Evaluation of therapeutic potential of *Moringa oleifera* leaves on acute liver failure (ALF) in dog

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OF

MASTER OF VETERINARY SCIENCE

IN

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BY

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COLLEGE OF VETERINARY SCIENCE AND ANIMAL HUSBANDRY

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(2023)

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This is to certify that the thesis entitled **"Evaluation of therapeutic potential of *Moringa oleifera* leaves on acute liver failure (ALF) in dog"** submitted by **Dr. Anupama Verma,** Enrollment No. V-1693/16 in partial fulfillment of the requirements for the award of the **Master of Veterinary Science** in **Veterinary Medicine** of the **U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan, Mathura (U.P.)** India, is a bonafide research work carried out by her under my supervision and guidance and no part of the thesis has been submitted for any other degree or diploma.

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Abbreviation	:	Stand For
%	:	Percent
/min.	:	Per minute
@	:	At the rate of
°C	:	Degree centigrade
10^3	:	Thousands
10^6	:	Million
A/G	:	Albumin/globulin
ALF	:	Acute liver failure
ALI	:	Acute liver injury
ALP	:	Alkaline phosphatase
ALT	:	Alanine aminotransferase
AMP	:	Adenosine Monophosphate
ANOVA	:	Analysis of variance
AST	:	Aspartate aminotransferase
b.wt.	:	Body Weight
BCG	:	Bromocresol Green
BD	:	Twice a day
CBC	:	Complete blood count
CRT	:	Capillary refill time
DLC	:	Differential leukocyte count
DNA	:	Deoxyribonucleic Acid
DUVASU	:	Deen Dayal Upadhyay Veterinary and Animal Science University
EDTA	:	Ethylene Diamine Tetra Acetic acid
et al.	:	Et alli/alia
Fig.	:	Figure
Fl	:	Femtolitre
G I	:	Group I
G II	:	Group II
G III	:	Group III
g/dl	:	Gram per decilitre

GB	:	Gall bladder
GGT	:	γ-Glutamyltransferase
GOD	:	Glucose Oxidase
GSD	:	Germen Shepherd
Hb	:	Hemoglobin
i.e.	:	that is
I/M	:	Intramuscular
I/V	:	Intravenous
INH	:	Isoniazid
Inj.	:	Injection
IU/L	:	International units per litre
IV	:	Intra venous
Kg	:	Kilogram
LDH	:	Lactate dehydrogenase
MCV	:	Mean corpuscular volume
mEq/L	:	Milli equivalent per litre
mg	:	milligram
mg/dl	:	Milligrams per decilitre
min	:	Minute
ml	:	Milli liter
MO	:	*Moringa oleifera*
NAC	:	N- Acetyl cysteine
No.	:	Number
OD	:	Once a day
ºF	:	Degree Fahrenheit
OTC	:	Over-the-counter
P NNP	:	p-Nitrophenyl Phosphate
PCV	:	Packed cell volume
PO	:	Per os
POD	:	Peroxidase
PT	:	Prothrombin time
PZA	:	pyrazinamide
RBC	:	Red blood cells

RMP	:	rifampicin
Rpm	:	Revolution per minute
S. No.	:	Serial number
SAMe	:	S-Adenosyl-L-Methionine
SE	:	Standard error
SEM	:	Standard error mean
SGOT	:	Serum Glutamic Oxaloacetic Transaminase
SGPT	:	Serum Glutamic Pyruvic Transaminase
Tab	:	Tablet
TEC	:	Total erythrocyte count
TLC	:	Total leukocyte count
TP	:	Total protein
TVCC	:	Teaching Veterinary Clinical Complex
U.P.	:	Uttar Pradesh
Viz	:	videre licet
WASAVA	:	World small animal veterinary association
WBC	:	White blood cells
Wt.	:	Weight
µg	:	Microgram
µl	:	Microlitre

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Date: 19 Dec 2023

Place: Mathura (U.P.)

(Anupama Verma)

Contents

ABSTRACT

The present study was conducted to investigate the occurrence of acute liver failure in dog and to evaluate the therapeutic potential of *Moringa oleifera* leaves on acute liver failure in dogs. For this, a total of 3881 dogs, irrespective of age, breed and sex presented to TVCC, DUVASU, Mathura were examined during a period of study *i.e.,* from February 2023 to July 2023. Total 205 dogs showed clinical signs concern to acute liver failure, out of which 31 dogs were found positive for acute liver failure by altered Hematology, altered serum biochemistry and diagnostic imaging abnormalities. The overall occurrence (hospital based prevalence) of acute liver failure in total dog population was 1.107 % (31/3881) whereas hospital based prevalence among suspected dogs was 15.12 % (31/205). Amongst different breeds, Labrador retriever had the highest occurrence 29.03 % and Shih Tzu possesses lowest occurrence of acute liver failure i.e. 5.26 %. Age wise prevalence was highest in dog age group 4-8 years and female dogs possessed more prevalence than male. Vomiting, anorexia, icterus, weakness, abdominal pain, wt. loss, fever, neurologic signs, hematemesis, inappetance, pale mucus membrane and polyuria/polydypsia are the important clinical findings in dogs with acute liver failure as per our study. Twelve (12) found positive for acute liver failure during screening were taken for therapeutic studyand randomly allocated into 2 groups, each group having 6 animals. In group II conventional treatment was given and in group III conventional treatment with combination of *Moringa oleifera* @ 30mg/kg body weight PO was administered for 14 days. Conventional treatment 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) @ 2mg/kg PO q12hrs if required; Inj metaclopramide @ 0.2-0.5 mg/kg every 8 to 12 hour interval if required; Amino acid supplementation orally @0.5ml/kg daily for 14 days if required;Inj B-complex @1-2ml i/m on alternate days for 14 days if required; according to clinical signs. The indigenous extract preparation comprised the leaves of *Moringa oleifera* which was air-dried under shade, grind to a fine powder and hydroalcoholic extract was prepared. Six apparently healthy dogs were placed in healthy control group (group I) after thorough physical examination and hemato-biochemical tests. Therapeutic evaluation was done on the basis of percent recovery assessment and hemato-biochemical alterations. Percent recovery was assessed by clinical improvement in terms of disappearance of clinical signs and alterations in the hemato-biochemical parameters on day 7^{th} and day 14^{th} post treatment.Complete clinical examination of all dogs of acute liver failure was made. Both conventional therapy as well as combination of *Moringa oleifera* with conventional therapy was found effective against acute liver failure in dog as evidenced by restoration of ALT, AST, ALP, GGT, total protein, cholesterol, bilirubin and glucose. Although, better recovery towards normalcy was noticed in group III. Based on results of the study it was concluded that *Moringa oleifera* (act as hepatoprotectant) may be advised as adjunct therapy along with conventional treatment for early recovery in acute liver failure in dog.

Introduction

CHAPTER-1

INTRODUCTION

The habit of owning a pet as dog is increasing in urban areas since dogs are known as man's best friend. Hence, the development of strong bond between the owner and the dog is mandatory. As the wellbeing of their pet become so important, visit of the owners to the clinics are escalating now a days due to various ailments. The liver's inability to operate properly is one of these illnesses. Since the liver is a highly compensating organ, clinical, biochemical, and technical approaches fall short in identifying the key symptoms of liver disorders (Popova *et al.*, 2020).

Liver, largest parenchymal organ of the body carries out numerous essential biochemical processes crucial for maintenance of homeostasis of normal body. Some of the major functions of liver include detoxification, synthesis of protein, fat and several blood clotting factors; metabolism of protein, fat and carbohydrate; synthesis and secretion of bile for digestion of food and storage of glycogen as an energy source. Liver's functioning in body made it uniquely susceptible to damage (Cynthia, 2013). Hepatic and biliary 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). Underlying etiologies of acute hepatic failure include metabolic, neoplastic, infectious or toxic processes (Sherding, 1985).

The prevalence of 3.01 percent was reported with hepatic disorders in demographic study (Vijayakumar *et al.,* 2003). Highest prevalence of hepatic disorders was found in dogs older than 8 years (34.86%), followed by those between 4 and 8 years (30.28%). This may be related to the indiscriminate use of medications like antibiotics and dewormers without proper consultation with a veterinarian, as well as the fact that as age increases, all body cells lose some of their physiological function and capacity to regenerate, including hepatocytes (Sameeksha *et al*., 2021).

A variety of clinical states can be presented, from the very ill to the asymptomatic in dogs with liver diseases (Dixit *et al*., 2010). In ALF, there is typically a history of acute onset of clinical signs (fewer than 8 weeks) in a previously healthy animal (Cooper and Webster, 2006). Anorexia, weakness, abdominal

discomfort, lethargic behaviour, nausea, and hematemesis are a few of the symptoms of liver disease that are frequently seen (Mehrotra and Tandan, 1973). In addition, anorexia, vomiting, diarrhea, constipation, yellowing of the skin and mucous membranes, hepatomegaly, ascites, fever, hindlimb edema, tachycardia and tachypnea, anemic appearance with pale mucous membranes, yellowish or coffee-colored Urine, melena, bloody stool, seizures, coma, skin lesions, coagulopathy, epigastric abdominal pain, polyuria and polydipsia, itching, weight loss, poor physical condition (Bera and Lodh, 2019).

The WSAVA liver standardization group recently categorized hepatic disorders in dogs into four main types i.e., vascular liver disorders, biliary tract disorders, parenchymal disorders and neoplasia (Brovida and Rothuizen, 2010). Contrary to other organs like the kidneys, the liver is particularly adept at regeneration; yet, major injury to the liver can result in acute liver failure, a rapid loss of function. Before liver failure develops, the liver normally has about 75% of its tissue destroyed (Mackenzie *et al*., 1975). 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. Acute liver injury (ALI) denotes acute hepatocellular damage and necrosis with retained hepatic function.

As the liver is diverse and has excellent regenerative capabilities, any single test fails to identify hepatic disease or its underlying cause. For this reason, bounteous tests must be used to diagnose the hepatobiliary disorders. A reasonable package of screening tests recommended for an animal suspected of having hepatobiliary disease includes a complete blood count (CBC), serum biochemical profile, urinalysis, coagulation studies. Survey radiography and Ultrasonography can also be used in diagnosing hepatobiliary affections (Kumar *et al*., 2012). Complete blood cell counts are often wide-ranging and non-specific (Grady et al., 1993). In acute hepatic necrosis, the leakage enzymes ALT and AST are typically the first values on standard blood work to increase. This may then be followed by hypoglycemia, prolonged PT, and hyperbilirubinemia. Hypoalbuminemia is typically a finding of end-stage liver failure (McCord and Webb, 2011). In the dog and cat, ALP activity increases 2- to 5-fold after acute, severe hepatic necrosis and then gradually decreases (Center, 2007). Hepatic ALP is found mainly in liver canalicular cell membranes and increases with

biliary disease, especially with cholestasis (Thapa and Anuj, 2007). The urinalysis provides limited information about the status of the liver but the finding of biurate crystals or calculi is strong evidence for the presence of a portosystemic shunt, and bilirubin crystals may confirm the presence of excessive amounts of urinary bilirubin (Hughes and King, 1995). Abdominal ultrasonography is considered the most practical diagnostic imaging procedure for detecting a hepatobiliary disease as it depicts the alterations in the liver as variances in echogenicity. This enables the identification of heterogeneous structures within the liver parenchyma and the comparison of liver tissue to other soft tissues. Radiography may be used in liver diagnostics mostly to evaluate the size and shape of the liver. Hepatic ultrasonography provides information regarding the size and shape of the organ, the echogenicity and echotexture of the parenchyma, along with information on the biliary tracts and main vessels (Webster *et al*., 2019).

Treatment of canine liver illnesses in frequently supportive and allows combinations of drugremedy and nutritional support (Bexfield and Watson, 2009). Generalized supportive care includes intravenous fluid therapy, liver protectants, nutritional management, anti-inflammatory drugs, antioxidants, diuretics and antibiotics (Pyleris and Dabos, 2010). Antioxidants including vitamin E, zinc, silymarin (milk thistle), N-acetyl cysteine (NAC) and SAMe are free radical scavengers and there is evidence for best efficacy the use of both SAMe and silymarin in dogs with acute toxic hepatopathies treatment (Uetsu *et al*., 2017; Pritt *et al*., 2010). Nutritional therapy is provided for hepatic repair and regeneration, and to prevent or manage complications of hepatic failure (Remillard and Saker, 2005).

Alternative and complementary medicine encompasses various healing philosophies that can easily integrate with veterinary medicine. Ayurveda, homeopathy, Acupuncture, traditional Chinese medicine and chiropractic are some of the widely practiced alternative treatments which when integrated with conventional therapy increased efficacy like enhancement in quality of life, minimization of symptoms, reduction or stabilization of disease and prolonged life.

Moringa oleifera, is a quick growing edible tree belonging to family Moringaceaeand is known by different names such as drumstick tree, kelor tree, Sajna (Bengali), Saijihan (Nepali), Shevga (Marathi), Marango, Sigru (Malayalam), horse radish tree (English), Soaanjna (Hindi), Shobhanjana (Sanskrit), Surajana(Punjabi),

etc. (Anwar and Bhanger, 2003). The tree is also noun 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). Mahmood et al. (2010) reported that Moringa leaves contain vitamin C, vitamin A, and high concentrations of essential amino acids. Among the 13 species, most of the studies focused on the leaves of the plants *Moringa oleifera*, *Moringa stenopetala*, *Moringa concanensi*s, and *Moringa peregrine*. Moreover, the availability of nutrients from *Moringa oleifera* is in nontoxic form and can be digested easily (Bey, 2010). It aids in the prevention of numerous diseases that affect the liver, eyes, gastrointestinal tract, respiratory system, cardiovascular system, and other organs. Leaves of *M. oleifera* have been shown to possess a high degree of biosafety with minimal or no adverse effects on humans and animals (Stohs and Hartman, 2015). The extract of *Moringa oleifera* leaves is also capable of reducing hyperglycemia and dyslipidemia (Mbikay, 2012). The leaves of *Moringa oleifera* has been reported to have antioxidant and hepatoprotective action in alcoholic extracts. Moringa extract lowers SGOT, SGPT, GGT, LDH and ALP level in serum along with inhibition of lipid peroxidation (Singh et al., 2014). The administration of aqueous Moringa extract with its antioxidant significantly restored the lead perturbations in male wistar rats through reduction of oxidative stress-induced DNA damage and reduced serum hepatic enzyme activities (Abdel et al., 2020). Administration of ethanolic extract of Moringa leaves showed protection against liver lipid changes caused by antitubercular drugs (INH+RMP+PZA), thereby denoting a broad spectrum of hepatoprotective effect (Pari and Kumar, 2002). *Moringa oleifera* has significant hepatoprotective value against paracetamol induced liver injury in rats (Islam *et al*., 2019). This study offers a theory for how *M. oleifera* would be most successful in the liver's anti-inflammatory and antioxidant activities while having the fewest negative effects.

Therefore, keeping in view the above facts, in order to evolve suitable herbal hepatoprotective drug for the therapeutic management of ALF in dogs using traditional herb as an adjunct therapy, the present investigation is planned with the following objectives.

Objectives:

1. To study the occurrence of acute liver failure in dogs.
2. To evaluate therapeutic efficacy of *Moringa oleifera* leaves on acute liver failure (ALF) in dog.

Review of Literature

CHAPTER -2

REVIEW OF LITERATURE

2.1 Hepatic disorders in Canines

It has been accounted that hepatic diseases account for three per cent of all diseases seen by veterinarians (Candlin, 1968).

Liver disease in pets as well as people is very complex. The liver disease may be annoying to diagnose for every practitioner. Liver disease in dogs can develop as a result of many different insults (Rutgers, 1996).

Chohan *et al.* (2007) studied liver dysfunction in 35 dogs and found that amongst the primary hepatopathies, acute hepatitis (42%) has formed the largest group, followed by chronic hepatitis (19%), hepatic venous outflow disorders (15%), cholecystitis (12%) and others as isolated cases of hepatic abscess, hepatic tumour and hepatic lipidosis (4% each).

Acute hepatitis (AH) and chronic hepatitis (CH), with or without cirrhosis, are common forms of primary hepatitis (PH) in dogs. The less common ones were eosinophilic hepatitis (EH), granulomatous hepatitis (GH), and lobular dissecting hepatitis. (Poldervaart *et al.*, 2009).

A prospective study on liver diseases in 140 dogs has reported that 58.60% had primary hepatopathy while 41.40% were having secondary hepatopathy. A thorough study was conducted on primary hepatitis in dogs and diagnosed 67 dogs with chronic hepatitis, 21 with acute hepatitis (Dixit *et al.*, 2010).

It is salient to account for the clinical presentation, laboratory test results, diagnostic imaging findings and the outcome of cytological and histopathologic evaluation together while assessing the patient with suspected hepatobiliary disease (Jonathan and Steiner, 2013).

2.2 Occurrence

Vijayakumar *et al.* (2003) recorded prevalence of 3.01 percent in a demographic study associated with hepatic diseases in dogs.

According to Boomkens *et al.* (2004), in the referred canine population, hepatitis affected about 1% of the animals. In addition, they mentioned that dogs had hepatitis more commonly than humans and experimental animals.

Apalkova (2012) reported the prevalence of 1.24% of all liver diseases diagnosed in dogs.

Saravanan *et al.* (2014) reported that from 2011 to 2012, in RVP, IVRI, Izatnagar, the prevalence of hepatic insuffiency in dogs was 2%.

An overall incidence of 1-2 percent is documented of hepatic disorders among clinical cases of dogs. (Tantary *et al.*, 2014).

Hiblu (2015) reported that out of 140 dogs with hepatic insufficiency, 12 (8.57%) dogs suffered from acute hepatitis.

Elhiblu *et al.* (2015) reported that among 140 dogs with hepatic insufficiency, 4.3 percent had liver cirrhosis.

Lathamani and Nalinikumari (2015) reported the prevalence of hepatic disorders among dogs as 1.93 percent in Tirupati, from May to November of 2006.

Kilpatrick *et al.* (2016) conducted a study on 70 confirmed cases of primary hepatopathy in dogs out of which 10 cases were diagnosed with acute hepatitis (14.29%).

Kumbhkar (2017) screened 1082 dogs for hepatic disorders on the basis of clinical observations and found 3.51% prevalence.

A preliminary study of hepatic disorders in dogs in Indore district reported a prevalence of 3.51% of liver disorders (Kumbhkar *et al.*, 2018).

Sameeksha *et al.* (2021) conducted a study on 10,204 dogs of which 109 (1.06%) were diagnosed with various liver disorders.

2.2.1 Breed predisposition

Tiwari (2002) documented that Pomeranian and German Shepherd were over presented in clinical cases studied for hepatic disorders.

Vijayakumar *et al.* (2003) recorded the most infected breeds with hepatic disorder were Doberman pinscher and Germen Shephard.

Hiblu (2015) reported that out of 140 dogs with hepatic insufficiency, Labrador Retrievers (50.71%) were highest affected, followed by German shepherd (15.71%), Mongrel (8.57%) and Pug (7.14%).

Lester *et al.* (2016) conducted a retrospective evaluation of acute liver failure in 49 dogs from 1995-2012 and reported that amongst 26 breeds affected, there were 7 Labrador Retrievers, 5 Golden Retrievers and 3 mixed breed dogs.

Kumbhkar (2017) documented hepatic disorders in 38 dogs in which 8 were Labrador Retriever (21.06%), 7 (18.42% each) were Spitz and non-descript, 4 (10.52%) each of German Shepherd and cross-breed, followed by 3 each (7.9%) of Rottweiler, Great Dane and 1 each (2.63%) of St. Bernard and Golden Retriever.

Murikipudi *et al.* (2017) found the highest occurrence of hepatic disorder in Labradors (35.55%), followed by Mongrels (26.66%), German Shepherd (17.77%), Spitz (11.11%) and Pug (4.44%).

Sameeksha *et al.* (2021) conducted a study on 10,204 dogs with various liver disorders and recorded highest prevalence in Labrador Retrievers (43.12%), followed by non-descript dogs (29.36%), Spitz (6.42%), German shepherd (5.51%). Golden Retriever, Rottweiler, Pug, Beagle, Great Dane, Lhasa Apso breeds showed prevalence of 1.84% each while the lowest, 0.91% was found in Shih Tzu, Boxer, Cocker Spaniel, St. Bernard, Siberian Husky.

2.2.2 Gender predisposition

Hiblu (2015) has studied that out of 140 dogs with hepatic insufficiency, males were more affected (64.86%) than females (35.14%).

Lester *et al.* (2016) conducted a retrospective evaluation of acute liver failure in 49 dogs from 1995-2012 and reported 28 male (57.14%) and 21 female dogs (42.86%) being affected.

Kumbhkar (2017) reported Female dogs (57.89%) were more affected than males (42.11%) with hepatic disorders.

Murikipudi *et al.* (2017) recorded higher occurrence of hepatic disorders in male dogs than females.

Jeena (2019) reported 61.11% occurrence of hepatic failure in males and 38.88% in females.

