



Ameliorative Effect of *Dalbergia sissoo*, *Aeglemarmelos* and *Punica granatum* on Clinical and Circulating IL-10, TNF- α , IFN- γ Status in Acute Undifferentiated Calf Diarrhoea

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ABSTRACT

Neonates diarrhoea is a serious problem and considered the most common disease inducing high morbidity and mortality rates in newborn calves and responsible for severe economic losses. The present study was conducted to determine the Ameliorative effect of *Dalbergia sissoo*, *Aeglemarmelos* and *Punica granatum* on clinical score and circulating cytokines status in acute calf diarrhoea. The clinical scores was recorded, before and after treatment. Proinflammatory (TNF- α , IFN γ) and anti-inflammatory (IL-10) cytokines were measured in serum of calve using bovine specific quantitative ELISA Kits and revealed that significant decrease in faecal consistency score, dehydration score and depression score on day 6th (post-treatment) in all the treatment groups with highest recovery was observed in treatment group IV (T3). Significant increase in serum IL10 concentration on day 6th (post-treatment) in all the treatment groups and significant decrease in serum TNF α concentration and serum INF γ concentration on day 6th (post-treatment) in all the treatment groups with highest recovery was observed in treatment group IV (T3).

HIGHLIGHTS

- ① Study the effect herbs on clinical and circulating IL-10, TNF- α , IFN- γ status in acute undifferentiated calf diarrhoea.
- ② *Dalbergia sissoo*, *Aeglemarmelos* and *Punica granatum* was found to have good success in treating acute undifferentiated calf diarrhoea.

Keywords: Diarrhoea, Cytokines, ELISA, Proinflammatory, Anti-inflammatory

Morbidity and mortality among the neonates have always proved a bottleneck and causes serious blow to the roots of dairy husbandry in India. In neonates diarrhoea is a serious problem and considered the most common disease inducing high morbidity and mortality rates in newborn calves and responsible for severe economic losses. Moreover, diarrhoea is a common problem in calves and other young ruminants, particularly in the first few months of life. Signs of diarrhoea include anorexia, loss of weight, and hemorrhagic and/or mucoid diarrhoea. In severe cases, feces are liquid, bloody and may contain strands

of intestinal mucosa, and animals may become emaciated, dehydrated, weak, and listless (Radostits *et al.*, 2007).

Cytokines, proteins of low-molecular weight, are the main components of the immune system, which contribute to signal transduction between cells and regulate the

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immune responses (Delirez *et al.*, 2016). Meanwhile, pro-inflammatory cytokines, such as interleukin (IL)-1 β , IL-6, and tumor necrosis factors alpha (TNF- α), play a major role in this regard. These cytokines are mainly produced by mononuclear phagocytes (Murtaugh *et al.*, 1996). According to the literature, pro-inflammatory cytokines can increase under many pathophysiological conditions (Kasimanickam *et al.*, 2013). The other type of cytokine, known as anti-inflammatory cytokine owing to its function, is produced by the immune cells to regulate the secretion of pro-inflammatory cytokines and control the associated tissue damage. The IL-10 is one of the anti-inflammatory cytokines released by many activated immune cells, controlling the inflammatory pathways in several diseases (Ouyang *et al.*, 2011). Therefore, this cytokine can be considered an appropriate indicator to monitor the activity of the immune system

In diarrhoea clinic-biochemical alterations are complex in nature characterized by imbalance of fluid, electrolyte and acid base status (Radostits *et al.*, 2009). The end result of diarrhoea is development of varying degrees of dehydration associated with severe electrolyte imbalance and acidosis. With worsening the condition calves will show more severe depression, may be unable to stand, will lose their nursing reflex and will drop to subnormal body temperature. Death in diarrhoea is due to severe dehydration and metabolic acidosis. In diarrhoea excess loss of intestinal fluid take place resulting in severe dehydration and electrolyte imbalances like hyponatraemia, hypochloraemia, hyperkalaemia and acid base imbalances like low blood pH, loss of bicarbonates and development of metabolic acidosis.

