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# Effect of herbal treatment on haemato-biochemical recovery in a *Colibacillosis* calves

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**Abstract**
The objective of the present study was to assess the *in-vitro* antimicrobial efficacy of *Punica granatum* rind and *Psidium guajava* leaf extract on enteropathogenic *Escherichia coli* and to evaluate its effectiveness in the treatment of *colibacillosis* in calf. The experiment was conducted at Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Birsa Agricultural University, Kanke Ranchi. A total no. of 60 *colibacillosis* positive calves (Escherichia *coli* positive) and six (06) clinically normal healthy calves (Healthy control) were taken to investigate the haemato-biochemical profiles. *E. coli* was isolated from the *colibacillosis* affected calves and the animals found positive were used for the study of haemato-biochemical profile. Faecal sample were cultured on MacConkey and EMB agar to diagnose *E. coli*. Analysis of blood and serum samples of the *colibacillosis* affected calves revealed significant increase in Haemoglobin, Total erythrocyte count, Total leukocyte count, Blood urea nitrogen, Total serum protein, Serum creatinine, and Serum potassium, while significant decrease in Blood glucose and Serum sodium. However, no significant changes were observed in Alanine transaminase.

**Keywords:** calf, *colibacillosis*, *E. coli*, haemato-biochemistry

## Introduction
*Colibacillosis* is a leading cause of calf mortality and morbidity during first few week of life (Radostits *et al.* 2000) [1]. *Colibacillosis* is one of the challenging tasks to veterinary practitioners. The various known causes of calf scour are grouped under infectious and noninfectious (Izzo *et. al.* 2011) [2]. Various antimicrobial agents have been the drugs of choice for the clinician but it has been observed that prolong use of oral antibiotics is detrimental to the intestinal mucosa resulting into mal-absorption diarrohea and even development of drug resistant (Mero *et. al.* 1985) [3]. Guava leaves and Pomegranate peels have been widely used as potent anti-diarrheal agent by rural peoples (Lutterodt, 1989; and Alnieida *et al.*1995) [4, 5]. Thus the present work has been designed to assess the treatment effect of *Psidium guajava* leaves and *Punica granatum* rind extract in restoration of haemato-biochemical changes in colibacillosis calves.

## Materials and Methods
A total of 60 *colibacillosis* affected calves presented or reported at Veterinary Clinical Complex (RVC), Instructional bovine farm of Ranchi Veterinary College, and from local organized or unorganized Dairy farm situated in an around Ranchi Veterinary College with the history of passage of soft fluid faeces, abnormal colour of faeces (white to yellowish green and mucoid) along with rapid loss of body weight, animal becoming lean and dehydrated was selected for the present study. The faecal sample were collected directly from the rectum of *colibacillosis* affected calves with the help of sterilized swab, the swab materials were then kept in a pre-enriched peptone water broth for its culture and isolation of bacteria. Pre-enriched sample were cultured on MacConkey Agar and on eosine methylene blue agar as per standard method Soulsby (1982) [6]. Gram's staining was done as per method described by Stokes *et al.* (1993) [7]. Further, biochemical test like IMViC test was done for its confirmation of isolates biochemical testing of *E. coli* was performed by using commercially available KB001 HiMedia *E. coli* Identification Kit (HiMedia Laboratories Pvt. Limited).

## Experimental design
On the basis of culture and sensitivity test the selected calves were randomly distributed into 3 equal groups i.e. group-A, group-B and group-C consist of 20 animals in each group.

Group-A was treated with Ofloxacin tab. orally, group-B was treated with ethanolic extract of *Punica granatum* rind orally and group-C was treated with ethanolic extract of *Psidium guajava* leaf orally. The entire treatments are continued for upto 7 days along with supportive therapy consisting of 5% DNS, B-complex inj. and liver tonic inj.

**Collection of Blood Samples**

Blood samples were collected on day 0 (pre- treatment) and on 2nd, 4th, 6th and 8th days of post-treatment for estimation of hemato-biochemical parameters. A total of 8 ml blood sample was collected aseptically through sterilized disposable syringe, directly from jugular vein. 2 ml of them were transferred to a test tube containing EDTA for its hematological examination (Hemoglobin, Total leukocyte count, Total erythrocyte count) by using Veterinary hematological Analyzer (Vet Scan HM5). Rest 6 ml of blood sample was transferred to a clean, dry, wide mouthed glass test tube having no anticoagulant, further, this test tube was allowed to stand in slanting position (for 30 minutes) at room temperature for serum separation. The collected serum was kept in refrigerator for its quantitative estimation of biochemical parameters (Blood glucose, Total serum protein, Blood urea nitrogen, Serum creatinine, Alanine transaminase) by using blood Auto analyzer (Model: EM 360) furthermore, Serum Sodium, Potassium were estimated using Easylyte plus (K/Na/Cl analyzer).

