

# Haemetobichemistry, Pathomorphology and Therapy of *Embelia tserium-cottom* toxicity in Malnad Gidda Cattle

NB Shridhar\*

Obscure Disease Research Center, Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, Karnataka, India

## Abstract

Toxicity episode of *Embelia tserium-cottom* was observed in Malnad Gidda cattle. The clinical signs were severe anorexia, constipation, ascites, anasarca, perineal edema, brisket edema and death in Malnad Gidda cattle which consumed the plant leaves. This study confirmed the plant as a cause for the clinical syndrome of perineal edema which was an obscure disease. There was a damage to kidney and liver with an increase in serum creatinine, BUN, AST and ALT. In post mortem it was observed that there was a damage to most of the vital organs like kidney, heart, liver, lungs had lesions followed by the histological changes. Ailing animals were treated with symptomatically with administration with balanced electrolytes, B-complex vitamins, activated charcoal, isoflupredone, frusemide etc with poor recovery rate.

**Keywords:** *Embelia tserium-cottom* • Toxicity • Hemetology, Biochemistry • Pathology • Perineal edema • Malnad Gidda Cattle • Obscure Disease

## Introduction

Plant toxicity is being reported recurrently from different parts of Karnataka. More than a dozen obscure diseases are prevalent in Malnad region of Western Ghats. For some of such diseases, toxic plant etiology has been suspected [1].

Malnad Gidda is a recently recognized dwarf breed of cattle distributed in Malnad area of Karnataka with population of 7-8 lakhs in Karnataka. Because of the edible nature of the plants like *Embelia tserium-cottom*, Malnad Gidda cattle do consume them and succumb to toxicity. Malnad Gidda cattle usually said to be resistant to bacterial and viral diseases but susceptible to plant toxicities because of their grazing nature. Body weight of these cattle range 100-150 kg and average milk yield is 2 liters [2].

*Embelia tserium-cottom* is one such plant belonging to the family Myrsinaceae. It is commonly known as the "Vayuvilanga" in Kannada and many other species including *Embelia ribes* are also called with same name [3]. *Embelia tserium-cottom* (Syn. *Embelia robusta*, Roxb) is a rambling shrub which is distributed in India, Sri Lanka, Myanmar, China, Thailand, Singapore and Malaysia. It is commonly distributed in Western Ghats and Eastern Ghats of India. The plant is a large climbing shrub commonly found in Western Ghats and in south interior Karnataka [4,5]. It is common plant in Western Ghat districts of Karnataka especially in Shivamogga, Uttara Kannada, Udupi, Chikkamagaluru and Dakshina Kannada districts. Hence the toxicity episodes may be reported more in these districts. The farmers put fire on the neighboring woodland known as "Soppina Betta" just before the monsoon rain begins to get rid of the weeds. The plant grows lush green to a height of one meter, and is easily accessible to grazing Malnad Gidda cattle. Apparently the seeds of this plant are a replacement source of *Embelia ribes* in local and Ayurvedic remedies and for adulterating pepper (*Piper nigrum*).

The plant has got many promising medicinal properties analgesic, anti-inflammatory, contraceptive, anti-infective, antioxidant, anti-diabetic, gastro-hepatoprotective, neuroprotective, radiation-protective and cancer

chemopreventive therapeutic properties which are attributed to its active principle embelin [5]. This plant was suspected to cause an obscure disease characterized by perineal and brisket edema and death in cattle [6].

The clinical signs, pathomorphology, hemeto biochemical changes and therapeutic regime of the toxicity of *Embelia tserium-cottom* documented in the present study. This is the first report of toxicity of *Embelia tserium-cottom* in Malnad Gidda cattle.

## Materials and Methods

### Place of conducting the study

In a span of 9 years (2010-2019), the study was conducted in Uttara Kannada and Udupi Shivamogga and Chikkamagaluru Districts of Karnataka where there is natural toxicity report was obtained by the veterinarians who reported with incidences of perineal edema in Malnad Gidda cattle. The respective places were visited, ailing animals were clinically examined, postmortem of the animals was conducted, symptomatic therapy was initiated and followed up with collection of the samples for laboratory investigation. The age of the affected animals ranged from 5-15 years with both sexes. Fifty eight adult Malnad Gidda cattle (100-150 kg) were examined for toxicity by accidental fresh aerial consumption of *Embelia tserium-cottom* with age ranging from 5-12 years of both sexes. The affected animals had a grazing history of the plants naturally present in the forest.

