



## Evaluation of Therapeutic Efficacy of *Moringa oleifera* Leaves on Acute Liver Failure in Dogs

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### ABSTRACT

In the present investigation, a total of twelve dogs found positive for acute liver failure and 6 healthy dogs were selected for therapeutic study. Six dogs were considered in apparently healthy control group (group I) after thorough physical examination and various diagnostic tests. Affected twelve dogs were randomly divided into two groups (group II and group III) six animal each. In group II conventional treatment was given and in group III conventional treatment with combination of *Moringa oleifera* @ 30 mg/kg body weight PO was administered for 14 days. Therapeutic evaluation was done on the basis of percent recovery assessment. Percent recovery was assessed by clinical improvement in terms of disappearance of clinical signs and alterations in the hemato-biochemical parameters on day 7<sup>th</sup> and day 14<sup>th</sup> post treatment. Complete clinical examination of all dogs of acute liver failure was made. ALF cases had significantly higher levels of ALT, AST, ALP, GGT, serum total bilirubin and cholesterol than the healthy animal group, whereas serum total protein, albumin and glucose found significantly in lower levels. Both conventional therapy as well as combination of *Moringa oleifera* with conventional therapy was found effective against acute liver failure in dogs as evidenced by restoration of ALT, AST, ALP, GGT, total protein, cholesterol, bilirubin and glucose. Based on results of the study it was concluded that *Moringa oleifera* may be advised as adjunct therapy along with conventional treatment for early recovery in acute liver failure in dogs.

### HIGHLIGHTS

- The study focused on therapeutic efficacy of *Moringa oleifera* leaves on acute liver failure (ALF) in dogs.
- *Moringa oleifera* helps in reduction of ALT, AST, ALP and GGT levels in dogs.
- It may be recommended as adjunct therapy in acute liver failure in dogs along with standard treatment for early recovery.

**Keywords:** Dogs, acute liver failure, *Moringa oleifera*

Acute liver failure is the clinical syndrome that results from rapid loss of liver function to the point that there is insufficient hepatic parenchyma to maintain synthetic and excretory homeostasis. According to Boomkens *et al.* (2004), in the referred canine population, hepatitis affected about 1% of the animals and dogs had hepatitis more commonly than humans and experimental animals. Hepatobiliary dysfunctions can be caused by various neoplastic diseases, infectious diseases, metabolic disorders, degenerative processes, congenital diseases, drug-induced hepatotoxicity, auto-immune diseases and even blunt trauma (Kumar *et al.*, 2012). The majority

of clinical signs associated with ALF are largely nonspecific, including vomiting, diarrhea, anorexia, lethargy, and abdominal pain (Ettinger, 2010). Diagnostic parameters include CBC, serum biochemical profile, urinalysis, coagulation studies, survey radiography and ultrasonography (Kumar *et al.*, 2012). Elevation

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in ALT and AST with hypoglycemia, prolonged PT, hyperbilirubinemia and hypoalbuminemia is typically a finding of liver failure (McCord and Webb, 2011).

Generalized supportive care includes intravenous fluid therapy, liver protectants, nutritional management, anti-inflammatory drugs, antioxidants, diuretics and antibiotics (Pylaris and Dabos, 2010). *Moringa oleifera* is known as “tree of life” or “miracle tree” due to its numerous medicinal properties. The different parts of *Moringa oleifera* possess various pharmacological properties such as anti-cancerous, anti-diabetic, antifertility, hepatoprotective, antihyperlipidemic, anti-ulcer, anti-convulsant activity, etc. as well as it contains various phytochemicals such as flavonoids, glycosides, sterols, proanthocyanidin, anthocyanins, etc. (Kesharwani *et al.*, 2014). Hence, it can be effectively used as liver- protectant.

## MATERIALS AND METHODS

### Study plan and sample collection

The present study was undertaken at Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura in association with Teaching Veterinary Clinical Complex, DUVASU, Mathura.

The selection of dogs of any breed, of any sex, of any age groups was done as per the history of weakness, depression; gastrointestinal tract signs such as anorexia, vomiting, or diarrhea with or without blood; or neurologic signs attributable to hepatic encephalopathy, yellowish skin, mucus membrane and sclera (duration of illness less than ten days) and altered hematology, serum biochemistry

and diagnostic imaging (ultrasonography) abnormalities.

Twelve dogs found positive for acute liver failure during screening along with six apparently healthy dogs were taken for therapeutic trial to access the therapeutic efficacy of *Moringa oleifera* leaves as per the below mentioned groups in table 1.