**Statistical analysis:** Statistical difference between respective means for various parameters was evaluated using tow way Anova as per the method described by Snedecor and Cochran (2004) [8].

**Result and Discussion**

**Hematological observation**

An increase in hemoglobin values (g/dl) of *colibacillosis* affected calves was observed among all the treatment group-A (13.250±2.962 g/dl), group-B (13.365±2.989 g/dl) and group-C (13.490±3.016 g/dl) on 0 day (pre-treatment) in comparison to healthy control calves (10.733±0.532 g/dl). Following different treatment regimen an increased hemoglobin followed a significant (p≤0.01%) decreasing trends towards restoration of normal hemoglobin within the same treatment group throughout the study periods. A normal hemoglobin level in the entire treatment group was achieved on 8th days of observation as 10.905±2.438 g/dl, 11.115±2.485 g/dl and 10.935±2.445 g/dl in the treatment group-A, group-B and group-C respectively. The haemocontration that have occurred in *colibacillosis* affected calves might be due to excessive loss of fluid through faeces which finally leads to hemoconcentration as well as decrease in per unit of blood volume. Hence due to decrease in blood volume and dehydration there is increase in hemoglobin values (Jain 1986; Santos *et al.*, 2002; Ghanem *et al.*, 2012; Kumar and Mandial, 2002) [9, 10, 11, 12]. These observation recorded are in agreement with Kumar and Mandial (2002) [12], Tikoo and soodan (2009) [13], Asati *et al.*, (2010) [14], Kumar *et al.*, (2010) [15], Tarunpreet *et al.*, (2018) [16] and Nayak *et al.*, (2019) [17].

An increase in TEC ($10^6$/µl) values in *colibacillosis* affected calves was observed among all the treatment group-A ($9.139\pm0.290x10^6$/µl), group-B ($9.265\pm0.340x10^6$/µl) and group-C ($8.861\pm0.401x10^6$/µl) on 0 day (pre-treatment) in comparison to healthy control calves $7.033\pm0.195x10^6$/µl. Following different treatment regimen an increased TEC followed a significant (p≤0.01%) decreasing trends towards restoration of normal TEC within the same treatment group. An early achievement in normal TEC value was noted on 6th days of observation in group-C ($7.668\pm0.342x10^6$/µl) whereas in group-A ($7.294\pm0.226x10^6$/µl) and group-B ($7.831\pm0.309x10^6$/µl) it was achieved on 8th days of observation. The main reason of increased in TEC values in *colibacillosis* affected calves might be the consequence of fluids loss which ultimately derived to hemoconcentration (Reddy, 1975; Boyd *et al.*, 1974) [18, 19]. Our trends of results are in agreement with the findings of Tikoo and Soodan (2009) [13], Mir *et al.*, (2010) [20], Kumar *et al.*, (2010) [15], Hassan *et al.*, (2013) [21], Tarunpreet *et al.*, (2018) [16], Nayak *et al.*, (2019) [17] and Manu *et al.*, (2019) [22].

An increase in TLC ($10^3$/µl) of *colibacillosis* affected calves was observed in group-A ($14.795\pm1.132x10^3$/µl), group-B ($14.278\pm0.861x10^3$/µl) and group-C ($14.469\pm0.7.3x10^3$/µl) on 0 day (pre-treatment) in comparison to healthy calves. Following different treatment regimen an increased TLC value followed a significant (p≤0.01%) decreasing trends towards restoration of normal TLC value ($10.550\pm0.670x10^3$/µl) within the same treatment group throughout the study periods. A normal TLC value in the entire treatment group was achieved on 8th days of observation as $11.309\pm0.713x10^3$/µl, $10.382\pm0.760x\ 10^3$/µl and $10.733\pm0.561x10^3$/µl in the treatment group-A, group-B and group-C respectively. An increase in TLC values of *colibacillosis* affected calves might be occurred due to reaction of body defense mechanism against *E. coli* infection as well as dehydration that causes an increase in the viscosity of blood (Tiko and Soodan 2009; Fernandes *et al.*, 2009) [23]. Our results of variation in TLC are in concurrence with the findings of other scientists Asati *et al.*, (2010) [14], Mir *et al.*, (2010) [13, 20], Kumar *et al.*, (2010) [15], Hassan *et al.*, (2013) [21], Mehesara (2018) [24], Tsarunpreet *et al.*, (2018) [16], Nayak *et al.*, (2019) [17] and Manu *et al.*, (2019) [22].