### Primary diagnosis of the toxicity

Primarily diagnosis of the toxicity did with clinical signs of sluggish and morose behavior, unable to get up, pellet like hard dung and characteristic perineal edema. The plant was subjected to botanical identification.

\*Address for Correspondence: NB Shridhar, Obscure Disease Research Center, Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, Karnataka, India, Tel: 251911796967; E-mail: shridharvet@gmail.com

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## Physical examination of affected animals

Physical examination and timely monitoring of the temperature, heart rate, pulse rate and respiratory rate was performed. Blood samples from the affected cattle were drawn in the daily period of once in two days following the onset of signs of toxicity. The blood was also drawn from a few unaffected Malnad Gidda cattle that belonged to both sexes to acted as control. Hematological parameters like haemoglobin, TC, DC and PCV and serum biochemical parameters viz. alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), blood urea nitrogen (BUN), serum creatinine (CRT), total protein, calcium, magnesium, phosphorus and glucose were estimated in serum of the affected animals.

## Treatment

The animals exhibiting the clinical signs of the toxicity were treated with IV administration of balanced electrolyte solution @ 5-10 ml/kg and B-complex vitamins IM twice in an interval of 10-12 h for three days and activated charcoal @ 2g/kg orally as 1:8 slurry twice at 10 to 12 h interval for two consecutive days. Isoflupredone was administered as 20-30 mg IM per animal once daily for three days and frusemide 0.5-1.0 mg / kg by IM as and when required. The animals were also provided with ruminatorics, purgatives (mineral oil, 1 l /500 kg), magnesium sulphate (450 g/400 kg) with massage with hot fomentation.

## Necropsy

Necropsy of died animals was conducted and gross/histology changes were recorded. The representative organ samples were collected in 10 % neutral buffered formalin (NBF) to study the microscopic lesions.

## Phytochemical analysis

The plant samples were screened for the presence of cyanide, nitrates and oxalates [7,8], Aqueous, petroleum ether, chloroform and methanol extracts were prepared according to standard extraction procedures [9]. Phytochemical analysis of the leaf as such and its extracts was conducted by the technique as explained by Harborne [10].

## Statistical analysis

All data are expressed as the Mean  $\pm$  Standard Error of Mean (SEM). GraphPad Prism Trial Version 8.0.0 software for Windows was used for statistical analysis. One-way ANOVA followed by Dunnett's multiple comparisons test was performed. Differences were deemed significant for values when  $P < 0.05$ .

## Results and Discussion

### Toxic dose

Toxicity of the plant *Embelia tserium-cottom* was studied in Malnad Gidda cattle consumed the leaf in the dose of 20-50 g / kg. This is also in the range of the dose as reported by Shridhar [6]. Mild toxicity signs were observed in those animals that might probably consumed less than this dose. Toxic dose of many other plants like *Mimosa invisa* which do cause similar clinical signs was also in the similar range of 25-50 g/kg in cattle and buffaloes [6,11,12]. Another herb, *Mimosa pudica* was also toxic to cattle and caused symptoms of perineal edema, but the toxic dose was higher and was 150, 200 and 250 g / kg respectively in calves, sheep and goats [13].

## Plant identification

The plant identity was botanically confirmed as *Embelia tserium-cottam* (Roem. and Schult.) by Dr K. Gopalakrishna Bhat, Retired Professor and Head, Department of Botany, Purnaprajna College, Udupi, Karnataka (Figure 1).



Figure 1: *Embelia tserium-cottom* plant.

## Physical examination of the animal

Physical examination of the affected animals revealed that the extremities of the affected animals were cold with decreased tail pinch reflex. The mucous membrane was pale. There was no change in the palpable lymph node size. There was hypothermia sometimes subnormal body temperature, tachycardia followed by bradycardia, weak and feeble pulse were observed. These clinical signs are common in many plant toxicities and mycotoxicosis as there will be reduced body metabolism [14,15].

## Hematological parameters

Hematological examination revealed decrease in total erythrocyte count (TEC), packed cell volume (PCV), hemoglobin concentration (Hb) and normal leukocyte count. Erythrocyte indices revealed elevated mean corpuscles volume (MCV), mean corpuscles hemoglobin (MCH) indicated tendency towards macrocytic hypochromic anemia (Table 1).

Table 1: Haematological profile in toxicity of *Embelia tserium-cottom* toxicity in Malnad Gidda cattle.