The therapeutic efficacy of above therapeutic regimens was evaluated on the basis of clinical recovery in term of abolishment of clinical signs after treatment and improvement in the altered values of the hemato-biochemical parameters towards normalcy (at par to the values in healthy control dogs) on day 7<sup>th</sup> and 14<sup>th</sup>. Complete Clinical examination of all cases were made, and following parameters viz. rectal temperature, heart rate, respiration rate and color of mucus membrane were recorded in individual cases.

### Preparation of medicaments

Fresh green leaves of plant *Moringa oleifera* was collected from medicinal herb garden, DUVASU, Mathura. The collected material was cleaned, washed, dried and powdered. Hydroalcoholic extract was prepared with extraction solvent (Ethanol: distilled water 1:1). The solvent was removed and extract was dried *in vacuo*. The extract was mixed with the vehicle and homogenous mixture was formed with the help of mortar and pestle then the formed powder was filled in the gelatin capsules at different doses i.e. 450 mg, 500 mg, and 600 mg (according to body weight).

**Table 1:** Therapeutic protocol to determine efficacy of *Moringa oleifera* leaves in acute liver failure dogs

Group (n=6)	Therapeutic regimens
Group I	Healthy dogs kept as control
Group II	Dogs with acute liver failure treated with conventional therapy*
Group III	Dogs with acute liver failure treated with hydro-alcoholic extract of <i>Moringa oleifera</i> leaves at 30 mg/kgp.o O.D along with conventional treatment

\*Conventional therapy includes Fluid therapy Inj. Dextrose 10% (as per dehydration status) Tab. Amoxicillin and clavulanic acid P.O at 22 mg/kg for 7-14 days. Diuretics (furosemide + spiranolactone) @ 2 mg/kg PO q 12 hrs if required. Injmetaclopramide @ 0.2-0.5 mg/kg every 8 to 12 hour interval if required. Amino acid supplementation orally @0.5 ml/kg daily for 14 days if required. Inj B-complex @1-2 ml I/M on alternate days for 14 days if required; according to clinical signs.

### Collection of blood

Five (5) ml of blood was collected aseptically from cephalic or saphenous vein of affected dogs using sterilized syringe and needle. Out of five ml blood, 2 ml was dispensed into EDTA (ethylene diamine tetra acetic acid) vial for hematology and remaining in clot activator vial for serum biochemistry.

### Estimation of hematological parameters

Following haematological parameters were estimated using Hematology Analyzer (Nihon Kohden): Haemoglobin (Hb, g/dl), Packed Cell Volume (PCV, %), Total Erythrocyte Count (TEC,  $\times 10^6/\mu\text{l}$ ), Total Leukocyte Count (TLC,  $\times 10^3/\mu\text{l}$ ), Differential Leukocyte Count (DLC, %) Neutrophils (%), monocytes (%), lymphocytes (%) and eosinophils (%) using standard methods.

### Estimation of biochemical parameters

The blood for serum required for biochemical parameters was collected in a 5 ml capacity clot activator vial and were allowed to stand undisturbed in a slant position for about 1 hour. The clots were retracted and the serum separated after rapid centrifugation (2000 rpm for 5 minutes). The obtained serum was stored in a deep freeze at  $-20^\circ\text{C}$  in serum collection tubes, which were properly capped and labeled with full details till analysis. Batch analysis was done to avoid repeated thaw and freeze cycles. Following biochemical parameters were estimated with the help of semi autoanalyzer using biochemical kits ARKRAY Healthcare Pvt. Ltd; Serum aspartate aminotransferase (AST)(U/L), Serum alanine aminotransferase (ALT) (U/L), Serum alkaline phosphatase (ALP)(U/L), Gamma glutamyltransferases (GGT)(U/L), Total serum protein (g/dl), Serum albumin (g/dl), Serum globulin (g/dl), A:G Ratio, Serum Bilirubin (Total, Direct) (mg/dl), Glucose (mg/dl) and cholesterol (mg/dl) using standard protocols.

### Ultrasonographic imaging

Ultrasonographic imaging of canine liver was carried out with Mindray machine Model DP-30 Vet using microconvex transducer with the frequency 5-6.5 MHz as per the procedure mentioned by Barr (1992) and Nyland *et al.* (1995). During the sweeps, ultrasonograms were

evaluated for information on liver size, shape, contour and internal architecture including alternations in echogenicity (focal or diffuse), intensity (an / hypo / normal / hyper echoic pattern), gallbladder and hepatic vessels. The ultrasonograms obtained were recorded.