**Biochemical observation**

A significant decrease in the blood glucose level (mg/dl) of *colibacillosis* affected calves among all the treatment group-A (38.625±0.791mg/dl), group-B (38.525±0.429 mg/dl) and group-C (39.720±0.887 mg/dl) on 0 day (pre-treatment) in comparison to healthy control calves 56.466±1.493 mg/dl. Following different treatment regimen the level of blood glucose followed a significant (p≤0.01%) increasing trend towards restoration of normal level within the same treatment group. The normal level of blood glucose was not achieved till end of treatment in all the groups-A, group-B and group-C. However, a maximum increased was observed in group-C 52.560±1.063 mg/dl) than group-B (51.890±0.859 mg/dl) and group-A (48.440±0.428 mg/dl) on 8th days of observation. The probable reason of decrease in blood glucose level (Hypoglycemia) in *colibacillosis* calves might be associated with anorexia, decreased intestinal absorption of glucose and reduce rate of conversion of lactic acid to glucose (Morris *et al.*, 1985) [25]. Our results of variation in blood glucose leves are in agreement with findings of Asati *et al.*, (2010) [14], Pal and Pachuri (2011) [26], Mehesare (2018) [24] and Tarunpreet *et al.*, (2018) [16].

An increase in mean values of blood urea nitrogen (mg/dl) of *colibacillosis* affected calves among the entire treatment group-A (28.910±1.061 mg/dl), group-B (30.547±0.819 mg/dl) and group-C (30.836±0.912 mg/dl) on 0 day i.e. before

start of treatment in comparison to healthy control animal (20.636±0.243 mg/dl). Following different treatment regimen an increase in blood urea nitrogen followed a significant (p≤0.01%) decreasing trends towards restoration of normal level within the same treatment group throughout the study periods. A nearer in normal blood urea nitrogen level was achieved on 8th days of observation. However, a maximum decreased was observed in group-C (21.927±0.813mg/dl) than group-B (22.298±0.811m/dl) and group-A (24.338±1.020mg/dl). The reason behind elevation in BUN level of *colibacillosis* affected calves might be due to catabolism of protein, fat and carbohydrates to generate water (Radiostitis *et al.,* 2007) [27]. These results are in agreement with the findings of Chagkjia (2002) [28], Seifi *et al.*, (2006) [29], Jesse *et al.*, (2016) [30] and Mehesare (2018) [24].

An increase in mean values of total serum protein (g/dl) of *colibacillosis* affected calves among all the treatment group-A (8.804±0.117 g/dl), group-B (8.446±0.415 g/dl) and group-C (8.013±0.259 g/dl) on 0 day (pre-treatment) in comparison to healthy control calves (7.223±0.197 g/dl). Following different treatment regimen an increased total serum protein followed a significant (p≤0.01%) decreasing trends towards restoration in normal total serum protein within the same treatment group. An early achievement of normal total serum protein level was noted in group-C (7.266±0.202 g/dl) than group-B (7.269±0.386 g/dl) on 6th days of observation, whereas it was achieved on 8th days of observation in group-A (7.453±0.309 g/dl). An increase in the mean values of total serum protein of *colibacillosis* affected calves might be due to dehydration which causes reduction in fluid volume leading to haemo-concentration as a result hyper-proteinaemia occurs (Kaneko 1989) [31]. Our findings of result are in similar trends with the findings of Asati *et al.,* (2010) [14], Mir *et al.,* (2010) [20], Singh *et al.,* (2014) [32], Tarunpreet *et al.,* (2018) [16] and Manu *et al.,* (2019) [22].

An increase in mean values of serum creatinine level (mg/dl) was observed among all the treatment group-A (2.382±0.090 mg/dl), group-B (2.30±0.087 mg/dl) and group-C (2.280±0.075 mg/dl) on 0 day (pre-treatment) in comparison to healthy control valves 1.323±0.149 mg/dl. Following different treatment regimen an increased serum creatinine level followed a significant (p≤0.01%) decreasing trends towards restoration of normal serum creatinine level within the same treatment throughout the study periods. A normal serum creatinine level as in group-B (1.354±0.061mg/dl) and group-C (1.363±0.071mg/dl) was noted on 8th days of observation whereas in group-A a nearer normal level (1.634±0.111mg/dl) was achieved till end of the treatment. An increase in serum creatinine values of *colibacillosis* affected calves could be due to insufficient perfusion pressure through kidneys which have occurred due to reduced blood volume as a result of severe dehydration (Radostitis *et al.,* 2007) [27]. Similar types of findings during present research work in *colibacillosis* affected calves are in corroboration with Changkjia (2002) [28], Seifi *et al.,* (2006) [29], Jesse *et al.,* (2016) [30] and Mehesare (2018) [24].

