement of immune system paves a way for early recovery of calves from diarrhea, which will reflect in reduced usage of antimicrobials and other drugs and can act as a preventive measure in calf diarrhea in young calves. Furthermore, other parameters could be included in future studies to prove other biochemical alterations in the body via these drugs.

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