Sameeksha *et al.* (2021) conducted a study on 10,204 dogs with various liver disorders and found out that male dogs (59.63%) suffered more in comparison to females (40.37%).

2.2.3 Age predisposition

Mean age of dogs with hepatic disorders was reported as 51.9 months containing hepatitis and cirrhosis both (Tiwari 2002).

Mandigers *et al.* (2004) reported that liver diseases were usually diagnosed in dogs of the age group four to six.

Schmucker (2005) opined that elderly dogs posed a higher chance of being affected by hepatic afflictions as the ageing liver greatly alters its morphology and functioning.

Shih *et al.* (2007) reported that the median age of Labrador retriever dogs suffering from hepatitis is 9.3 years (range, 3.9-14.0 years).

Hiblu (2015) studied 140 dogs with hepatic insufficiency and stated age groups ranged from 6 months to 14 years with majority of the cases (44.29%) in young age group (0-4 years), followed by middle age (4-8 years) group (35%) and geriatric age (> 8 years) group (20.71%).

Lester *et al.* (2016) conducted a retrospective evaluation of acute liver failure in 49 dogs from 1995-2012 and age group of affected dogs ranged between 1 month to 13 years.

Jeena (2019) reported highest number of hepatic failure cases in dogs above 8 years of age (33.33%), followed by 4 to 8 year old dogs (27.78%), less than 1 year old (22.22%) and 1 to 4 year old age group (16.67%).

2.3 Clinical signs

According to Hughes and King (1995), clinical signs of acute liver failure in dogs include weakness, depression, gastrointestinal signs such as anorexia, vomiting, diarrhoea, neurologic signs, polydipsia/polyuria, icterus etc. Physical examination may reveal organomegaly, cranial abdominal pain or a peritoneal effusion.

According to Shih *et al.* (2007) most of the dogs of chronic hepatitis had vague signs of decreased appetite, vomiting, lethargy, weight loss which are somewhere asymptomatic except for increases in serum liver enzymes.

Clinical signs of hepatopathies in dogs are extremely variable due to the liver's extensive interaction with other organs and its unusual regenerative capacity (Dial 1995: Fleming 2011).

Clinical signs in liver diseases were vague and varying from decreased appetite, anorexia, nausea, vomiting, ascites, weakness, weight loss, pale mucosa, epigastric pain, pyrexia, bilateral hind limb edema, constipation, diarrhoea, melena, icterus, encephalopathy, polyuria, polydipsia to depression indifferent combination (Dixit *et al*., 2010).

Ettinger (2010) quoted that the majority of clinical signs associated with ALF are largely nonspecific, including vomiting, diarrhea, anorexia, lethargy, and abdominal pain. These signs may be due to the underlying etiology or related to the numerous complications that can occur in the more advanced stages of ALF including cerebral edema and sepsis.

Lester *et al.* (2016) performed retrospective evaluation of acute liver failure in 49 dogs and found anorexia (28/49, 57%), vomiting (25/49, 51%), neurologic abnormalities (17/49, 35%), and polydipsia/polyuria (10/49, 20%) as majority clinical signs.

Schmid and Hovda (2016) have reported vomiting and hypoglycemia in a Dog after xylitol ingestion due to acute hepatic failure.

Clinical symptoms associated with hepato biliary disorders in dogs could be enlisted as decreased activity, inappetance, anorexia, vomiting, diarrhea, constipation, haematemesis, yellowish tinge in skin and mucus membrane, ascites, fever, oedema of hind limbs, tachycardia and tachypnoea, anaemic appearance with pale mucus membrane, yellowish or coffee colored urine, haematochezia, seizures, coma, cutaneous lesions, coagulopathy, epigastric abdominal pain, polyuria and polydipsia, pruritis, weight loss and depreciating body condition (Saravanan *et al.*, 2014; Elhiblu *et al.*, 2015; Lawrence, 2015; Pradeep *et al.*, 2017; Bera and Lodh , 2019; Jeena *et al*., 2020; Sameeksha, 2021).

2.4 Hematological profile

Tiwari *et al.* (2001) reported dogs with hepatobiliary disorder shows poikilocytosis and anisocytosis.

Ettinger and Feldman (2005) observed thrombocytopaenia in dogs with hepatic disorders. Their findings coincided with those observed by Tiwari *et al.* (2001) and Pradeep *et al.* (2017).

According to Bera (2016), in acute hepatic disorder, there is decrease in mean haemoglobin, TEC, PCV values and lymphocytes; meanwhile increase in mean TLC value and neutrophils.

Lester *et al.* (2016) studied 49 dogs with acute liver failure shows thrombocytopenia 26/49 dogs [53%], leukocytosis 17/49 [34%], and leukopenia in 1 dog. Anemia (PCV <39%) was identified in 14/49 (28%) and hemo-concentration in 5/49 (10%) dogs.

Several mechanisms have been suggested for thrombocytopenia in patients with liver disease, including (1) increased platelet sequestration in the spleen as a result of congestive splenomegaly; (2) reduced production of thrombopoietin by the liver; (3) increased platelet breakdown due to auto-antibodies and (4) increased consumption resulting from low-grade DIC (Witters *et al.*, 2008).

According to Sameeksha (2021), the increased values of TLC in hepatic diseases were due to hepatocellular injury, sepsis, infection or the absorption of intestinal bacterial toxins.

Liver disorders are responsible for 14.17% cases of anaemia reported among domestic dogs (Panchal *et al*., 2022)

2.5 Biochemical profile

Hughes and King (1995) reported marked elevation in ALT and AST values in acute liver failure.

Compared to ALT and AST, there was a minimal ALP rise in the plasma after a severe and acute insult. (Meyer and Harvey, 1998).

Previous clinical experience indicated that interpreting the two aminotransferases may offer more information to help evaluate specific hepatic

disease types. Because of their different plasma half-lives and cellular locations, the serum AST activity would return to normal more quickly (hours to days) than the serum ALT activity (days) following an acute injury that resulted in a moderate to substantial elevation in the serum ALT and AST activities. (Hess and Bunch, 2000 and Webb *et al.*, 2002)

Richter (2003) observed that albumin concentration decreased with severe hepatic ailments because of decreased albumin production, increased ammonia levels which inhibits albumin release from hepatocytes, abnormal insulin and glucagon concentrations resulting in prohibition of albumin release of hepatocytes along with increased volume of distribution of albumin in the presence of ascites.

Hall and German (2005) stated that the minimum serum biochemical parameters comprises of ALT, AST, ALP, GGT, BUN, creatinine, total bilirubin, total protein, glucose, albumin, globulin, and cholesterol in suspected hepatic affections in canine and feline.

Toulza *et al.* (2006) in their study concluded that estimation of Protein C and combining it with the other parameters increased the chances of diagnosis of hepatic disorders including hepatic failure. It also reflected on the severity of the disease when combined with the results of hyperbilirubinemia and low antithrombin activity.

Sharma (2010) deduced that total and direct bilirubin was markedly increased in acute inflammatory hepatic diseases, cholecystitis, chronic hepatic disorders, tumour and acute liver dysfunctions.

Chapman and Hostutler (2013) found common biochemistry findings in ALF including decreased serum blood urea nitrogen, hypoglycemia, hypocholesterolemia, hypoalbuminemia and hyperbilirubinemia due to hepatocyte dysfunction and intrahepatic cholestasis.

Elevated ALT and AST activity are sensitive markers of acute liver injury, according to Weingarten and Sande (2015).

Lester *et al.* (2016) studied and observed that the most common serum biochemical findings of acute liver failure were hyperbilirubinemia (49/49), increased serum alkaline phosphatase activity (45/47), aspartate aminotransferase activity (45/49), alanineaminotransferase (ALT) activity (45/49), gamma glutamyltrans-

peptidase activity (35/45), hypoalbuminemia (23/49)(45%) and serum hypoglycemia 10/49 (20%).

Prebavathy *et al.* (2020) reported that canine clinical cases suspected for hepatic diseases were screened with the help of clinical examination, radiography, ultrasound scan and haemato-biochemical examination. Out of 10,921 cases, 52 clinical cases were diagnosed to be suffering from hepatic disorders and elevated ALT and AST activity was recorded.

Bhatt (2021) revealed significant increase in the values of liver enzymes, namely, AST, ALT, ALP, total, direct and indirect bilirubin levels in acute hepatitis.

2.6 Ultrasonography

Hughes and King (1995) studied the echogenicity of liver parenchyma in dogs with acute hepatic failure and reported that the echogenicity ranged from normal to diffusely mottled to decreased echogenicity.

Lamb (1995) observed that large liver lobes in hepatomegaly in acute hepatic insults often have rounded margins, whereas the normal liver lobes have sharp edges seen in radiographic image.

Konde and Pugh (1996) reported that ultrasonography is one of the best noninvasive techniques to study liver parenchyma. It helps to differentiate focal disease from diffuse disease, cysts from solid masses and obstructive jaundice from non- obstructive jaundice.

Resende *et al.* (2011) studied ultrasonographic images of dogs with acute hepatic failure and observed hepatomegally with normal, increased or decreased echogenicity of the parenchyma where in the discrete hepatic borders are not visualized.

Webster *et al.* (2019) reported that hepatic ultrasonography gives information regarding the size and shape of the organ, the echogenicity and echotexture of the parenchyma, along with the information on the biliary tracts and main vessels. However, in that study it was also derived that because of the location of the liver being under the ribs and its variations in conformation among various breeds, the type of equipments used and modality being highly operator dependent, the results may vary in different published works.

2.7 Treatment of hepatic disorder

Varshney (2001) opined that the single most effective therapy for hepatic disorders is dietary therapy. A traditional dietary approach consisting of low fat, low sodium, high carbohydrates and high protein has been adopted to alter the course of hepatic diseases (low aromatic amino acid). The researcher also reported that diuretic combination of furosemide and spironolactone was more effective than furosemide alone.

Honeckman (2003) suggested therapy with various widely used antibiotics in case of hepatic disorders including metronidazole, fluroquinolone, ampicillin, amoxicillin and clavulanic acid.

Silverstein and Beer (2015) stated that IV fluid therapy is an integral part of the management of the ALI/ALF patient, used to restore and maintain perfusion, to correct dehydration, and to maintain euhydration in patients that are not amenable to oral fluid administration.

Lester *et al.* (2016) conducted retrospective study on ALF in dogs and given antimicrobial therapy, gastroprotectants, intravenous n-acetylcysteine, ursodeoxy-cholate and S-adenosylmethionine for treatment.

2.8 Therapeutic efficacy of *Moringa oleifera*

No study has been conducted till date for evaluating the efficacy of *Moringa oleifera* in acute hepatic failure in dogs, however effect of other herbal plants have been studied in hepatic disorders. In Ayurveda, Homeopathy and Unani system of medicine, *Moringa oleifera* and/or its extracts and/or parts and/or constituents are being used since ancient times for therapeutic purposes for treating different ailments in human as well as in animals. Majority of such work has been conducted in laboratory animals like rats and mice. Due to scanty literature available for the same, this study was conducted for the first time in dogs.

2.8.1 Hepatoprotective activity of *Moringa oleifera*

Pari and Kumar (2002) reported hepatoprotective Activity of ethanolic extract of *Moringa oleifera* (150, 200 or 250 mg/kg body weight for 45 days) against Antitubercular Drug-Induced Liver Damage in Rats, namely isoniazid (7.5 mg/kg B.W), rifampicin (10 mg/kg B.W) and Pyrazinamide (35mg/kg B.W). They concluded

that co-administration of the leaf extract in conjunction with these drugs ameliorate the increases in the serum enzyme levels, bilirubin, tissue lipids, and lipid peroxidative markers. The degree of protection was observed maximally with the highest dose of the extract.

Toppo *et al.* (2015) concluded that supplementation of hydro-alcoholic extracts of leaves of *M. oleifera* (500 mg/kg), daily oral for 28 days shows protection against cadmium-induced hepatotoxicity in wistar albino rats. This attributed to the antioxidant and free radical scavenging property of *Moringa oleifera*, which could be helpful in reducing the oxidative stress caused by cadmium, by reducing the ROS production, maintaining the antioxidant potential, and significantly reducing elevated serum biomarker levels in the body.

El-Gannam *et al.* (2018) studied hepatoprotective effects of *Moringa oleifera* extracts on acetaminophen-induced oxidative damage in rats. In the hepatoprotective study, either leaves or pods extracts (300mg/kg bw or 600mg/kg bw stomach tube orally) were administrated to rats. The hepatoprotective activity of MO leaves and pods extracts were followed for 21 days by observed in the levels of liver markers such as ALT and the levels of oxidative damage markers including superoxide dismutase (SOD) and malondialdehyde (MDA) and catalase (CAT). The outcome of these parameters indicates reduction in the severity of liver damage in group treated with MO extracts + APAP and compared to those treated with APAP alone.

Islam et al. (2019) demonstrated liver protective activity of *Moringa oleifera* bark extract in paracetamol induced hepatotoxicity in rats. The ethanolic extract of MO was given orally at 250 and 500mg/kg body weight for one week with Silymarin (100mg/kg body weight) as standard hepatoprotective drug. The level of hepatic injury recovery was determined by the estimation of liver enzymes like SGPT, SGOT, ALP, Bilirubin, Total protein and Albumin. They concluded that treatment with MO extract as well as standard hepatoprotective agent silymarin ameliorated the increased plasma levels of these hepatic enzymes and indicated the hepatoprotective potential of the extract.

Abdel *et al.* (2020) studied hepatoprotective effect of *Moringa oleifera* leaves aquatic extract (200 mg/kg B.W orally for 4 weeks) 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 and levels of Total protein and albumin elevated in MOLE + LA–administered rats in comparison with LA-intoxicated rats.

Materials and Methods

CHAPTER-3

MATERIAL AND METHOD

3.1 Place of work

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. Dogs were screened for acute liver failure and further therapeutic potential of *Moringa oleifera* leaves on acute liver failure in dog was evaluated in the study.

3.2 Selection criteria for Animals

Clinical cases of dogs irrespective of breed, sex and age presented to TVCC showing signs suggestive of acute hepatic disorders (duration of illness less than ten days) such as 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 was chosen for the experimental study.

3.3 Screening of animal

Dogs having clinical findings suggestive of hepatic insufficiency with special reference to acute liver failure were screened for experimental trial based on altered hematology, serum biochemistry and diagnostic imaging (ultrasonography) abnormalities.

3.3.1 Clinical Examination

All the dogs were thoroughly examined and different clinical parameters were recorded in each dog:

- **Rectal temperature (˚F):** The rectal temperature of dogs was recorded using clinical thermometer.
- **Pulse rate (beats per minute):** Pulse rate was recorded by digital palpation of the femoral artery up to 1 minute.
- **Respiration rate (breaths per minute):** Respiration rate in each dog was recorded by observing the movement of the chest for up to 1 minute.

- **Mucous membrane examination:** Variation in color changes of conjunctival, penile, vulvar, oral mucous membranes was examined.

3.3.2 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. Blood samples were put in an ice box and brought to the laboratory for analysis.

Examination of blood

- **Haematology:** Complete blood count (CBC) was estimated by automatic haematology analyzer (Fig. 1) as per manufacturer's instruction.
- **Serum biochemistry:** Liver function tests including ALT, AST, ALP, GGT, total protein, albumin, globulin, A/G ratio,bilirubin (total, direct and indirect), glucose and cholesterol were estimated by biochemical analyzer as per manufacturer's instruction (Fig. 2)

3.3.2.1Estimation of haematological parameters-

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

3.3.2.2Estimation 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). Extreme care was taken to prevent hemolysis of the serum sample. The obtained serum was stored in a deep freeze at -20°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, which might lead to changes in biochemical values of the serum samples.

Various biochemical parameters were done with the help of semi autoanalyzer using biochemical kits ARKRAY Healthcare Pvt. Ltd. Serum globulin was estimated by subtracting the values of serum albumin from the estimated value of serum total proteins (Total proteins-Albumin).

- **Serum aspartate aminotransferase (AST)(U/L)**

The values were estimated by Modified UV (IFCC), Kinetic Assay method using kits Arkray Healthcare Pvt. Ltd.

- **Serum alanine aminotransferase (ALT)(U/L)**

The values were estimated by Modified UV (IFCC), Kinetic Assay method using kits Arkray Healthcare Pvt. Ltd.

- **Serum alkaline phosphatase (ALP)(U/L)**

The values were estimated by pNPP-AMP (IFCC), Kinetic Assay method using kits of Arkray Healthcare Pvt. Ltd.

- **Serum Gamma glutamyltransferases (GGT)(U/L)**

The values were estimated by Carboxy substrate method using kits of Arkray Healthcare Pvt. Ltd.

- **Total serum protein (g/dl)**

The values were estimated by Modified Biuret, End Point Assay method using kits of Arkray Healthcare Pvt. Ltd.

- **Serum albumin (g/dl)**

The values were estimated by Bromocresol Green (BCG), End Point assay method using kits of Arkray Healthcare Pvt. Ltd.

- **Serum globulin (g/dl)**

The values were estimated by subtracting serum albumin (g/dl) from total serum protein (g/dl) values.

- **A:G Ratio**

The values were estimated by computation of serum albumin (g/dl) divided by serum globulin (g/dl) values.

- **Serum Bilirubin (Total, Direct) (mg/dl)**

The values were estimated by Jendrassik and Groff, End Point assay method using kits of Arkray Healthcare Pvt. Ltd.

- **Indirect Bilirubin (mg/dl)**

The values were estimated by subtracting serum direct bilirubin (mg/dl) from serum total bilirubin (mg/dl) values.

- **Serum glucose (mg/dl)**

The values were estimated Glucose Oxidase - Peroxidase (GOD-POD), kinetic assay method using kits of Arkray Healthcare Pvt. Ltd.

- **Serum cholesterol (mg/dl)**

The values were estimated by CHOD-PAP, Enzymatic End Point Assay method using kits of Arkray Healthcare Pvt. Ltd.

3.3.3 Ultrasonographic imaging

- Ultrasonographic imaging of canine liver was to be 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) (Fig. 3).

3.3.3.1 Preparation and restraining of the animal

The animal was placed in supine position with one assistant securing the rear legs while another one assisted to maintain the required positioning by restraint of the front legs and head. The area between xiphisternum and the umbilicus, extending several centimeters on each side of the umbilicus was shaved. A liberal quantity of acoustic gel was applied for effective sound transmission.

3.3.3.2 Imaging technique

The liver was imaged using 3.5 MHz or 5.0 MHz transducer. The selection of frequency was based on the body size of the animal i.e. lower frequency transducer was selected for bigger body size. The transducer was placed directly under the sternum with firm but gentle pressure. In this position, the transducer was placed in the midline and angled craniodorsally to image a transverse section of the liver. To

visualize all parts of the liver, multiple sweeps through the organ were made by directing the beam dorsally and ventrally in the sagittal plane and to the right of left in the transverse plane. Intercostals views were taken to augment the visualization of peripheral parts of the liver.

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.(Fig. 4, 5, 6)

3.4 Occurrence study

The hospital based prevalence of acute hepatic failure was studied in dogs on the basis of breed, age and sex along with total prevalence.

3.5 To evaluate the therapeutic efficacy of *Moringa oleifera* leaves on acute liver failure (ALF) in dog.

3.5.1 Collection and processing of plant material

Fresh green leaves of plant *Moringa oleifera* was collected from medicinal herb garden, DUVASU, Mathura. The collected material was cleaned manually to remove coarse impurities; cut into pieces; wash with distilled water to remove the dirt, air dried under shade at a well-ventilated place and uniformly powdered using an electric mixer grinder. The powder formed was stored in air tight container (Fig. 7,8 and 9).

3.5.2 Hydoalcoholic extract Prepration

The powdered plant material was placed inside a clean container. The extraction solvent (Ethanol: distilled water 1:1) is then poured on top of the drug material at room temperature (37˚C), soaked, and kept for 72 hrs.and then the extract was filtered using filter paper (Whatman no. 40). The solvent was removed by using rotary evaporator. The extract was dried *in vacuo* and stored refrigerated (Fig. 10, 11 and 12).

3.5.3 Capsules Formation

Gelatin capsules of *Moringa oleifera* leaves extract were made in Department of Veterinary Pharmacology and Toxicology, DUVASU, Mathura. After extraction of moringa leaf extract, it 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 600mg (according to body weight) (Fig 13 and 14).

3.5.4 Therapeutic regimen

Dogs found positive for acute liver failure during screening were taken for therapeutic trial to access the therapeutic efficacy of *Moringa oleifera* leaves as per the below mentioned groups. Six dogs were considered in apparently healthy control group (group I) after thorough physical examination and various diagnostic tests. Minimum twelve dogs were randomly divided into two groups (group II and group III) six animal each. Group II animals were treated with conventional therapy for 14 days. Hydro-alcoholic extract of *Moringa oleifera* leaves was given orally at dose of 30mg/kg for 14 days along with conventional therapy twice daily in group III animals. Therapeutic efficacy was evaluated at weekly interval for 14 days by hemato-biochemical analysis and abolishment in clinical signs.