An increase in mean values of ALT level in *colibacillosis* affected calves was observed among all the treatment group-A (18.590±0.44 IU/L), group-B (18.030±0.430 IU/L) and group-C (18.560±0.799 IU/L) on 0 day (pre-treatment) in comparison to the values of healthy control calves 17.296±0.825 IU/L. Following treatment on subsequent days a non-significant difference was noticed within and between different treatment groups, however in different treatment regimen, the level of ALT showed decreasing trend in both the herbal treatment group-B and group-C and near normal level such as 17.360±0.455 IU/L and 17.580±0.819 IU/L was archived in both these group-B and group-C respectively. This decrease in ALT level was more in herbal treatment group then the antibiotics treatment group-A which remains to 18.320±0.498 IU/L at the end of treatment i.e. on 8th days of observation. In the present study a non-significant change in ALT activities has indicated the absence of marked hepatic damage (Lewis *et al.,* 1975) [33]. Our findings of ALT are in corroboration with the findings of Lewis *et al.,* (1975) [33]; Singh *et al.,* (2014) [32] and Shekhar *et al.,* (2017) [34].

A decrease in mean values of serum sodium concentration (mEq/L) of *colibacillosis* affected calves was observed among all the treatment group-A (118.414±0.865 mEq/L), group-B (118.769±0.585 mEq/L) and group-C (119.927±0.740 mEq/L) on 0 day (pre-treatment) in comparison to healthy control calves 127.913±2.800 mEq/L. Following different treatment regimen a decreased serum sodium level followed a significant (p≤0.01%) increasing trends towards restoration of normal serum sodium level within the same treatment group. An early achievement in normal serum sodium level was noted on 4th days of observation in group-C (127.071±0.670 mEq/L) whereas in group-A (127.632±0.623 mEq/L) and group-B (128.323±0.536 mEq/L) it was achieved on 8th days of observation. The reason of decrease in the mean values of serum sodium concentration of *colibacillosis* affected calves might due to loss of sodium bicarbonate (normally present in intestinal contents) through faeces which ultimately leads to fall in sodium ion concentration (Benjamin1985) [35]. Our findings of results are in agreement with the findings of Asati *et al.,* (2010) [14], Mir *et al.,* (2010) [20], Mehesare (2018) [24], Tarunpreet *et al.,* (2018) [16] and Manu *et al.,* (2019) [22].

An increase in mean values of serum potassium concentration (mEq/L) of *colibacillosis* affected calves was observed among all the treatment group-A (6.725±0.243 mEq/L), group-B (6.845±0.180 mEq/L) and group-C (6.795±0.222 mEq/L) on 0 day (pre-treatment) in comparison to healthy control calves 4.953±0.881. Following different treatment regimen an increased serum potassium level followed a significant (p≤0.01%) decreasing trends towards restoration of normal serum potassium level within the same treatment group throughout the study periods. A normal serum potassium level among the entire treatment group was achieved on 8th days of observation as 5.110±0.226 mEq/L, 5.075±0.123 mEq/L and 4.710±0.195 mEq/L in the treatment group-A, group-B and group-C respectively. The main reason of increase in the mean values of serum potassium concentration of *colibacillosis* affected calves might be due to metabolic acidosis, which might have stimulates the transfer of potassium ion from intracellular space to extracellular space because excess hydrogen ions are buffered intra-cellularly, therefore potassium ion along with sodium ion leave to maintain neutrality (Brobst 1986) [36]. The results of present findings are in close agreement with the findings of Asati *et al.,* (2010) [14], Mir *et al.,* (2010) [20], Mehesare (2018) [24], Tarunpreet *et al.,* (2018) [16] and Manu *et al.,* (2019) [22].

## Conclusions

After, critical analyzing the various features of *colibacillosis*, it was concluded that treatment with *Psidium guajava* leaf extract@ 40mg/kg b.wt. for upto 7th days can prove to be more beneficial than *Punica granatum* rind extract and Ofloxacin in achieving 100% recovery in hemato-biochemical

changes that have occurred due to *colibacillosis* in calves.

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