Study of bone marrow stimulating property of N-acetylcysteine and Desmopressin for therapeutic management of aplastic pancytopenia associated with canine hemoprotozoan diseases

Thesis

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Izatnagar - 243 122 (U.P.), India

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy
(Veterinary Medicine)

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Dedicated To...

Sacrificed Rats,

My Beloved Family

and

Guide

भारतीय पशु चिकित्सा अनुसंधान संस्थान
(सम विश्वविद्यालय)
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Dated: 11-07-2022

Certificate

This is to be certified that the research work embodied in this thesis entitled **"Study of bone marrow stimulating property of N-acetylcysteine and desmopressin for therapeutic management of aplastic pancytopenia associated with canine hemoprotozoan diseases"** *submitted by* **Dr. Narayani Yadav, Roll No. P-2226,** *for the award of* **Doctor of Philosophy** *Degree in* **Veterinary Medicine** *at Indian Veterinary Research Institute, Izatnagar, is the original work carried out by the candidate herself under my supervision and guidance.*

It is further certified that **Dr. Narayani Yadav, Roll No. P-2226,** *has worked for more than 30 months in the Institute and has put in more than 300 days attendance under me from the date of registration for the Doctor of Philosophy Degree in this Deemed University, as required under the relevant ordinance.*

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Certificate

We the undersigned members of Advisory Committee of **Dr. Narayani Yadav, Roll No. P-2226,** *a candidate for the degree of* **Doctor of Philosophy** *with the major discipline* **Veterinary Medicine,** *agree that the thesis entitled* **"Study of bone marrow stimulating property of N-acetylcysteine and desmopressin for therapeutic management of aplastic pancytopenia associated with canine hemoprotozoan diseases"** *may be submitted in partial fulfillment of the requirement for the degree.*

We have gone through the contents of the thesis and are fully satisfied with the work carried out by the candidate, which is being presented for the award of **Doctor of Philosophy** *Degree of this Institute.*

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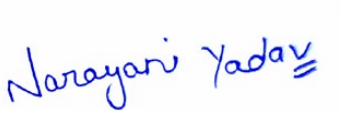

Date: ***(Narayani Yadav)***

Place: *ICAR-IVRI, Izatnagar*

ABBREVIATIONS

%	:	Percent
/	:	Per
<	:	Lesser than
=	:	Equal to
>	:	Greater than
µ	:	Micro
µg	:	Microgram
µl	:	Microliter
A: G ratio	:	Albumin : Globulin Ratio
ALP	:	Alkaline phosphatase
ALT	:	Alanine transaminase
AST	:	Aspartate transaminase
A. platys	:	*Anaplasma platys*
B. canis	:	*Babesia canis*
B. gibsoni	:	*Babesia gibsoni*
BM	:	Bone marrow
Ca^{2+}	:	Calcium
CBC	:	Complete blood count
CHD	:	Canine hemoprotozoan disease
CME	:	Canine monocytic ehrlichiosis
$CO3^{-}$	:	Carbon trioxide ion
CP	:	Cyclophosphamide
CV	:	Central vein
CVBD	:	Canine vector-borne diseases
DDAVP	:	1-deamino-8-D-arginine vasopressin
DPPH	:	1,1 diphenyl 2, picrylhydrazyl
dl	:	Deciliter
DNA	:	Deoxyribonucleic acid
E. canis	:	*Ehrlichia canis*
DIC	:	Disseminated intravascular coagulopathy
DLC	:	Differential leucocyte count
EDTA	:	Ethylene diamine tetraacetic acid
ELISA	:	Enzyme linked immunosorbent assay
et al.	:	*et ali*
E. chaffeensis	:	*Ehrlichia chaffeensis*
E. ewingii	:	*Ehrlichia ewingii*
FAO	:	Food agricultural organization
FRAP	:	Ferric reducing ability of plasma
g	:	Gram
GB	:	Gallbladder
GIT	:	Gastrointestinal tract

GSH	:	Glutathione
h	:	Hour
Hb	:	Haemoglobin
HBD	:	Hepatobiliary disease
H. canis	:	*Hepatozoan canis*
H. longicornis	:	*Hemophysalis longicornis*
H & E	:	Hematoxylene & eosin
HR	:	Heart rate
H_2O_2	:	Hydrogen peroxides
HPC	:	Hemopoeitic Progenator Cell
HSC	:	Hemopoeitic Stem Cell
HUVEC	:	Human vascular endothelial cells
i.e	:	That is
Interleukin- 1	:	Interleukin- 1
IL- 6	:	Interleukin- 6
IL-8	:	Interleukin- 8
I/V	:	Intravenous
IM	:	Intramuscular
iPSCs	:	Induced pluripotent stem cells
IMHA	:	Immune mediated haemolytic anemia
ISTH	:	International Society on Thrombosis and Hemostasis
Kg	:	Kilogram
L	:	Litre
L. infantum	:	*Leishmania infantum*
MDA	:	Malondialdehyde
MDS	:	Myelodysplastic syndrome
mg	:	Milligram
ml	:	Millilitre
MODS	:	Multiple organ dysfunction syndrome
Na^+	:	Sodium
NAC	:	N- acetylcysteine
NBF	:	Neutral Buffered Formalin
NO	:	Nitric oxide
NO^2	:	Nitrogen dioxide
O^2	:	Oxygen
O2-	:	Superoxide anions
OH-	:	Hydroxyl radical
P	:	P- value
PCOP	:	Plasma colloid osmotic pressure
PCR	:	Polymerase chain reaction
PCV	:	Packed cell value
PMN	:	Polymorphonuclear cell
PO	:	Per os
PT	:	Prothrombin time

PV	:	Portal vein
RBC	:	Red blood cell
R. sanguineus	:	*Rhipicephalus sanguineus*
ROS	:	Reactive oxygen species
RNS	:	Reactive nitrogen species
Rpm	:	Rotations per minute
SC	:	Subcutaneous
SE	:	Standard error
SIRS	:	Systemic inflammatory response syndrome
T. evansi	:	*Trypanosoma evansi*
TEC	:	Total erythrocyte count
TLC	:	Total leucocyte count
TNF-α	:	Tumour necrotic factor alpha
TP	:	Total protein
TPTZ	:	2,4,6- tripyridyl-s- triazine
TTBDs	:	Tick and tick borne disease
U/L	:	Unit/litre
UL-vWF	:	Ultralarge multimers of vWF
USG	:	Ultrasonography
vWF	:	Von willebrand factor
WBC	:	White blood cell
WPBs	:	Weibel-Palade bodies

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Introduction

1 INTRODUCTION

Ticks and tick born diseases (TTBDs) are broadly scattered worldwide (FAO, 2004) and are the major hindrance for livestock industry as well as companion animal health care, mainly in subtropical and tropical regions and even in temperate climate regions (Irwin and Jefferies, 2004). Companion animals such as dogs are associated with different haemoparasites and can pose serious health concern worldwide (Manandhar and Rajawar, 2008). In India, climatic conditions are mostly suitable for the growth and multiplication of arthropod born pathogen which act as vector for many diseases in animals including canine (Jadhav *et al.*, 2011). Due to large population of stray dogs in our country, certain disease transmission occurs very rapidly because these stray dogs act as maintenance of host for certain tick borne pathogens which transmits many hemoprotozoan disease (Azaziah *et al.*, 2010). In India dog population is around to be 25 million (Menezes, 2008) and about 5 million dogs within the 'pet' category are suffered from tick infestation (Sudarshan *et al.*, 2006). Ticks spreads a vast majority of pathogenic microorganisms like protozoa, rickettsiae, spirochaetes and viruses (Lee *et al.*, 2005) affecting livestock, humans and companion animals.

Tick transmitted diseases are emerging problem in dogs. In spite of causing severe disease in tropical and semi-tropical regions they also increasing rapidly and recognized as the cause of the disease in dogs in temperature climates and urban environment. Babesiosis, Ehrlichiosis and Hepatozoonosis are the most common arthropod borne diseases of dogs that are prevalent in India (Jadhav *et al.*, 2011). Veterinarians were able to get scarce information regarding the prevalence, epidemiology, diagnosis and management of canine vector-borne diseases (CVBD) (Irwin and Traub, 2006). Clinical manifestations of haemoprotozoan diseases

varies from mild to acute form leading to death if not diagnosed early and treated properly (Guan *et al.,* 2010). High temperature (sometimes hypothermia in severely pancytopenic dogs), anorexia, pale mucus membrane, depression, lethargy, lymphadenomegaly, splenomegaly, ocular abnormalities, aplastic pancytopenia and bleeding tendency are typical clinical manifestations in the naturally-occurring tick born disease. (Mylonakis *et al.,* 2011). Diagnosis of these infections are based on the integrated evaluation of history like prevalence of tick infestation, living in or traveling to the areas where ticks are constantly present or clinical and pathological compatibility.

Oxidative stress develops due to imbalance between radical generating system and scavenging mechanism (Omar *et al.,* 2015). As the liver is a richly blood supplied organ thus it has complex tasks for both synthesis and detoxification. Liver sets to be a productive organ for harbouring infections and infestations. Hepatobiliary diseases are characterized by hepatopathy, ascites, jaundice, weight loss, vomiting and abdominal pain (Sumathi *et al.,* 2017).

Hypo echogenicity of liver, splenomegaly, hepato-splenomegaly, gall bladder distension and ascites in different tick borne intracellular diseases like ehrlichiosis, babesiosis, hepatozoonosis, anaplasmosis and in mixed infection are common during ultrasonographic examination of abdominal region (Sarma *et al.,* 2016).

Tick borne diseases mainly attacks spleen, bone marrow and lymph nodes but may also attacks other internal organs like lungs, liver and kidney (Jacobson and Clark 1994; Sarma et al 2016). Multiple organ failure along with liver and spleen involvement are common in babesiosis (Mylonakis *et al.,* 2010), ehrlichiosis (Ganguly and Mukhopadhayay 2008), *H. canis* infection (Baneth 2006) and anaplasmosis (Carrade *et al.,* 2009)

Immuno-suppressed dogs are unable to eliminate the parasite in acute stage and infection develops into chronic stage resulting into bone marrow (BM) aplasia, peripheral blood pancytopenia and high deaths which may be due to severe bleeding or septicemia (Mylonakis *et al.,* 2004). Aplastic pancytopenia is the most obvious finding in canine hemoprotozoan diseases characterized by hypoplasia of the bone marrow, cytopenias in various cell lines and replacement of bone marrow with adipose tissue with or without fibrosis (Weiss, 2002).

Thrombocytopenia may be due to destruction of platelets by immune mediated reactions, excessive consumption due to mild vasculitis, splenic sequestration, or by BM (Bone Marrow) failure (Mylonakis *et al.,* 2006; Waner and Harrus 2013; Harrus *et al.,* 1998).

Pancytopenia usually represents a diagnostic challenge for presenting a high variability of etiologies. For this reason, when the diagnosis is not evident, the biopsy or bone marrow aspiration cytology is necessary (Weinzierl and Arber, 2013). The cytological examination allows demonstrating a more detailed cellular morphology and is indicated for neoplastic staging and diagnosis, iron store estimate and unknown fever origin evaluation (Stokol, 2000). Bone marrow (BM) aspirate alone is not adequate for confirmatory diagnosis, as hypocellular marrow lacking of hemopoietic cells so mainly capitulates a little material. So biopsy of bone marrow and histopathologic examination are mandatory for confirmatory diagnosis of aplastic anaemia (Young *et al.,* 2006). Quantitative alterations should be analysed by myelogram and also require concurrent assessment of peripheral blood for essential findings. Pancytopenic dogs should be evaluated for developing sepsis or this being the pancytopenia cause. The neutrophils left shift in complete blood count (CBC) might be the point in predicting whether sepsis is the cause or a consequence of hematologic condition. Its absence, as in the animal mentioned earlier, suggests that bone marrow suppression is linked to another cause and sepsis condition is secondary. Once when sepsis is the primary pancytopenia cause, there is an increase in leucopoiesis and mature cell mobilization, resulting in a left shift (Weiss, 1999).

In the pancytopenic severely ill dog, supportive treatment is crucial if the limited chances for survival are to be pursued which includes the administration of balanced crystalloid solutions and or periodic blood-typed and cross-matching of packed RBC or whole blood (20 ml/kg) transfusions. Platelet components may also be given, but they are impractical in the clinical setting. A fresh whole blood unit may increase the platelet count of a 20 Kg dog by approximately 20,000-30,000/ µl and may be of help to stop the ongoing hemorrhages (Levi, 2004). Iron sulphate supplements (100-300 mg, daily, per os x 3-5 months, at least 2 hours before, or after Doxycycline per orally), are also indicated, as in dogs suffered with chronic pancytopenia there is iron depletion, presumably due to the chronic hemorrhagic tendency (Mylonakis *et al.,* 2010). In canine monocytic ehrlichiosis (CME) associated pancytopenia, the initially choice

of antibiotic is mainly depend on its effectiveness and adequecy to reach in intestine which decontaminate or reduces Gram negative and Gram positive aerobic bacteria but produces no effect on anaerobic intestinal flora). The choice of antibiotic should have lesser effect on platelet function so that less chances of toxicity to an already compromised BM (Abrams-Ogg, 2000). In this respect, Sulfonamides, Chloramphenicol and Penicillin are to be avoided in the myelo-suppressive CME (Weiss, 2002; Stokol, 2000).