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 *Moringa oleifera* leaves at 30mg/kg p.o O.D along with conventional treatment

***Conventional therapy:** 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) @ 2mg/kg PO q12hrs if required. Injmetaclopramide @ 0.2-0.5 mg/kg every 8 to 12 hour interval if required. Amino acid supplementation orally @0.5ml/kg daily for 14 days if required. Inj B-complex @1-2ml I/M on alternate days for 14 days if required; according to clinical signs.

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^{th} and 14^{th}.

3.6 Statistical analysis

The data will be expressed as mean ± SEM. Standard error of mean and p-values will be used to determine whether there is any significant difference among different treatment groups using one-way analysis of variance (ANOVA) following standard protocol (Snedecor and Cochran, 1994).

Fig. 1: Automated hematology analyzer

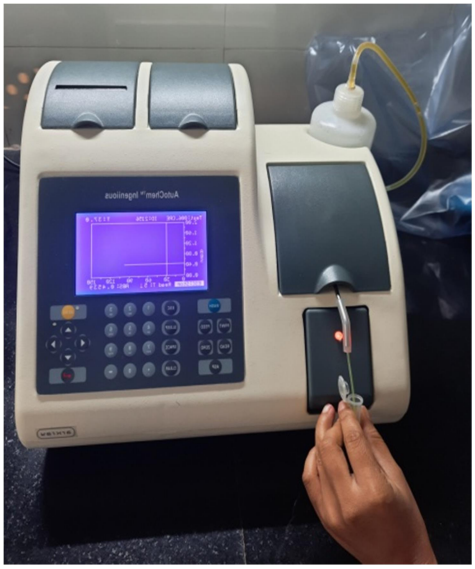


Fig. 2: Biochemical Analyzer

Fig. 3: Ultrasound machine

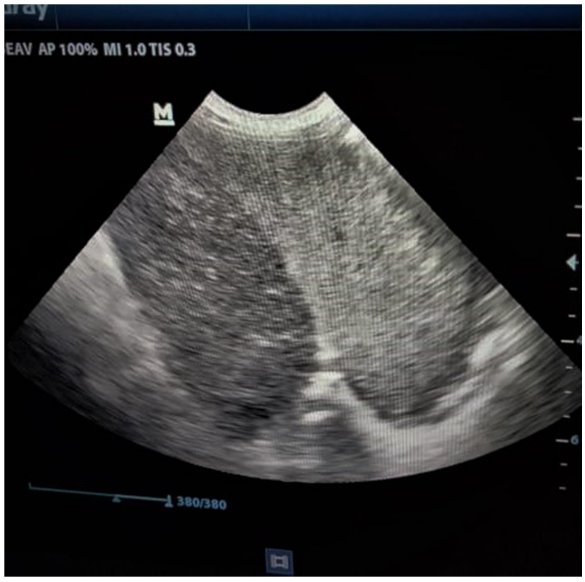


Fig. 4: Mixed echogenicity of hepatic parenchyma and rounded liver margins

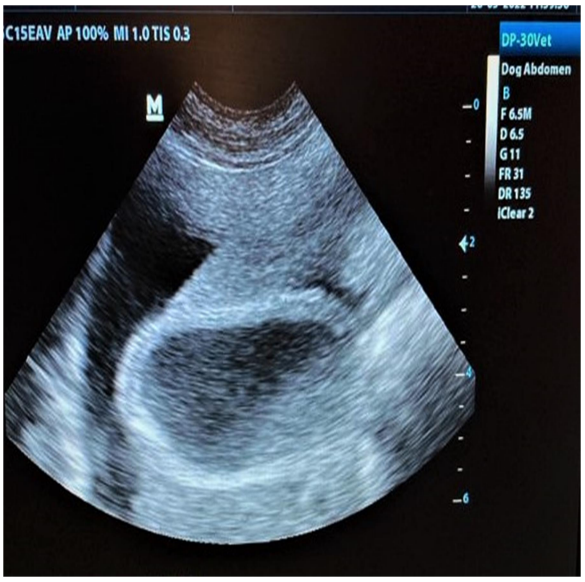


Fig. 5: Increased parenchymal echogenicity with hyperechoic double rim appearance of GB wall

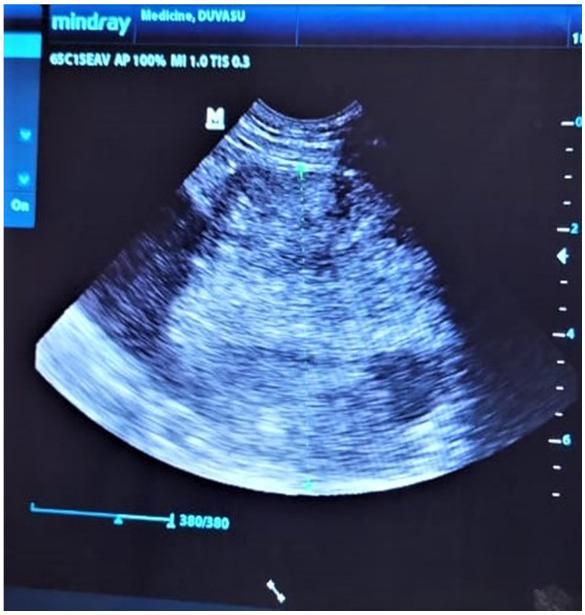


Fig. 6: Hepatomegaly with increased parenchymal echogenicity where the discrete hepatic borders are not visualized

Fig. 7: Fresh leaves of *Moringa oleifera* on day 1

Fig 8: Dried leaves of *Moringa oleifera* after two months

Fig. 9: Moringa oleifera leaves powder

Fig. 10: Moringa leaves extract on day 0 and after 72 hrs.

Fig. 11: Filtration of Moringa oleifera leaves extract

Fig. 12: Moringa oleifera leaves Extract after evaporation of solvent

Fig. 13: Steps showing homogeneous mixture formation with the help of Mortar & pestle

Fig. 14: Filling of homogenous moringa powder in gelatin capsules

Results

CHAPTER-4

RESULTS

The present study was conducted to investigate the occurrence of acute liver failure in dog and to evaluate the therapeutic potential of *Moringa oleifera* leaves on acute liver failure in dog presented to Teaching Veterinary Clinical Complex, DUVASU, Mathura. The data obtained were statistically analysed and presented.

4.1 Occurrence of acute liver failure in dogs

4.1.1 Hospital based prevalence

Total 205 dogs showed clinical signs concern to acute hepatic disorders (duration of illness less than ten days), out of which 31 dogs were found positive for acute liver failure on the basis of altered hematology, altered serum biochemistry and diagnostic imaging abnormalities viz. alternations in hepatic parenchymal echogenicity; size and shape of liver.

The overall prevalence of acute liver failure during the period from February (2023) to July (2023) in total dog population was 1.107 % (31/3881) whereas prevalence among suspected dogs was 15.12 % (31/205) (Table 2).

Table 2: Occurrence of acute liver failure in dogs

Total No. of Dogs	No. of dogs examined	No of dogs positive for acute liver failure	Suspected Prevalence (%)	Overall Prevalence(%)
3881	205	31	15.12	1.107

4.1.2 Prevalence of acute liver failure in dogs in relation to breed

The breed wise hospital based prevalence of acute liver failure was highest in Labrador Retrievers 29.03 % (09/31), followed by German Shepherd 17.95 % (07/39), Golden Retriever 13.51 % (04/37), Mongrel or mix breed 11.76 % (04/34), Pug 9.52 % (02/21), German Spitz 8.33 % (02/24), Pomeranian 5.55 % (01/18) and Shih Tzu 5.26% (02/19). The results are shown in Table 3 and Graph 1.

Table 3: Prevalence of acute liver failure in dogs in relation to breed

S. No.	Breed	No. of dogs suspected	No. of dogs affected	Prevalence %
1	Labrador Retriever	31	9	29.03
2	German Shepherd	39	7	17.95
3	Golden Retriever	37	4	13.51
4	Mongrel	34	4	11.76
5	Pug	21	2	9.52
6	German Spitz	24	2	8.33
7	Pomeranian	18	1	5.55
8	Shih Tzu	19	2	5.26
	Total	**205**	**31**	**15.12**

4.1.3 Prevalence of acute liver failure in dogs in relation to age

The age wise hospital based prevalence of acute liver failure is depicted in Table 4 and Graph 2. The age wise hospital based prevalence among different aged group dogs *viz* less than 1 years, 1-4 years, 4-8 years and above 8 years was 12 % (03/25), 13.46 % (07/52), 19.67 % (12/61), and 14.06 % (09/64), respectively. In this study, the highest occurrence of acute liver failure was in the age group 4-8 years and lowest occurrence in less than 1 year.

Table 4: Prevalence of acute liver failure in dogs in relation to age

Age	No. of dogs suspected	No. of dogs affected	Prevalence %
< 1 year	25	3	12
1 to 4 year	52	7	13.46
4 to 8 year	61	12	19.67
8 years and Above	64	9	14.06
Total	**205**	**31**	**15.12**

4.1.4 Prevalence of acute liver failure in dogs in relation to sex

The sex wise hospital based prevalence of acute liver failure is summarized in Table 5 and Graph 3. In the present study, hospital based prevalence of acute liver failure was more in female dogs (36.66%) than in male dogs (11.42%).

Table 5: Prevalence of acute liver failure in dogs in relation to sex

Sex	No. of dogs screened	No. of dogs suspected	No. of dogs affected	Prevalence (%)	Overall Prevalence (%)
Male	2618	175	20	11.42	0.764
Female	1263	30	11	36.66	0.87
Total	3881	205	31	15.12	0.799

4.2 Clinical findings and their frequency distribution in dogs with acute liver failure

Observed clinical symptoms in dogs with acute liver failure (Fig. 15 to 20) and their frequency distribution are presented in Table 6 and Graph 4. Important clinical symptoms exhibited by dogs with acute liver failure in order of decreasing frequency were vomiting (96.77%), anorexia (90.32%), icterus (87.09%), weakness (77.41%), abdominal pain (67.74%), wt. loss (38.70%), fever (22.58%), neurologic signs (16.12%), hematemesis (12.9%), inappetance (9.67%), pale mucus membrane (6.45%) and polyuria / polydypsia (3.22%).

Table 6: Clinical findings and their frequency distribution in dogs with acute liver failure

S. No.	Parameters	No. of positive cases (n=31)	Frequency (%)
1	Vomiting	30	96.77
2	Anorexia	28	90.32
3	Icterus	27	87.09
4	Weakness	24	77.41
5	Abdominal Pain	21	67.74
6	Wt. loss	12	38.70
7	Fever	7	22.58
8	Neurologic signs	5	16.12
9	Hematemesis	4	12.9
10	Inappetance	3	9.67
11	Pale mucus membrane	2	6.45
12	Polydypsia/ Polyuria	1	3.22

Objective II: Therapeutic efficacy of *Moringa oleifera* leaves on acute liver failure in dog

Therapeutic evaluation was done on the basis of percent recovery assessment and hemato-biochemical alterations. Percent recovery was assessed by clinical improvement in terms of disappearance of clinical signs and alterations in the hemato-biochemical parameters on day 7^{th} and day 14^{th} post treatment.

4.5.1 Alterations in the Physiological parameters

4.5.1.1 Rectal temperature (^{0}F)

The Mean±SE values of rectal temperature (^{0}F) 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 rectal temperature in both the treatment groups at day 7^{th} and 14^{th}. (Table 7 and graph 5) However, all the mean values in different groups and at different intervals remained within the physiological range.

Table 7: Alterations inrectal temperature (^{0}F) of dogs in different treatment groups at different intervals

Parameter	Group	Day 0	Day 7	Day 14
Rectal temperature (^{0}F)	Group I	101.45^{aA}±0.13	101.27^{aA}±0.12	101.32^{aA}±0.14
	Group II	102.58^{bB}±0.12	102.0^{aB}±0.02	101.92^{aB}±0.09
	Group III	102.70^{bB}±0.12	102.12^{aB}±0.06	101.87^{aB}±0.05

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 $P<0.05$.

4.5.1.2. Pulse rate (beats/min)

The Mean±SE value of pulse rate (beats/min) in both the treatment groups was significantly higher than that of healthy control group (group A) on the day of presentation (day 0). There was significant decrease in the mean value of pulse rate (beats/min) on day 7 followed by day 14 post-treatment in both the treatment groups.

Although, the pulse rate of all groups was within the physiological range (60-140 beats/min) (Table 8 and graph 6)

Table 8: Alterations in pulse rate (beats/min) of dogs in different treatment groups at different intervals

Parameter	Group	Day 0	Day 7	Day 14
Pulse rate (beats/min)	Group I	81.17^{aA}±2.46	81.17^{aA}±2.93	83.50^{aA}±3.08
	Group II	112.83^{cB}±1.4	104.17^{bB}±1.38	98.50^{aB}±1.18
	Group III	115.00^{cB}±1.03	104.33^{bB}±1.28	96.50^{aB}±0.85

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.

4.5.1.3 Respiration rate (breaths/min)

Statistical analysis revealed the respiration rate (breaths/min) in dogs having acute liver failure (Group II and Group III) were significantly higher than that of control group on the day of presentation (day 0). There was a significant decrease in the mean respiration rate (breaths/min) on day 7 followed by on day 14 post-treatment in all treatment groups. All the mean values of respiration rate in all groups and on all days were within the physiological range (Table 9 and Graph 7).

Table 9: Alterations in respiration rate (breaths/min) of dogs in different treatment groups at different intervals

Parameter	Groups	Day 0	Day 7	Day 14
Respiration rate (breaths/min)	Group I	22.17^{aA}±1.17	22.17^{aA}±1.19	21.00^{aA}±0.77
	Group II	38.50^{cB}±1.40	34.50^{bB}±1.23	30.66^{aB}±0.50
	Group III	37.83^{cB}±1.58	33.00^{bB}±0.97	28.83^{aB}±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.

4.5.2 Alterations in the haematological parameters

4.5.2.1 Haemoglobin (gm/dl), total erythrocyte counts (TEC)($\times 10^6$/µl) and packed cell volume (PCV) (%)

The Mean ± SE values of haemoglobin (gm/dl), total erythrocyte count ($\times 10^6$/µl) and packed cell volume (%) in dogs at various interval of study is depicted in Table 10. The mean concentration of haemoglobin (Graph 8), TEC (Graph 9) and PCV (Graph 10) decreased significantly in both the treatment groups (Group II and Group III) with respect to healthy dogs at day 0 (Group I) of study. However, there was a significant increase ($p< 0.05$) in the Hb ,TEC and PCV concentration at day 7^{th} and day 14^{th} after the treatment in both treatment groups with highest recovery in group III followed by group II. Therefore, in terms of improvement in haemoglobin, TEC and PCV concentration in treated groups of dog better recovery were assessed in group III followed group II.

Table 10: Alterations in haemoglobin (gm/dl, total erythrocyte count ($\times 10^6$/µl) and packed cell volume (%) of dogs in different treatment groups at different intervals

Parameters	Groups	Day 0	Day 7	Day 14
Haemoglobin (g/dl)	Group I	$13.11^{aB}\pm0.31$	$13.03^{aC}\pm0.27$	$13.28^{aC}\pm0.35$
	Group II	$9.78^{aA}\pm0.19$	$10.14^{abA}\pm0.17$	$10.65^{bA}\pm0.27$
	Group III	$9.53^{aA}\pm0.10$	$11.04^{bB}\pm0.17$	$12.45^{cB}\pm0.13$
Total erythrocyte count (TEC) ($\times 10^6$/µl)	Group I	$6.88^{aB}\pm0.14$	$6.96^{aB}\pm0.15$	$6.74^{aB}\pm0.09$
	Group II	$4.98^{aA}\pm0.08$	$5.36^{aA}\pm0.26$	$5.81^{bA}\pm0.06$
	Group III	$4.77^{aA}\pm0.08$	$5.50^{bA}\pm0.16$	$6.47^{cB}\pm0.17$
Packed cell volume (PCV) (%)	Group I	$40.50^{aB}\pm0.45$	$40.23^{aC}\pm0.39$	$39.55^{aC}\pm0.48$
	Group II	$29.31^{aA}\pm0.57$	$30.30^{abA}\pm0.42$	$31.53^{bA}\pm0.61$
	Group III	$28.60^{aA}\pm0.29$	$33.18^{bB}\pm0.55$	$37.40^{cB}\pm0.42$

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 $P<0.05$.

4.5.2.2 Total leukocyte count (×10^3/µL)

The Mean ± SE value of total leukocyte count (×10^3/µL) in dogs at various interval of study is depicted in Table 11 and Graph 11. The mean value of total leukocyte count (TLC) increased significantly at day 0 in diseased dogs (Group II and Group III) as compared to that of healthy dogs (Group I). The Mean ± SE value of TLC decreased significantly in both the treatment groups; however in Group IIIit becomes statistically similar to healthy dog. Therefore, in terms of improvement in total leukocyte count in treated groups of dog better recovery was assessed in group III followed by group II.

4.5.2.3 Differential leucocyte count

4.5.2.3.1 Lymphocytes (%)

The Mean±SE (Table 11 & Graph 12) values of lymphocytes on day 0 were found to be significantly low ($p< 0.05$) in the both the treatment groups (Group II and Group III) in comparison to healthy control (Group I). However, there was a significant increase ($p< 0.05$) in the lymphocytes count at day 7^{th} and day 14^{th} after the treatment in both the treatment groups (Group II and Group III) with higher recovery in group III followed by group II. Therefore, in terms of improvement in lymphocyte count in treated groups of dog better recovery were assessed in group III followed by group II.

4.5.2.3.2 Neutrophils

The Mean±SE (Table 11, graph 13) values of neutrophils on day 0 were found to be significantly increase ($p<0.05$) 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 neutrophil count at day 7^{th} and 14^{th} after the treatment in both the treatment groups (Group II and Group III) with higher recovery in group III followed by group II. Therefore, in terms of improvement in neutrophil count in treated groups of dog better recovery were assessed in group III followed by group II.

4.5.2.3.3 Monocytes (%), Eosinophils (%) and Basophils (%)

The other components of differential leukocyte count viz. monocytes (%) and eosinophils (%) and basophils (%) varied non-significantly and data were statistically

similar among different groups throughout the study period (Table 11, graph 14,15 and 16).

Table 11: Alterations in total leukocyte count (×10^3/µL) and differential leukocyte count (DLC) (%) of dogs in different treatment groups at different intervals

Parameters	Groups	Day 0	Day 7	Day 14
Total leukocyte count TLC (×10^3/µl)	Group I	13.80 ^{aA}±0.50	13.79 ^{aA}±0.43	13.15 ^{aA}±0.41
	Group II	20.60^{bB}±0.84	17.84^{aB}±0.78	16.30^{aB}±0.57
	Group III	20.05^{cB}±0.79	17.14^{bB}±0.77	13.45 ^{aA}±0.20
Neutrophils (%)	Group I	66.75 ^{aA}±0.57	66.98 ^{aA}±0.53	67.22 ^{aA}±0.61
	Group II	77.12 ^{bB}±0.44	76.03 ^{bB}±0.46	73.58^{aB}±0.84
	Group III	78.49^{cB}±0.39	72.71^{bC}±0.69	68.39 ^{aA}±0.49
Lymphocytes (%)	Group I	27.87^{aB}±0.50	27.38^{aC}±0.28	27.58^{aC}±0.59
	Group II	17.27^{aA}±0.43	18.26^{aA}±0.45	20.82^{bA}±0.77
	Group III	17.01^{aA}±0.20	21.18^{bB}±0.59	25.51^{cB}±0.56
Monocytes (%)	Group I	4.40±0.13	4.41±0.15	4.59±0.07
	Group II	4.51±0.08	4.63±0.09	4.70±0.05
	Group III	4.78±0.36	4.48±0.08	4.06±0.10
Basophils (%)	Group I	0.20±0.06	0.20±0.08	0.27±0.11
	Group II	0.32±0.07	0.38±0.12	0.32±0.07
	Group III	0.22±0.03	0.60±0.09	0.28±0.09
Eosinophils (%)	Group I	0.61±0.11	0.54±0.12	0.38±0.16
	Group II	0.77±0.10	0.52±0.10	0.59±0.14
	Group III	0.57±0.21	0.55±0.11	0.68±0.12

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.

3.5.3 Alterations in the Biochemical Parameters

3.5.3.1. Alanine aminotransferase (ALT) (U/L)

The mean alanine aminotransferase concentration (U/L) in all groups is presented in Table 12 & graph 17. Significantly higher values were observed in both the treatment groups (Group II and Group III) on day 0 (pre-treatment), notably above the upper limit of the physiological range as compared to healthy control (Group I). However, there was a significant decrease ($p< 0.05$) in the ALT level at day 7th and day 14th after the treatment in both the treatment groups (Group II and Group III).Early improvement was observed in Group III from day 7 (post-treatment), with continued reduction till day 14. Therefore, in terms of improvement in ALT level in treated groups of dog better recovery were assessed in group III followed by group II.

Table 12: Alterations in Alanine aminotransferase (ALT) (U/L) 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^{aA}±3.99	28.98^{aA}±2.64	30.96^{aA}±3.31
	Group II	256.29^{cB}±20.91	176.20^{bB}±26.06	101.80^{aB}±10.51
	Group III	264.39^{bB}±49.30	117.07^{aB}±18.38	62.54^{aAB}±6.65

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 $P<0.05$.