Group	Control animals (n=6)	Animals with onion toxicity (n=6)	Reference values
RBCs ( $10^6/\mu\text{l}$ )	7.15 $\pm$ 1.10	2.8 $\pm$ 0.48**	5.0-10.0
Hb (g/dl)	12 $\pm$ 0.20	5.77 $\pm$ 0.28 *	Aug-15
PCV (%)	32.15 $\pm$ 2.5	13.24 $\pm$ 0.78*	24-46
MCV (fl)	46.12 $\pm$ 5.4	54.63 $\pm$ 6.8*	40-60
MCH (pg)	13.10 $\pm$ 2.4	23.30 $\pm$ 4.2*	Nov-17
WBCs ( $10^3/\mu\text{l}$ )	11.50 $\pm$ 1.3	11.97 $\pm$ 4.38	4.0-12.0
Platelets ( $10^3/\mu\text{l}$ )	280.47 66.57	$\pm$ 253.26 $\pm$ 47.94	100-800
Lymphocytes (%)	58.15 $\pm$ 4.5	50.15 $\pm$ 4.30	62-63
Neutrophils (%)	28.17 $\pm$ 5.4	39.89 $\pm$ 1.7	15-33

Eosinophils (%)	11.79 ± 0.33	10.87 ± 0.43	0-20
Monocytes (%)	5.75 ± 0.5	4.77 ± 1.28	0-8

Similar change in the haematological parameters were reported by many workers in sheep in which hemoglobin concentration, packed cell volume, total erythrocyte count reduced than the reference range in onion toxicity [16,17].

### Biochemical parameters

The serum biochemical findings revealed significant increase ( $P < 0.05$ ) in AST, ALT, BUN, CRT and decrease in calcium, magnesium, phosphorus and glucose (Table 2).

The significant increase ( $P < 0.05$ ) in ALT and AST values observed in animals consumed the *Embelia tserium-cottom* might be related to a degree of hepatic damage. In the liver AST, ALT show high activity and are most often determined if there is a suspicion of acute and chronic liver disease. In cattle, increased AST will be accompanied by an elevated serum concentration of hepatic biochemical marker enzymes indicative of liver damage [18]. Increased AST, ALT and GGT activity in the serum is a sensitive marker of liver damage, and in the present study both values are increased indicating a confirmative liver damage [19,20]. The liver damage was confirmed by the increase in the concentrations of AST, ALT, ALP, GGT, BUN and creatinine were noticed by Srikanth and Kumar [21] and many others in plant toxicity of cattle where there was elevated AST, GGT and bile acids indicative of increased hepatocellular release owing to damage and decreased serum clearance of these enzymes [22,23].

One of the ingredient of the *Embelia tserium-cottom* is tannin as reported in the present study also earlier reports [5]. These changes may have been directly caused by an attempt by hepatocytes to detoxify tannin or other metabolic products absorbed from the intestine, probably exacerbated by the abomasitis and enteritis caused by tannins [24].

There was a significant ( $P < 0.05$ ) increase in the concentration BUN and creatinine (Table 2). An increase in BUN and serum creatinine concentration above the normal value implied that there is a damage to nephrons [25]. This finding is in accordance with the finding in toxicity of *Mimosa invisa* in Malnad Gidda cattle where there was increased BUN and creatinine concentrations. The decrease in the micro and macromineral concentration did indicate that the kidney damage might have also contributed to the deficiency [6,11,12].

### Clinical signs of the toxicity

The clinical signs exhibited by the affected animals after 5-6 days of consumption of the plant leaves were sluggish ruminal motility, lateral deviation of head, sunken eyeballs, ascites, polyurea, bruxism and dyspnoea. In few animals there was sub-mandible and brisket edema. The affected animals started passing pellet like hard dung on or after day 5-6 depending based on plant quantity consumed. The gradual to severe development perineal edema within of the plant was also after day 5 of consumption of plant was observed. Abdominocentesis revealed ascites by free fluid flow from a punctured needle. Most of the animals died within a span of 14 days of after onset of the clinical signs. (Figures 2 and 3).



Figure 2: Cattle with toxicity exhibiting the signs of severe perineal edema.



Figure 3: Signs of brisket edema.

Similar clinical signs are reported earlier where there will be involvement of multiple organ failure in which there will be ruminal atony, ascites, hard dung etc in Haemorrhagic Bowel Syndrome (HBS) in dairy cattle [26]. Ascites will be caused in multiple organ damage due to exudation of the fluid from the endothelial cell damage in peritoneal cavity. Clinical signs like pellet like dung, ascites were also exhibited by the cattle in oak leaf poisoning as reported by Garg et al. [27]. This was caused by dehydration due to edema which can result from excessive leakage of fluid from blood vessels or from impaired lymph drainage [28,29]. Multiple organ damage in cattle induces these clinical signs due to endothelial cell damage due to several types of toxic compounds [30,31]. The renal damage might be attributed to the embelin, one of the major ingredient of the plant *Embelia tserium-cottom*. Embelin toxicity was assessed in cycling female rats in dose of 20 mg/kg which did not cause severe toxicity but caused marked tubular damage was observed in the kidneys as seen in present study also [29].