In the recent years, there have been several drugs which increases the platelet production in the BM and were used for supportive therapy in chemotherapy induced thrombocytopenia or pancytopenia. Important agents like eltrombopag, romiplostim are thrombopoietin receptor agonists having favourable results and have less role in immunogenicity. Instead of this, it is not clear whether use of eltrombopag is beneficial for avoiding adverse effect of chemotherapy in cancer patients or cost limits is affordabile and accessable (Kuter, 2015). Hence, there is requirement to inspect other option to treat chemotherapy induced thrombocytopenia or pancytopenia.

Cyclophosphamide (CP) is a chemotherapeutic alkylating agent widely used against a variety of malignant tumors and some immune diseases. Also, it also been used as an immunosuppressive agent for organ transplantation, multiple sclerosis, and systemic lupus erythematosus (Feng *et al.,* 2016). Like other chemotherapeutic drugs, cyclophosphamide has wide network of adverse effects such as the severe reduction in the platelet count, white blood cells and red blood cells (WBCs, RBCs). It can cause severe thrombocytopenia, as well. CP not only shows impact on neoplastic cells but also influences healthy cells, and it can results into more free radicle formation and nitric oxide (NO), leading to peroxynitrite generation which damages the cellular proteins, DNA, and lipids (Abdel-Hafez *et al.,* 2017). Thymus and spleen are two main immune organs that play vital role in cellular and humoral immunity. The functions of thymus and spleen can be embarrassed by cyclophosphamide (Zhao and Liu, 2009).

Disease specific therapy is targeted to remove the infective origin of various hemoprotozoan diseases which may be or may not be heals the certain pathological condition

like bone marrow stimulating activity which is required to overcome evolved pancytopenia. For effective stimulation of bone marrow activity, therapy requires disease oriented interventions with the aim for elimination of causative factors and controlling physiological complications that arises in the course of disease. However, specific therapy has often been not much effective or takes longer duration for stimulation of bone marrow (BM) activity when damage to bone marrow is of greater degree or of continuing nature. Attention has been recently focused on bone marrow stimulating activity as the major candidate factor in aplastic pancytopenia. Medicines like N - acetylcysteine (NAC) and Desmopressin may accelerate bone marrow stimulating activity.

N - acetylcysteine (NAC), a glutathione (GSH) precursor mucolytic agent (Mokhtari, et. al. 2017) helps in kidney and liver damage by detoxification (Amaral *et al.,* 2016) and improves immune function, inhibits inflammation (Raffaele, et al ,2018) and act as nutritional supplement, powerful antioxidant (Pei *et. al.,* 2018). It also improves viability and give rise to indefinitely more stem cells of same cell type (Shaban *et. al.,* 2017), increases production of bone marrow B Cells (Palmer, 2011) and improves hematopoietic differentiation by protecting induced pluripotent stem cells (Berniakovich *et al.,* 2012).

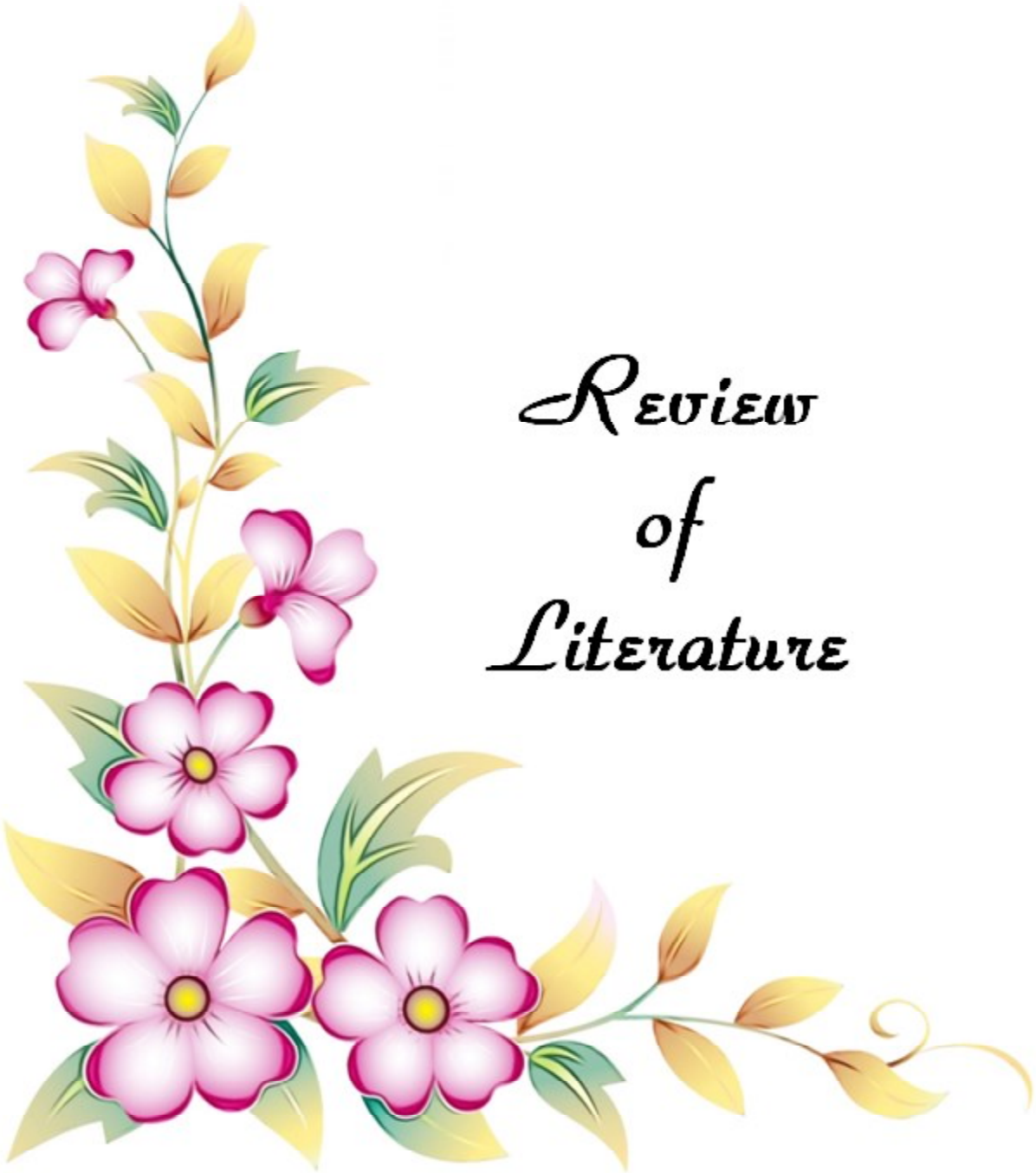
Desmopressin used in human patients with mild alteration in platelet function (Collucci *et al.,* 2014). It enhances selectively and markedly formation of pro-coagulant platelets (Rosemarie *et al.,* 2003), increases platelet-dependent thrombin generation by enhancing Na(+)/Ca(2+) mobilization (Pier and Armando. 2012) and plays a considerable effect on haemostasis by releasing von willebrand factor from the storage sites - endothelial cells. It also releases large molecular weight VWF multimers, which increases the activation of platelets & helps in thrombus formation in post surgery (Swieringa *et.al.,* 2015).

Several past studies have proven that antioxidants not only helps in alleviating oxidative stress or improvement in survival of stem cell but also have affects on the strength and differentiation of stem cells. Colucci *et al.* (2014) hypothesized that Desmopressin (DDAVP) not only favors the haemostatic process by releasing von willebrand factor (VWF) from the storage site i.e endothelial cells, but also increases the procoagulant activity of platelets that

also plays an essential role in hemostasis. It is impossible to prepare standardized therapeutic strategies, that are applicable in all situation of aplastic pancytopenia and hepatobiliary disease (HBD) and the preferred approach is to analyze thoroughly the situation and apply the "best bet" options effectively as a combination. Despite substantial recent advances in knowledge regarding biology of tick borne diseases and its immuno-pathogenesis, diagnostic testing, chemotherapy and blood transfusion, we are still unable to attain novel therapeutic approaches for aplastic pancytopenia in various hemoprotozoan diseases in canine.

Considering all these facts, the present work has been envisaged with the following objectives:-

- **To study bone marrow stimulating property of N-acetylcysteine & Desmopressin.**
- **To develop novel therapeutic regimen for management of aplastic pancytopenia associated with canine hemoprotozoan diseases**.



Review of Literature

2 REVIEW OF LITERATURE

2.1. Canine hemoprotozoan diseases

2.1 Background

At about ten to fifteen thousand years ago, dog was the first domesticated pet animal and act as best companion animal for human beings due to its faithfulness, usefulness and intelligence like qualities. In india, dog population is around 25 million (Menezes, 2008). Like other animals, dogs also suffer from many infectious diseases like bacterial, viral and many parasitic diseases. Various type of ecto and endoparasites attacks dog. Stray animal may also act as source of infection to pet dogs. Out of various ectoparasites, ticks are the main vector for many hemoprotozoan diseases in canines as they sucks blood of animal and causes annoyance, irritation to dogs. These tick and tick born diseases are more prevailing in subtropical and tropical countries (Irwin and Jefferies, 2004). As these tropical and subtropical regions contains moist and humid climatic conditions that favors the growth and development of ticks (Jadav *et al.*, 2011). Among all the tick born diseases, hemoprotozoan infections are of great importance because it causes severe loss due to their high pathogenicity. Babesiosis, Ehrlichiosis and Hepatozoonosis are the major arthropod carrying hemoprotozoan infection in dogs prevalent in India (Jadhav *et al.,* 2011).

2.2 Major hemoprotozoan diseases in canines

Babesia and Ehrlichia are the major spp that causes most of the tick born hemoprotozoan diseases in canines. Co-infection with other species may also occur like *Babesia with bartonella Ehrlichia, Hepatozoon, Leishmania and Rickettsia* species (O'Dwyer *et al.*, 2001).

2.2.1 Babesiosis

Canine babesiosis is the major tick born hemoprotozoan disease across the world in both domesticated and wild Canidea that parasitize the red blood cell which leads to progressive anemia. Babesia spp are morphologically categorized into small form (*Babesia gibsoni)* and larger form *(Babesia canis). Babesia gibsoni* is highly pathogenic and is transmitted by *Rhipicephalus sanguineus* (Senthil *et al*., 2009). Dogs may also gains the infection by ingestion of infected ticks that contains sporulated oocyst (Soulsby, 1982). This infection also occurs in dogs by blood transfusion. The major clinical signs associated with this babesia infection are anorexia, lethargy, vomition , jaundice, anemia, enlargement of spleen, Tachycardia , tachypnea and weight loss.

2.2.2 Canine hepatozoonosis

It is the hemoprotozoan disease caused by *Hepatozoon canis* and is communicated by ingesting infected tick *R. sanguineus* but not transmitted by the biting of ticks, as the infective stages don't reach to salivary glands of the ticks (Baneth *et al.,* 2001). In India, the prevalence of *Hepatozoon canis* was studied by Pawar and Gatne (2005); Lakshmanan and John (2007) and Palanivel *et al.* (2010). The clinical signs of *H. canis* infection ranges from subclinical to serious life-threatening state (Baneth *et al.*, 2003). The infection of H. canis mainly affects the blood forming tissues or organs such as the spleen, bone marrow and lymph nodes. In severe clinical conditions, high temperature, anorexia, weight loss, hyperglobulinaemia that results into hepatitis, anaemia, glomerulonephritis and pneumonia. Ehrlichia, Leishmania and parvovirus occurs concurrently with *H. canis* (Baneth and Weigler, 1997). *Ehrlichia. canis* and *Babesia.gibsoni* also present concurrently with *H. canis* infection that resulting into thunder attack of ehrlichiosis and babesiosis in canines (Harikrishnan *et al.,* 2005). The preventive measures of *H. canis* infection is depends upon the productive tick control program. Regular cleaning and combing of dogs would prevent them tick infestation.