### STATISTICAL ANALYSIS

The data was expressed as mean  $\pm$  SE using one-way analysis of variance (ANOVA) following standard protocol (Snedecor and Cochran, 1994).

### RESULTS AND DISCUSSION

#### Alterations in the Physiological parameters

The Mean  $\pm$  SE values of rectal temperature( $^{\circ}\text{F}$ ), pulse rate (beats/min) and respiration rate (breaths/min) on day 0 were found to be significantly high ( $p < 0.05$ ) in both the treatment groups (Group II and Group III) in comparison to healthy control (Group I). However, there was a significant decrease ( $p < 0.05$ ) in the all the parameters in both the treatment groups at day 7<sup>th</sup> and 14<sup>th</sup>. However, all the mean values in different groups and at different intervals remained within the physiological range. A similar result has also been reported by Hiblu (2015) in dogs with acute hepatic dysfunction (Table 2).

Increase in pulse and respiration rate is a compensatory process for body, for compensation of the oxygen demand in anaemic and/or icteric conditions; the respiration rate might be elevated. Pyrexia might be resulting from hepatocellular damage, infection, sepsis or absorption of intestinal bacterial toxins (Hiblu, 2015).

#### Alterations in the Hematological parameters

The mean concentration of hemoglobin, TEC and PCV decreased significantly in both the treatment groups (Group II and Group III) with respect to healthy dogs at day 0 (Group I) of study. Similarly, Lester *et al.* (2016) reveal Anemia (PCV  $< 39\%$ ) in dogs with acute liver failure However, there was a significant increase in the Hb, TEC and PCV concentration at day 7<sup>th</sup> and day 14<sup>th</sup> after the treatment in both treatment groups with highest recovery in group III followed by group II. Therefore, in terms of improvement in hemoglobin, TEC and PCV

**Table 2:** Alterations in physiological parameters of dogs in different treatment groups at different intervals

Parameter	Group	Day 0	Day 7	Day 14
Rectal temperature (°F)	Group I	101.45 <sup>aA</sup> ±0.13	101.27 <sup>aA</sup> ±0.12	101.32 <sup>aA</sup> ±0.14
	Group II	102.58 <sup>bB</sup> ±0.12	102.0 <sup>aB</sup> ±0.02	101.92 <sup>aB</sup> ±0.09
	Group III	102.70 <sup>bB</sup> ±0.12	102.12 <sup>aB</sup> ±0.06	101.87 <sup>aB</sup> ±0.05
Pulse rate (beats/min)	Group I	81.17 <sup>aA</sup> ±2.46	81.17 <sup>aA</sup> ±2.93	83.50 <sup>aA</sup> ±3.08
	Group II	112.83 <sup>cB</sup> ±1.4	104.17 <sup>bB</sup> ±1.38	98.50 <sup>aB</sup> ±1.18
	Group III	115.00 <sup>cB</sup> ±1.03	104.33 <sup>bB</sup> ±1.28	96.50 <sup>aB</sup> ±0.85
Respiration rate (breaths/min)	Group I	22.17 <sup>aA</sup> ±1.17	22.17 <sup>aA</sup> ±1.19	21.00 <sup>aA</sup> ±0.77
	Group II	38.50 <sup>cB</sup> ±1.40	34.50 <sup>bB</sup> ±1.23	30.66 <sup>aB</sup> ±0.50
	Group III	37.83 <sup>cB</sup> ±1.58	33.00 <sup>bB</sup> ±0.97	28.83 <sup>aB</sup> ±0.60

Group I: healthy control group; Group II: conventional treatment group and Group III: conventional treatment + *Moringa oleifera*. Values (Mean±SE) within same column for a particular parameter (capital letters) and in same row (small letter) bearing similar superscript do not differ at P<0.05.