4.5.3.2 Aspartate transaminase (AST) (U/L)

The AST concentration (U/L) in dogs of all groups was recorded on day 0 (pre-treatment) and on days 7 and 14 (post-treatment). Significantly higher values were observed in both the treatment groups (Group II and Group III) on day 0 (pre-treatment) as compared to healthy control (Group I). However, there was a significant decrease ($p< 0.05$) in the AST level at day 7th and day 14th after the treatment in both the treatment groups (Group II and Group III) and in Group III it becomes relatively similar at 14th day of treatment to healthy dog. Therefore, in terms of improvement in AST level in treated groups of dog better recovery were assessed in group III followed by group II (Table 13 & graph 18).

Table 13. Alterations in Aspartate aminotranferase (AST) (U/L) of dogs in different treatment groups at different intervals.

Parameters	Groups	Day 0	Day 7	Day 14
Aspartate aminotransferase (U/L)	Group I	27.27^{aA}±4.56	21.04^{aA}±3.86	20.50^{aA}±3.82
	Group II	137.77^{cB}±15.37	84.60^{bB}±8.35	51.55^{aB}±9.55
	Group III	126.71^{cB}±10.94	74.76^{bB}±7.10	41.81^{aAB}±5.46

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.

4.5.3.3 Alkaline phosphatase (ALP) (U/L)

Both the treatment groups (Group II and Group III) revealed significantly higher values on day 0 (pre-treatment), markedly higher than the upper limit of the normal range in dogs (Table 14 & Graph 19). Both the treatment groups revealed a declining trend towards restoration of near normalcy from day 7 till day 14. However, non-significant difference was noted between the both treatment groups.

Table 14: Alterations in Alkaline phosphatase (ALP) (U/L) of dogs in different treatment groups at different intervals

Parameters	Groups	Day 0	Day 7	Day 14
Alkaline phosphatase (U/L)	Group I	64.50^{aA}±11.82	62.52^{aA}±11.82	62.61^{aA}±10.76
	Group II	727.83^{bB}±357.66	405.32^{aA}±143.72	170.08^{aA}±33.29
	Group III	630.22^{bB}±125.51	300.80^{abA}±48.27	104.28^{aA}±5.16

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.

4.5.3.4 Gamma-glutamyltransferase (GGT) (U/L)

Both the treatment groups (Group II and Group III) revealed significantly higher values on day 0 (pre-treatment), markedly higher than the upper limit of the normal range in dogs as compared to healthy control (Group I) (Table 15 & Graph

20). Both the treatment groups revealed a declining trend towards restoration of near normalcy from day 7 till 14. However, significant difference was noted between both the treatment groups and better recovery noticed in Group III. Therefore, in terms of improvement in GGT level in treated groups of dog best recovery were assessed in group III followed by group II.

Table 15: Alterations in Gamma-glutamyltransferase (U/L) of dogs in different treatment groups at different intervals

Parameters	Groups	Day 0	Day 7	Day 14
Gamma-glutamyltransferase (U/L)	Group I	6.78^{aA}±1.41	5.83^{aA}±1.71	6.18^{aA}±0.93
	Group II	59.40^{bB}±7.17	50.44^{bBb}±5.61	32.77^{aB}±2.92
	Group III	55.90^{cB}±7.65	33.58^{bC}±4.56	17.69^{aA}±3.17

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.

4.5.3.5 Total protein (g/dl)

Both the treatment groups (Group II and Group III) had slightly lower values on day 0, compared to the healthy control group (Group I), but within the physiological range. On different days of post treatment the values were found to be significantly improving in the treatment group III, however no such variations were noticed in group II. Therefore, in terms of improvement in total protein in treated groups of dog better recovery were assessed in group III followed by group II.

Table 16: Alterations in Total protein (gm/dl) of dogs in different treatment groups at different intervals.

Parameters	Groups	Day 0	Day 7	Day 14
Total protein (gm/dl)	Group I	5.93^{abA}±0.12	6.05^{bB}±0.06	5.69^{aA}±0.11
	Group II	5.44^{aA}±0.12	5.64^{aA}±0.08	5.82^{aA}±0.07
	Group III	5.70^{aA}±0.13	6.07^{bB}±0.03	6.36^{cB}±0.10

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.

4.5.3.6 Albumin

The Mean±SE (Table 17 & Graph 22) values of albumin on day 0 were found to be significantly low ($p<0.05$) in both the treatment groups (Group II and Group III) in comparison to healthy control (Group I). However, there was a significant increase ($p< 0.05$) in the albumin at day 7^{th} and day 14^{th} after the treatment in both the treatment groups (Group II and Group III). However, non-significant difference was noted between the both treatment groups.

Table 17: Alterations in Albumin (gm/dl) of dogs in different treatment groups at different intervals.

Parameters	Groups	Day 0	Day 7	Day 14
Albumin (gm/dl)	Group I	3.45^{aC}±0.10	3.46^{aB}±0.13	3.39^{aA}±0.10
	Group II	2.56^{aA}±0.19	3.07^{bA}±0.03	3.28^{bA}±0.07
	Group III	2.89^{aB}±0.03	3.08^{aA}±0.04	3.55^{bA}±0.17

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 $P<0.05$.

4.5.3.7 Globulin and A: G Ratio

The mean value of globulin (Graph 23) and albumin/globulin ratio (Graph 24) varied non-significantly and data were statistically similar among different groups throughout the study period.

Table 18: Alterations in Globulin and A:G ratio of dogs in different treatment groups at different intervals.

Parameters	Groups	Day 0	Day 7	Day 14
Globulin (gm/dl)	Group I	2.47±0.13	2.49±0.17	2.30±0.03
	Group II	2.88±0.22	2.57±0.07	2.54±0.05
	Group III	2.81±0.13	2.78±0.06	2.80±0.24
A:G Ratio	Group I	1.42±0.09	1.38±0.13	1.47±0.05
	Group II	0.98±0.12	1.19±0.03	1.29±0.04
	Group III	1.04±0.06	1.04±0.03	1.33±0.16

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 $P<0.05$.

4.5.3.8 Serum total bilirubin (mg/dl)

Statistically significant difference among both treatment groups (Group II and Group III) was observed on day 0 (pre-treatment) and the values remained higher than the upper limit of the physiological range. Decrease in the value was observed in both the treatment groups (Group II and Group III) on day 7 and the trend persisted till day 14 (post-treatment). Therefore, in terms of improvement in serum total bilirubin in treated groups of dog better recovery were noticed in group III followed by group II. The results are summarized in Table 19 and depicted in graph 25.

Table 19: Alterations in Serum total bilirubin (mg/dl) of dogs in different treatment groups at different intervals.

Parameters	Groups	Day 0	Day 7	Day 14
Total Bilirubin (mg/dl)	Group I	$0.49^{aA}\pm0.09$	$0.46^{aA}\pm0.07$	$0.58^{aA}\pm0.11$
	Group II	$4.71^{aAB}\pm2.16$	$3.28^{aA}\pm1.40$	$1.84^{aA}\pm0.54$
	Group III	$8.70^{bB}\pm3.05$	$3.99^{aA}\pm1.24$	$0.92^{aA}\pm0.25$

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 $P<0.05$.

4.5.3.9 Serum direct bilirubin (mg/dl)

Both the treatment groups (Group II and Group III) exhibited higher than the physiological range on day 0 (pre-treatment) as compared to healthy control (group I). The values declined significantly in both treatment groups on day 7 which persisted till day 14. (Table 20 & graph 26). However, non-significant difference was noted between the both treatment groups. But, in terms of improvement in direct bilirubin in treated groups of dog best recovery were assessed in group III followed by group II.

Table 20: Alterations in Serum direct bilirubin (mg/dl) of dogs in different treatment groups at different intervals

Parameters	Groups	Day 0	Day 7	Day 14
Direct Bilirubin (mg/dl)	Group I	$0.18^{aA}\pm0.02$	$0.15^{aA}\pm0.02$	$0.20^{aA}\pm0.05$
	Group II	$3.93^{aB}\pm1.96$	$1.90^{aA}\pm0.78$	$0.97^{aA}\pm0.32$
	Group III	$6.38^{bB}\pm1.99$	$2.64^{aA}\pm0.99$	$0.24^{aA}\pm0.14$

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 $P<0.05$.

4.5.3.10 Serum indirect bilirubin (mg/dl)

Group III exhibited higher values of the mean indirect bilirubin concentration on day 0 (pre-treatment) than the normal physiological range. Group II demonstrated non-significant decline in the values from day 7 till 14 after treatment (Table 21 & graph 27) whereas Group III reveals significant early decline after treatment. Therefore, in terms of improvement in indirect bilirubin in treated groups of dog best recovery were assessed in group III followed by group II.

Table 21: Alterations in serum indirect bilirubin (mg/dl) of dogs in different treatment groups at different intervals.

Parameters	Groups	Day 0	Day 7	Day 14
Indirect Bilirubin (mg/dl)	Group I	$0.31^{aA}\pm0.09$	$0.31^{aA}\pm0.09$	$0.38^{aA}\pm0.09$
	Group II	$0.79^{aA}\pm0.29$	$1.38^{aA}\pm0.62$	$0.86^{aA}\pm0.28$
	Group III	$2.31^{bB}\pm1.14$	$1.35^{aA}\pm0.42$	$0.67^{aA}\pm0.16$

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 $P<0.05$.

4.5.3.12 Serum Cholesterol(mg/dl)

The Mean±SE (Table 22 & Graph 28) values of cholesterol 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 cholesterol concentration at day 7th after the treatment in both the treatment groups (Group II and Group III). But, in terms of improvement in cholesterol concentration in treated groups of dog better recovery were assessed in Group III followed by group II.

Table 22: Alterations in serum cholesterol (mg/dl) of dogs in different treatment groups at different intervals

Parameters	Groups	Day 0	Day 7	Day 14
Cholesterol (mg/dl)	Group I	187.61^{aA}±2.37	187.42^{aA}±2.61	187.67^{aA}±2.63
	Group II	287.4^{cB}±6.12	254.37^{bB}±9.17	211.5^{aB}±7.17
	Group III	280.87^{cB}±6.6	228.32^{bC}±7.3	197.75^{aAB}±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 $P<0.05$.

4.5.3.13 Serum glucose (mg/dL)

On perusal of Table 23 & graph 29 values of serum glucose on day 0 were found to be significantly low ($p< 0.05$) in both the treatment groups of dogs (Group II and Group III) in comparison to healthy control(Group I). However, there was a significant increase ($p< 0.05$) in the serum glucose concentration at day 7th after the treatment which persisted till day 14 in both the treatment groups. But, in terms of improvement in serum glucose concentration in treated groups of dogs, better recovery was assessed in Group III.

Table 23: Alterations in Serum glucose (mg/dL) of dogs in different treatment groups at different intervals.

Parameters	Groups	Day 0	Day 7	Day 14
Serum glucose (mg/dl)	Group I	$93.80^{aB}\pm2.26$	$94.72^{aA}\pm1.88$	$95.84^{aB}\pm1.12$
	Group II	$79.94^{aA}\pm1.98$	$88.64^{bA}\pm1.17$	$87.55^{bA}\pm3.32$
	Group III	$80.26^{aA}\pm1.01$	$88.08^{bA}\pm3.10$	$91.43^{bAB}\pm1.86$

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 $P<0.05$.

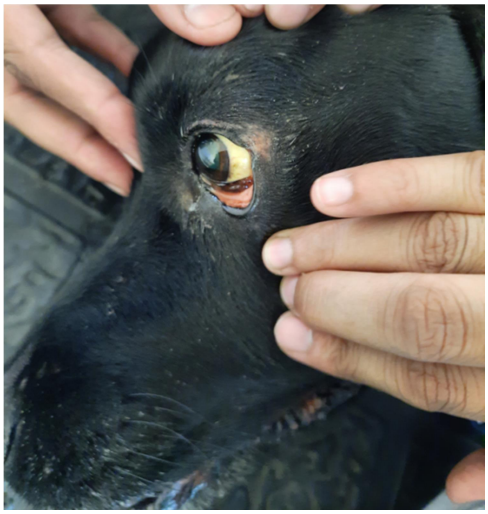


Fig. 15: Icteric conjuctival membrane of a dog

Fig. 16: Pale penile mucus membrane of a dog

Fig. 17: Weakness in GSD dog

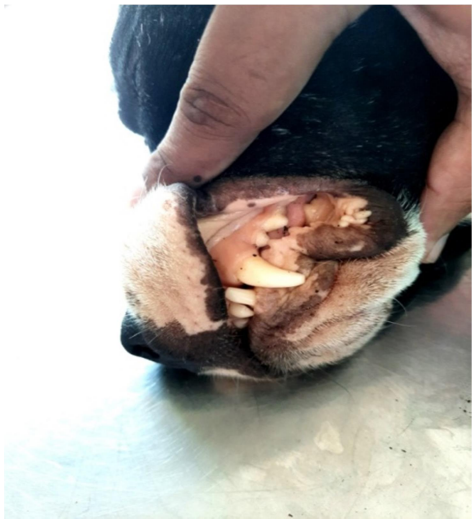
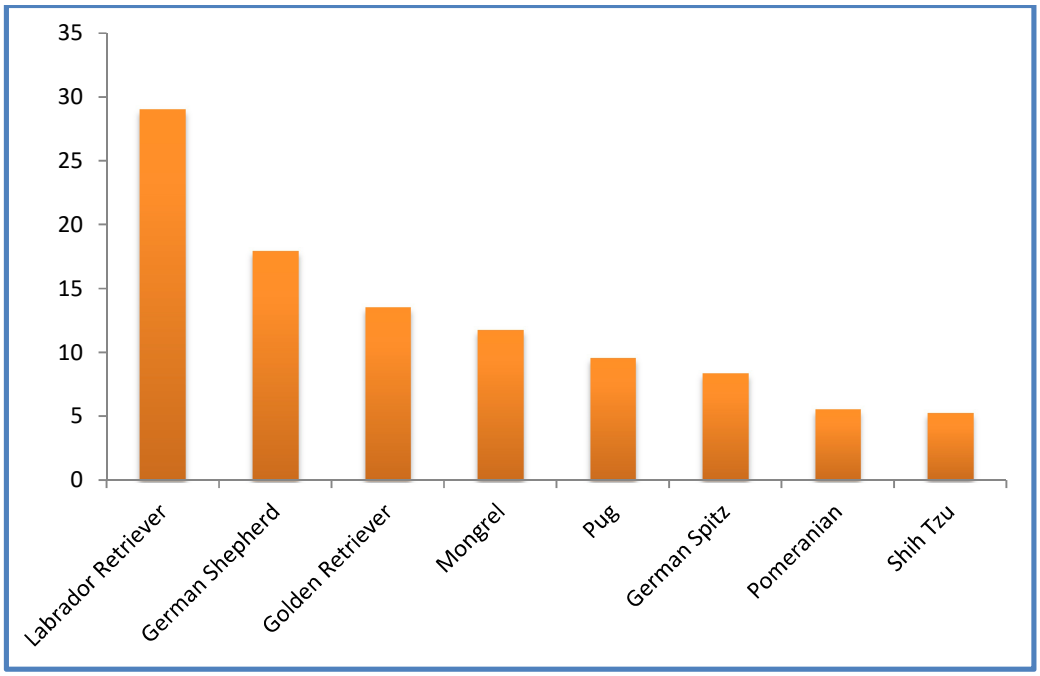


Fig. 18: Pale mucus membrane of gum in acute liver failure dog

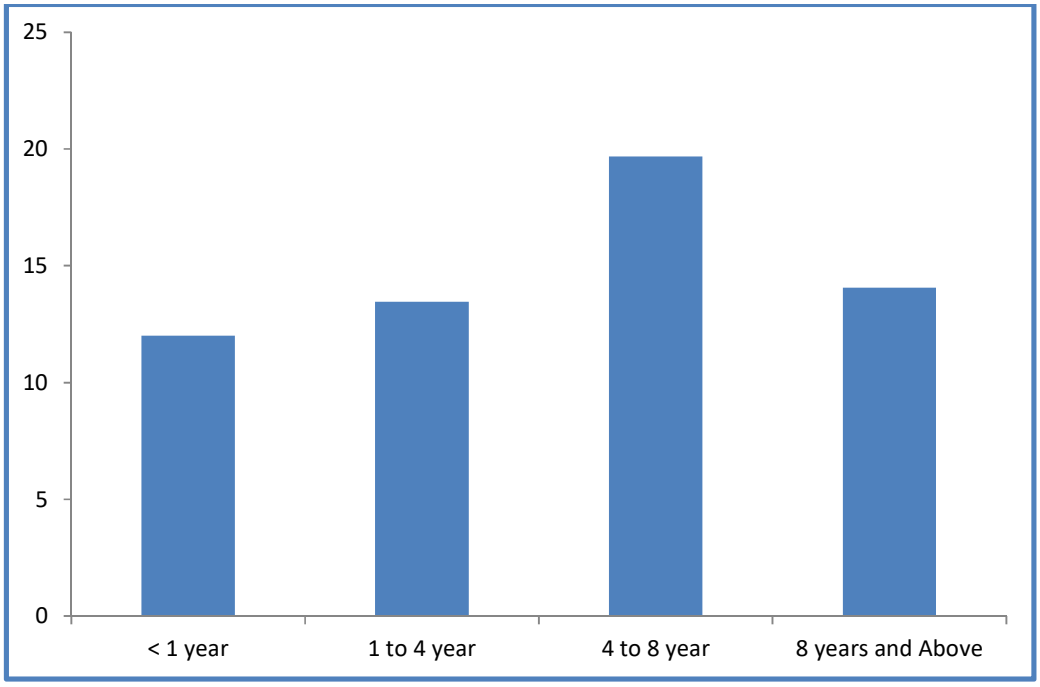
Fig. 19: Vomitus of dog suffering from acute liver failure

Fig. 20: Icteric skin in acute liver failure in Labrador

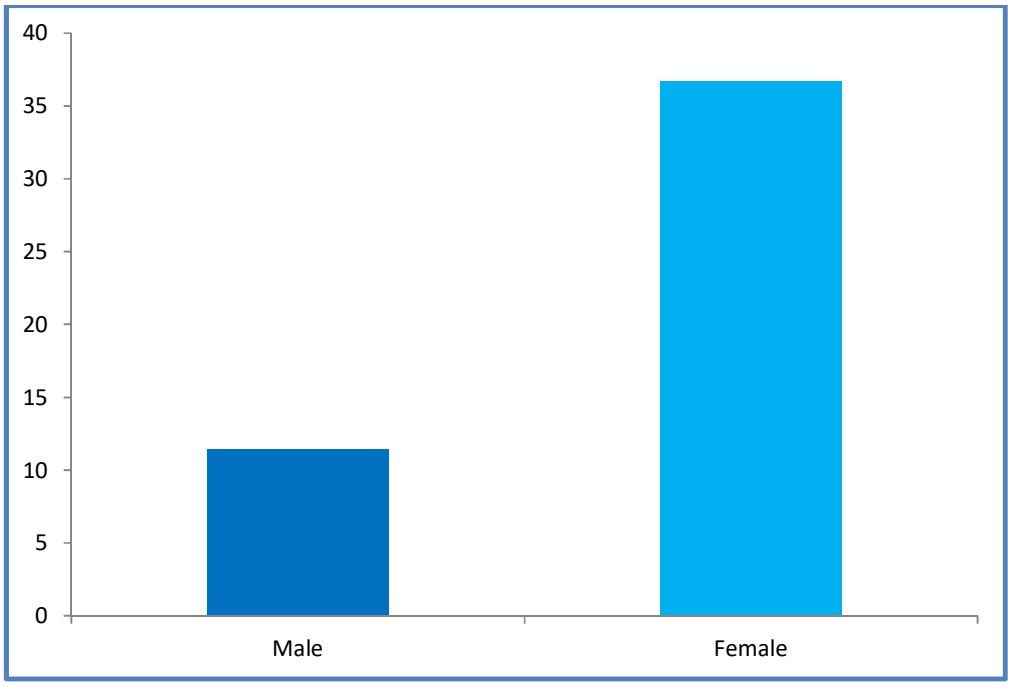
Graph 1: Hospital based prevalence of acute liver failure in dogs in relation to breed