2.2.3 Canine trypanosomosis

It is a worldwide disease of the Western Hemisphere and is endemic in Central America and Southern regions affecting animals and humans (Barr *et al.*, 1991). In dogs, this disease is

mechanically transmitted and also by consumption of the infected animal carcass. *Trypanosoma evansi* infection produces severe disease in dogs reflecting with the clinical signs of restlessness, high temperature, opacity of cornea, pale mucus membrane, enlarged liver with generalized edema which rapidly progress to death. Thick or thin blood films and buffy coat smears are used for observing trypomastigotes stage. Diagnosis of canine trypanosomiasis is also done in tissue by mouse inoculation. However, microscopy examination may under-diagnose the disease as in chronic infection parasitemia level is very low (Da Silva et al, 2009). Recently, a TaqMan Polymerase Chain Reaction (PCR) assay using ribosomal DNA has been used to confirms *T. evansi* infection (Taylor *et al.,* 2008). This parasites have also been seen in clinically normal dog blood, which suggests that the *T. evansi* does not always cause severe disease and subclinical infection may occur (Irwin and Jefferies 2004).

2.2.4 Ehrlichiosis

Ehrlichia is an alpha-proteobacteria and belongs to the Ehrlichiaceae family. It is a pleomorphic, gram negative, obligate intracellular bacteria belonging to the genus Ehrlichia which mainly affects monocytes, lymphocytes and macrophages by forming intracytoplasmic, membrane-bound bacterial aggregates, called morulae (Sainz *et al.,* 2015). In dogs, *E. canis* causes tropical canine pancytopaenia, *E. ewingii* causes canine granulocytic ehrlichiosis, and *E. chaffeensis* causes monocytic ehrlichiosis in humans (Anderson *et al.*, 1991). *E. canis* is the most commonly found species that affects dogs and causes serious disease. *E. canis* infection is broadly scattered across subtropical and tropical regions and its prevalence depends upon the distribution of tick vector *Rhipicephalus sanguineus* (Shaw *et al.*, 2001).

2.2.5 Mixed infection

Mixed infection with Leptospira and *E. canis* was also found in a dog (Sangeeta et al., 2019)**.** Candidatus Mycoplasma haematoparvum and Mycoplasma haemocanis are the two spp that have been suggested in dogs. These two spp shows their clinical effect mainly in splenectomised or immunocomprised dogs which varies from asymptomatic infections to serious haemolytic syndrome (Brinson and Messick 2001; Sykes *et al.,* 2005). In India, the disease caused by Mycoplasma haemotropic (Dharaskar *et al*., 2003) and A. platys infections (Kumar

and Varshney 2007) have been reported but the prevalence and dispersal of these microrganisms remains largely undected. Infections related with multiple tick pathogens may occur in animals in endemic areas, because the same tick may act as vector for several pathogens (Kledmanee *et al.,* 2009).

2.3 Prevalance of hemoprotozoan infection in India

Several studies have been carried out for investigating the prevalence of various hemoprotozoan infection in India. Nair *et al.* (1979) found that the occurance of babesiosis in canine in New Delhi was 30.8 % while in Madras, Lakshmanan and John (2007) suggested incidence of *B. canis* and *B. gibsoni* in 4.34% of dogs and in 8.69% of dogs suffered from *Ehrlichia* and *Hepatozoon* infection coincidently. In Chennai only 0.1% of dogs were suffered from *Babesia gibsoni* infection (Sunder et al, 2004). Other studies reported that 9% and 22% dogs in UP and Assam affected with babesia infection respectively (Chaudhari, 2006). *B. vogeli B. gibsoni* these both species are endemic in **I**ndia and transmitted by *R. sanguineus* and *H. longicornis* (Abd Rani et al, 2011). Gadahi *et al.* (2008) in Hyderabad area reported the prevalence of blood-parasites in stray and pet dogs and also investigated an overall prevalence of blood-parasites as 11.66 % and of which 5% was *Babesia canis* infected dogs. Godara *et al.* (2010) suggested that 16.39% was the prevalence of haemoprotozoan infection in dogs overall, out of which 13.1% were Babesia spp. and 4.9% were *Ehrlichia canis*. From 2006-2011, in Madras Veterinary College Teaching Hospital, Vairamuthu *et al.* (2014) observed that 13.25% was the prevalence of blood protozoan diseases among dogs. Among all the blood borne infection in dogs, *B. gibsoni* infection was highest i.e 56.65 % followed by *E. canis* (23.21 %) and *H. canis* (11.23 %). In Ludhiana, Punjab an overall prevalence of canine babesiosis was 7.47% out of which 0.93% dogs infected with *B. canis* and 6.54% of dogs infected with *B.gibsoni* using Giemsa-stained peripheral thin blood smears (Singh *et al.* (2014). Chowdhury *et al.* (2005) performed a survey of trypanosomosis on dogs in Kolkata and reported 1.72% of infection. 10.54% was the overall prevalence of haemoprotozoan parasites in Bhubaneswar, Odisha. Out of which 4.81% was of *Babesia gibsoni*, followed by 3.33% *Hepatozoon canis* and then by 1.66% *Babesia canis* and by 0.74% *Trypanosome evansi*, respectively (Sahu *et al.,* 2014). Selvaraj *et al.* (2010) reported

11.74% of dogs were found positive for haemoprotozoan infections in Chennai. Abdrani *et al.* (2011) investicated that 39% of cases were of **c**oncurrent infection with more than one hemoparasites**.**

2.4 Pathogenesis of hemoprotozoan diseases

2.4.1 Ehrlichia canis

Ehrlichia canis is naturally transmitted disease which spreads transstadially by the tick *Rhipicephalus sanguineous* (Sainz *et al*., 2015). Incubation period of *Ehrlichia canis* is of 8-20 days. The course of this infection completed into acute, subclinical and chronic phage (Harrus *et al.,* 2012). Acute phage persists for 2-4 weeks and subclinical for several months to years. Immunocompetent dogs may shows clinical recovery from acute or subclinical phases of infection (Codner and Farris-Smith 1986; Breitschwerdt *et al*., 1998), but some dogs will eventually enters into the chronic stage of infection, characterized by aplasia of bone marrow (BM) , myelosuppression , pancytopenia and high mortality due severe bleeding or septicemia (Mylonakis *et al*., 2004). German Shepherd breed are more susceptible to *E. canis* infection and other vector-borne pathogens concurrently (e.g. Leishmania infantum, Anaplasma spp., Babesia spp., Rickettsia spp., Bartonella spp. Cellular immunity plays a crucial role in protecting the dogs against *E. canis* but the exuberant humoral immunity confers no protective response, but in fact may be harmful to the host (Breitschwerdt *et al*., 1998).

The other manifestations of this disease includes, including glomerulonephritis, uveitis, thrombocytopenia, anemia, lymphocytic-plasmacytic infiltration of many parenchymal organs, polyclonal hyperglobulinemia and antiplatelet antibodies due to an immune-mediated complexes in the circulation. (Hildebrandt *et al.,* 1973; Harrus *et al.,* 1996; 1998; Mylonakis *et al.,* 2006; Waner and Harrus 2013). Bleeding tendency is related with impaired primary hemostasis which is the hallmark of *E. canis* infection occurs due to decrease platelet count, mild vasculitis and thrombocytopathy (De Castro *et al.,* 2004; Mylonakis *et al.,* 2004). Thrombocytopenia may be related with immune-mediated destruction of platelets, excessive consumption secondary to mild vasculitis, overexpression of a platelet migration inhibition factor, splenic sequestration and BM failure in the myelosuppressive CME (Harrus *et al*., 1996, 1998).

2.4.2 Pathogenesis of Babesia spp

Babesia spp. mainly cause disease in young dogs, but dogs of all age group can be affected. The incubation period for canine babesiosis ranges from 10-28 days. After feeding on their host for about one week, female ticks left their host for disease to develops. The pathogenicity of the disease depends upon age and immune status of the animal, species of *Babesia* and *the* presence of concurrent infections. The clinical signs include fever, anemia, depression, less feed intake, increased heart rate (HR), respiratory rate, weakness and splenomegaly. Clinical signs are related with anemia and systemic inflammatory response syndrome (SIRS) which may be due to tissue hypoxia and marked cytokine release. The severity of disease is marked by haemolytic anaemia, acid-base abnormalities and secondary multiple organ failure such as acute kidney failure, hepatic failure with marked icterus, decreased glucose level, acute respiratory distress and cerebral edema. A small number of dogs show high haematocrits value (relative haemoconcentration) despite vigorous haemolysis, due to presumed shifting of fluid from the intravascular to the extravascular component.

2.4.3 Pathogenesis of Hepatozoon canis

The main gross pathological finding in dogs, infected with *Hepatozoon canis* is cachexia. Muscle atrophy, anaemia, mildly icteric mucous membrane and slightly enlarged spleen and liver are observed. Congestive changes in the lung parenchyma and the mucosa of stomach, pale kidneys and lymphadenopathy are also communicated (Gevrey 1993; Craig 1990). On histologicl examination, schizonts are found in the skeletal muscles, cardiac muscles, lymph nodes, spleen, liver and kidneys etc. (Baneth, 2006; Craig 1984). 2 kind of schizonts are noticed: microschizonts and macroschizonts. Microschizonts contain micromerozoites and macroschizonts occupies macromerozoites. Microschizonts are observed more frequently in various organs. On schizont formation, no cellular reaction occurs. But on the release of merozoites, a vigorous cell response is detected, containing an equal amounts of neutrophils and macrophages but varying number of eosinophils. In the skeletal muscle, cysts are detected. They form clusters of neutrophils in the muscles that cause pain, fever and periosteal proliferations.

2.5 Hematobiochemical changes

Baneth and Weigler (1997) studied decrease in hemoglobin concentration, total RBC count, hematocrit, platelet count and total neutrophil count in *Hepatozoon canis* infected dogs. The Complete Blood Count (CBC) in Babesia infected dogs shows a decrease RBC count. There may also be elevated (leukocytosis), normal, or decreased (leukopenia) white blood cell count (WBC), that mostly depends on the etiology of the liver problem and how long it has been present (Jain, 1986). The most common abnormality in 100% of dogs suffering from babesiosis was thrombocytopenia. The mechanism related to thrombocytopenia in babesiosis is not yet fully described; various mechanisms, including platelets sequestration in the spleen, immunemediated destruction of platelet and progression of disseminated intravascular coagulation (DIC) (Boozer and Macintire, 2003). Salakij *et al.* (1999) found that most of the dogs infected with haemoprotozoan infection exihibits anemia, lymphopenia, hypoproteinemia, leukopenia and eosinopenia. Marked increase in serum ALT activity may develop in association with haemoprotozoan diseases (Hoe and Jabara, 1967). Ongoing hepato-cellular damage is characterized by consistent elevated serum alanine amino transferase (ALT) activity (Sterczer *et al.,* 2001). Soja (2003) reported increased serum ALT activity in dogs with hepatopathy. Hyperproteinemia due to hyperglobulinemia, hypoalbuminemia and mildly increased ALT and alkaline phosphatase (AP) activities are common biochemical alterations in *E. canis* infection (Mylonakis *et al.,* 2011; Frank and Breitschwerdt 1999). Pancytopenic dogs mostly have lesser total protein (TP), total globulin and γ-globulin values as compared to their non-pancytopenic dogs . Alterations in Liver parameters may be primarly or secondarly related to hypoxia, septicemia or intrahepatic hemorrhage in the myelosuppressive *E. canis* infection (Decastro *et al.,* 2004; Mylonakis *et al.,* 2010).

2.6 Oxidative- Antioxidant status in CHD

Hemoprotozoan parasite**s** have concerned with the oxidative level inside their host cells. Reactive oxygen species (ROS) are very reactive or excited metabolites of molecular oxygen (O2) produces in the cytosol or in mitochondria or the peroxisomes by aerobic metabolism that causes oxidative stress (Andreyev *et al.*, 2005). These oxygen metabolites

are superoxide anions (O2-), hydrogen peroxides (H_2O_2) or the highly reactive hydroxyl radical (OH^-) that is formed in the presence of metal ions via the Fenton and/or HaberWeiss reactions (Massimine *et al.,* 2006). Increase in the reactive species or free radicle ROS can induce oxidative stress in the DNA, proteins, and lipids (Trachootham *et al*.,2008), which ultimately results into to cellular death. Oxidative stress is noticed when oxidants level exceeds than that of antioxidants (Lykkesfeldt and Svendsen 2007). Variation of oxidative stress indices have been noticed in various parasitic diseases (Dede *et al*., 2002; Pabon *et al*., 2003).

Malondialdehyde (MDA) is a product of lipid peroxidation that can be used as an ideal marker of oxidative stress (Kuhn and Borchert 2002). Malondialdehyde, reacts with elements of cell membrane and thereby increases the permeability of cell and enzyme which leads to destruction of erythrocytes. Erythrocytic peroxidation plays a crucial role in the pathological progress of haemoparasitic infestations (Otsuka *et al*., 2002; Nazifi *et al*., 2008). Superoxide dismutase, reduced glutathione and catalase are the antioxidants that varies in various parasitic infestations and plays vital in counteract ROS damage (Nazifi *et al*., 2011; De *et al*., 2012; Omar *et al*., 2015). Recently in blood parasites studies, strong confirmation were found between resultant anemia and oxidative damage due to lipid peroxidation of red cells (Nazifi *et al*., 2011).