**Table 3:** Alterations in haematological parameters of dogs in different treatment groups at different intervals

Parameters	Groups	Day 0	Day 7	Day 14
Hemoglobin (g/dL)	Group I	13.11 <sup>aB</sup> ±0.31	13.03 <sup>aC</sup> ±0.27	13.28 <sup>aC</sup> ±0.35
	Group II	9.78 <sup>aA</sup> ±0.19	10.14 <sup>abA</sup> ±0.17	10.65 <sup>bA</sup> ±0.27
	Group III	9.53 <sup>aA</sup> ±0.10	11.04 <sup>bB</sup> ±0.17	12.45 <sup>cB</sup> ±0.13
Total erythrocyte count (TEC) (×10 <sup>6</sup> /μl)	Group I	6.88 <sup>aB</sup> ±0.14	6.96 <sup>aB</sup> ±0.15	6.74 <sup>aB</sup> ±0.09
	Group II	4.98 <sup>aA</sup> ±0.08	5.36 <sup>aA</sup> ±0.26	5.81 <sup>bA</sup> ±0.06
	Group III	4.77 <sup>aA</sup> ±0.08	5.50 <sup>bA</sup> ±0.16	6.47 <sup>cB</sup> ±0.17
Packed cell volume (PCV) (%)	Group I	40.50 <sup>aB</sup> ±0.45	40.23 <sup>aC</sup> ±0.39	39.55 <sup>aC</sup> ±0.48
	Group II	29.31 <sup>aA</sup> ±0.57	30.30 <sup>abA</sup> ±0.42	31.53 <sup>bA</sup> ±0.61
	Group III	28.60 <sup>aA</sup> ±0.29	33.18 <sup>bB</sup> ±0.55	37.40 <sup>cB</sup> ±0.42
Total leukocyte count TLC (×10 <sup>3</sup> /μl)	Group I	13.80 <sup>aA</sup> ±0.50	13.79 <sup>aA</sup> ±0.43	13.15 <sup>aA</sup> ±0.41
	Group II	20.60 <sup>bB</sup> ±0.84	17.84 <sup>aB</sup> ±0.78	16.30 <sup>aB</sup> ±0.57
	Group III	20.05 <sup>cB</sup> ±0.79	17.14 <sup>bB</sup> ±0.77	13.45 <sup>aA</sup> ±0.20

Group I: healthy control group; Group II: conventional treatment group and Group III: conventional treatment + *Moringa oleifera*. Values (Mean±SE) within same column for a particular parameter (capital letters) and in same row (small letter) bearing similar superscript do not differ at P<0.05.

concentration in treated groups of dog best recovery were assessed in group III followed group II. Similarly, Osman et al. (2012) ; Aljohani and Abduljawad (2018) reported RBCs and PCV numbers were increased significantly (P < 0.05) in rabbits fed *Moringa oleifera* leaves in addition to diet.

The mean total leucocyte count increased significantly in dogs with acute liver failure than those of apparently healthy dogs before initiation of any treatment. Whereas, after treating the dogs with conventional treatment as well as combination of *Moringa oleifera*, the values were decreased significantly. The elevated levels of TLC could be explained as representation of the ongoing inflammatory process in the ailing the hepatobiliary system (Brempeleis

and Crispe, 2016; Lecoindre and Arpaillange, 2010). Anti-inflammatory and antioxidant properties of *Moringa oleifera* may be attributable to the reported response of treatment (Table 3).

#### Alterations in the Biochemical Parameters

A significant increase in concentration of ALT, AST, GGT and ALP, was recorded in dogs with acute liver failure (Group II and Group III) as compared to apparently healthy control dogs (Group I) before administration of any treatment. Conventional therapy as well as combination of *Moringa oleifera* significantly decreased the concentration of ALT, AST, GGT and ALP in affected dogs but better recovery was noticed in Group III.

Within 1-2 days of severe and acute hepatocellular insult, the enzyme activity increases upto 100-fold which may lead to increase concentration of ALT, AST and GGT (Weingarten and Sande, 2015). Similar results were obtained by Abdel *et al.* (2020) who studied hepatoprotective effect of aquatic extract of *Moringa oleifera* leaves against lead acetate-induced liver injury in male wistar rats and stipulated that the activity of liver enzymes AST, ALT, and ALP were significantly reduced in MO treated rats.

There was significantly decreased concentration of TP and albumin in acute liver failure dogs as compared to apparently healthy control dogs before administration of any treatment. The conventional therapy as well as combination with *Moringa oleifera* significantly increased the TP and albumin concentration in dogs. Hypoalbuminemia may occur in ALF secondary to concurrent vasculitis or blood loss (Cooper and Webster, 2006). These findings are in agreement with Meel *et al.* (2018) who studied the effect of *Moringa oleifera* leaves feeding on sirohi goat kids.

There was significant increase in pre-treatment concentration of total bilirubin and direct bilirubin in acute liver failure dogs as compared to apparently healthy dogs. Conventional treatment as well as combination of *Moringa*

*oleifera* significantly decreased the concentration of bilirubin with respect to their pre-treatment concentration. In the setting of ALF, hyperbilirubinemia is often the result of hepatocyte dysfunction and intrahepatic cholestasis leading to impaired uptake, conjugation, and excretion of bilirubin (Chapman and Hostutler, 2013). Similarly, decreased serum bilirubin level was observed after the administration of *M. oleifera* leaves extract in rats (Pari and Kumar, 2002).