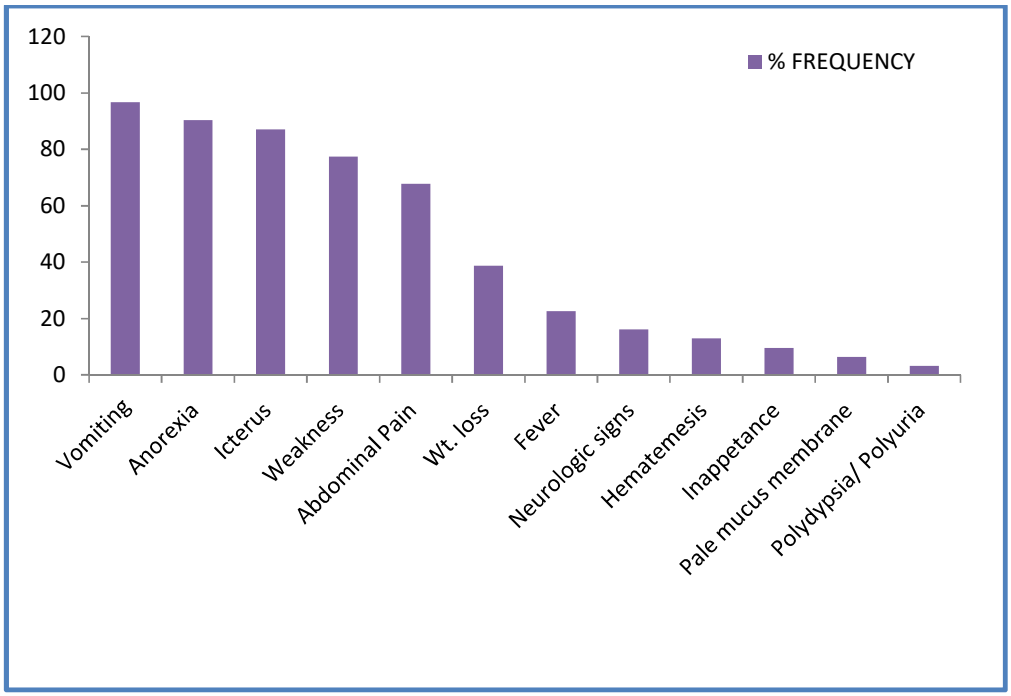
Graph 2: Hospital based prevalence of acute liver failure in dogs in relation to age



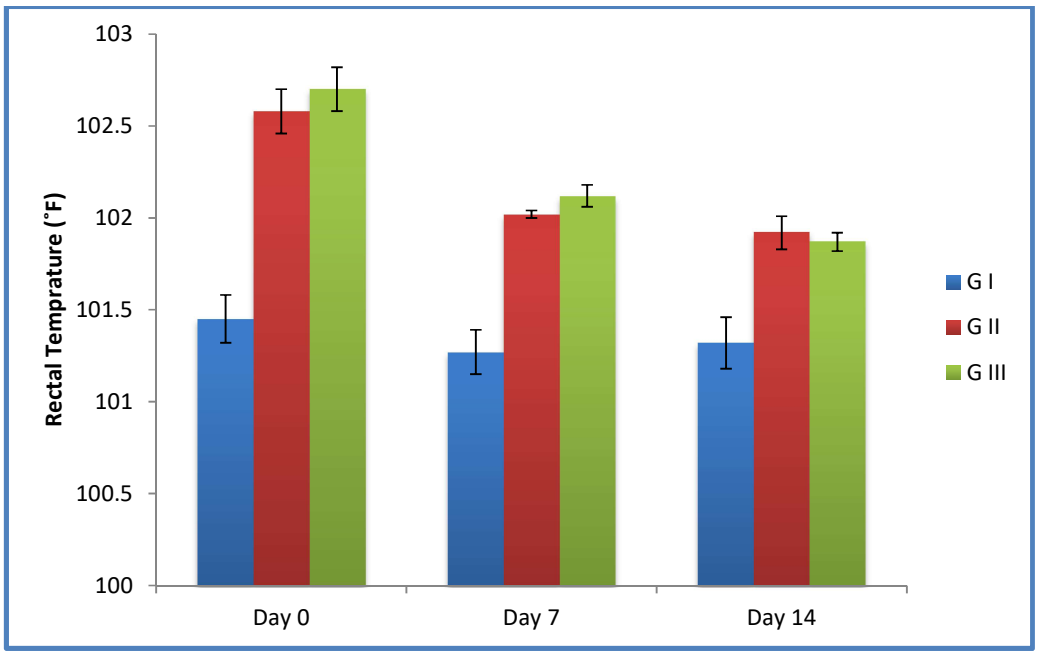
Graph 3: Hospital based prevalence of acute liver failure in dogs in relation to sex



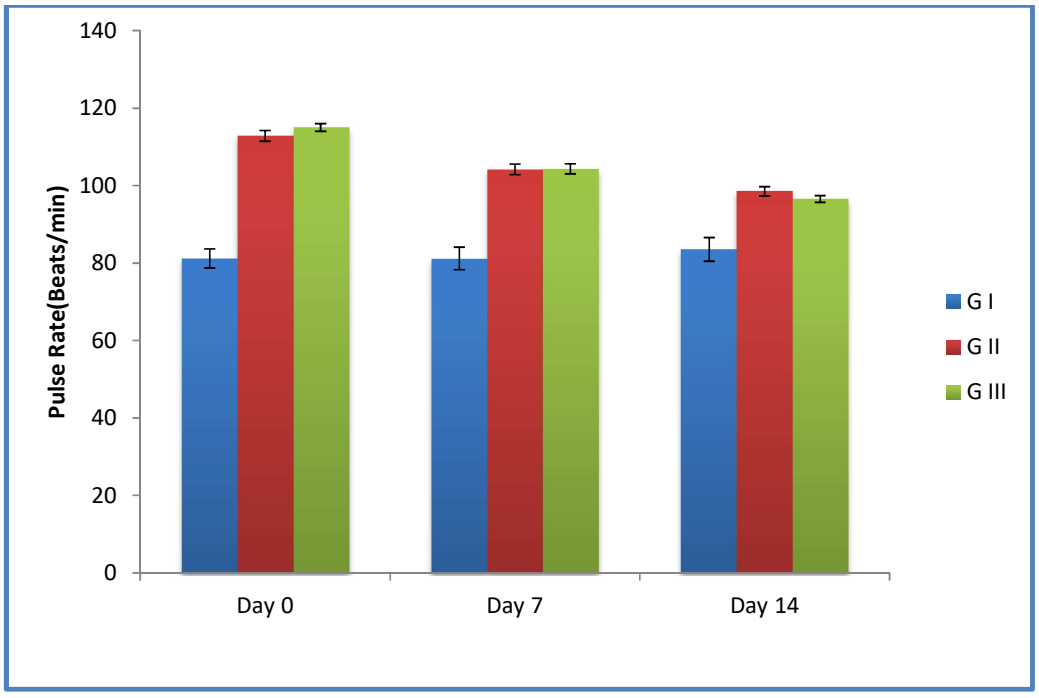
Graph 4: Clinical findings and their frequency distribution in dogs with acute liver failure



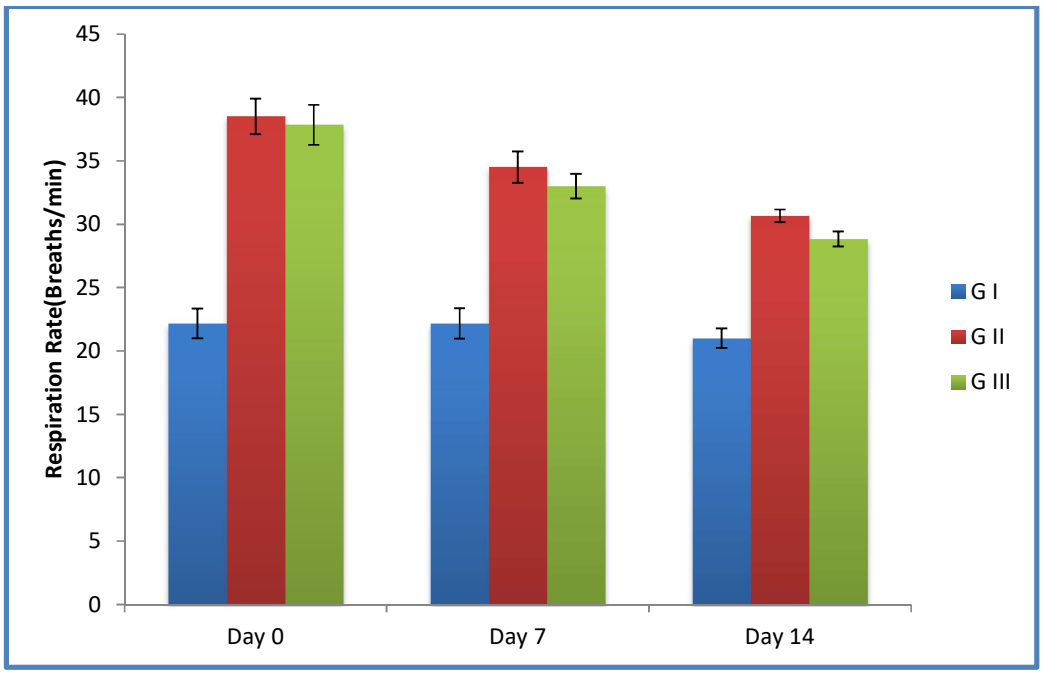
Graph 5: Rectal temperature (^{0}F) of dogs in different treatment groups at different intervals.



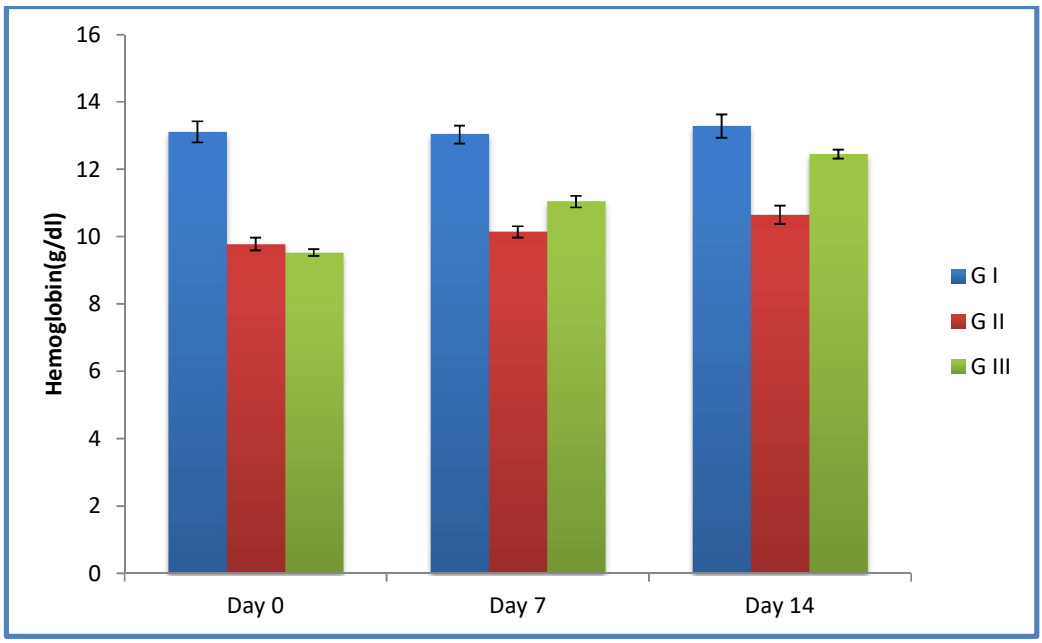
Graph 6: Pulse rate (beats/min) of dogs in different treatment groups at different intervals.



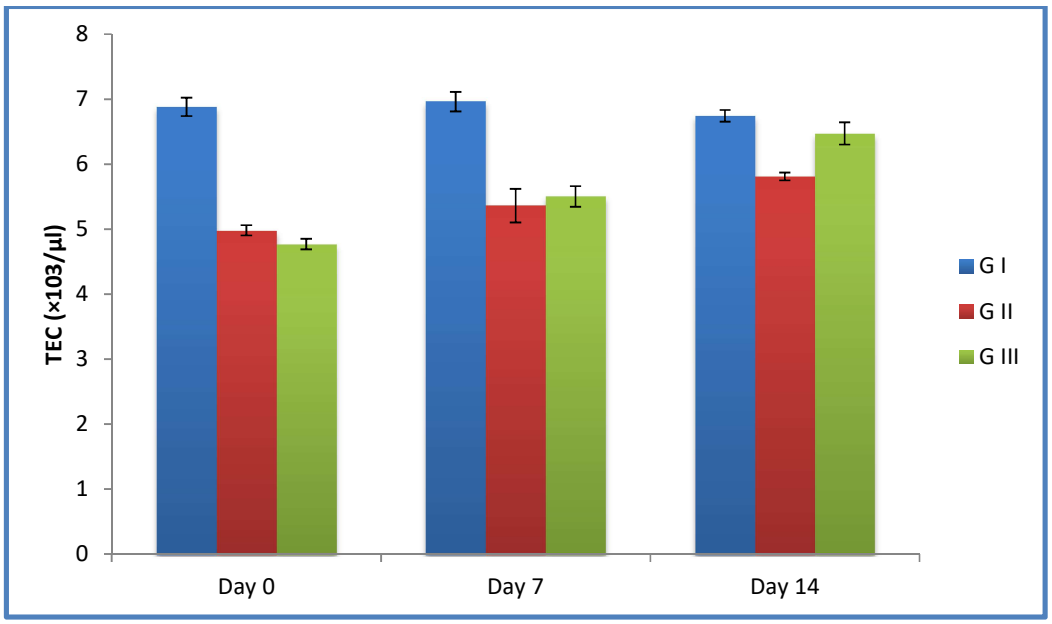
Graph 7: Respiration rate (breaths/min) of dogs in different treatment groups at different intervals.



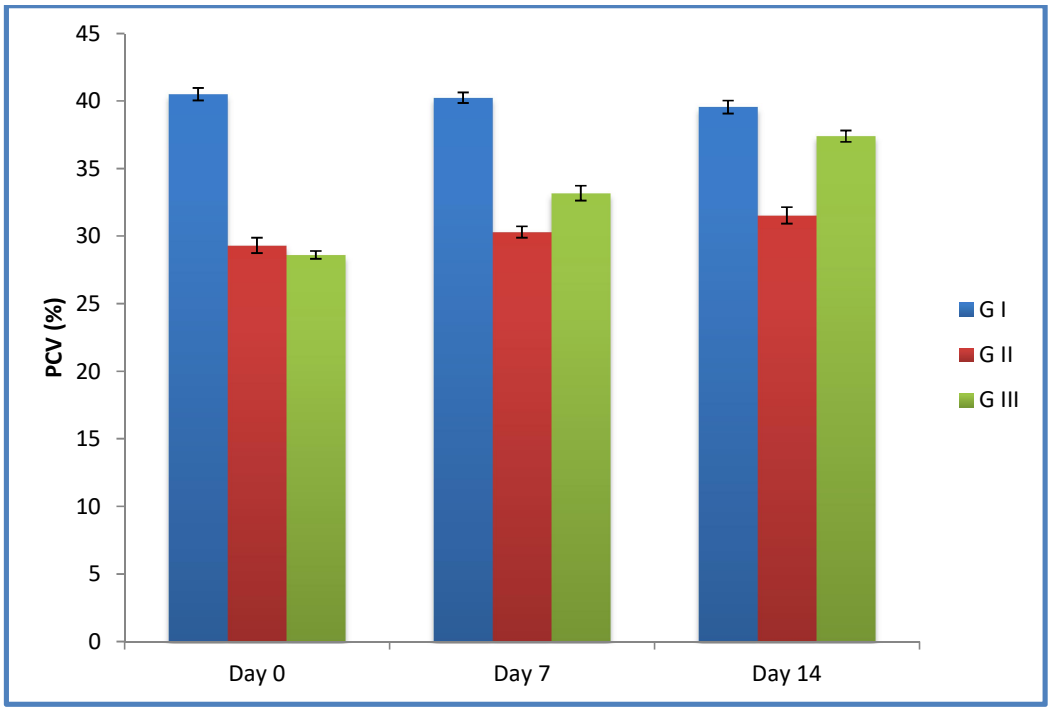
Graph 8: Haemoglobin concentration (gm/dL) of dogs in different treatment groups at different intervals.



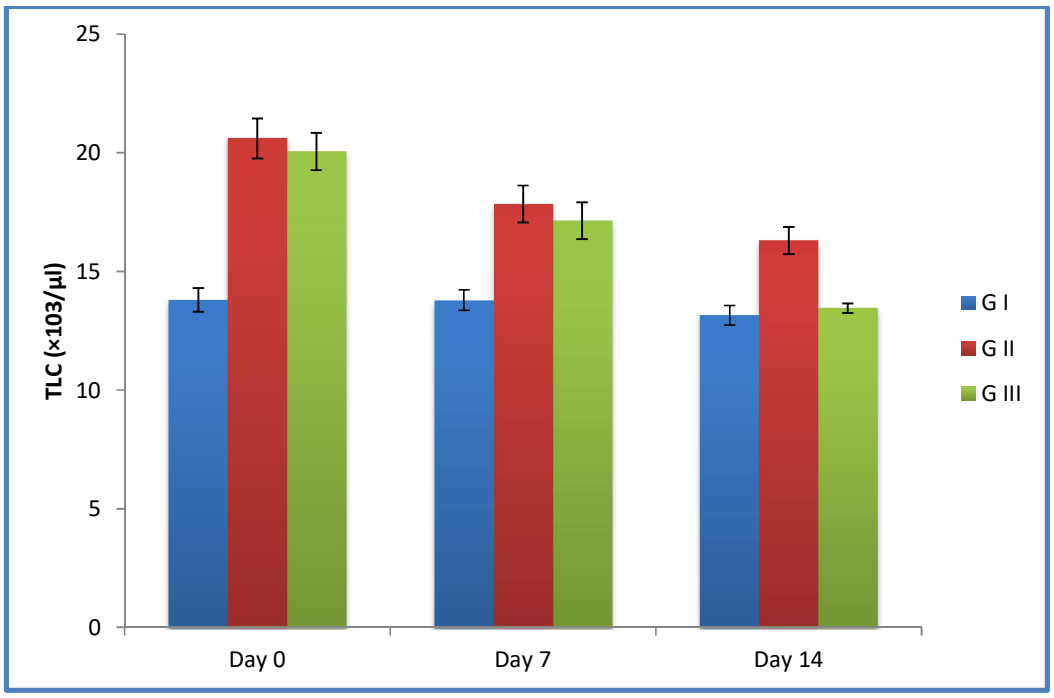
Graph 9: Total erythrocyte count (× 10^6/µl) of dogs in different treatment groups at different intervals.



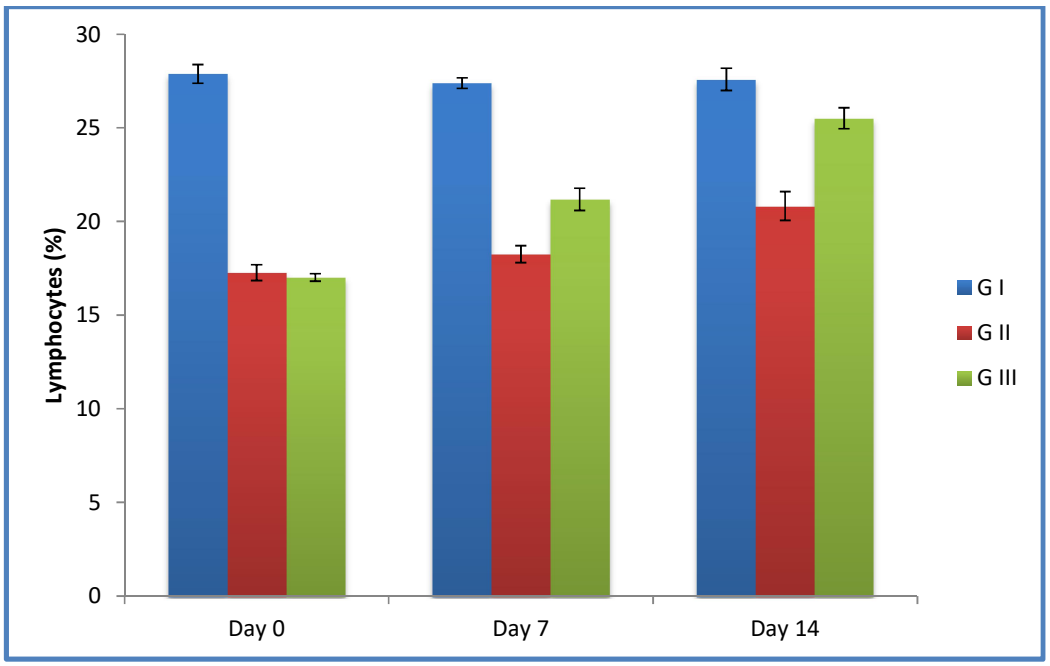
Graph 10: Packed cell volume (%) of dogs in different treatment groups at different intervals.