India has a rich wealth of local and traditional knowledge of herbal medicine. Medicinal plants are playing a vital role in the treatment of human and livestock ailments (Megersa and Tamrat, 2022). For treating common and complex ailments both in humans and animals over 7500 species of plants are being used. To treat diarrhoeal syndrome further, various medicinal plants have been used for years in daily life. It will not only help to fasten the recovery but economical also. WHO has also emphasized the need to integrate traditional indigenous health care system with modern facilities. Shisham (*Dalbergia sissoo*) leaves, Bael (*Aegalmarmelos*) unripe fruit, Anar (*Punica granatum*) bark etc. was reported to be used in diarrhoea and dysentery with very good results in laboratory animals.

Dalbergia sissoo Roxb. (Fabaceae), known as Indian rosewood, is reported to be useful in many conditions including fever, ulcers, digestive disorders, and skin diseases. It is also known to be effective against diarrhoea and dysentery. *Punica granatum* Linn. (Punicaceae) commonly known as 'Dalim' (Bengali) and 'Anar' (Hindi) is a shrub cultivated throughout the India. Almost all parts of this plant are used in traditional medicine for the treatment of various ailments. Bark and rind of the fruit are used in dysentery, diarrhoea, piles, bronchitis, to reduce the risk of cardiovascular disease, and as an anthelmintic (Senthamarai *et al.*, 2011; Akter *et al.*, 2013). Half-ripe fruit of *Aegle marmelos*, Linn (Bael) have remarkable astringents activity (Singh *et al.*, 2007) and subside irritation in the digestive tract of diarrhoeic calves (Mir, 2009) arising from histopathological degenerative changes in the lining epithelium (Ghanem *et al.*, 2012).

MATERIALS AND METHODS

Study plan and sample collection

The present work was conducted in the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, U.P. Pandit Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishvavidyalay Evam Go-Anusandhan Sansthan (DUVASU), Mathura. The healthy and diarrhoeic cow calves at LFC and VCC of DUVASU, Mathura (U.P.) and other gaushalas in Mathura were utilized for the investigation.

Table 1: Different therapeutic regimens

Groups (n=6)	Therapeutic regimens
I	Healthy cow calves kept as control
II	Diarrhoeic cow calves treated with amoxicillin@ 10 mg/kg, intramuscularly bid for five days*
III	Diarrhoeic cow calves treated with amoxicillin@ 10 mg/kg, intramuscularly along with powder <i>Aegalmarmelos</i> fruits at the decided dose bid for five days*
IV	Diarrhoeic cow calves treated with powders of <i>Punica granatum</i> peel, <i>Aegalmarmelos</i> fruits and <i>Dalbergia sissoo</i> leaves at the decided doses bid for five days*

*Supportive medication instituted in all the diarrhoeic calves viz. Ringer' lactate @ 25 ml/kg b wt iv as per the need along with anti-inflammatory and multivitamins.

Clinical observations and blood sampling was done on the day of occurrence of diarrhoea and on day 6th post treatment. Clinical signs viz. general condition, rectal temperature, heart rate, respiration rate, consistency of faeces, depression, dehydration etc. was recorded.

Preparation of medicaments

The indigenous preparation in the present study comprised of *Dalbergia sissoo* leaves powder, *Aegle marmelos* fruit powder, *Punica granatum* peel powder. It was prepared by collecting fresh *Dalbergia sissoo* leaves, *Aegle marmelos* fruit, *Punica granatum* peel, which were dried under shed and grinded to fine powder. It was administered @ 50 gm BID orally twice daily for five days.

Clinical scores in diarrhoeic calves

The clinical scores (0-3 basis) for faecal consistency, clinical depression and dehydration was recorded before treatment (on day 0) and (on day 6th) post treatment. (Table 2).

