



COMBINED EFFECT OF *Emblica officinalis* AND *Tinospora cordifolia* ON HEMATO-BIOCHEMICAL PROFILE OF MURRAH BUFFALO CALVES

Pragati Patel*, H.S. Singh, Aditya Mishra and M.A. Quadri

Department of Veterinary Physiology & Biochemistry, College of Veterinary Science & Animal Husbandry,
Nanaji Deshmukh Veterinary Science University, Jabalpur – 482 001, Madhya Pradesh (India)

*e-mail: pragatip23@gmail.com

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ABSTRACT

The present study was aimed to assess the performance of neonatal buffalo calves based on haematological and biochemical profile upon supplementation of *Emblica officinalis* and *Tinospora cordifolia*, well known anti-anaemic, adaptogenic and anti-stressor herbs. Twelve calves aged 14 d were randomly divided into 2 groups of 6 each. One group was maintained as control and other group supplemented with a combination of dry powder of *E. officinalis* and *T. cordifolia* (2:1) @ 450 mg kg⁻¹ body weight once a day orally for 28 days. Blood samples were collected aseptically from control and experimental animals on 28th and 42nd day age of calves from jugular vein. The treatment group showed significantly higher ($p < 0.05$) total erythrocyte count, packed cell volume and haemoglobin concentration in blood. Total leukocyte count and L:N was significantly higher in treatment group than control group. No significant changes were observed in blood glucose, total protein, serum cholesterol and serum triglycerides during the experimental period.

Key words: *Emblica officinalis*, Haemato-biochemical profile, Murrah buffalo calves, *Tinospora cordifolia*

INTRODUCTION

The importance of haemato-biochemical indices in animal husbandry is well known. The changes in haematological constituents are important indicators of physiological or pathological state of animal (Hassan *et al.*, 2012). Complete blood count (CBC) is a powerful diagnostic tool as a component of a minimum database. It can be used to monitor response to therapy to gauge the severity of illness or as a starting point for formulating a list of differential diagnosis. Variables such as breed, physiological stage, age, reproductive/ lactation stage and environmental conditions/season influence the physico-biochemical parameters (Chandra *et al.*, 2012). Determination of normal values for haematological and blood biochemical values are important for clinical interpretation of laboratory data.

Emblica officinalis (Amla) belongs to family *Euphorbiaceae* and is one of the strongest rejuvenatives, particularly for blood, bone, liver and heart (Singh *et al.*, 2006). It is an exceptionally rich source of vitamin C. *Tinospora cordifolia* is commonly known as Giloy or Guduchi (Guduchi is a Sanskrit name which means the one which protects the entire body belonging to family *Menispermaceae*). It stimulates reticulo-endothelial system, bone marrow cellularity, proliferation of stem cells and enhances the haemopoietic growth factor and IL-3 (Upadhyay *et al.*, 2010). In

ayurvedic literature, there is synergistic effect for the combination of these plants. The present study was carried out to evaluate the combined impact of both herbal plants on hemato-biochemical profile of neonatal Murrah buffalo calves to prevent calf mortality.

MATERIALS AND METHODS

The experiment was conducted in the Department of Veterinary Physiology & Biochemistry, NDVSU, Jabalpur (India). Twelve Murrah buffalo calves of 14 day age were selected and kept under the same feeding and management system. The experiment was conducted in the year 2014. The herbal powder of *Emblica officinalis* and *Tinospora cordifolia* was procured from Department of Aromatic and Medicinal Plants, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (India). Treatment group was supplemented with both *E. officinalis* and *T. cordifolia* (2:1) @ 450 mg kg⁻¹ body weight once a day orally for 28 days. Blood samples (5 mL) were collected aseptically from control and all the experimental animals on day 28th and 42nd day age of calves from jugular vein. The blood samples were collected in sterilized glass vials containing 10% aqueous solution of EDTA for haematology as well as without anticoagulant for separation and collection of serum for biochemical study.

Complete haematology of calves was determined using Auto-Cell Counter and differential leukocyte count estimated by using cell counter by the manual methods as per Feldman *et al.* (2002). Lymphocyte, mid cells (inclusive of monocytes, eosinophils and basophils) and granulocyte were separated through auto-cell counter and the results further ascertained by staining blood smears of calves with Leishman's stain and manually counting the different types of leucocytes as per the method of Feldman *et al.* (2002). At least 200 cells were counted and results expressed in percent of different leukocytes types. The selected biochemical parameters were estimated using serological auto-analyzer for accurate and precise quantification. Blood glucose was estimated by Trinder's method (Pileggi and Szuskeiweiz, 1974) using diagnostic kits (Erba Diagnostics, Mannheim GmbH, Germany). The concentration was expressed in mg dL⁻¹ of blood glucose. The total serum protein was estimated by Biuret method (Tietz, 1986) using the kits procured from Erba Diagnostics, Germany. The concentration of total serum protein was expressed as g dL⁻¹ serum. The concentration of triglycerides in serum was determined by modified Wako method (Fossati, 1982) using readymade kits procured from Erba Diagnostics, Germany. The concentration of cholesterol in serum was determined by modified method of Roeschlau *et al.* (1974) using readymade kits procured from Erba Diagnostics, Germany. The experimental data was analyzed by analysis of variance using hierarchical design as described by Snedecor and Cochran (1989). Various groups were compared by using Duncan multiple range test (DMRT).