2.7 Hepatobiliary dysfunction and Ultrasonographic study

Hepatobiliary dysfunction occurs in many of acute and chronic infections such as drug related hepatotoxicity, many infectious diseases, metabolic disorders, congenital or neoplastic diseases and certain vascular injury. Hepatopathy is a common problem noticed in canine babesiosis (Jacobson 2004; Máthé *et al.,* 2006). Liver disease may be primarly or secondarly related with hypoxia, hemorrhage within hepatic parenchyma, or septicemia in the myelosuppressive canine monocytic ehrlichiosis (De Castro *et al.,* 2004; Mylonakis *et al.,* 2010). Non regenerative anemia which is mainly normocytic and normochromic observed in dogs suffered from babesiosis is often associated with inadequate use of systemic iron store (Webster, 2005) is an important factor that causes hypoxia and hypoxic liver injury, which can result in an increase in ALT, AST, and ALP activities (Reyers *et al.,* 1998; Taboada and

Lobetti 2006). On histopathological examination, the liver section showed severe fatty degeneration of hepatocytes with infiltration of few inflammatory cells in *H. canis* onfection (Raguvaran *et al.,* 2018). Cholestasis is probably caused by hepatomegaly, may be the one of reasons of the marked increase in ALP activity. Ascites in certain hemoprotozoan diseases may result due to severe hypoproteinaemia, fall of plasma colloid osmotic pressure (PCOP), renal, cardiac or hepatic insufficiency and protein losing enteropathy etc. of which liver disorder is one of the important causes of this problem (Chakrabarti, 2006). The clinical signs related with heptobiliary disorders directly or indirectly reflects the gastrointestinal, haematopoietic and renal system disorders.

The main target organs affected in various tick borne diseases are BM, spleen, lymph nodes and many other internal organs such as the kidney, liver and lungs (Jacobson and Clark 1994). Multiple organ dysfunction along with with liver and spleen is common finding in canine ehrlichia (Ganguly and Mukhopadhayay 2008), babesia (Mylonakis *et al.,* 2010), anaplasma (Carrade *et al.,* 2009) and H. canis infection (Baneth 2006). In tick borne haemoparasitic diseases, Ultrasonographic examination of liver and spleen may be used as an important diagnostic tool which is apart from other diagnostic procedure. Ultrasonography examination of abdomen in various tick born diseases and in mixed infection revealed hyperechogenicity and hypoechogenicity of liver, hepato-splenomegaly, splenomegaly, gall bladder distension and ascites (Kumar 2004).

In babesiosis, USG of abdominal region showed hypoechoic images with hepatomegaly, splenomegaly (Eduardo *et al.,* 2011) and enlargement of gallbladder (GB) (Adaszek *et al.,* 2009). According to Kohn et al. (2008), hyper echogenicity of liver with splenomegaly is noticed in case of anaplasmosis. Enlargement of liver and spleen may be due to multiplication of pathogens within circulating mononuclear cells and phagocytic tissues of liver, spleen, and lymphnode (Hildebrandt *et al.,* 1963). In case of tick born diseases, hepatic enlargement may be due to passive venous congestion, hyperplasia of reticuloendothelial cell or infiltrative diseases mediated through cytokines (Meyer and Twedt 2000). Splenomegaly may be due to hyperplasia of reactive lymphoid cells and synchronous with extramedullary hematopoiesis (Egenvall *et al.,* 2000).

2.8 Sepsis

It is the main cause of pancytopenia. Septicemia can affect bone marrow by endotoxin production which causes the destruction of BM precursors (Weiss 2002). In septicemia the hematologic findings includes an inflammatory leukogram with a degenerative left shift, neutropenia, mild to moderate anemia, and thrombocytopenia.

2.9 Disseminated Intravascular Coagulation (DIC)

DIC occurs due to excessive activation of coagulation which results into intravascular formation of fibrin and thrombotic plagues resulting into occlusion of small and medium size blood vessels. Intravascular coagulation hampers the blood supply to various organs leads to multiple organ failure. Haemoprotozoan diseases like *B. gibsoni* infection may cause disturbance in the blood coagulation mechanism which results in disseminated intravascular coagulopathy (DIC) and further leads to multiple organ failure (Bick *et al.,*1999). In haemoprotozoan diseases, platelet count decreases and prothrombin time prolonged. The most hyperfibrinolysis is caused by high offense factors or low defense factors. Recently, hypercoagulability results into organ failure which is considered to be an important factor in the pathogenesis of DIC. Diagnosis is done mainly by knowing abnormalities in atleast 3 or 4 laboratory parameters such as thrombocyte count, prothrombin time (PT), fibrinogen and fibrin degradation products (Wade *et al.,* 2003). Recently, many animal experiments and clinical studies showed that certain factors such as chemical mediators, leukocyte activation and vascular endothelial injury exhibits multi-organ failure (Ten Cate *et al.,* 1999).

2.10 Physiology of Von Willebrand Factor (VWF)

Plasma level of FVIII decreases rapidly without the VWF depending on the severity of the disorder which resulting into a haemostatic defect similar to haemophilia A. (Ginsburg 2001). VWF liberates into plasma as a large multimeric glycoprotein molecule, which is immediately cleaved down by a protease into several multimers of high, intermediate and low molecular sizes. The protease that breaks VWF immediately release into the plasma, and its deficiency results into the pathological process of thrombotic thrombocytopenic purpura (Levy *et al.,* 2005). In this disorder, aggregation of platelet occurs near and around unusually large

VWF molecules in circulation, leading to disseminated thrombosis which results into multiorgan dysfunction. *B. gibsoni* parasite damages the endothelium of blood vessels which release VWF from preformed stores (Weibel-Palade Bodies), in ultralarge multimers of vWF (UL-vWF) (Ribes *et al.,* 1987). UL-vWF multimers are more active in inducing coagulation that leads DIC which results into multiorgan dysfunction (Levy *et al.,* 2005).

2.11 Effect of CHD on Bone marrow (BM)

It is the primary lymphoid tissue and a major hemopoeitic organ in the body of animal. BM is is infected with many tick born pathogens like *Hepatozoon canis*, *E. canis***,** Leishmania and Anaplasma therefore BM is a sensitive tissue for identification of these pathogens (Solano-Gallego *et al.,* 2011; Cardoso *et al.,* 2014). The presence of these tick borne pathogens in BM causes substantial alternations in numbers and function of erythrocyte**,** granulocyte, lymphocyte, monocyte and thrombocyte. Due to peripheral cell destruction, the bone marrow is often hypercellular in acute stages. Excessive destruction and consumption of platelets and sequestration may leads to thrombocytopenia. In the acute stage of infection, suppression of erythrocyte production and destruction may leads to progressive anemia. In chronic infections, aplastic anemia can occurs; the mechanism behind this BM suppression and subsequent hypoplasia of all precursor cells of BM is not well described (Neer *et al.,* 2002).

Alterations in BM related with L. infantum infections included emperipolesis, BM aplasia and megakaryocytes dysplasia, whereas *E. canis* infection cause myelosuppression. The typical BM alterations related with *H. canis* infections were dysmegakaryocytopoiesis and dysgranulopoiesis. In dogs suffered with *A. platys* infection, hyperplasia and dysplasia of megakaryocytic cells were found even in absence of thrombocytopenia, whereas L.infantum infection was associated with erythroid BM suppression, atypical mitosis and the BM infiltration with lymphocytes and plasma cells. Severe BM depression was found in dogs infected with more than one tick borne pathogens (Anna *et al.*, 2014).

2.11.1 Aplastic Pancytopenia

It is a rare immune-mediated myelodysplastic syndrome of dogs characterized by pancytopenia (reduced red and white cell numbers) in peripheral blood, associated with bone

marrow hypocellularity (Weiss *et al.,* 1999). It is derived from Greek word "pan" means all and is defined as decreased in number of all type of marrow cells i.e myeloid, erythroid, and megakaryocytic cells. The clinical findings associated with pancytopenia are pallor mucus membrane, petechiae hemorrhage which is related to anemia and thrombocytopenia. The causes of decresed production of marrow cells are due to hypoplasia or aplasia, necrosis, fibrosis/sclerosis of BM, myelophthisis, and myelodysplastic syndrome (MDS). BM aplasia and hypoplasia is believed to be due to destructions of stem cells or genetic defects. The aplastic term is used when all marrow hematopoietic cells are markedly reduced or absent whereas in hypoplastic and aplastic marrow, Stromal cells, like adipocytes, reticuloendothelial cells, macrophages, plasma cells as well as lymphocytes may be still present despite the decrease in other cell lines (Harvey 2001; Searcy 2001). Many infectious agents, neoplastic conditions and inflammatory reactions, or immune-mediated diseases results into decreased production of cell lines by various mechanisms (Weiss, 2002).

2.12 Effect of chemicals on Bone Marrow (BM)

Various immune mediated reactions and certain drugs like cyclophosphamide, vincrystine, doxarubicine and phenylbutazone etc also causes BM suppression. The severity of BM suppression associated with drugs depends upon dose, idiosyncratic, or with hypersensitivity or an immune-mediated reaction. Hematopoietic tissues may either affected directly by various chemicals or its metabolites or indirectly affected by damaging other organ systems or metabolic pathways. Lesions in the BM due to chemical exposure may be observed on paraffin-embedded sections. As the blood forming organ are the major target for chemical exposure so blood and bone marrow evaluation are the vital component for toxicity studies and safety assessment. CP is a pro-drug with its two active metabolites, phosphoramide mustard (PM) and acrolein. PM is an anticancer metabolite, whereas acrolein is a toxic metabolite, responsible for hematological toxicity and myelotoxicity (Brock and Hohorst 1967). CP or its metabolite reacts with glutathione (GSH), restricts its antioxidant activity, increases the production of reactive oxygen species, and causes lipid peroxidation that leads to oxidative stress (Patra *et al.,* 2012; El-Naggar *et al*., 2018). The evaluation of hematopoietic system is done by tissue sectioning of BM which gives information about tissue architecture of BM, iron

stores in the BM and identification of pigment, infectious agents, proliferative or neoplastic disorders. The histopathological studies of BM gives great information about the blood forming system that might be overlooked by peripheral examination of blood alone.

2.13 Diagnosis

The traditional method for identifying the hemoprotozoan parasite in infected animals is by microscopic examination of thick and thin blood films. In acute infections, parasites are most readly found in blood and tissue than chronic infection. Thick films can be mostly used in identifying small numbers of parasites, but identification of species is best detected in thin blood films. Babesia can be detected in giemsa stained blood smears under oil immersion. However, for hepatozoonosis and Trypanosomiasis, diagnosis is done by microscopic examination of Giemsa stained thin blood smear or buffy coat smears. Various serological tests can be used for diagnosis of hemoprotozoan parasites. Among serological tests, immunofluroscent antibody test can be used for detecting serum antibodies that reacts with hemoprotozoan organisms. However disparity between species and sub species creates hindrance due to cross reactive antibodies in some infected animals. To overcome this hindrance molecular (PCR based) diagnosis can be used. Among the molecular diagnostic techniques, conventional PCR, PCR with RFLP, RAPD-PCR and Multiplex PCR can be easily used as these techniques are more sensitive and analysis large number of samples at single time (Dey and Singh, 2009).

2.14 Treatment of Aplastic Pancytopenia

The treatment of aplastic anemia is very less effective and mainly depends upon the elimination of the etiological agent. The approach to treatment is mainly supportive care that is based on hospitalization, oxygen therapy, blood transfusions, intravenous fluids, and antibiotics for secondary infections. If pancytopenia is due to autoimmune disorder then immune-suppressive medications, such as cyclosporine, prednisone, azathioprine, or intravenous immune globulin, may be indicated, if Hematinics such vitamin B12, folic acid, irons, and crude liver extract does not play a great role in the treatment of aplastic anemia (Wintrobe and Bithe, 1970). Some dogs suffered with aplastic pancytopenia may recover but prognosis is guarded.

2.15 Conflicts in therapy of canine hemoprotozoan disease

The main aim are to remove the pathogen and alter the severe anaemia. After initial parasitemia, the immune system does not fully remove the infection, and animal goes into the chronic carrier state. Reoccurance of infection may occur after several months to years, and resulting into the sequelae of glomerulonephritis or polyarthritis (Wozniak *et al.,* 1997; Casapulla *et al.,* 1998; Lobetti, 1998). No single drugs have been demonstrated to clear hemoprotozoan infection like *B. gibsoni* (Asian genotype) infections from dogs (Vercammen et al., 1996; Wulansari *et al.,* 2003; Birkenheuer *et al.,* 2004; Vial and Gorenflot, 2006; Matsuu *et al.,* 2008; Sakuma *et al.,* 2009). Small Babesia spp are generally more difficult to treat (Boozer and Macintire, 2003). Successful treatment of *B. gibsoni,* has been particularly demanding (Wulansari *et al.,* 2003). Many drugs such as babesiacidal agents (imidocarb diproprionate, diminazene aceturate), antibiotics (doxycycline, clindamycin, azithromycin, metronidazole) and an antiprotozoal agent (Atovaquone) have been used in the management of babesiosis. Several studies showed that outcome of *B. gibsoni* infection in animals resulting into chronic infections, relapses, persistant parasitemia and animal shows poor response to therapy (Meinkoth *et al.,* 2002).