Table 2: Clinical Score in diarrhoeic calf

Score	Faecal Consistency Score	Clinical Depression Score	Clinical Dehydration Score
0	Normal, well-formed faeces	Normal, vigorous Suckling	Normal, bright eyes, pliable skin
1	Pasty faeces	Mild depression, calf suckles but not vigorously	Mild dehydration, eyes not recess into orbits, slight loss of skin elasticity, skin tents < 3 seconds
2	Semi liquid faeces still with a solid component	Moderate depression, calf able to stand, suckling is weak or disorganized	Moderate dehydration, eyes slightly recess into orbit, skin tent > 3 seconds but < 10seconds
3	Watery faeces	Severe depression, unable to stand and suckle	Severe dehydration, eyes markedly recess into orbits, skin tents > 10 seconds

Estimation of Serum interleukin 10, tumor necrosis factor α , interferon gamma (IL10, TNF α , IFN γ) Status

For the assessment of pattern of cytokine production serum

was separated and stored at -20°C for estimation of cytokine (IL-10, TNF- α , IFN γ). Proinflammatory (TNF- α , IFN γ) and anti-inflammatory (IL-10) cytokines were measured in serum of calve using bovine specific quantitative ELISA Kits (Bioassay Technology Laboratory, Shanghai, China) and standard procedure of estimation was followed as provided in kits literature. Curve expert basic version 1.4 software was used to draw standard curve for ELISA.

STATISTICAL ANALYSIS

Statistical analysis of all the data to test significance of means was done as per the method described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Clinical score

Under the clinical score, faecal consistency, dehydration score, depression score in respect of each calf were recorded on day 0 (pre-treatment) and day 6th (post-treatment) and scoring will be done as per Mir (2009) with slight modifications.

Faecal consistency score

On perusal of Table 3, the Mean \pm SE values of faecal consistency score (0-3) of various treated groups were found to be significantly higher in all treated groups of calves than healthy control group on day 0 (pre-treatment). There was significant decrease in faecal consistency score on day 6th (post-treatment) in all the treatment groups with highest recovery was observed in treatment group IV (T3). Therefore, in term of improvement in faecal consistency score in treated groups of calves best recovery were assessed in group IV (T3)

Table 3: Faecal consistency of diarrhoeic calves in different treatment groups at different intervals

Group (n=6)	Treatment intervals (days)	
	Day 0	Day 6
Hc	0.00 ^b \pm 0.00	0.00 \pm 0.00
T1	2.66 ^{Aa} \pm 0.21	0.50 ^B \pm 0.22
T2	2.66 ^{Aa} \pm 0.21	0.33 ^B \pm 0.21
T3	2.66 ^{Aa} \pm 0.21	0.16 ^B \pm 0.16

Dehydration score

On perusal of Table 4, The Mean \pm SE values of dehydration score (0-3) of various treated groups were found to be significantly higher in all treated groups of calves than healthy control group on day 0 (pre-treatment). There was significant decrease in dehydration score on day 6th (post-treatment) in all the treatment groups with highest recovery was observed in treatment group IV (T3). Therefore, in term of improvement in dehydration score in treated groups of calves best recovery were assessed in IV (T3).

Table 4: Dehydration score of diarrhoeic calves in different treatment groups at different intervals

Group (n=6)	Treatment intervals (days)	
	Day 0	Day 6
Hc	0.00 ^b \pm 0.00	0.00 \pm 0.00
T1	2.00 ^{Aa} \pm 0.25	0.50 ^B \pm 0.22
T2	2.50 ^{Aa} \pm 0.22	0.33 ^B \pm 0.21
T3	2.33 ^{Aa} \pm 0.33	0.16 ^B \pm 0.16

Depression score

On perusal of Table 5, The Mean \pm SE values of depression score (0-3) of various treated groups were found to be significantly higher in all treated groups of calves than healthy control group on day 0 (pre-treatment). There was significant decrease in depression score on day 6th (post-treatment) in all the treatment groups with highest recovery was observed in treatment group IV (T3). Therefore, in term of improvement in depression score in treated groups of calves best recovery were assessed in IV (T3).