RESULTS AND DISCUSSION

The combined effect of *Emblica officinalis* and *Tinospora cordifolia* on haematological parameters, markers for anaemia, are shown in Table 1. A significantly higher erythrocyte count and haemoglobin was observed in treatment group than control group but no significant change was observed in packed cell volume in both experimental groups. The possible reason for this may be their effect in potentiating bone marrow viability and stimulating haemopoietic system. The findings are in consonance with Pawar and Somkumar (2006) and Sahu (2007) who concluded that *E. officinalis* is a potent anti-anaemic and stimulator of haemopoiesis. However, no significant

change was observed by supplementation of *T. cordifolia* to peripartum crossbred cows by Mallick and Prakash (2010). Sharma and Pandey (2010) found significant increase ($P < 0.05$) in haemoglobin and packed cell volume levels in *T. cordifolia* treated mice to protect against lead intoxication.

In present study, the pattern of changes in total leukocyte count and differential leukocyte count during neonatal life was similar to those observed in earlier studies in calves; however a significantly higher TLC, lymphocyte count and lymphocyte-neutrophil ratio were observed in *E. officinalis* and *T. cordifolia* treated group as compared control group whereas the monocyte count did not differ significantly between the groups. There was significantly higher neutrophil and eosinophil count in control than treatment group. Mathew and Kuttan (1999) reported that the administration of alcoholic extract of *T. cordifolia* stem in mice stimulated proliferation of stem cells causing an increase in total white blood cells. Sahu (2007) reported that L:N ratio increased significantly ($P < 0.01$) in calves from day 21 to day 60 after supplementation with *E. officinalis*. Suja (2009) observed significant increase in total leukocyte count and per cent lymphocyte distribution in mice after treatment of amla. Mallick and Prakash (2010) found an increase in total leukocyte, lymphocyte and neutrophil counts in Guduchi supplemented cows in comparison to untreated cows. Sharma and Pandey (2010) observed that *T. cordifolia* supplementation have protective action against lead intoxication by scavenging lead-induced haematological alterations to some extent such as by enhancing TLC, DLC and some other haematological parameters. These findings are in consonance with our observations and may be correlated with ability of these herbal plants to stimulate haemolymphopoietic system.

Table 1: Effect of combined use of *Embllica officinalis* and *Tinospora cordifolia* on various haematological parameters in buffalo calves (overall mean $\pm$ SEM)