### Treatment

The number of animals recovered after onset of perineal edema was lower. However, the treatment with balanced electrolyte solution @ 5-10 ml/kg, B-complex vitamins IM, twice in an interval of 10-12 h for three days, activated charcoal @ 2g/kg orally for two days as 1:8 slurry, isoflupredone @ 20-30 mg IM per animal once daily for three days and frusemide 0.5-1.0 mg / kg by IM whenever edema is there could save few of the animals.

The affected cattle were treated with slow administration of 5-10 ml/kg balanced electrolyte with dextrose solution. Fluid administration is a well known therapeutic technique for toxin dilution and enhanced toxin excretion from the body. B-complex vitamins are vital dietary supplements. Thiamine is an important vitamin in the B complex family which is needed for metabolism of carbohydrates. In carbohydrate metabolism, for formation of thiamine pyrophosphate, thiamine is a cofactor in carbohydrate metabolism and produces energy. In the present study, B-complex vitamins containing



thiamine with administration of dextrose might augmented the recovery of the toxicity affected cattle [32,33].

Activated charcoal was administered orally as a 1:8 slurry at a dosage of 2 g / kg which might have adsorbed the toxins from the gut and made the animals recover faster. Activated charcoal is administered in early stage toxicity before the toxin / s are absorbed into circulation or before the onset of toxicity clinical signs to inhibit further absorption of toxicants in the gut. Many plant toxicities are treated effectively by administration of activated charcoal in similar dose with examples of Lantana toxicity [34,35] and Yellow tulp (Moraea pallid) toxicity in cattle [36].

Treatment with isoflupredone potent corticosteroid is beneficial for the treatment of animals with toxemia or shock due to a number of reasons. In addition, it will not cause abortions in pregnant cattle as much as it is appropriate to treat them in toxicity emergencies [37,38]. Use of high ceiling diuretics like frusemide is indicated in the treatment of edema of cardiac or renal origin [39].

Along with suggested treatment, ruminatorics, purgatives or magnesium sulfate or polyethylene glycol (1 g/kg/day) may be effective if administered early in the course of disease which may help in getting the rid of toxins. The palliative therapy of massages with hot fomentation on perineal edema region increases blood flow and extra fluid accumulated will be removed [40].

## Postmortem examination

In post mortem examination, petechial to to extensive extensive hemorrhages on the vital organs such as the kidney, heart, lungs and liver indicated the multiple organ damage. Necropsy also revealed severe anasarca, ascites etc. Parietal peritoneum had hemorrhages of extensive nature. Large quantity of ascetic fluid (serous), mildly blood mixed was found flowing upon opening the cavity. Adhesions were evident in dorsal lumbar areas in kidney. Peri renal edema with semi-organised haemorrhagic fat, adherent to capsule was seen. Sub-capsular venous congestion with gelatinous appearance of surrounding area was evident. Extensive cortical hemorrhages, cortico-medullary congestion was also evident.

Liver had nutmeg appearance and blood was oozing upon sectioning. Gall bladder extremely distended containing approx. 1-1.5 liters of bile. In heart, there was fibrinous pericarditis with extensive echymotic haemorrhages on pericardium and epicardium. Similar post mortem findings were also seen in the Mimosa invisa toxicity in which there was multiple organ damage with a perineal edema, anasarca, ascites etc which were also seen in the present study as reported in earlier toxicities [6,11-13].

Ascites in the present study might be attributed to the seepage of the plasma and fluids through the capillaries due to damage to endothelial cells. This is evident by the petechial to extensive hemorrhages present on all the visceral organ and peritoneum. The ascites might be due to an increase in hydrostatic pressure in capillaries which might be due to cardiac insufficiency, congestive heart failure or passive venous congestion with inefficient lymphatic drainage system. Ascites might be attributed to all three reasons in this study, as the plant toxin could be responsible for all of them. This type of pathophysiology was also explained by the earlier workers who studied the oak poisoning in animals [24,27,28].