Medications such as erythropoietin or granulocyte stimulating factor can be used in few patients for stimulating the BM, but it is unclear whether they provide beneficial effects. Whereas people suffering with aplastic anemia may go for transplantation of BM, but this method is not commonly used for animals (Merrill 1970) Some dogs suffered with aplastic pancytopenia may retrieved but prognosis is guarded. The main conflicts in treating thrombocytopenia by platelets transfusion is short-life of platelet (a few hours) which rises the count for short period and platelet have less chances of survival post-transfusion. The potential risks and complications related to blood transfusion may sometimes exceed to benefits and can cause damaging to the recipient animal. The improper facilities and techniques affects the storage life of blood. Storage of whole blood for long term also causes storage lesions. Glucocorticoids are commonly used as immune-suppressive agent (Ferguson *et al*., 2017). Systemic glucocorticoid treatment predisposes the animal to secondary infection which results into immunosuppression (Torres *et al.*, 2005). Use of systemic glucocorticoid may also leads to GIT bleeding.

2.16 N- acetylcysteine (NAC)

NAC is a Glutathione precursor and prodrug of cysteine that scvange free radicals and bind metal ions into complex (Atkuri *et al.*, 2007). It is used as sputum liquefier (inhalation) and acetaminophen toxicity antidote (oral). NAC is also used as a nutritional supplement that posses hepatoprotective effect by replenising intracellular cysteine and glutathione levels or by improving vascular tone that may improve oxygen supply in acute liver failure (Webster and Cooper 2009). NAC also effects on the energy metabolism of hepatic mitochondrial, and posses anti-inflammatory actions by blocking adhesion of polymorphonuclear cell (PMN) on endothelial surface, PMN activation, and cytokine release (TNF-a) (Zafarullah *et al.*, 2003).

Intracellular oxidative stress occurs when production of reactive oxygen species (ROS)/ reactive nitrogen species (RNS) exceeds than that of antioxidation capacity of cell's. Excessive oxidative stress occurs due to oxidative modification of proteins, lipids, DNA, and subsequent cell death (Hybertson *et al.*, 2011). N-Acetylcysteine acts as direct antioxidant by interacting with the electrophilic moiety of free radicals through its free thiol side-chain and rapidly reacts with hydroxyl radical (·OH), nitrogen dioxide ($\cdot NO_2$), and carbon trioxide ion ($CO3^-$), it removes the free radicle produced by leukocytes (Akca *et al.*, 2005). Apart from its direct antioxidant property, NAC also act as an indirect antioxidant by replenishment of intracellular GSH, which is the major antioxidant of body with versatile cellular functions (Sies 1999). NAC plays a predominant role of antioxidant by maintaining GSH levels in the intracellular environment [Gibson *et al.*, 2009]. NAC also increases the osteogen level mainly in bone regeneration therapies. NAC diminished anti-TB drug and many more drugs induced liver injury (DILI) by scavenging free radicals that are formed during the metabolism of these drugs and also increasing the synthesis of GSH (Rana *et al.*, 2006).

NF-κB, the transcription factor plays an essential role in inflammation process and immune response by regulating the expression of related genes (Yamamoto and Gaynor 2001). NAC posses anti-inflammatory activity by decreasing NF-κB activity; and also suppresses ubiquitination and degradation of I-κB (an inhibitor of NF-κB) and thereby blocks NF-κB nuclear translocation and activation (Oka *et al.*, 2000; Pajonk *et al.*, 2002). NAC prevents

the release of proinflammatory cytokines such as interleukin-1β (IL-1β), IL-6 and IL-8, tumor necrosis factor-alpha (TNF-α), and transforming growth factor β (TGF- β) in macrophages [Karapinar *et al.,* 2016]. N-Acetylcysteine reduces the inflammation in cytotoxicity studies of resinous materials [Celik et al., 2017].

In oxidative stress, developing B cells present in the BM are affected resulting into significant reduction in the percentage of bone marrow B220+ CD432 B cells (which includes pre-B, immature, and mature B cells). NAC counteracts the oxidative stress by neutralizing ROS and enhances the stimulation of pro- and pre-B cells in the BM (Palmer *et al.,* 2011). NAC causes the demarcation of induced pluripotent stem cells (iPSCs) in the hematopoietic system which is long lasting process. NAC provides cytoprotection to the hematopoietic cells, because it preserves all the blood profile parameters and hemoglobin level and prevents the cellular death after carmustine adminstration in rats, (El-Sayed et al., 2010) and inhibits the oxidative stress in peripheral blood mononuclear cells in humans on exposure to pesticides (Ahmed *et al.,* 2014). NAC has good radioprotective qualities and on irradiation it also decreases cytotoxicity and genotoxicity effects in BM of different animal species. It also enhances clonogenic functions and improves long-lasting engrafting of Hemopoeitic Stem Cell (HSC) and Hemopoeitic Progenator Cell (HPC) (Demirel *et al.,* 2009). NAC improves the long term self-renewal capacity of HSC (Ito *et al.,* 2006). It also reduces the cellular death and ROS level in HSC (Tothova *et al.,* 2007). ROS are physiologically required for the maintenance of self-renewal capacity of stem cells and providing protection against invading microbes (Le *et al*., 2011; Kobayashi and Suda *et al.*, 2012; Pérez *et al.*, 2014; Lyublinskaya *et al.,* 2015).

2.17 Desmopressin (DDAVP)

In 1977 Desmopressin (1-deamino-8-D-arginine vasopressin, abbreviated as DDAVP), is a synthetic analog of the antidiuretic hormone vassopresin. In 1977, desmopressin for the first time, was used in hemophilia A and von Willebrand disease (VWD) suffered patients, which are the most commonly congenital bleeding disorders (Mannucci *et al.,*1977). In the late 1970s and early 1980s, desmopressin plays a crucial role in raising factor VIII and

von Willebrand factor (VWF) in plasma without the requirement of blood products. In 1772, it was found that blood collected under stress conditions clotted rapidly [Hewson 1886]. They showed that clotting of blood rapidly under stress conditions may be due to release of adrenaline in plasma (Cannon and Mendenhall 1914). In 1957, Marciniak found a mechanism for rapid clotting of blood after adrenaline injection in rabbits due to increase in coagulation factor VIII. Vasopressin and insulin were also shown to increase factor VIII in plasma [Mannucci *et al.,* 1972], but their side effects were more than those of adrenaline, making clinical use unrealistic. An important step behind the desmopressin was that, it increases VWF and factor VIII in normal individuals [Cash *et al.,* 1974; Mannucci *et al.,* 1975). Unlike other natural diuretic hormone vasopressin, desmopressin produces little or no constrictory effect on blood vessels, uterus or GIT and causes no increase in blood pressure, so that it is good tolerated by humans (Cash *et al.,* 1974; Mannucci *et al.,* 1975).

It is not only used in bleeding disorders like hemophilia and VWD but also used in platelet function defects and abnormalities of hemostasis which was associated with chronic kidney and liver disease. Prophylactically desmopressin has also been used in those patients who requires significant blood transfusion in various surgical procedures like cardiac and orthopedic surgery. Desmopressin treatment after cardiothoracic surgery increases the levels of large molecular weight VWF multimers, which leads to thrombus formation and platelet activation under flow conditions. This can help to restore hemostasis in these patients.

Desmopressin decreases the bleeding time and prolonged activated partial thromboplastin time by increases factor VIII and VWF which plays a rate-accelerating step in primary hemostasis and intrinsic coagulation (Mannucci *et al.,* 1976). Desmopressin plays no drastic changes on platelet count or aggregation, but increases the adhesion of platelet to the vessel wall (Barnhart *et al.,* 1983; Sakariassen *et al.*, 1984). Desmopressin was effectively used in thrombocytopenic patients for improving hemostasis (Castaman *et al.,* 1997; Noris *et al.,* 1998; Balduini *et al.,* 1999**).** DDAVP has been used to improve congenital or acquired platelet dysfunction which are found in liver cirrhosis, uremia and drug-induced bleeding related to heparin, hirudin, dextran, aspirin, or ticlopidine **(**Mannucci 1988**).** Desmopressin also release greater amounts of tissue plasminogen activator in the plasma but this effect is short lived

(Cash *et al.,* 1974; Mannucci *et al.,* 1975). Plasminogen activator produces plasmin *in vivo,* but most of the plasmin forms complex with α2 –antiplasmin, and that's why plasmin do not cause fibrinogenolysis in circulating blood (Levi *et al.,* 1992).

The storage site and release of factor VIII is less well described as compared to VWF (Kaufmann and Vischer 2003). The storage site for VWF are platelets and Weibel-Palade bodies (WPBs) in endothelial cells (Wagner *et al.,* 1982; Sporn *et al.,* 1985). Desmopressin mediates the release of these factors from the storage sites. This fact is carried by the examination in rats, after injections of desmopressin which exhibits biological reaction that are associated with the endothelial cell activation, and subsequent margination of leukocytes (Kanwar *et al.,* 1995). *In vitro,* administration of desmopressin to cultured endothelial cells does not release VWF (Booyse *et al.,* 1981). This visible contradiction was resolved by the proof that desmopressin does not have direct effect on human vascular endothelial cells (HUVEC) which is explicable to the truth that these cells do not posses the V2 receptor (V2R) (Kaufmann *et al.,* 2000). For the rise of factor VIII in plasma after desmopressin adminstration, factor VIII and VWF must be co-localised which is indicated by the examination that, after transplantation of liver, patients with hemophilia A undergoes infusion of desmopressin showed the anticipated increase of VWF but found no change in plasma factor VIII (Lamont and Ragni 2005). Because factor VIII is synthesized only in the transplanted liver, this examination supports the fact that co-localization of factor VIII and VWF in extrahepatic cells is crucial for release of factor VIII *in vivo* after desmopressin adminstration (Lamont and Ragni 2005).

Materials and Methods

3 MATERIALS AND METHODS

The present study aimed to study about bone marrow stimulating property of N-acetylcysteine and Desmopressin for therapeutic management of Aplastic Pancytopenia associated with Canine Hemoprotozoan Diseases. The study was conducted in 03 distinct phases:-

Phase I: Experimental study of bone marrow (BM) stimulating property of N-acetylcysteine & Desmopressin in rats.

i) Induction of Aplastic Pancytopenia by bone marrow suppression in rat *(Pilot study to establish experimental* bone marrow suppression *model)*

Experimental Animal: Wister Rat 200 gm (N = 06 x 6 Grs = 36)

Bone marrow suppression: 2 doses of Cyclophosphamide @75mg/kg B Wt S/C on alternate days

Duration of Study: 02 weeks

ii) *In vivo* study in rat to assess bone marrow stimulating properties of N-acetylcysteine & Desmopressin

Therapeutic agent:

i) N-acetylcysteine @25 & 50mg/kg B Wt orally

ii) Desmopressin @ 7.5 & 15µg/kg B Wt S/C

Parameters of study:

i) Hematological Parameters

Hemoglobin: Hemoglobin was done as per (Jain, 1986). Haemoglobin was estimated by using Sahle's Haemoglobinometer with double prism standard and expressed in g/dl.

Packed Cell Volume (PCV): Packed cell volume was measured by Wintrobe haematocrit method.

Total Erythrocytes Count (TEC): Total erythrocytes count was estimated by using improved Neubauer Chamber with the help of Gower's RBC diluting solution (Crystalline sodium- 0.25g, sodium chloride- 0.5g and distilled water 100ml) and the result were expressed in million per microlitre of blood.

Total Leucocytes Count (TLC): TLC was estimated by using improved Neubauer Chamber of haemocytometer with WBC diluting fluid and the results were expressed in thousand per microlitre of blood.

Differential Leucocytes Count (DLC): The Differential leucocytes counts was expressed by the method of parallel stripe from the smears prepared from the fresh blood after staining with Giemsa stain.

Platelets count: platelets were counted per 100 WBCs in the Geimsa stained blood smears and calculations were made using the following formula: Platelets/µl=Number of Platelets x TLC/100WBC

ii) Blood biochemical and Liver profile

Serum Alanine Amino Transferase (ALT): Serum ALT activity was estimated by Reitman and Frankel method (1957) using standard kit (Span diagnostic Ltd.).

Serum Aspartate Amino Transferase (AST): Serum AST activity was estimated by Reitman and Frankel method (1957) using standard kit (Span diagnostic Ltd.).