Table 5: Depression score of diarrhoeic calves in different treatment groups at different intervals

Group (n=6)	Treatment intervals (days)	
	Day 0	Day 6
Hc	0.00 ^b \pm 0.00	0.00 \pm 0.00
T1	1.66 ^{Aa} \pm 0.21	0.33 ^B \pm 0.21
T2	2.16 ^{Aa} \pm 0.30	0.33 ^B \pm 0.21
T3	2.00 ^{Aa} \pm 0.25	0.16 ^B \pm 0.16

During the present investigation the calf with acute diarrhoea showed faecal consistency, dehydration and depression score (0-3) of various treated groups were

found to be significantly higher in all treated groups of calves than healthy control group on day 0 (pre-treatment). There was significant decrease in these scores on day 6th (post-treatment) in all the treatment groups with highest recovery was observed in treatment group IV (T3). Clinical symptoms of dullness, depression and anorexia in calves suffering from mild to moderate diarrhoea. (Cho and Yoon, 2014), reported moderate dehydration as a constant feature in diarrhoeic calves with slow suckling reflex and semi solid to watery faeces, whereas longer diarrhoea with loss of suckling reflex have been reported. These observations are in agreement with the observations of the present study. All sick calves had the usual yellow and watery diarrhoea. Calves with 4% to 8% dehydration (moderate) had a weak suckling reflex, dry mucus membrane, warm mouth and partly good muscular tone (Jaiswal *et al.*, 2019). Calves with 10% and above dehydration (severe) were unable to stand and had no suckling reflex and cold mouth with other general clinical symptoms.

In present study significant improvement in these clinical score suggests that therapeutic regimens applied are effective in treating the condition but best recovery was observed in the treatment group IV (T3) where *Dalbergia sissoo*, *Aegle marmelos* and *Punica granatum* were given. These findings indicate that these herbs found effective and proved to be a potent anti-diarrhoeal in diarrhoeic calves. The findings observed in present investigation are in corroboration with the findings earlier reported (Mehesare *et al.*, 2017 and Wankhade *et al.*, 2019).

Serum IL 10(ng/L) estimation

On perusal of Table 6, Values of serum IL10 concentration (Mean \pm SE) of various treated groups were found to be significantly lower in all treated groups of calves than healthy control group on day 0 (pre-treatment).

Table 6: Serum Interleukin 10 (ng/L) of diarrhoeic calves in different treatment groups at different intervals

Group (n=6)	Treatment intervals (days)	
	Day 0	Day 6
Hc	488.33 ^b \pm 1.43	489.00 ^a \pm 1.52
T1	263.33 ^{Aa} \pm 3.28	350.33 ^{Bb} \pm 12.97
T2	258.33 ^{Aa} \pm 8.81	353.33 ^{Bb} \pm 11.83
T3	253.67 ^{Aa} \pm 11.97	487.33 ^{Ba} \pm 1.45

There was significant increase in serum IL10 concentration on day 6th (post-treatment) in all the treatment groups with highest recovery was observed in treatment group IV (T3). Therefore, in term of improvement in serum IL10 concentration in treated groups of calves best recovery were assessed in IV (T3).

Serum TNF α (ng/L) estimation

On perusal of Table 7, The Mean \pm SE values of serum TNF α concentration of various treated groups were found to be significantly higher in all treated groups of calves than healthy control group on day 0 (pre-treatment). There was significant decrease in serum TNF α concentration on day 6th (post-treatment) in all the treatment groups with highest recovery was observed in treatment group IV (T3). Therefore, in term of improvement in serum TNF α concentration in treated groups of calves best recovery were assessed in IV (T3).

Table 7: Serum Tumor necrosis factor alpha (ng/L) of diarrhoeic calves in different treatment groups at different intervals

Group (n=6)	Treatment intervals (days)	
	Day 0	Day 6
Hc	196.67 ^{Ab} \pm 37.11	170.00 ^B \pm 36.05
T1	213.33 ^{Aab} \pm 20.27	136.67 ^B \pm 3.33
T2	233.33 ^{Aab} \pm 12.01	190.00 ^B \pm 5.57
T3	236.67 ^{Aab} \pm 14.52	173.33 ^B \pm 8.81

Serum Interferon gamma INF γ (ng/L) estimation

On perusal of Table 8, the Mean \pm SE values of serum INF γ concentration of various treated groups were found to be significantly higher in all treated groups of calves than healthy control group on day 0 (pre-treatment). There was significant decrease in serum INF γ concentration on day 6th (post-treatment) in all the treatment groups with highest recovery was observed in treatment group IV (T3). Therefore, in term of improvement in serum INF γ concentration in treated groups of calves best recovery were assessed in IV (T3).