In present study, mean values of glucose concentration were found to be significantly low while value of cholesterol concentration was found to be significantly high in dogs with acute liver failure than those of apparently healthy dogs before initiation of any treatment. Whereas, after treating the dogs with conventional treatment as well as combination of *Moringa oleifera*, the values become relatively similar to apparently healthy dogs. These findings are in correlation with Garcia *et al.* (2015); Kassem *et al.* (2020) who reported significant decrease in lipid profile after MO extract treatment in dogs in other study. Pari and Kumar (2002) also reported that *Moringa* leaves showed hypocholesterolemic activity. However, in contrast Abakpa *et al.* (2017) reported that aqueous extract of *Moringa oleifera* leaves has a hypoglycemic effect in dogs (Table 4).

**Table 4:** Alterations in various biochemical parameters of dogs in different treatment groups at different intervals

Parameters	Groups	Day 0	Day 7	Day 14
Alanine aminotransferase (U/L)	Group I	30.90 <sup>aA</sup> ±3.99	28.98 <sup>aA</sup> ±2.64	30.96 <sup>aA</sup> ±3.31
	Group II	256.29 <sup>cB</sup> ±20.91	176.20 <sup>bB</sup> ±26.06	101.80 <sup>aB</sup> ±10.51
	Group III	264.39 <sup>bB</sup> ±49.30	117.07 <sup>aB</sup> ±18.38	62.54 <sup>aAB</sup> ±6.65
Aspartate aminotransferase (U/L)	Group I	27.27 <sup>aA</sup> ±4.56	21.04 <sup>aA</sup> ±3.86	20.50 <sup>aA</sup> ±3.82
	Group II	137.77 <sup>cB</sup> ±15.37	84.60 <sup>bB</sup> ±8.35	51.55 <sup>aB</sup> ±9.55
	Group III	126.71 <sup>cB</sup> ±10.94	74.76 <sup>bB</sup> ±7.10	41.81 <sup>aAB</sup> ±5.46
Alkaline phosphatase (U/L)	Group I	64.50 <sup>aA</sup> ±11.82	62.52 <sup>aA</sup> ±11.82	62.61 <sup>aA</sup> ±10.76
	Group II	727.83 <sup>bB</sup> ±357.66	405.32 <sup>aA</sup> ±143.72	170.08 <sup>aA</sup> ±33.29
	Group III	630.22 <sup>bB</sup> ±125.51	300.80 <sup>abA</sup> ±48.27	104.28 <sup>aA</sup> ±5.16
Gamma-glutamyltransferase (U/L)	Group I	6.78 <sup>aA</sup> ±1.41	5.83 <sup>aA</sup> ±1.71	6.18 <sup>aA</sup> ±0.93
	Group II	59.40 <sup>bB</sup> ±7.17	50.44 <sup>bB</sup> ±5.61	32.77 <sup>aB</sup> ±2.92
	Group III	55.90 <sup>cB</sup> ±7.65	33.58 <sup>bC</sup> ±4.56	17.69 <sup>aA</sup> ±3.17
Cholesterol (mg/dl)	Group I	187.61 <sup>aA</sup> ±2.37	187.42 <sup>aA</sup> ±2.61	187.67 <sup>aA</sup> ±2.63
	Group II	287.4 <sup>cB</sup> ±6.12	254.37 <sup>bB</sup> ±9.17	211.5 <sup>aB</sup> ±7.17
	Group III	280.87 <sup>cB</sup> ±6.6	228.32 <sup>bC</sup> ±7.3	197.75 <sup>aAB</sup> ±4.51

Group I: healthy control group; Group II: conventional treatment group and Group III: conventional treatment + *Moringa oleifera*. Values (Mean±SE) within same column for a particular parameter (capital letters) and in same row (small letter) bearing similar superscript do not differ at P<0.05.

## CONCLUSION

The Evaluation of the therapeutic potential of *Moringa oleifera* leaves on acute liver failure (ALF) in dogs in different treatment groups were studied and it was observed that acute liver failure cases under treatment groups III in which *Moringa oleifera* extract (act as hepatoprotectant) was given found to be most efficacious as evident by the improvement in clinical recovery in term of abolishment of clinical signs after treatment and improvement in the altered values of the hemato-biochemical parameters towards normalcy. Therefore it may be recommended as adjunct therapy in acute liver failure in dogs along with standard treatment for early recovery.

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