Alkaline Phosphatase: Serum Alkaline Phosphatase was estimated by PNPP method using standard test kit (Autospan diagnostic Ltd.).

Serum Total protein (TP) : Serum total protein was calculated by modified Biuret method (Varley *et al.*, 1980) by using standard kit (Autospan diagnostic Ltd.)

Serum Albumin: Serum albumin was estimated by Dumas method (Varley et al., 1980) by using standard kit (Autospan diagnostic Ltd.) Albumin: Globulin ratio was calculated by substracting albumin from total protein.

iii) Histopathology of decalcified bone marrow

Fixation- Bone and calcified tissue were cut into smaller pieces with saw before fixation. Bone specimens fixed in 10% NBF (Neutral Buffered Formalin) overnight with multiple incisions to facilitate penetration.

Decalcification (By Formic Acid- Sodium Citrate Method)

1) Decalcifed the bone for 5-14 days in Formic Acid- Sodium Citrate solution. Change the solution daily for better results

Solution A

Sodium Citrate	50 gm
Distilled Water	250 cc

Solution B

Formic Acid (90%)	125 cc
Distilled Water	125 cc

Mix solution A and B

2) Wash in running water for 4-8 hrs

3) Dehydrate, clear and embed

HE Staining- HE staining was conducted according to routine protocols (Guo *et al.*, 2016).

Experimental Design

Gr.	Treatment	Experiment Protocol (n= 06)	Duration
Gr.I	Healthy Control	Standard feeding & managemental condition as per IAEC norms	No treatment
Gr.II	Disease Control	Induction of aplastic pancytopenia by 02 dose of Cyclophosphamide @75mg/kg B Wt S/C alternate day *(Feng et. al 2016)*	No treatment*(Therapy at the end of experiment as per IAEC norms)*
Gr.III	N- acetylcysteine (low dose)	Induction of aplastic pancytopenia *(Feng et. al 2016)* + N-acetylcysteine (NAC) @ 25mg/kg B. Wt orally *(Sacrifice as per IAEC norms on day 15th to study blood biomarker & bone marrow histopathology)*	Treatment from day 4th daily for 10 days
Gr.IV	N- acetylcysteine (high dose)	Induction of aplastic pancytopenia *(Feng et. al 2016)* + NAC @ 50mg/kg B Wt orally *(Sacrifice as per IAEC norms on day 15th to study blood biomarker & bone marrow histopathology)*	Treatment from day 4th daily for 10 days
Gr.V	Desmopressin (low dose)	Induction of aplastic pancytopenia *(Feng et. al 2016)* + Desmopressin (DDAVP) @ 7.5µg/kg B Wt PO *(Sacrifice as per IAEC norms on day 15th to study blood biomarker & bone marrow histopathology)*	Treatment from day 4th daily for 10 days
Gr.VI	Desmopressin (high dose)	Induction of aplastic pancytopenia *(Feng et. al 2016)* + DDAVP @ 15µg/kg B Wt PO *(Sacrifice as per IAEC norms on day 15th to study blood biomarker & bone marrow histopathology)*	Treatment from day 4th daily for 10 days

Phase II: Screening, diagnosis and therapy of aplastic pancytopenia and or hepatobiliary dysfunction (HBD) associated with canine haemoprotozoan diseases (CHDs) in RVP, IVRI.

i) Clinical study

The study was conducted to screen the clinical aplastic pancytopenia and or hepatobiliary dysfunction (HBD) associated with canine haemoprotozoan diseases (CHDs) presented at

Referral Veterinary Polyclinic (RVP), Indian Veterinary Research Institute, Izatnagar showing the symptoms of canine haemoprotozoan diseases. History of each dog was noted in relation to age, sex, breed, body weight etc for epidemiological study. Efforts was made to categorize hepatopathies and pancytopenia associated with CHDs. This may serve as the key indicator for disease specific hepatopathies and pancytopenia. A module was developed with detailed clinical signs and laboratory findings of individual ailing cases for critical analysis.

Collection of blood

Blood will be collected from sephanous/cephalic or jugular vein in clean dry sterilized non-heparinized vial for biochemical analysis. After one hour of clotting, blood will be subjected to centrifugation at 3000 rpm for 5 minute. Collected serum will be stored in deep freeze at (–) 20 ^{0}C for biochemical estimation.

Preparation of blood smear: Blood smear was prepared from the ear margin capillary bed and was stained with Giemsa for microscopical examination of intracellular organism namely babesiosis, Ehrlichiasis and hepatozoonosis. Babesia gibsoni is a small organism measuring 1-3.2 micrometer and appeared as signet ring shape under microscope. Babesia canis a large form measuring 4-5µm long and appeared pyriform in shape, pointed one end and round other end.

Cytological Examination:

Cytological examination of peripheral blood was carried out as per the standard method described by Jain, 1986.

Division of Medicine

ICAR - IVRI, Izatnagar, Bareilly (UP), India

Module for screening, diagnosis & therapy of aplastic pancytopenia & or HBD associated with CHDs

Case No_____ Date._____

Owner's Name, Address & phone No. _____

Age _____ Sex_____Breed_____

History/Owner's perception:

Clinical observation

Temperature:_____ Pulse _____ Resp.rate _____

Symptom	**Observed**
1. Anorexia/ Inappetance	
2. Lethargy/ weakness/ depression	
3. Diarrhea/ vomiting / urination/thirstiness	
4. Visible mucous membrane)	
5. Dehydration	
6. Discharge (nose/eyes/rectum/vagina	
7. Specific ocular signs	
8. Enlarged lymph nodes	
9. Arthritis/ Abnormal gait/ Posture	
10. Neurological signs	

A. Blood smear study for Haemoprotozoa:

B. Haematological value:

Parameter	**Value**
Hb (gm%)	
PCV (%)	
TEC (m/cumm)	
TLC (thousand/cumm)	
Neutrophil (%)	
Lymphocyte (%)	
Monocyte (%)	
Eosinophil (%)	
Basophil (%)	
Platelets (103/µL)	
Clotting time	

C. LFT & Enzyme study

Parameter	**Value**
ALT (IU/L)	
AST (IU/L)	
ALP (g/dl)	
Bilirubin (g/dl)	
Total protein (g/dl)	
GSH (µg/mg protein)	
SOD	
GPx	
CAT(U/mg)	

D. USG / X-RAY (if any):

Tentative/Confirmatory Diagnosis

Treatment

ii) Parameters of study:

a) Hematological Parameters

Hemoglobin: Hemoglobin was done as per (Jain, 1986). Haemoglobin was estimated by using Sahle's Haemoglobinometer with double prism standard and expressed in g/dl.

Packed Cell Volume (PCV): Packed cell volume was measured by Wintrobe haematocrit method.

Total Erythrocytes Count (TEC): Total erythrocytes count was estimated by using improved Neubauer Chamber with the help of Gower's RBC diluting solution (Crystalline sodium- 0.25g, sodium chloride- 0.5g and distilled water 100ml) and the result were expressed in million per microlitre of blood.

Total Leucocytes Count (TLC): TLC was estimated by using improved Neubauer Chamber of haemocytometer with WBC diluting fluid and the results were expressed in thousand per microlitre of blood.

Differential Leucocytes Count (DLC): The Differential leucocytes counts was expressed by the method of parallel stripe from the smears prepared from the fresh blood after staining with Giemsa stain.

Platelets count: platelets were counted per 100 WBCs in the Geimsa stained blood smears and calculations were made using the following formula: Platelets/µl=Number of Platelets x TLC/100WBC

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Serum Albumin: Serum albumin was estimated by Dumas method (Varley et al., 1980) by using standard kit (Autospan diagnostic Ltd.) Albumin: Globulin ratio was calculated by substracting albumin from total protein.

c) Antioxidant potential of Canine blood

Antioxidant activity of canine blood was assessed by following methods

Free radical scavenging activity (DPPH method)

The free radical scavenging activity of canine blood was measured by DPPH (1,1 diphenyl 2, picrylhydrazyl) assay with slight modifications (Brand- Williams et al., 1995). It measures the free radical scavenging activity in terms of hydrogen donating ability or radical scavenging property of any biological fluid using the stable free radical DPPH solution. Serum sample (100µl) was mixed with 2 ml of DPPH solution (0.2Mm) prepared in methanol. The mixture was allowed to incubate at room temperature for 30 minutes. After completion of incubation period, 1 ml of chloroform was added and centrifuged at 3000× g for 5 min. The absorbance of clear solution was measured at 517 nm. A 100Mm of DPPH solution prepared in methanol was used as a control. The percentage inhibition of DPPH free radical (scavenged %) was calculated based on reading of control solution by employing the following equation:

$$\text{Scavenging activity}(\%) = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

FRAP assay to determine Total Antioxidant Activity

To determine total antioxidant capacity of serum, a modified FRAP assay was used with slight modifications (Benzie and Strain, 1996). The fresh working FRAP reagent was prepared by mixing 10 volume of 300mmol/L acetate buffer (3.1g of CH3COONa and 16 ml of CH3COOH), ph 3.6 with 1 volume of 10 mmol/L TPTZ (2,4,6- tripyridyl-s- triazine) in 40mmol/L Fecl3. Serum sample (50 µl) was mixed with 1.5 ml of FRAP reagent and kept at dark for 10 min. The resulting intense blue colouration (Ferrous tripyridyltriazine complex)

was subsequently measured at 593nm. Aqueous solution of $FeSO_4$. $7H_2O$ (100-1000 µM) was used as standard curve. The data was expressed as FRAP values (µM/ml Fe (II)

d) Ultrasonography (USG)

Ultrasonographic study in dogs selected on the basis of clinical signs was performed using a 5- 7.5 MHz curvilinear transducer on a Chison IVIS 60 expert Vet® Colour Doppler ultrasound system. Ventral abdomen of the animal was shaved or clipped and the animal was put in dorsal recumbency on a 'V' top table. Subjective and quantitative evaluation of the organs was made as per the standard protocol (Nyland and Matoon 2002). For ultrasonographic examination of liver, transducer was kept on the midline just immediately behind the xiphisternum and angled cranio dorsally to view a transverse section of liver. Then the transducer was moved gradually caudally remaining on the midline and head of transducer was rotated at 90 degree to view a longitudinal section of liver on midline. For right transverse oblique screen of the gall bladder, transducer was kept approximately 6-8 cm cranial to xiphoid and 4-5 cm dorsal to the sternum on right side. The transducer was angled towards midline between costal cartilages. For left transverse oblique scan of the gall bladder, the head of transducer was placed on right 10^{th}- 11^{th} inter costal space, 5-10cm ventral to the spine.

iii) Prognostic Score Card: Prognostic score card for therapeutic efficacy was developed against the degree of aplastic pancytopenia and level of HBD associated with CHDs based upon human patient Child - Pugh score (Durand and Valla, 2008) after necessary modifications in ailing dogs and was recorded.

Parameters	**Prognosis**		
	Prognosis + 3 (Good)	**Prognosis + 2 (Moderate)**	**Prognosis + 1 (Poor)**
Hb (12- 18 gm/dl)	10- 12	8- 10	5-8
RBC Count (5- 8 × 10^6/ µl)	4- 5	2- 4	<2
WBC Count (5-14 × 103/ µl)	10- 14	5- 10	< 5
Reticulocyte Count(60-80,000/µl)	60-80	40- 60	<30
Platelet Count (lakh/cumm) (1.5- 4)	2.5- 4	0.5-2.5	0.1-0.5
ALT (0- 40 IU)	(40- 50)	50- 70	70- 90
Total Score	13-15	10-12	7-9

A total score of 13-15 was considered Grade A, (+3) (Good Prognosis); 10-12 was Grade B, (+2) (Moderate Prognosis); and 7-9 was Grade C, (+1) (Poor Prognosis).

Phase III: To evolve novel therapeutic regimen against aplastic pancytopenia and hepatobiliary dysfunction (HBD) associated in CHDs

Therapeutic evaluation of *in vivo* standardized drug / chemical for suitable management of aplastic pancytopenia and HBD associated in CHDs along with disease specific therapy.

Experimental Design

Gr	Treatment	Experiment design	Duration
Gr.I	Standard Therapy	Disease Specific Therapy + Symptomatic Supportive Therapy with standard dose	10 days therapy followed by correlation of clinico-diagnostic tools with canine reference values. (*INFORMED CLIENT CONSENT TRIAL*)
Gr. II	Specific Therapy	Disease Specific Therapy + Best of Phase - I trial + Symptomatic Supportive Therapy with standard dose	

Parameters of study: Clinico-diagnostic tools performed during Phase II screening study was utilized for therapeutic evaluation and was compared with canine reference values.

Statistical analysis

The analysis of the data was evaluated using appropriate statistical tests as per the statistical methods.