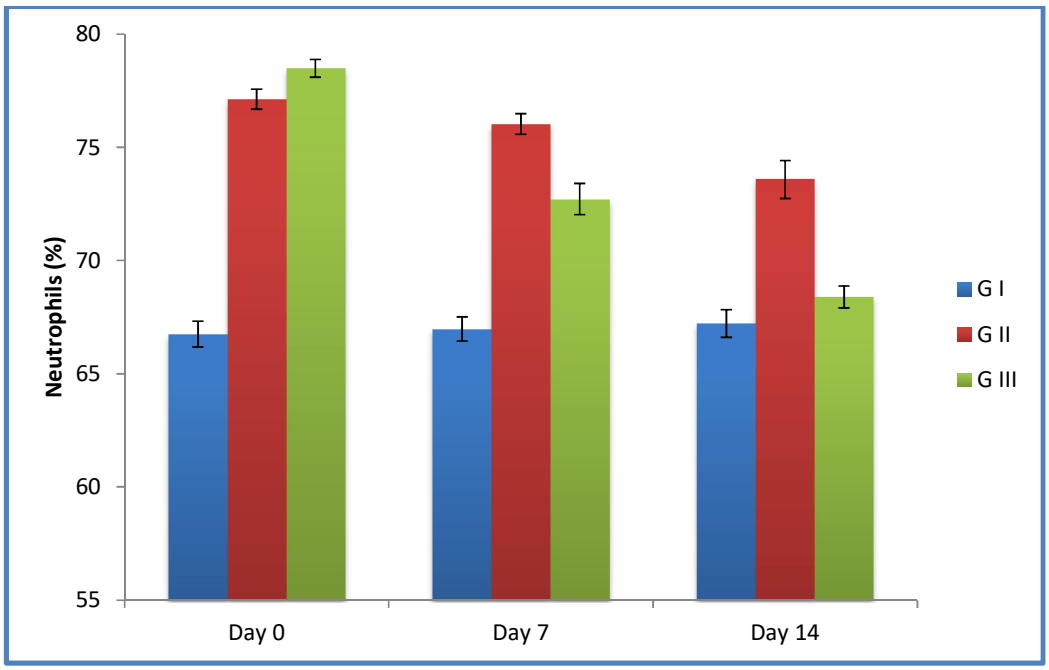
Graph 11: Total leucocyte count ($\times 10^3/\mu L$) of dogs in different treatment groups at different intervals.



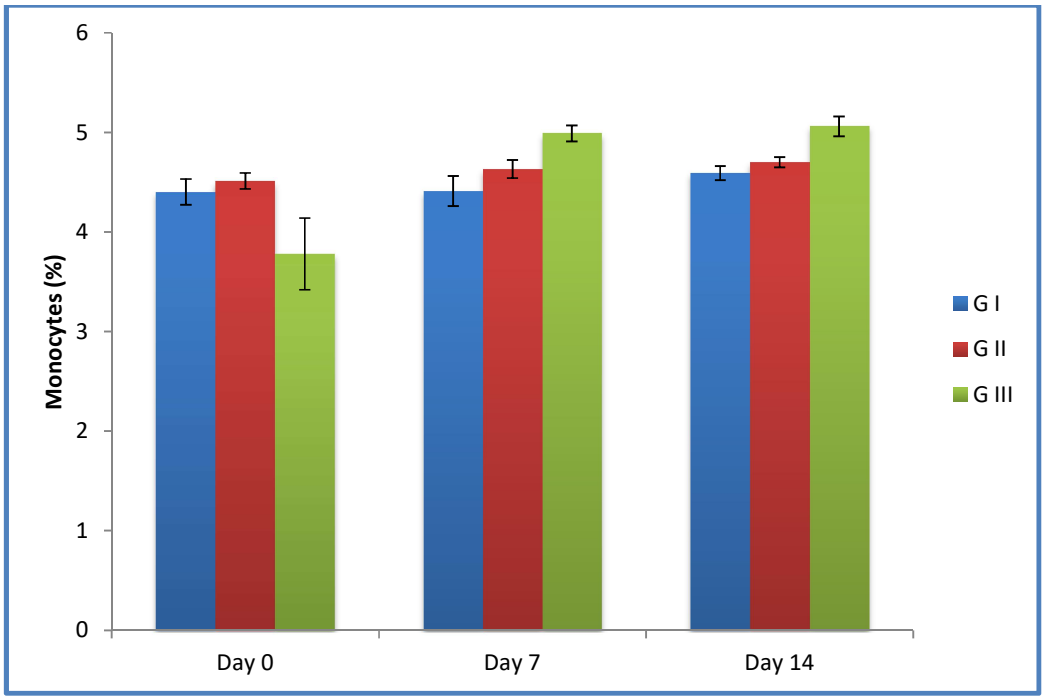
Graph 12: Lymphocytes (%) of dogs in different treatment groups at different intervals.



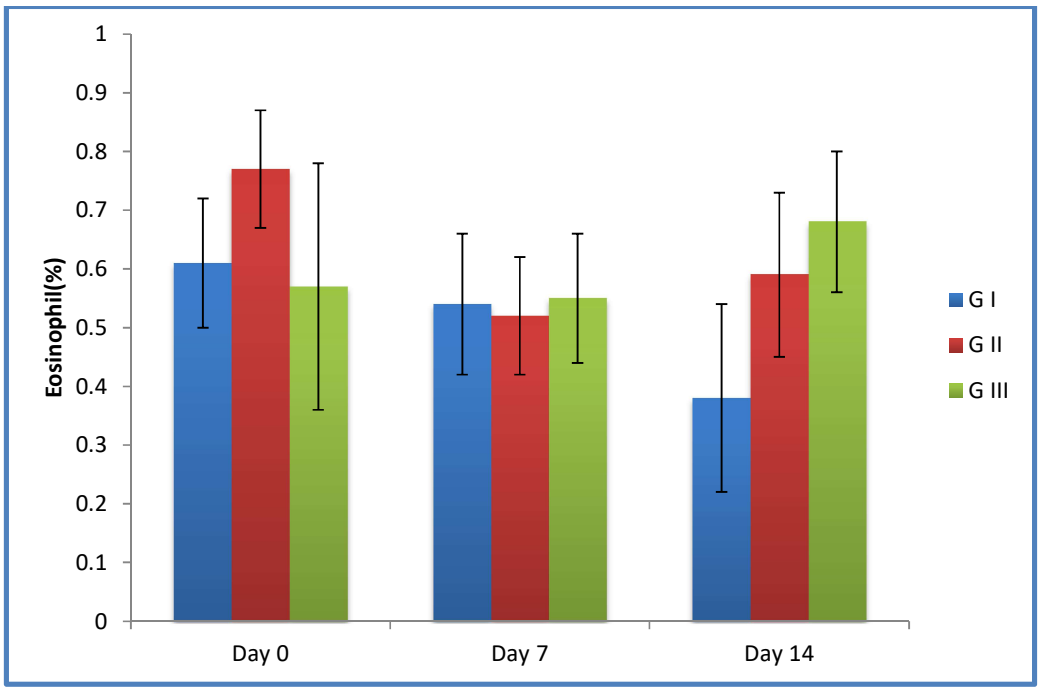
Graph 13: Neutrophils (%) of dogs in different treatment groups at different intervals.



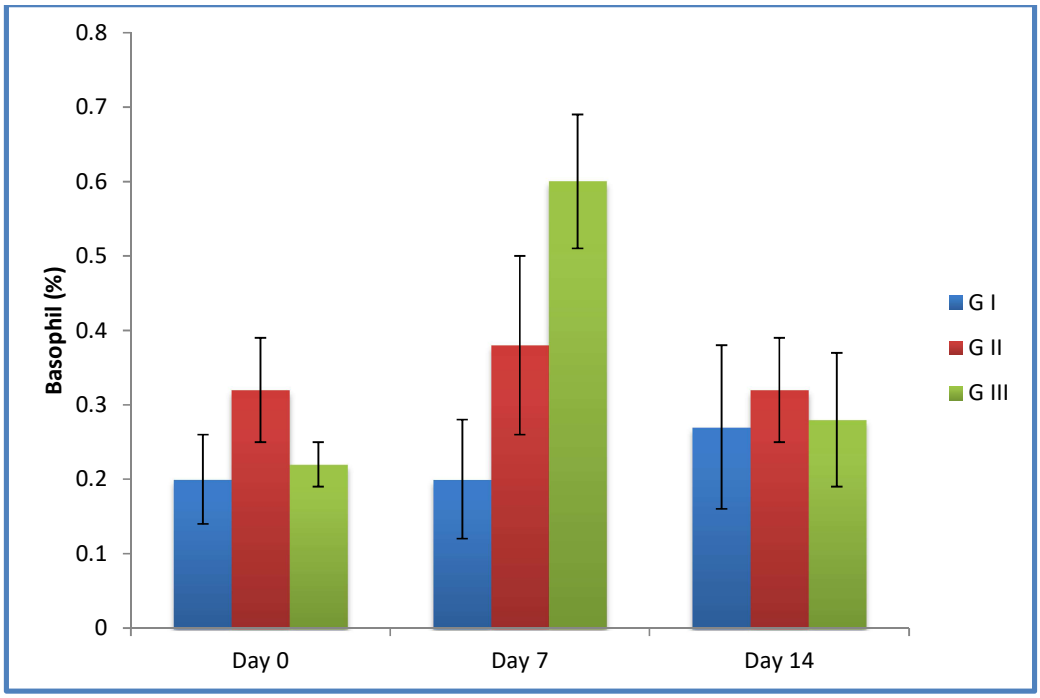
Graph 14: Monocyte (%) of dogs in different treatment groups at different intervals.



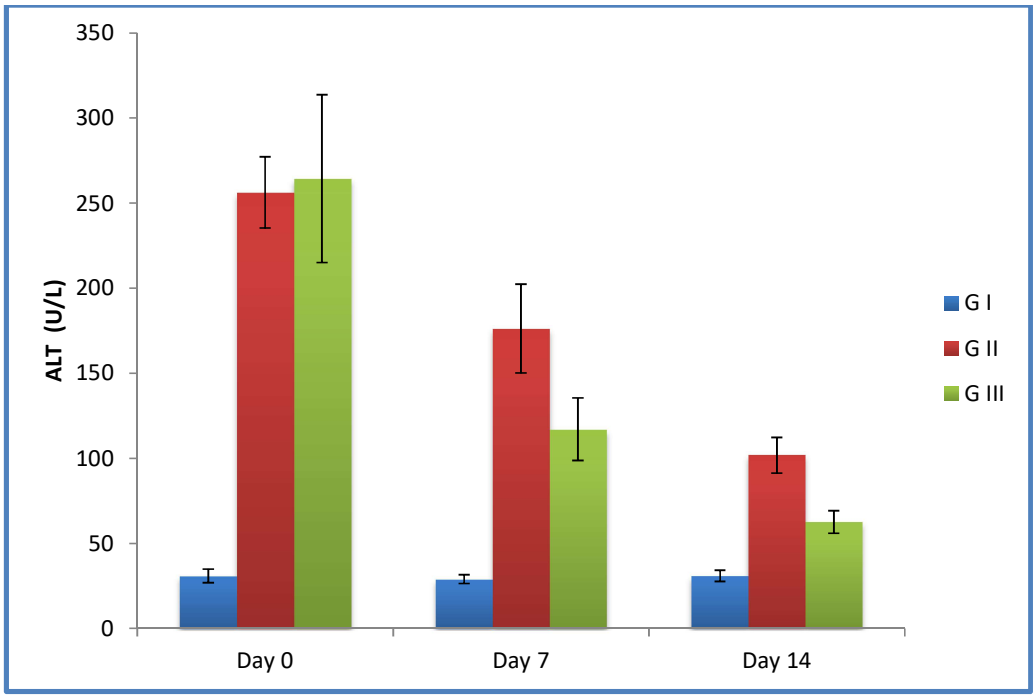
Graph 15: Eosinophils (%) of dogs in different treatment groups at different intervals.



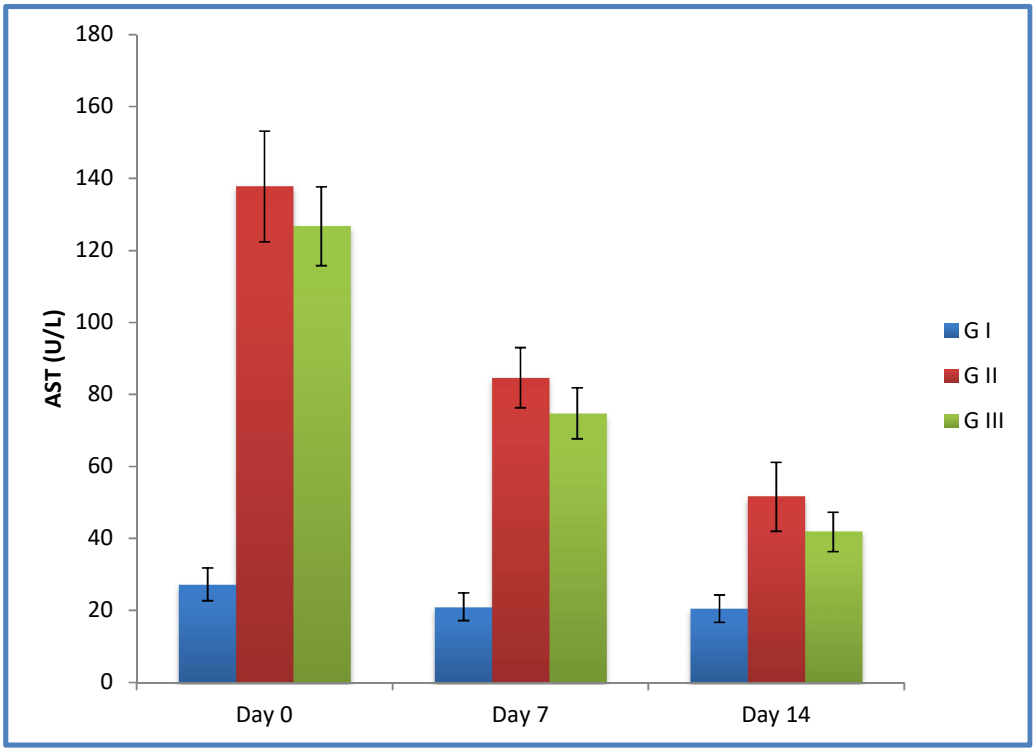
Graph 16: Basophils (%) of dogs in different treatment groups at different intervals.



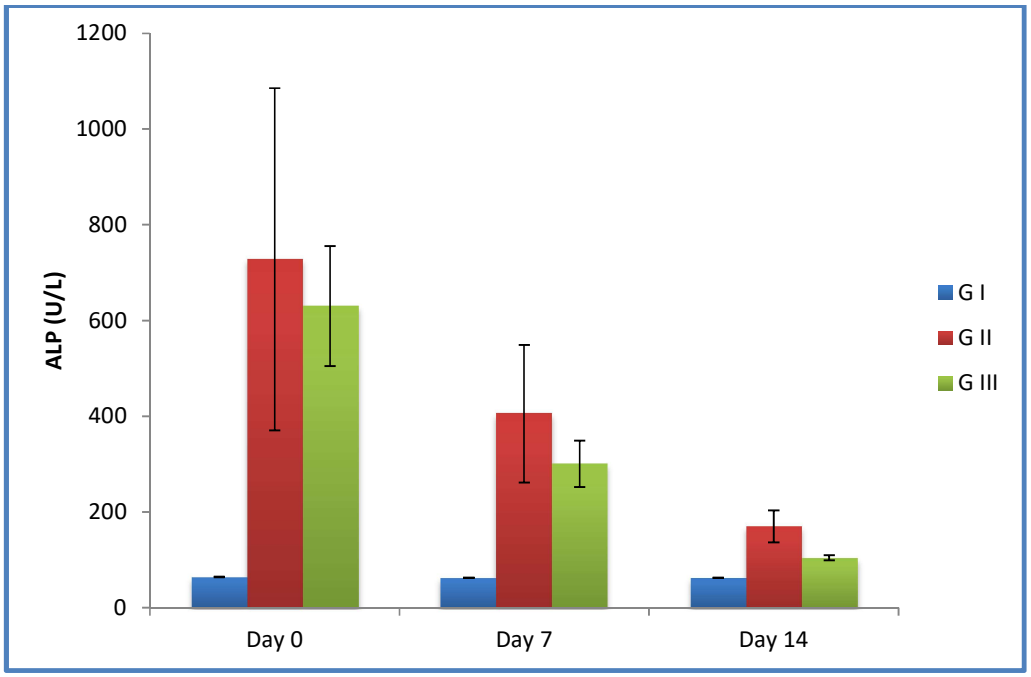
Graph 17: ALT (U/L) of dogs in different treatment groups at different intervals.



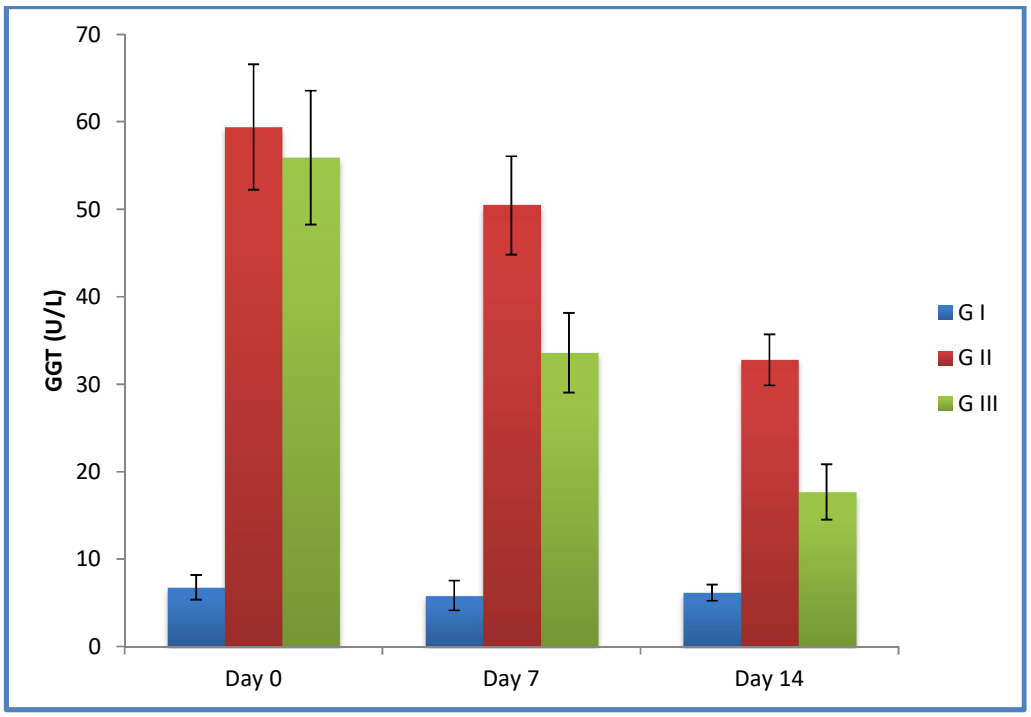
Graph 18: AST (U/L) of dogs in different treatment groups at different intervals.



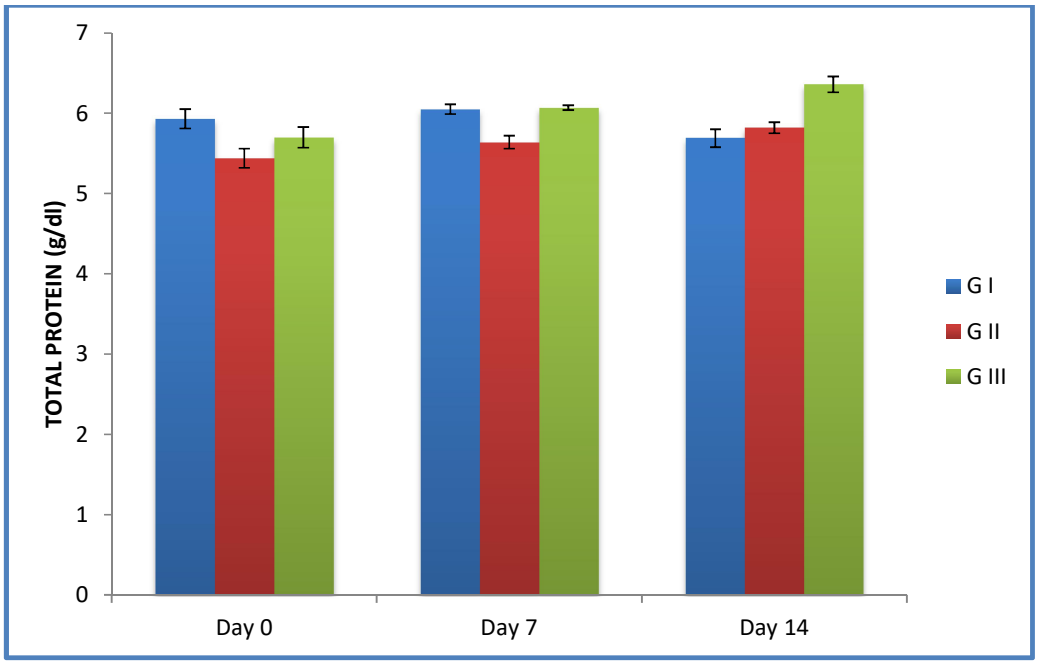
Graph 19: ALP (U/L) of dogs in different treatment groups at different intervals.