The perineal edema seen in most of the affected animals might be due to the seepage of the ascetic fluid through the loose connective tissue of perineal region. As the animals lie down, due to pressure put on the abdominal cavity and gravity, more and more ascetic fluids percolate beneath the subcutaneous tissue. These fluids also percolate into the inguinal region. The edema in brisket and submandibular region might be due to the cardiac problem along with liver insufficiency. This was evident from the gross and histological changes supported by increased AST, ALT and GGT values. The pathophysiology of pellet like dung might be attributed to the severe dehydration and fluid loss, and accumulation body fluids in the form of ascetic fluid. This might passively drawn the fluid content from large and

small intestine leading to hard feces. The histological features of the *Embelia tserium-cottom* exhibited the cytoplasmic vacuolation of endothelial cells in the glomeruli and the tubules with varying degrees of lymphoid cell infiltration in the interstitium. These changes are suggestive of tubular interstitial nephritis [41]. Gross and histological changes in the heart, and the lungs were degenerative changes indicating toxic nature of the plant on these organs as observed in toxicity of Mimosa invisa in cattle and buffaloes [6,11-13] (Figures 4-7).



Figure 4: Nephrotoxicity



Figure 5: Extensive haemorrhages in heart and kidney with necrosis.

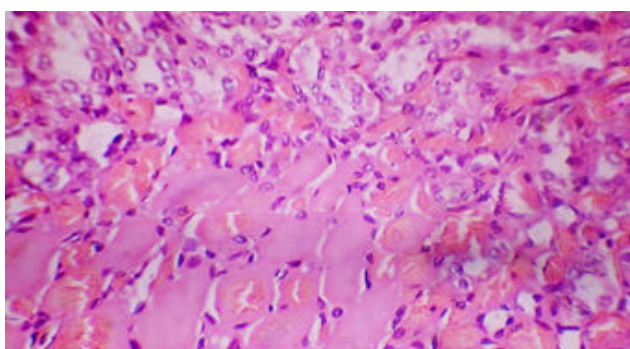


Figure 6: Section of kidney showing severe congestion of inter tubular vessels and focal areas of tubular necrosis with loss of architecture (100 x H&E).

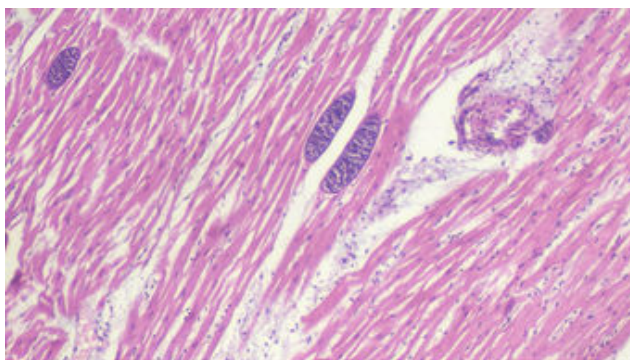


Figure 7: Section of heart showing multifocal sarcocystosis observed with congestion (100 x H&E).

## Phytochemical analysis

In phytochemical screening, the major phytotoxins like cyanide and nitrates and oxalates were absent. This is in accordance with the finding of other researchers [42,43]. Tannins, cardiac glycoside, flavonoids, phenols, terpenoids and saponins are present in the plant leaf extract. Embelin is

benzoquinone a phenolic compound in the different parts of the plant and present to the extent of 1.50 % and responsible for various medicinal properties. This is also in accordance with the findings of Chandrappa et al. [44] and Ananth et al. [45] who screened various extracts of *Embelia tserium-cottom* for the presence of different phytochemical constituents which varied in different parts of plant. This is further supported by the findings of Pandey and Vijayalakshmi [46] and Manisha and Uday [47] who also screened the different parts of *Embelia tserium-cottom* for the content of embelin. However, embelin was not so toxic to rats in the short term toxicity studies as per report of Prakash [29], However, same may be responsible for toxicity in ruminants due to species variation and difference in its kinetic pattern in other animals like ruminants [48].

Further study is required to confirm the role of each phytoconstituent in causing the toxicity in animals. Effort is also needed to elucidate the suitable therapeutic regimen for saving the animals.

## Conclusion

The present study revealed the toxic nature of the *Embelia tserium-cottom* which caused severe perineal edema which was an obscure disease and death in Malnad Gidda cattle consumed the plant leaves. Hematology revealed the anemia. There was multiple organ damage to kidney, liver, heart and lungs etc with increased in serum AST, ALT, GGT, creatinine and BUN which was confirmed in histopathological findings. Few of the animals could recover with symptomatic therapy with administration of fluid, B-complex vitamins, activated charcoal isoflupredone and frusemide.

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