Results

4 RESULTS

4.1 Experimental study of bone marrow (BM) stimulating property of N-acetylcysteine and Desmopressin in rats

4.1.1 Induction of aplastic pancytopenia by bone marrow suppression in rat *(Pilot study to establish experimental* bone marrow suppression *model)*

Pilot study was conducted to establish the experimental bone marrow suppression model in 3 rats. Firstly bone marrow suppression was done by using 2 doses of cyclophosphamide @75mg/Kg B.wt SC route on alternate days. Out of three rats two succumbed by 10th day of trial and postmortem examination revealed extensive hemorrhage with severe aplastic pancytopenia. Hence it was decided to go with single half dose of cyclophosphamide @37.5mg/Kg B.wt SC. In the revised pilot study, rats received cyclophosphamide @37.5mg/Kg B.wt SC single dose were pancytopenic after 4th day of induction and all the rats were alive even after 14 days of induction. Finally it was concluded that single dose of cyclophosphamide @37.5mg/Kg B.Wt SC used for induced bone marrow suppression and to establish aplastic pancytopenia.

4.1.2 *In vivo* study in rat to assess bone marrow stimulating properties of N-acetylcysteine and Desmopressin.

Thirty six rats were selected for this study. These 36 rats were divided randomly into 6 groups and each group had 6 rats (n=6). Duration of study was 2 weeks. Rats of Gr.I and Gr.II served as healthy and disease control rats, respectively. Gr.III and Gr.IV rats were treated with N- acetylcycteine @25 and 50 mg/Kg B.Wt orally from 4th day after induction. Gr.V and Gr.VI rats were treated with Desmopressin @ 7.5 & 15µg/Kg B.Wt orally from 4th

day after induction. All rats were sacrificed as per IAEC norms on day 15th for study of blood biomarkers and bone marrow histopathology.

4.1.3 Parameters of Study

4.1.3.1 Hematological parameters

Pre and post treatment changes in the hematological parameters have been given in table 1. Hemoglobin (Hb), Packed Cell Volume (PCV), total erythrocyte count (TEC), platelet values were significantly ($p<0.05$) decreased in all the groups on day 15th except Gr. I. But, total leucocyte count (TLC) values were non- significant at the end of therapy in all groups except Gr. II. Significantly ($p<0.05$) reduced Hb level noticed at the end of therapy in Gr. II. Hb levels of rats treated with low (Gr.III) and high dose (Gr.IV) of n-acetylcysteine (NAC) and rats treated with low (Gr.V) and high dose (Gr.VI) of Desmopressin were significantly ($p<0.05$) increased after 15th day of therapy as compared to disease control group (Gr. II). Significantly ($p<0.05$) decreased PCV value was noticed on day 15 in Gr. II as compared to healthy and treated groups. PCV and TEC values were significantly ($p<0.05$) decreased in all treated groups at the end of therapy as compared to Gr. I, whereas PCV and RBC values of Gr. IV rats were significantly ($P<0.05$) higher than Gr. II (disease control). TLC was significantly ($p<0.05$) increased on day 15 in Gr. II, whereas it was non-significant in all other groups. The platelet count was significantly ($P<0.05$) decreased in all the groups except Gr. I on day 15. Among the treated groups, hematological parameters (Hb, TEC, PCV) were significantly ($P<0.05$) increased on day 15 in Gr. IV as compared to Gr. II. But these values were lesser than Gr. I (healthy control).

Critical analysis of the data revealed better efficacy in animals supplemented with NAC @50mg/Kg B.Wt with respect to Hb, PCV and TEC.

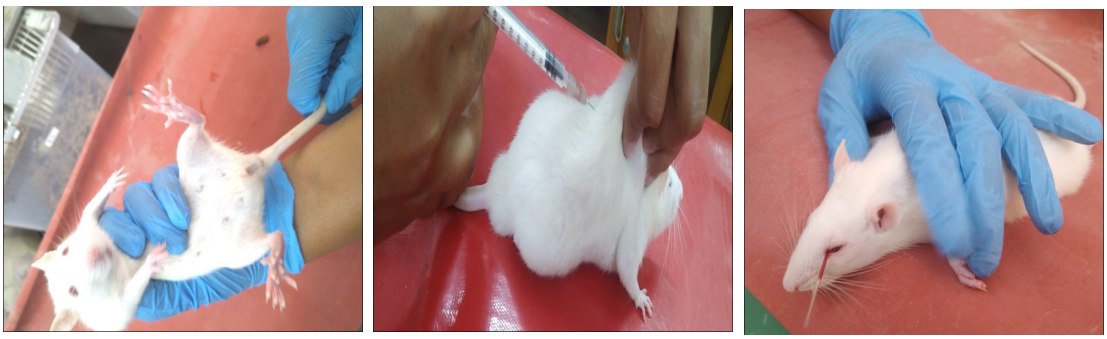


Fig. 1: Handling, induction and blood collection of rats

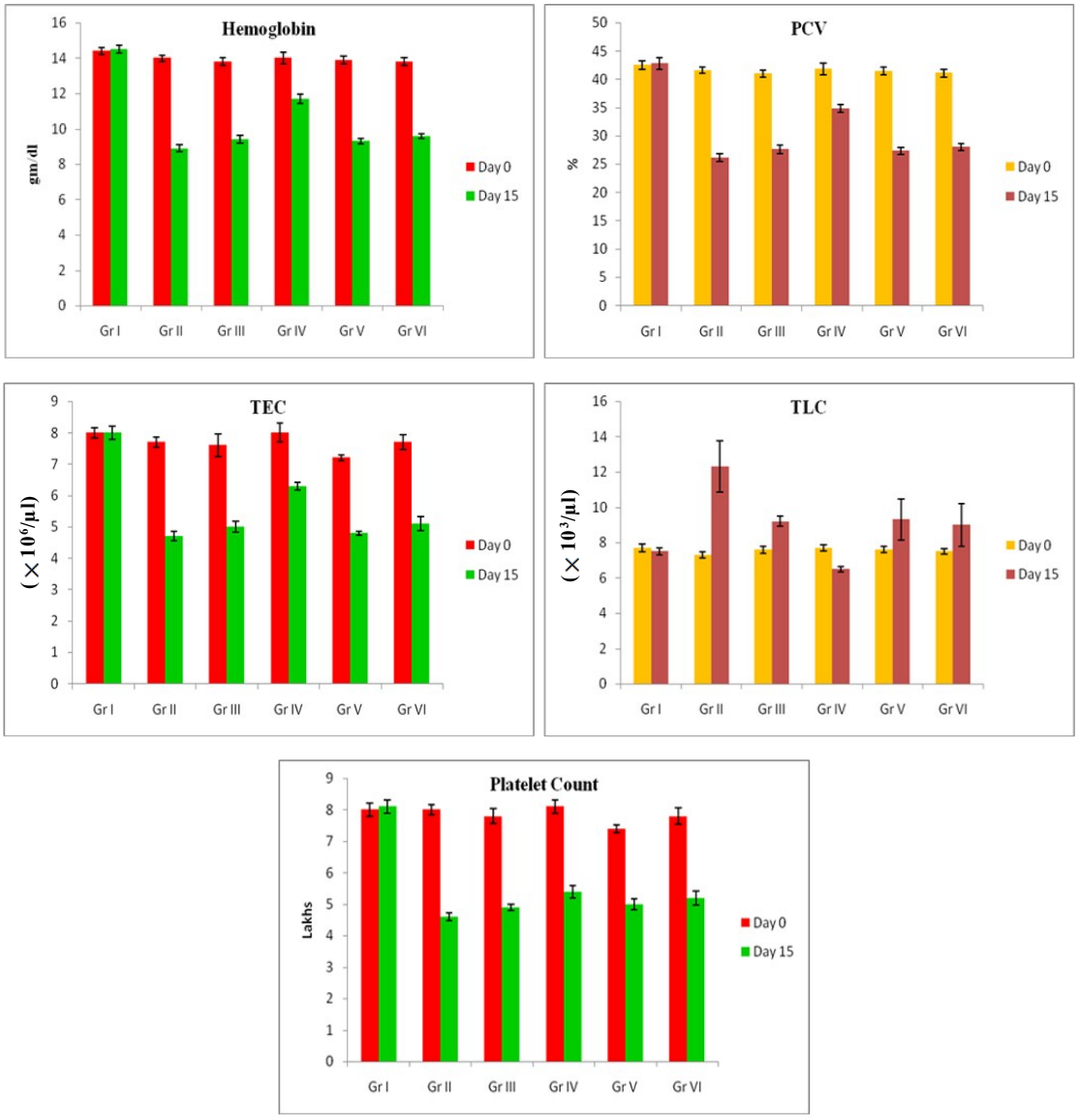


Fig. 2: Complete Blood Count (CBC) in rats received different treatments

Table 1. Mean±SE values of Complete Blood Count (CBC) in rats received different treatments

Groups*	Hb (g/dl)		PCV (%)		RBC ($\times 10^{6}$/µl)		WBC ($\times 10^{3}$/µl)		Platelet (lakhs)	
	0 Day	15Day	0 Day	15Day	0Day	15Day	0Day	15Day	0Day	15Day
I	14.4 ± 0.18^{aA}	14.5 ± 0.21^{aA}	42.5 ± 0.76^{aA}	42.8 ± 1.01^{aA}	8.0 ± 0.16^{aA}	8.0 ± 0.22^{aA}	7.7 ± 0.21^{aA}	7.5 ± 0.19^{aB}	8.0 ± 0.21^{aA}	8.1 ± 0.20^{aA}
II	14.0 ± 0.18^{bA}	8.9 ± 0.20^{aB}	41.6 ± 0.61^{bA}	26.1 ± 0.70^{aB}	7.7 ± 0.17^{bA}	4.7 ± 0.15^{aB}	7.3 ± 0.17^{bA}	12.3 ± 1.46^{aA}	8.0 ± 0.15^{bA}	4.6 ± 0.12^{aB}
III	13.8 ± 0.21^{bB}	9.4 ± 0.21^{aC}	41.0 ± 0.63^{bA}	27.6 ± 0.67^{aB}	7.6 ± 0.37^{bA}	5.0 ± 0.18^{aB}	7.6 ± 0.20^{aA}	9.2 ± 0.28^{aAB}	7.8 ± 0.24^{bA}	4.9 ± 0.09^{aB}
IV	14.0 ± 0.33^{bB}	11.7 ± 0.27^{aD}	41.8 ± 1.01^{bA}	34.8 ± 0.70^{aC}	8.0 ± 0.30^{bA}	6.3 ± 0.12^{aC}	7.7 ± 0.17^{aA}	6.5 ± 0.15^{aB}	8.1 ± 0.21^{bA}	5.4 ± 0.20^{aB}
V	13.9 ± 0.22^{bB}	9.3 ± 0.17^{aE}	41.5 ± 0.67^{bA}	27.3 ± 0.67^{aB}	7.2 ± 0.09^{bA}	4.8 ± 0.06^{aB}	7.6 ± 0.19^{aA}	9.3 ± 1.15^{aAB}	7.4 ± 0.13^{bA}	5.0 ± 0.17^{aB}
VI	13.8 ± 0.22^{bB}	9.6 ± 0.14^{aE}	41.1 ± 0.70^{bA}	28.0 ± 0.63^{aB}	7.7 ± 0.24^{bA}	5.1 ± 0.22^{aB}	7.5 ± 0.14^{aA}	9.0 ± 1.20^{aAB}	7.8 ± 0.26^{bA}	5.2 ± 0.22^{aB}

Mean±SE values within same column for a particular parameter (Capital letter) and in same row (small letter) bearing similar superscript do not differ at $P<0.05$

* Gr.I: Healthy control, Gr.II: Disease control, Gr. III: Low dose(LD) NAC, Gr.IV: High dose(HD) NAC, Gr. V: Low dose(LD) Desmopressin, Gr.VI: High dose (HD) Desmopressin.

4.1.3.2 Serum Biochemistry

Pre and post treatment changes in the serum biochemical parameters have been given in table 2. ALT, AST, ALP, TP and Albumin values were non-significant in Gr. I on pre and post treatment. ALT, AST and ALP values of Gr. II were significantly ($P< 0.05$) increased on day 15 as compared to Gr. I. ALT values was non-significantly decreased in Gr. III and IV and non- significantly increased in Gr.V and Gr.VI on day 15. Similar kind of trend was noticed with regards to AST level, AST values were non- significantly decreased in Gr. III and IV and non- significantly increased in Gr. V and Gr.VI on day 15. Non- significantly decreased ALP values were noticed in all the treated groups on day 15. There was significantly ($P< 0.05$) decreased TP and the Albumin values noticed in Gr. II on day 15 as compared to Gr. I whereas it was non- significant in all the treated groups except in Gr. V and VI there was significantly ($P< 0.05$) decreased albumin level noticed on day 15 as compared to Gr. I. However, protein profile in treated groups was significantly ($p<0.05$) higher than disease control group. Among the treated groups Gr. IV showed better results in serum biochemical parameters.

Critical analysis of the data revealed better efficacy in animals supplemented with NAC @50mg/Kg B.Wt with respect to ALT and TP.