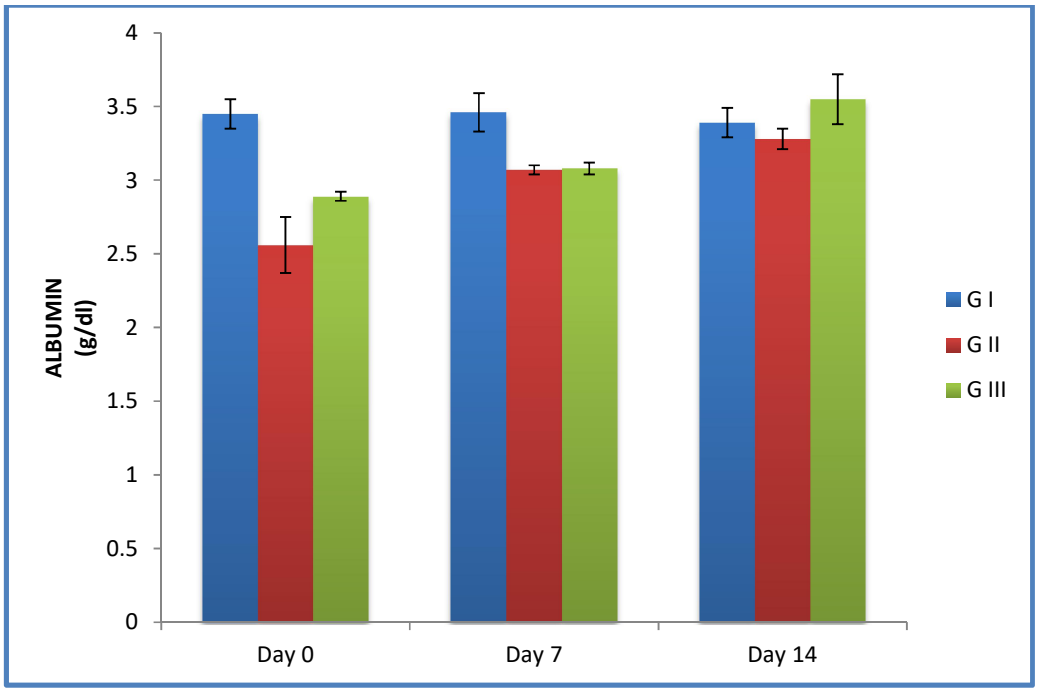
Graph 20: GGT (U/L) of dogs in different treatment groups at different intervals.



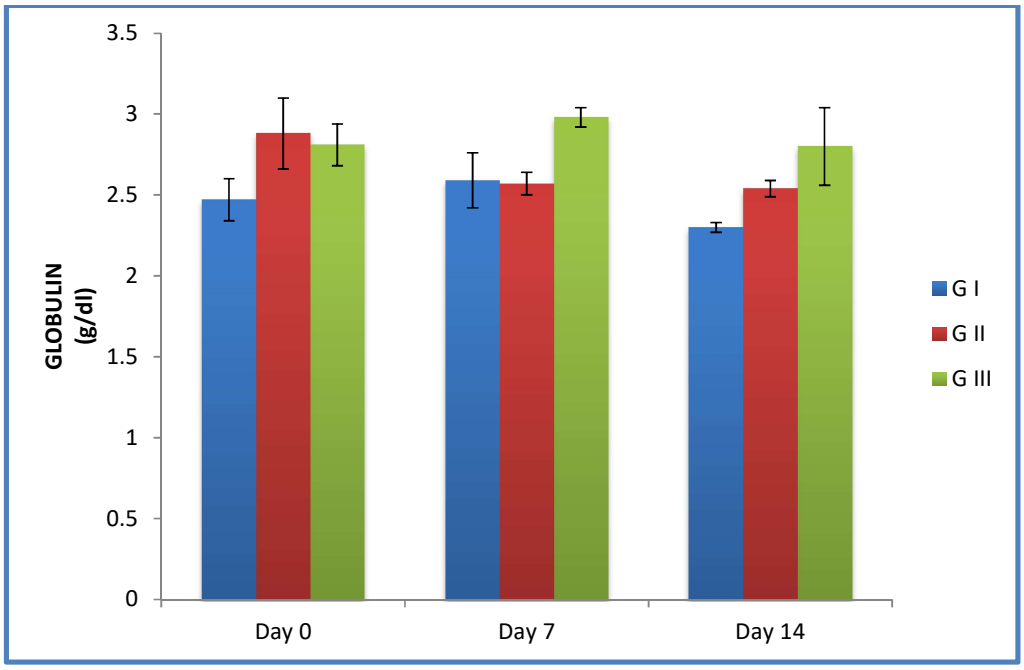
Graph 21: Total protein (g/dl) of dogs in different treatment groups at different intervals.



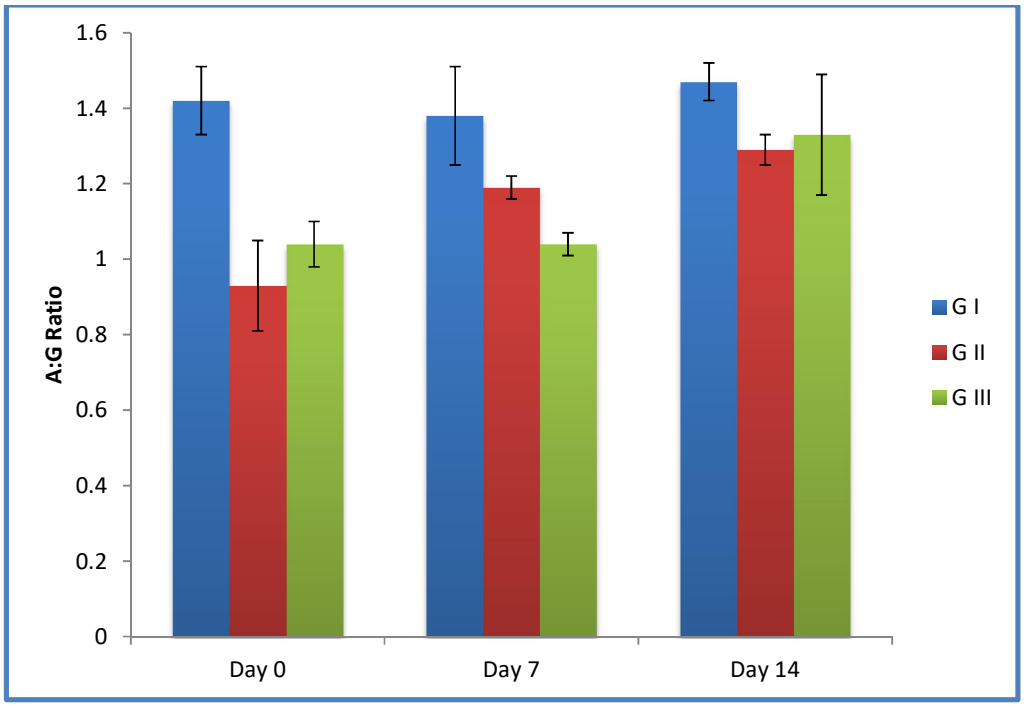
Graph 22: Albumin (g/dl) of dogs in different treatment groups at different intervals.



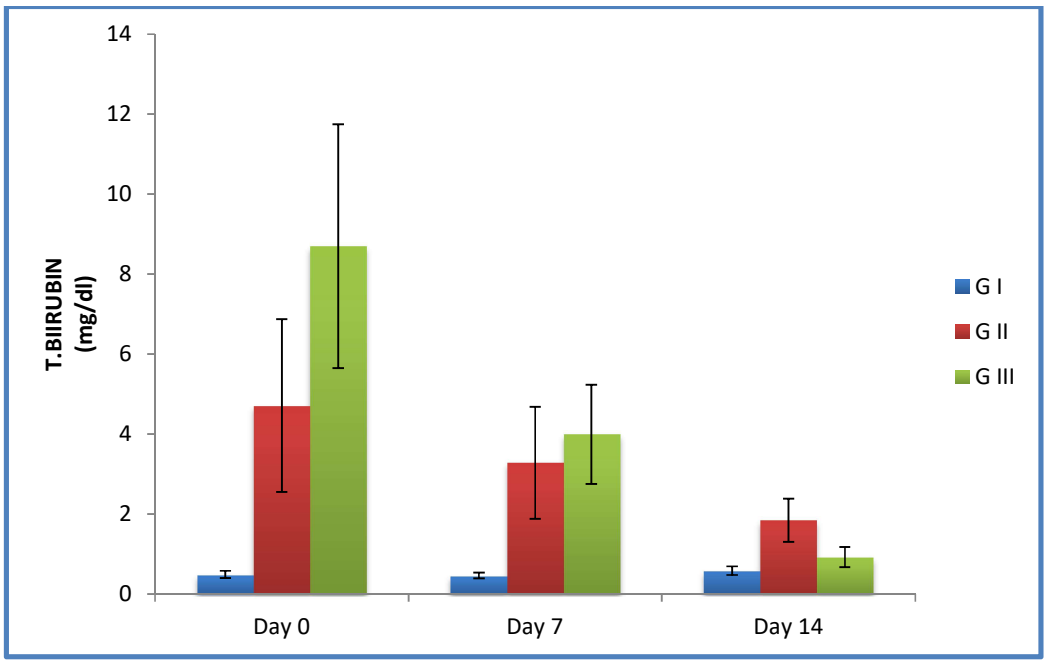
Graph 23: Globulin (g/dl) of dogs in different treatment groups at different intervals.



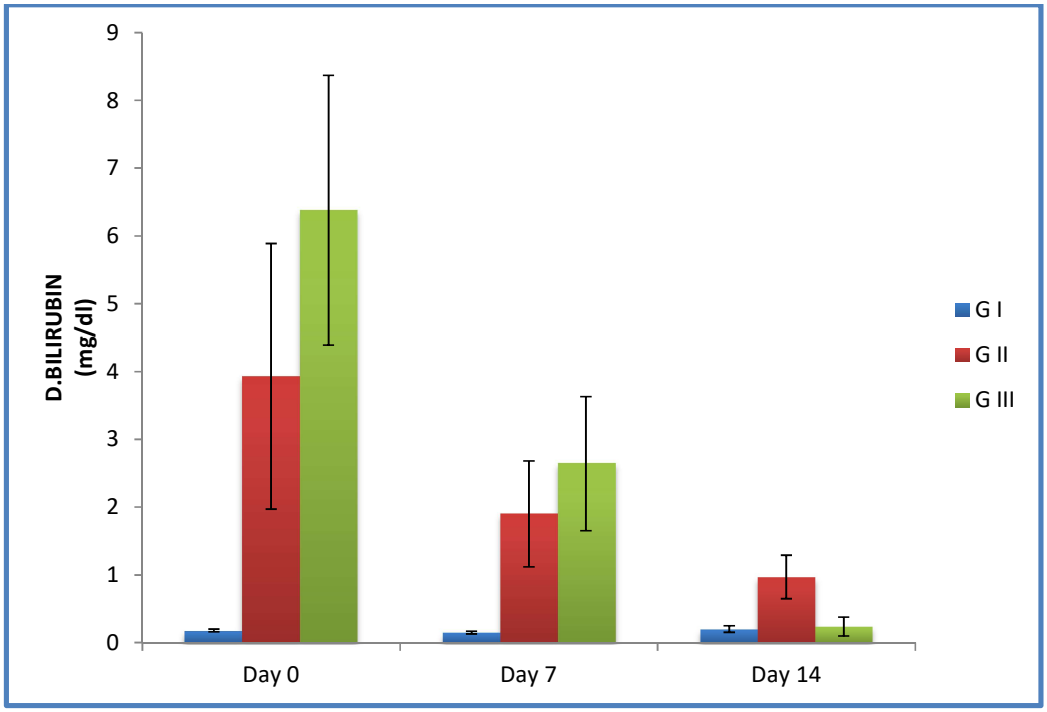
Graph 24: A:G Ratio of dogs in different treatment groups at different intervals.



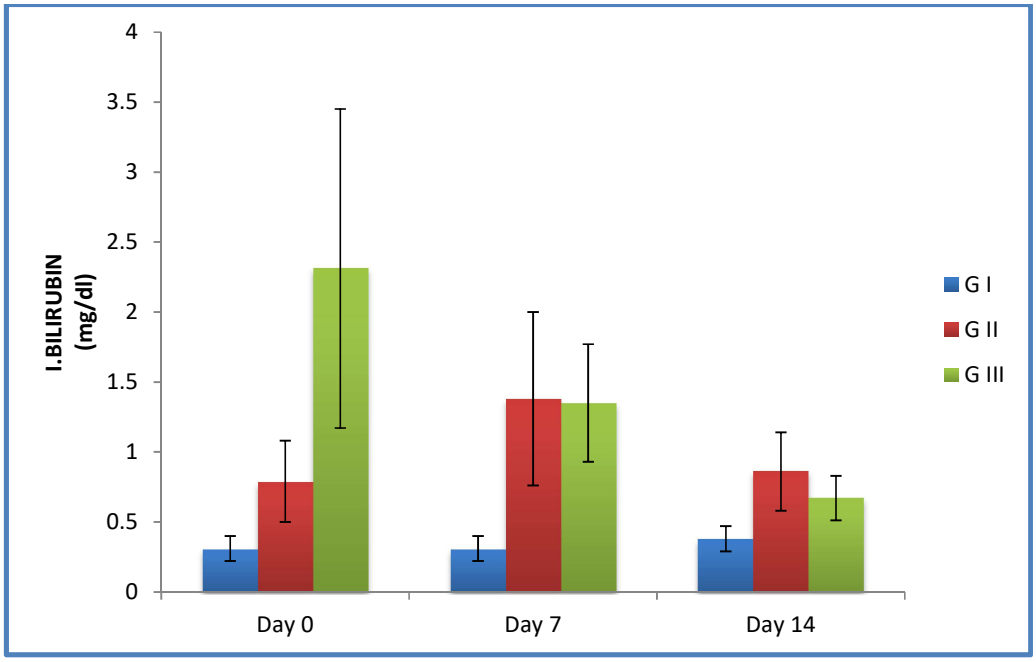
Graph 25: Total bilirubin (mg/dl) of dogs in different treatment groups at different intervals.



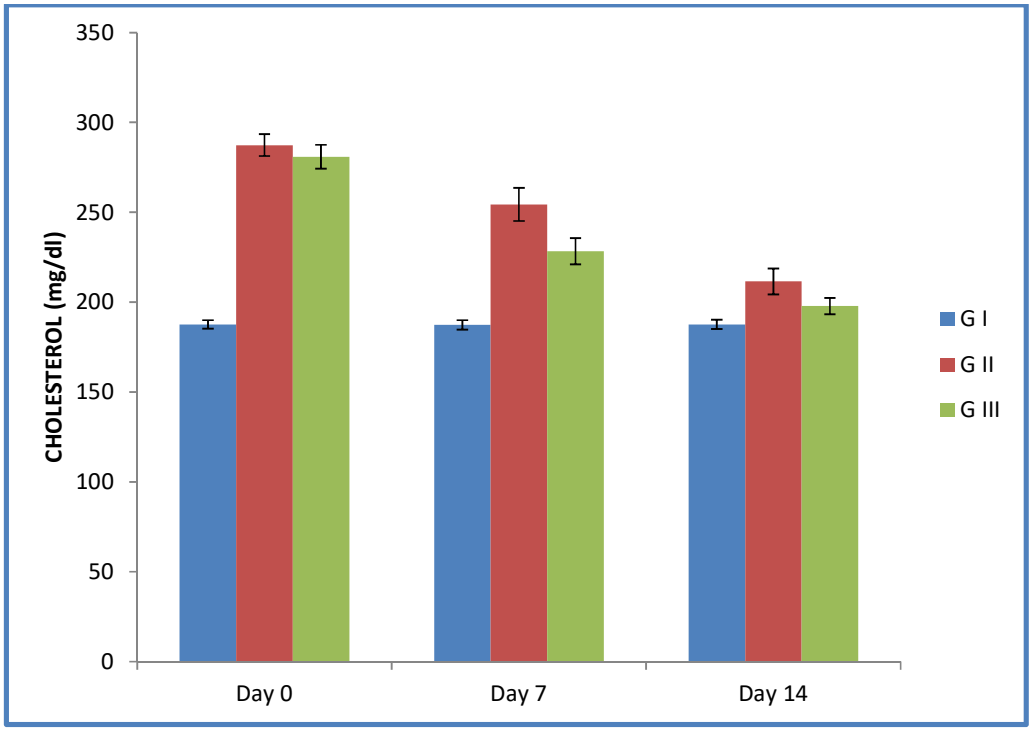
Graph 26: Direct Bilirubin (mg/dl) of dogs in different treatment groups at different intervals.



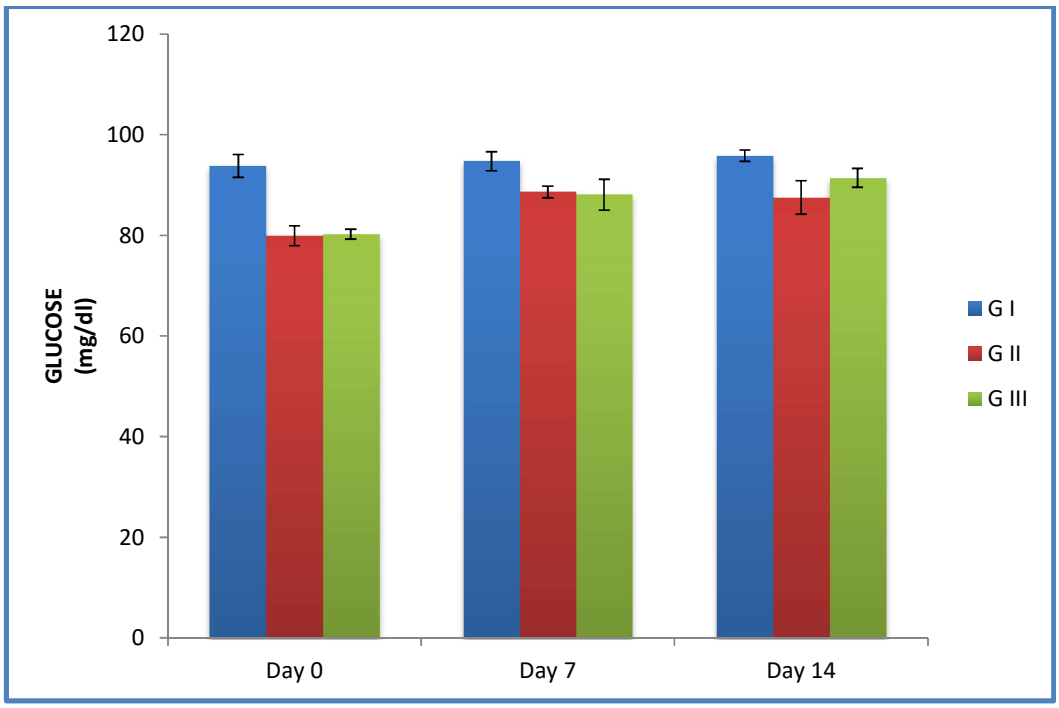
Graph 27: Indirect bilirubin (mg/dl) of dogs in different treatment groups at different intervals.



Graph 28: Cholesterol (mg/dl) of dogs in different treatment groups at different intervals.



Graph 29: Glucose level (mg/dl) of dogs in different treatment groups at different intervals.



Discussion

CHAPTER -5

DISCUSSION

The present investigation entitled "Evaluation of therapeutic potential of *Moringa oleifera* leaves on acute liver failure in dog" was undertaken to screen dogs for acute hepatic failure and to evaluate the associated haemato-biochemical alterations. In addition to this, therapeutic response of *Moringa oleifera* leaves on acute liver failure was also evaluated. In present investigation, a total of 205 dogs were screened for acute liver failure based on clinical signs, haemato-biochemical analysis and diagnostic imaging abnormalities. Out of 205 dogs, 31 dogs were positive for acute liver failure. A total 12 dogs were taken for therapeutic study, which were randomly divided into 2 groups having equal number of dogs and therapeutic efficacy of *Moringa oleifera* leaves on acute liver failure was evaluated as per therapeutic protocol.

5.1 Occurrence

In the present study, a total of 3881 dogs, irrespective of age, breed, sex and month presented at the Teaching Veterinary Clinical Complex (TVCC), DUVASU, Mathura, U.P. were examined during a period of six months i.e., from February 2023 to July 2023. Total 205 dogs showed clinical signs pertaining to acute liver failure, out of which 31 dogs were found to be positively affected as per hemato-biochemical analysis. The overall occurrence of acute liver failure in total dog population was 1.107 % (31/3881) whereas occurrence among suspected dogs was 15.12 % (31/205). These observations are in accordance with those of earlier reports of Sultana *et al.* (2022), Sameeksha *et al.* (2021), Telagar (2017) and Apalkova (2012) who reported the overall clinical prevalence % of liver disorders in dogs were 0.73%, 1.06%,0.75% and 1.24% respectively.