The values of Serum IL-10 concentration of various treated groups were found to be significantly lower in all treated groups of calves than healthy control group on day 0 (pre-treatment). There was significant increase in serum

IL-10 concentration on day 6th (post-treatment) in all the treatment groups. While values of serum cytokine TNF- α (ng/ml) and IFN γ (Pg/mL) concentration of various treated groups were found to be significantly higher in all treated groups of calves than healthy control group on day 0 (pre-treatment). There was significant decrease in serum TNF- α and IFN γ concentration on day 6th (post-treatment) in all the treatment groups. Studies have shown that pro-inflammatory cytokines can increase in many pathophysiological conditions.

Table 8: Serum Interferon gamma (ng/L) of diarrhoeic calves in different treatment groups at different intervals

Group (n=6)	Treatment intervals (days)	
	Day 0	Day 6
Hc	341.67 ^b \pm 4.40	346.67 ^b \pm 13.01
T1	460.00 ^{Aa} \pm 17.55	341.67 ^{Bb} \pm 7.26
T2	430.00 ^{Aa} \pm 11.54	356.67 ^{Bb} \pm 8.81
T3	455.00 ^{Aa} \pm 16.07	345.00 ^{Bb} \pm 7.63

In addition, IL-10 as one of the most important anti-inflammatory cytokines contributes to the inhibition of pro-inflammatory cytokines. TNF- α lead to the migration of leukocytes to the infection site. Increased TNF- α production is associated with the suppression of appetite and loss of weight in inflammatory conditions (Kasimanickam *et al.*, 2013). The increased plasma concentration of TNF- α is probably due to its viral infections, LPS-induced inflammatory response, and protozoal agents (Sohn *et al.*, 2007). Meanwhile, pro-inflammatory cytokines, such as interleukin (IL)-1 β , IL-6, and tumor necrosis factors alpha (TNF- α), play a major role in this regard (Beheshtipour and Raeeszadeh, 2020). Several in vitro studies have shown that high temperature (42°C) and temperature-humidity index (THI) value of > 72 can reduce the proliferation of IL-10-secreting cells (e.g., monocytes and regulatory T cells), compared to low temperature (38.5°C) and a THI value of < 72 (Lacetera *et al.*, 2005). It has been established that IL-10 has a regulatory role in inflammatory conditions (Ouyang *et al.*, 2011). The IL-10 directly inhibits proinflammatory cytokines and reduces their damaging effects. In conclusion, the findings of this study showed that pro-inflammatory cytokines were increased in diarrhoea syndrome. Therefore, these cytokines can be used to recognize the immune system response. Another important finding of the present study was the negligible

role of IL-10 as an important anti-inflammatory cytokine in controlling the pro-inflammatory cytokines. Significant improvement in cytokine profile towards normalcy suggests that therapeutic regimens applied are effective in treating the condition but best recovery was observed in the treatment group IV (T3) where *Dalbergia sissoo*, *Aegle marmelos* and *Punica granatum* were given.

CONCLUSION

The present study was conducted to determine the effect of *Dalbergia sissoo*, *Aeglemarmelos* and *Punica granatum* on clinical and circulating IL-10, TNF- α , IFN- γ Status in acute Undifferentiated calf diarrhoea. The clinical scores, Proinflammatory (TNF- α , IFN γ) and anti-inflammatory (IL-10) cytokines was recorded, before and after treatment revealed that significant decrease in faecal consistency score, dehydration score and depression score on day 6th (post-treatment) in all the treatment groups with highest recovery was observed in treatment group IV (T3). Significant increase in serum IL10 concentration on day 6th (post-treatment) in all the treatment groups and significant decrease in serum TNF α concentration and serum INF γ concentration on day 6th (post-treatment) in all the treatment groups with highest recovery was observed in treatment group IV (T3). The conclusion of the study was revealed that the clinical scores, pro inflammatory (TNF- α , IFN γ) and anti-inflammatory (IL-10) cytokines were effectively improved after the use of the combination of *Dalbergia sissoo*, *Aeglemarmelos* and *Punica granatum*.

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