Parameters	Groups	Days		Overall average
		28	42	
Total erythrocyte count (million μL^{-1})	Control	8.82 $\pm$ 0.70	9.03 $\pm$ 0.62	8.93 ^b $\pm$ 0.45
	Treatment	10.50 $\pm$ 0.36	11.05 $\pm$ 0.28	10.77 ^a $\pm$ 0.23
Packed cell volume (%)	Control	44.73 $\pm$ 1.45	41.73 $\pm$ 1.63	42.88 $\pm$ 1.10
	Treatment	48.77 $\pm$ 2.18	45.52 $\pm$ 2.24	47.14 $\pm$ 1.57
Haemoglobin (g dL^{-1})	Control	13.33 $\pm$ 0.91	13.23 $\pm$ 0.76	13.28 ^b $\pm$ 0.57
	Treatment	15.38 $\pm$ 0.68	15.32 $\pm$ 0.89	15.35 ^a $\pm$ 0.53
Total leukocyte count ($10^3 \mu\text{L}^{-1}$)	Control	4.70 $\pm$ 0.59	4.83 $\pm$ 0.85	4.77 ^b $\pm$ 0.50
	Treatment	8.05 $\pm$ 1.20	8.37 $\pm$ 0.85	8.21 ^a $\pm$ 0.70
Differential leukocyte count				
Lymphocyte (%)	Control	48.17 $\pm$ 0.60	50.33 $\pm$ 0.88	49.25 ^b $\pm$ 0.61
	Treatment	62.67 $\pm$ 0.76	64.17 $\pm$ 0.60	63.42 ^a $\pm$ 0.51
Neutrophil (%)	Control	42.17 $\pm$ 0.60	40.33 $\pm$ 0.88	41.25 ^a $\pm$ 0.58
	Treatment	30.33 $\pm$ 0.76	30.50 $\pm$ 0.76	30.42 ^b $\pm$ 0.51
Eosinophil (%)	Control	6.33 $\pm$ 0.76	6.33 $\pm$ 1.12	6.33 ^a $\pm$ 0.64
	Treatment	3.83 $\pm$ 0.31	2.50 $\pm$ 0.22	3.17 ^b $\pm$ 0.27
Monocyte (%)	Control	3.33 $\pm$ 0.49	3.00 $\pm$ 0.73	3.17 $\pm$ 0.42
	Treatment	3.83 $\pm$ 0.31	3.00 $\pm$ 0.37	3.42 $\pm$ 0.27
Lymphocyte and neutrophil ratio (L: N)	Control	1.14 $\pm$ 0.01	1.25 $\pm$ 0.01	1.20 ^b $\pm$ 0.02
	Treatment	2.07 $\pm$ 0.08	2.08 $\pm$ 0.08	2.08 ^a $\pm$ 0.05
Mean corpuscular volume (fl)	Control	50.97 $\pm$ 2.95	46.94 $\pm$ 2.58	48.96 ^a $\pm$ 1.96
	Treatment	46.45 $\pm$ 1.28	42.05 $\pm$ 0.95	44.25 ^b $\pm$ 1.01
Mean corpuscular haemoglobin pg)	Control	15.35 $\pm$ 1.09	14.75 $\pm$ 0.53	15.05 $\pm$ 0.59
	Treatment	14.65 $\pm$ 0.43	13.83 $\pm$ 0.59	14.24 $\pm$ 0.37
Mean corpuscular haemo-globin concentration (%)	Control	30.17 $\pm$ 1.37	31.64 $\pm$ 0.95	30.90 $\pm$ 0.83
	Treatment	31.55 $\pm$ 0.25	33.03 $\pm$ 1.41	32.29 $\pm$ 0.72

Overall average with different superscripts (a and b) differ significantly

Table 2: Effect of combined use of *Embllica officinalis* and *Tinospora cordifolia* on various biochemical parameters of experimental buffalo calves (overall mean $\pm$ SEM)

Parameter	Groups	Days		Overall average
		28	42	
Glucose (mg dL ⁻¹)	Control	48.58 $\pm$ 5.99	64.09 $\pm$ 6.42	56.34 $\pm$ 4.80
	Treatment	57.23 $\pm$ 11.89	64.19 $\pm$ 7.34	60.71 $\pm$ 6.75
Total protein (mg dL ⁻¹)	Control	7.11 $\pm$ 0.48	8.34 $\pm$ 0.69	7.73 $\pm$ 0.44
	Treatment	10.88 $\pm$ 1.78	8.38 $\pm$ 1.15	9.63 $\pm$ 1.08
Triglycerides (mg dL ⁻¹)	Control	80.62 $\pm$ 3.48	79.55 $\pm$ 3.92	80.09 $\pm$ 2.51
	Treatment	75.56 $\pm$ 4.81	86.04 $\pm$ 3.26	80.80 $\pm$ 3.19
Cholesterol (mg dL ⁻¹)	Control	77.99 $\pm$ 4.93	69.96 $\pm$ 3.31	73.97 $\pm$ 3.08
	Treatment	62.52 $\pm$ 2.60	63.24 $\pm$ 1.93	62.88 $\pm$ 1.55

Biochemical constituents of blood reflect the ongoing metabolic events and the sum total reactions going on within a body system. The study revealed no significant difference in blood glucose in both groups of calves indicating that supplementation of *E. officinalis* and *T. cordifolia* have no effect on serum glucose. It is a tightly regulated biochemical parameter in blood which is important for metabolic homeostasis. The results are in agreement with Sahu (2007) in calves and Mir *et al.* (2013) in Murrah buffaloes.

In present study the total protein remained almost constant throughout the experiment. These findings were in complete agreement with Sahu (2007) in calves. There was no significant change in serum triglycerides and cholesterol which was significantly higher in both groups. Our findings are in agreement with Jain *et al.* (2007) who found lower triglycerides in 1st week after birth and increased thereafter in buffalo calves. This hyperlipidemia in calves might be the result of hypothyroidism in neonatal calves. These observations are in line with Capan and Martin (2003). Combined supplementation of *E. officinalis* and *T. cordifolia* enhanced TEC and haemoglobin concentration so reveals its anti-anaemic effect. Higher TLC, lymphocyte count and lymphocyte-neutrophil ratio in treatment group indicate the stimulation of haemo-lymphopoietic system or by stimulation of proliferation of stem cells causing an increase in total white blood cells, indicate adaptogenic and antistressor property of these herbal plants. *E. officinalis* and *T. cordifolia* supplementation has anti-anaemic, adaptogenic and antistressor property during neonatal life which help to combat alien environmental stress.

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