In present investigation, prevalence of acute liver failure was found highest in Labrador breed (29.03 %) followed by Germen shepherd (17.95 %), Golden Retriever (13.51%), Mongrel or mix breed (11.76%), Pug (9.52%), German Spitz (8.33%), Pomeranian (5.55%) and Shiz Tzu (5.26 %). These observations are in line with those of the earlier reports of Sameeksha (2021), Kumbhkar (2017), Telagar (2017), Murikipudi *et al*. (2017), Pradeep *et al*. (2017), Lester *et al*. (2016), Hiblu (2015),

Dixit et al. (2010), Sharma (2010) and Andersen and Sevelius (1991) who recorded highest occurrence of hepatic disorders in Labrador Retriever. However, these findings are in contrast with those of Vijayakumar *et al.* (2003) and Tiwari (2002) who recorded highest occurrence in Germen Shepherd breed.

One reason for the common encounter of acute liver failure in Labrador breed in Mathura region could be attributed to its higher popularity in this geographical region. The Labrador breed is also found susceptible to pyometra, leptospirosis and other systemic diseases (Tufani *et al*., 2015). The potential variability in the prevalence among the breeds may be attributed to the difference in genetic resistance, hygiene, management systems, and technical knowledge of the investigators along with methods used for the diagnosis of the disease. The total sample size and number of animals taken among different breeds also play vital role for variation of percentage of hospital based prevalence of acute liver failure in different breeds of dog. Hoffman et al. (2006) explained the higher incidences of hepatic disorders in Labradors as manifestation of hereditary mechanism. Because labrador retrievers are among the most popular pet breeds in Mathura and adjoining regions, it is also the breed that is most represented in the population studied for this study.

In present investigation, the higher occurrence of acute liver failure was observed in female dogs than male dogs. The present findings are in compliance with the work of Kumbhkar (2017), Dixit *et al.* (2010), Vijayakumar *et al.* (2003) and Rutgers and Haywood (1988) who reported dominance of fernale gender in cases of acquiring hepatic disorders. However, Lester *et al*. (2016), Hiblu (2015), Selgas *et al.* (2014), Sharma (2010) and Andersen and Sevelius (1991) reported contrasting results of higher occurrence of hepatic disorders in male dogs than female. Also, Chohan *et al*. (2007) observed no significant difference between male and female dogs in relation to liver disorders.

In present study, the highest occurrence of acute liver failure was observed in the age group of 4-8 years (19.67 %), followed by 8 years and above (14.06 %), 1-4 years (13.46 %) and lowest in <1 year of age (12 %). The findings of this study are similar with other studies who reported the highest incidence in middle-aged dogs (4 and 6 years of age) (Sultana *et al.*, 2022; Mandigers *et al.*, 2004). The number of cases being highest in the age group of 4 to 8 years could likely due to the fact that the cases presented at the centre of study were mostly of that age group.

5.2 Clinical sign frequency in dogs

Predominant signs in present study were vomiting, anorexia, icterus, weakness, abdominal pain, wt. loss, fever, neurologic signs, hematemesis, inappetance, pale mucus membrane and polyuria / polydypsia. Vomiting was found to be the most predominant clinical sign in present investigation. The present findings are in compliance with the work of Sameeksha (2021), Bera (2016), Lester *et al*. (2016), Rondeau (2015), Tantary *et al*. (2014), Sharma (2010), Kearns (2009) and Hughes and King (1995).The degree of liver damage completely determines the patient's clinical profile and vomiting is a consistent sign in acute hepatic injury, irrespective of the cause (Kumar *et al*., 2012 and Rothuizen, 2011). Inappetence to anorexia observed in many dogs in our study, is a very common non-specific sign in canine hepatic disorders (Sameeksha, 2021). Releasing of pyrogens from necrotic tissues and decreased clearance of endotoxins and bacteria from portal blood can lead to pyrexia (Rothuizen, 2011).Hyperbilirubinemia manifesting as icterus in ALF is frequently caused by intrahepatic cholestasis, which can result from leakage of bile canaliculi, swelling of hepatocytes, or necrosis of hepatocytes. Icterus from intrahepatic cholestasis only manifests when the liver is significantly affected due large reserve capacity of liver (Rothuizen, 2009)

5.3 Ultrasonography

Imaging techniques can be extremely useful for distinguishing animals with acute hepatotoxicity from those that have acute manifestation of a chronic disease and also evaluating the overall size and architecture of the liver. In the current study, common sonographic changes included hepatomegaly, variable echogenecity of hepatic parenchyma with cholestasis and rounded liver margin. Ultrasonographic findings are variable in cases of ALF. Typically, the liver is enlarged with normal to hypoechoic parenchyma. (Biller *et al*., 1992). Acute hepatic failure can be identified by diffuse parenchymal abnormalities in which the distinct hepatic borders are not apparent combined with hepatomegaly with normal, increased, or decreased parenchymal echogenicity (Resende *et al*., 2011). Large liver lobes in hepatomegaly in acute hepatic insults often have rounded margins, whereas the normal liver lobes have sharp edges (Lamb, 1995).

5.4 Therapeutic study

Dogs found positive for acute liver failure during screening were taken for therapeutic trail to accesses therapeutic efficacy of *Moringa oleifera* leaves. Six apparently healthy dogs were placed in healthy control group (group I) after thorough physical examination and hemato-biochemical analysis. Twelve dogs screened for acute liver failure were randomly divided into two groups (group II and group III) containing six animals each. Group II animals were treated with the conventional treatment (as per clinical findings and requirement) for 14 days. Group III animals were treated with the combination of *Moringa oleifera* leaves powder (30mg/kg) along with conventional treatment for 14 days. Therapeutic evaluation was done on the basis of percent recovery assessment and hemato-biochemical alterations. Percent recovery was assessed by clinical improvement in terms of disappearance of clinical signs and alterations in the hemato-biochemical parameters on day 7^{th} and day 14^{th} post treatment.

5.4.1 Physiological parameters

In present study, rectal temperature, pulse rate and respiration rate were significantly high in animals with acute liver failure as compared to healthy animals before initiation of any treatment. A similar result has also been reported by Hiblu (2015) in dogs with acute hepatic dysfunction. However, Sharma (2010) observed non-significant variation in the rectal temperature in hepatitis with jaundice group compared to the healthy control group. Also, Bera (2016) reported the respiration rate of 29.5±1.12 in dogs with acute hepatic disorder.

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).

5.4.2 Haematological Parameters

In the present study, mean haemoglobin, PCV and total erythrocyte count decreased significantly in dogs with acute liver failure (group II and group III) than those of apparently healthy dogs (group I). Similarly, Lester et al. (2016) reveal findings included Anemia (PCV <39%) in dogs with acute liver failure. Also, presence of anaemia is a prominent feature of cases reported to have other

hepatobiliary disorders (Sultana et al., 2022; Bhatti, 2020; Lakshmi *et al.*, 2018; Telagar, 2017; Kumar *et al.*, 2013; Chaturvedi *et al.*, 2013; Shrivastava *et al.*, 2010; Vijayakumar *et al.*, 2004; Sharma *et al.*, 2001 and Tiwari *et al.*, 2001). Anorexia and inappetance in hepatic disorders could be the cause of this anaemia (Watson and Bunch, 2009).

Whereas, after treating the animals with conventional treatment as well as combination of *Moringa oleifera*, the values were increased significantly. A similar increase in haemoglobin, packed cell volume and total erythrocyte count has also been reported when concentrate feed was replaced with 100% *Moringa oleifera* leaves in diet of sirohi goat kids (Meel *et al.,* 2018). Also, Mandal *et al.* (2015) reported significant increase in Hb% and PCV after supplementation of dried *M. oleifera* fruit powder to fluorosis affected calves. Also, Osman et al. (2012) and 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. However, these observations are in contrast with those of Nurhayati *et al.* (2023) who reported non-significant change in haemoglobin, PCV and total erythrocyte count on consumption of Moringa leaf powder in male wistar rats.

In present study, 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 (Brempelis and Crispe, 2016; Lecoindre and Arpaillange, 2010)

In present study, mean values of lymphocytes were found to be significantly low while values of neutrophils were 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.The neutrophils values are high in cases of severe, or acute inflammatory processes (Hughes and King, 1995). Lester *et al.* (2016) noted a similar haemopoietic response in acute liver failure. In the Present study no significant differences were recorded in mean concentration of monocyte %, eosinophils % and basophils % either

between the groups or within groups at different observation periods. Non-significant changes in basophil and eosinophil counts in present study corroborates with the findings in acute hepatic disorder by Bera (2016).

5.4.3 Serum Biochemistry Analysis

A significant increase in concentration of ALT, AST, GGT and ALP, was recorded in dogs with acute liver failure as compared to apparently healthy control dogs 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.

These findings are in corroboration with similar findings of Lester *et al.* (2016). Alanine aminotransferase is a biomarker of loss of structural and functional patency of the plasma membrane with high circulatory levels encountered in hepatocyte degeneration (Hughes and King, 1995). The increase in value truely reflects the magnitude of traumatic insult to hepatocytes. Sameeksha (2021) stated that the increase in ALT level suggests leakage from the hepatocytes, secondary hepatopathies, and the enzyme is also liberated by the regenerating hepatocytes. Therefore rise in the levels for an extended period of days to weeks following acute injury does not necessarily signify a poor prognosis. Within 1-2 days of severe and acute hepatocellular insult, the enzyme activity increases upto 100-fold (Weingarten and Sande, 2015). Marked elevation in serum ALP concentration is observed in the clinical cases of acute hepatitis and cholecystitis in dogs in carbon tetrachloride induced experimental hepatic insult (Sharma, 2010). In dogs, increased ALP (U/L) value 2-5 fold is reported in acute, severe hepatic necrosis which then subside gradually (Weingarten and Sande, 2015). Because alkaline phosphatase is present on both the hepatocyte biliary membrane and bileduct cells and g-glutamyltransferase is found primarily on the latter, larger increases in these enzymes occur in ALF syndromes associated with cholestasis (Center, 1996). In dogs and cats with experimental acute, severe, diffuse hepatocellular necrosis, GGT activity remains unchanged or only mildly increased (Chapman and Hostutler, 2013).

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 bilrubin 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 and 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 hypoglycaemic effect in dogs.

5.5 *Moringa oleifera*

Medicinal plants are the backbone of traditional medicine and more than 3.3 billion people in the countries utilizes medicinal plants on a regular basis (Davidson, 2000). Various bioactive components of *Moringa oleifera* leaves contributes to its hepatoprotective effects as noticed in present study. A previous study by Selvakumar and Natarajan (2008) reported hepatoprotective effect of *M. oleifera* was due to presence of Quercetin and kaempferol. Flavanoids have antioxidant potential that

scavenges free radicals thus stabilizing hepatocytes cell membrane and prohibiting enzyme seepage as postulated earlier (Pari and Karthikesan 2007). *M. oleifera* leaves serve as a rich source of *b*-carotene and are used as a hepatoprotectant and hepatostimulant (Praveen *et. al*., 1993).

The findings of the present study revealed restoration of ALT, AST, ALP, GGT, total protein, cholesterol, bilirubin and glucose. Since, better recovery towards normalcy was noticed in group III. Therefore, the therapeutic regimen adopted in the treatment of acute liver failure under treatment group III in which *Moringa oleifera* leaf extract (act as hepatoprotectant) was given found to be efficacious.

Summary and Conclusions

CHAPTER- 6

SUMMARY AND CONCLUSIONS

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. It denotes acute hepatocellular damage and necrosis with retained hepatic function, ALF occurs once hepatocellular damage is so extensive that it compromises hepatic synthetic, excretory and regulatory functions. Because of the large functional reserve of the liver, loss of more than 70% of the hepatocellular mass is required before clinical signs of acute liver failure become apparent.

Common clinical signs include anorexia, weakness, abdominal discomfort, lethargic behaviour, vomiting, diarrhea, constipation, yellowing of the skin and mucous membranes, hepatomegaly, fever, hindlimb, tachycardia and tachypnea, anemic appearance with pale mucous membranes, melena, seizures, skin lesions, coagulopathy, epigastric abdominal pain, polyuria and polydipsia, weight loss and poor physical condition. Diagnosis is based on clinical signs, haematological examinations, biochemistry analysis including abnormally high liver function activity and diagnostic imaging abnormalities.

The present study titled '**Evaluation of therapeutic potential of *Moringa oleifera* leaves on acute liver failure (ALF) in dog'** was conducted to evaluate the occurrence of acute liver failure in dog and to evaluate the therapeutic potential of *Moringa oleifera* leaves on acute liver failure in dogs. For this, a total of 3881 dogs, irrespective of age, breed and sex presented to TVCC, DUVASU, Mathura were examined during a period of study *i.e.,* from February 2023 to July 2023. Total 205 dogs showed clinical signs concern to acute liver failure, out of which 31 dogs were found positive for acute liver failure by altered hematology, altered serum biochemistry and diagnostic imaging abnormalities. The overall occurrence of acute liver failure in total dog population was 1.107 % (31/3881) whereas hospital based prevalence among suspected dogs was 15.12 % (31/205). Amongst different breeds, Labrador retriever had the highest occurrence 29.03 % and Shih Tzu possesses lowest occurrence of acute liver failure i.e. 5.26 %. Age wise prevalence was highest in dog

age group 4-8 years and lowest occurrence in less than 1 year age group of dogs. Sex wise prevalence of acute liver failure was higher in female (36.66%) than that of males (11.42%). Vomiting, anorexia, icterus, weakness, abdominal pain, wt. loss, fever, neurologic signs, hematemesis, inappetance, pale mucus membrane and polyuria/polydypsia are the important clinical findings in dogs with acute liver failure as per our study.

Twelve (12) found positive for acute liver failure during screening were taken for therapeutic study and randomly allocated into 2 groups, each group having 6 animals. In group II conventional treatment was given and in group III conventional treatment with combination of *Moringa oleifera* @ 30mg/kg body weight PO was administered for 14 days. Six apparently healthy dogs were placed in healthy control group (group I) after thorough physical examination and hemato-biochemical tests.

The physiological parameters including rectal temperature, pulse rate and respiration rate were significantly higher in both the treatment groups of acute liver failure (group II and group III) than in the healthy control group (group I) on day 0 (pre-treatment). There was significant decrease in rectal temperature, pulse rate and respiration rate on day 7^{th} and 14^{th} (post treatment).

Haematological parameters revealed significant decrease in Hb (g/dl), TEC (10^6/µl), PCV(%), lymphocytes (%) and increased concentration of TLC (10^3/µl) and neutrophils (%) on day 0 (pre-treatment) dogs having acute liver failure (group II and group III) than apparently healthy control dogs (group I). However, treatment with conventional drugs as well as combination therapy with *Moringa oleifera* significant improvements was observed in the values of Hb, TEC, PCV, neutrophils, lymphocytes and TLC with the highest recovery was observed in treatment group III. No any significant differences in mean concentration of monocytes, eosinophils and basophils either between the groups or within groups at different observation periods of study before and after therapy was noticed.

Biochemical parameters revealed significant increase in the activities of ALT, AST,ALP, GGT, serum total bilurubin, cholesterol and decrease in serum total protein, albumin, glucose on day 0 (pre-treatment) dogs having acute liver failure (group II and group III) than apparently healthy control dogs (group I). However, on day 14 (post-treatment), treatment with conventional drugs as well as combination

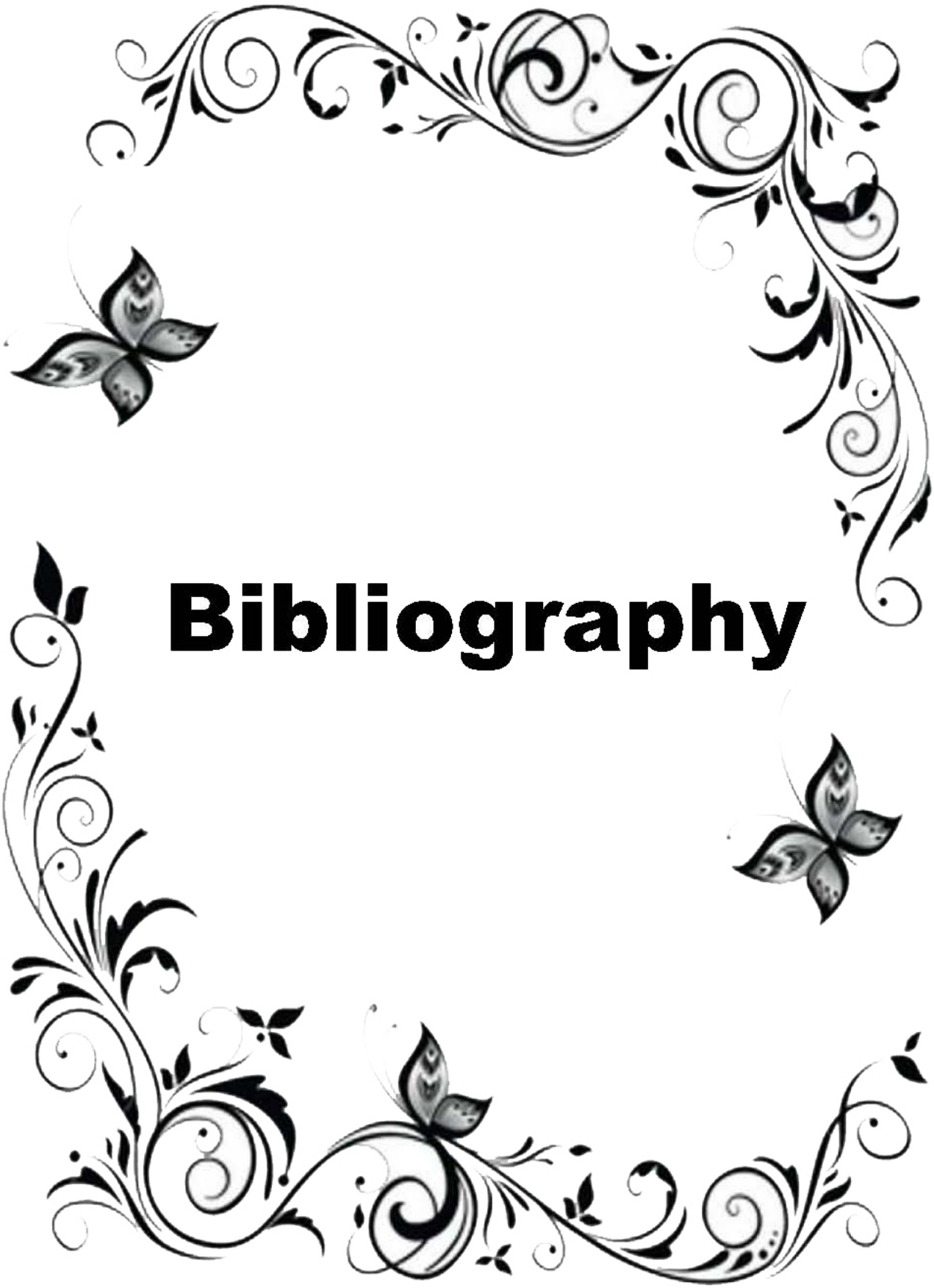
therapy with *Moringa oleifera* significant improvements was observed in the values of ALT, AST, ALP, GGT, serum total bilurubin, total protein, albumin, glucose and cholesterol with the highest recovery observed in treatment group III. No any significant differences in mean concentration of globulin and A:G ratio either between the groups or within groups at different observation periods of study before and after therapy was noticed.

Therefore, the therapeutic regimen adopted in the treatment of acute liver failure under treatment group III in which *Moringa oleifera* leaf extract (act as hepatoprotectant) was given found to be efficacious.

CONCLUSIONS

Based on results of the study "Evaluation of therapeutic potential of *Moringa oleifera* leaves on acute liver failure in dog"the following conclusions were made:

- The overall prevalence of acute liver failure in total dog population was 1.107 % whereas prevalence among suspected dogs was 15.12 %.
- The breed wise prevalence of acute liver failure was found to be highest in Labrador Retrievers and lowest in Shih Tzu.
- The age wise prevalence of acute liver failure was found to be highest in the age group 4-8 years and lowest in less than 1 year.
- Prevalence of acute liver failure was found to be more in female dogs than in male dogs.
- Most predominant signs (>75% frequency) in acute liver failure in dogs were found to be vomiting, anorexia, icterus and weakness.
- Both conventional therapy as well as combination of *Moringa oleifera* with conventional therapy was found effective against acute liver failure in dog. Although, better recovery was observed in combination of conventional therapy with *Moringa oleifera* as evidenced by restoration of ALT, AST, ALP, GGT, total protein, cholesterol, bilirubin and glucose values towards normalcy.
- *Moringa oleifera* (act as hepatoprotectant) may be advised as adjunct therapy along with conventional treatment for early recovery in acute liver failure in dog.



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UNDERTAKING OF COPY RIGHT

I, **Dr. Anupama Verma**, Enrollment No. **V-1693/16**, undertake that I give copy right to the DUVASU, Mathura of my thesis entitled **"Evaluation of therapeutic potential of *Moringa oleifera* leaves on acute liver failure (ALF) in dog".**

I also undertake that patent, if any, arising out of research work conducted during the programme shall be filed by me with due permission of the competent authority of U. P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go-Anusandhan Sansthan (DUVASU), Mathura (UP).

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