4.1.3.3 Histopathology of decalcified bone, liver and spleen

The histopathology of bone marrow of different groups of rats is shown in Fig. 4, 5, 6, 7, 8, 9 and were graded in the scale of 0-3. (Table 3). The bone marrow hematopoietic tissue of healthy control (Gr. I) rats revealed normal cellularity of the myeloid and erythroid precursor cells in the form of sheet and graded as 3 by histopathological score system (HPS). Bone marrow section of disease control rats (Gr. II) showed decrease in the cellularity of the myeloid and erythroid precursor cells as compared to Gr. I, and was assigned grade 0. Gr. III showed compact sheets of hematopoietic cells (myeloid and erythroid lineages) at places which is more denser than the Gr. II and assigned grade 2 by HPS system. Histopathology of bone marrow of Gr. IV showed completely packed bone marrow with hemopoetic cells and the presence of megakaryocytes and was assigned grade 2.5. Histopathology of bone marrow of Gr.V and Gr.VI showed decrease density of hematopoetic cells population in epiphysis portion

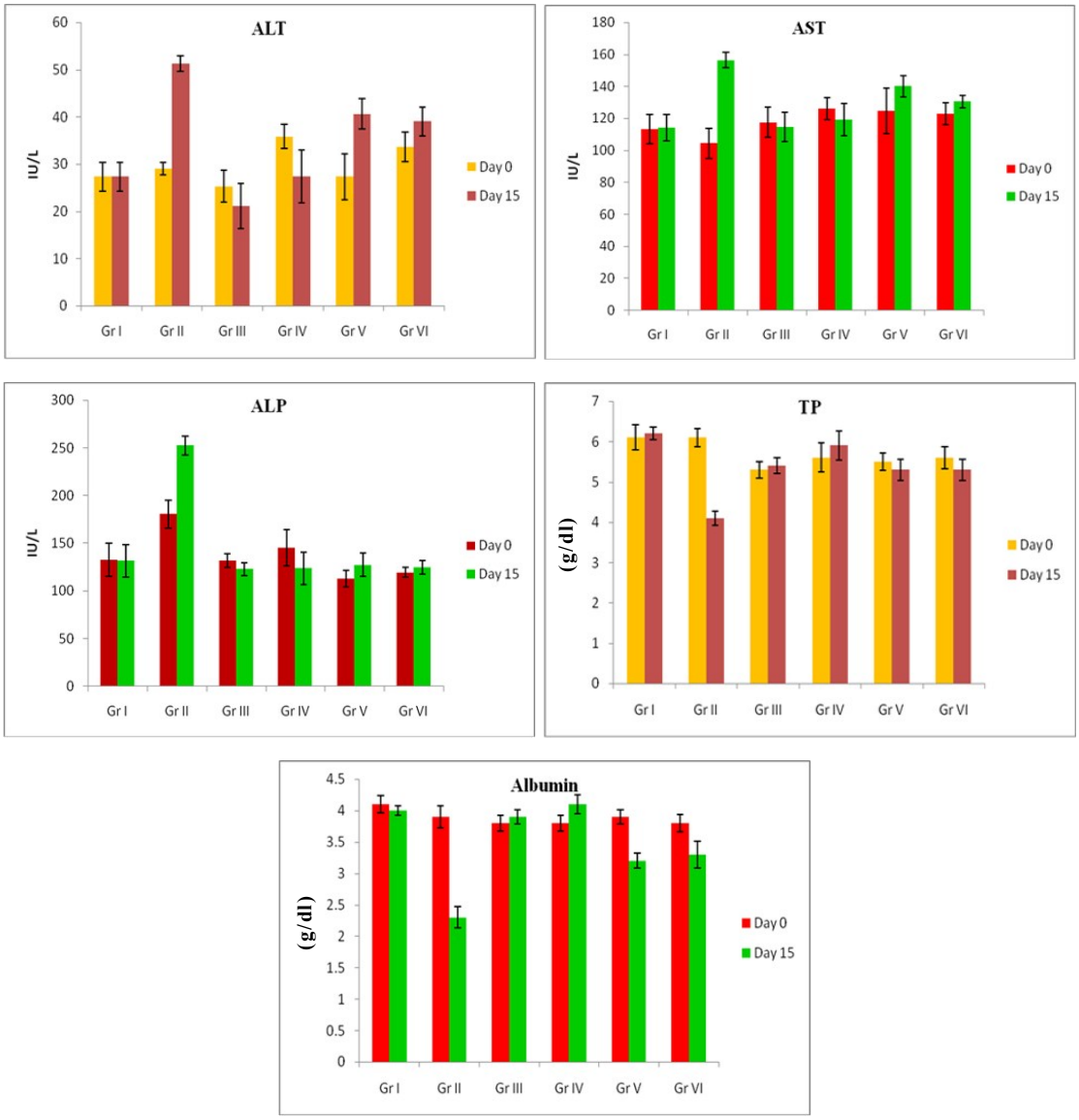


Fig. 3: Serum Biochemical Profile in rats received different treatments

Table 2. Mean±SE values of serum biochemical profile of rats received different treatments

Groups*	ALT (IU/L)		AST (IU/L)		ALP (IU/L)		TP (g/dl)		Albumin (g/dl)	
	0 Day	15Day	0 Day	15Day	0Day	15Day	0Day	15Day	0Day	15Day
I	27.3±2.99^{aA}	27.3±2.99^{aB}	113.3±9.11^{aA}	114.3±8.17^{aB}	132.6±17.32^{aB}	131.5±17.19^{aB}	6.1±0.31^{aA}	6.2±0.15^{aA}	4.1±0.14^{aA}	4.0±0.08^{aA}
II	29.0±1.34^{bA}	51.3±1.63^{aA}	104.5±9.37^{bA}	156.3±4.81^{aA}	180.5±14.79^{bA}	252.5±9.73^{aA}	6.1±0.23^{bA}	4.1±0.17^{aB}	3.9±0.18^{aA}	2.3±0.17^{bB}
III	25.3±3.38^{aA}	21.1±4.85^{aC}	117.6±9.30^{aA}	114.8±9.12^{aB}	132.0±7.07^{aB}	122.8±6.94^{aB}	5.3±0.20^{aB}	5.4±0.20^{aC}	3.8±0.12^{aA}	3.9±0.11^{aA}
IV	35.8±2.57^{aA}	27.4±5.64^{aB}	126.0±6.75^{aA}	119.3±10.06^{aB}	145.1±18.74^{aB}	123.6±17.03^{aB}	5.6±0.36^{aB}	5.9±0.36^{aA}	3.8±0.13^{aA}	4.1±0.15^{aA}
V	27.3±4.86^{aA}	40.6±3.20^{aA}	124.8±14.13^{aA}	140.1±6.75^{aA}	113.1±8.67^{aB}	127.3±12.34^{aB}	5.5±0.22^{aB}	5.3±0.27^{aC}	3.9±0.11^{aA}	3.2±0.12^{bC}
VI	33.6±3.16^{aA}	39.0±3.09^{aA}	123.0±6.98^{aA}	130.5±3.82^{aA}	119.1±5.13^{aB}	124.5±7.37^{aB}	5.6±0.27^{aB}	5.3±0.27^{aC}	3.8±0.14^{aA}	3.3±0.21^{bC}

Mean±SE values within same column for a particular parameter (Capital letter) and in same row (small letter) bearing similar superscript do not differ at $P<0.05$

* Gr.I: Healthy control, Gr.II: Disease control, Gr. III: Low dose(LD) NAC, Gr.IV: High dose(HD) NAC, Gr. V: Low dose(LD) Desmopressin, Gr.VI: High dose (HD) Desmopressin

of femur bone as compared to Gr. III and IV. However, bone marrow cellularity is more than disease control group and assigned grade 1 and 1.5.

The histopathology of liver section of different groups of rats is shown in Fig.10, 11, 12, 13, 14, 15 and graded in the scale of 0-3. The liver section of healthy control (Gr. I) revealed normal architecture of hepatic lobules including well organized hepatocytes as cords, sinusoids, inactive kuppfer cells, central vein, and portal triad and graded as 3 by histopathological score system (HPS). Liver section of disease control rats (Gr. II) showed swollen hepatic cells with fatty changes in periportal and midzonal areas; engorged sinusoids around central veins; mild fibrocellular reaction in periportal connective tissue, and necrotic hepatic cells at places and graded 0. The liver tissue of Gr. III showed improvement in lesions as compared to disease control group with prominency of kuffer cells, degeneration of hepatocytes with fatty changes, mild fibrocellular infiltration in the portal connective tissue and was graded 2. The liver tissue of Gr. IV showed normal architecture of the hepatic lobules compared to Gr. III. Gr. IV and graded 2.5. The liver tissue of Gr. V showed disorganized cords and degenerated hepatic cells, sinusoids dilatation and few mononuclear cells in the sinus spaces and around the portals and graded 1. Histopathology of Gr.VI showed degenerated hepatocytes and aggregates of hematopoetic cells scattered in the hepatic lobules and graded 1.5 .

The histopathology of spleen section of different groups of rats is shown in Fig.16, 17, 18, 19, 20, 21 and graded in the scale of 0-3. The spleen section of healthy control (Gr. I) revealed normal architecture of white and red pulp region with normal lymphoid tissue cells or macrophages and normal splenic cords and graded 3 by histopathological score system (HPS). Spleen section of disease control rats (Gr. II) showed reduced lymphoid cells in white pulp and increased number of macrophages with neutrophils in the red pulp sinuses and cords as compared with Gr. I. and graded 0. The spleen of group Gr. III showed hyperplasia of lymphoid tissue in Periarteriiolar lymphoid sheath (PALS) and follicles, activated macrophages and neutrophils in red pulp as compared to disease control and graded 2. The spleen of Gr. IV showed extramedullary hematopoesis with collection of erythroid precursors cells, myloid precursors cells and plenty of megakaryocytes in red pulp and white pulp zones. The white

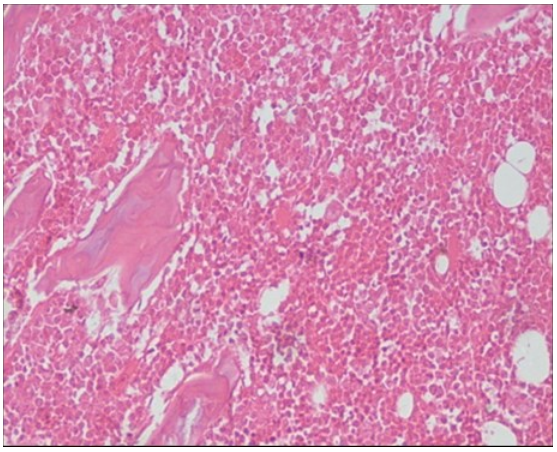


Fig. 4: Long Bone group I: Normal cellularity of the myeloid and erythroid precursor cells in the form of sheet (H & E 20X)

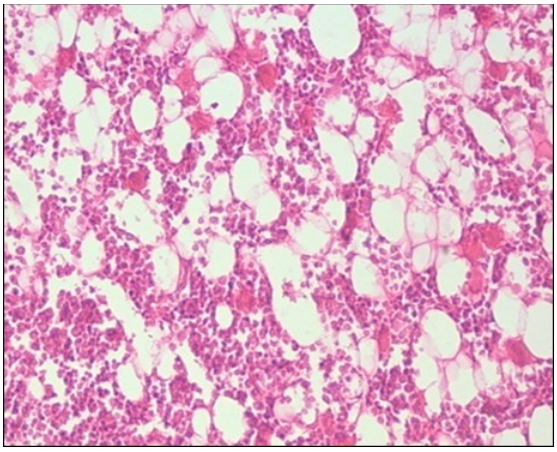


Fig. 5: Long Bone group II: Decrease in the cellularity of the myeloid and erythroid precursor cells as compared to healthy control (H & E 20X)

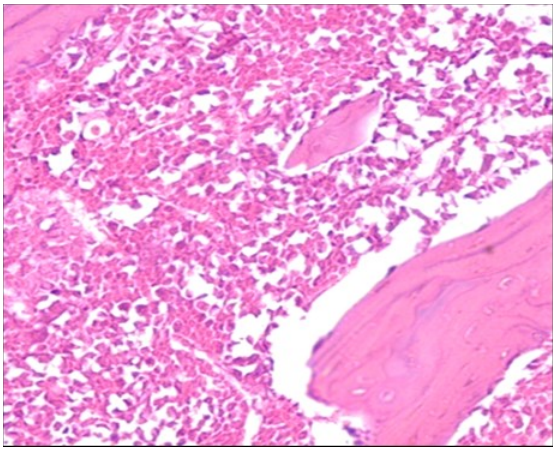


Fig. 6: Long Bone group III: Compact sheets of hematopoietic cells (myeloid and erythroid lineages) at places (H & E 20X)

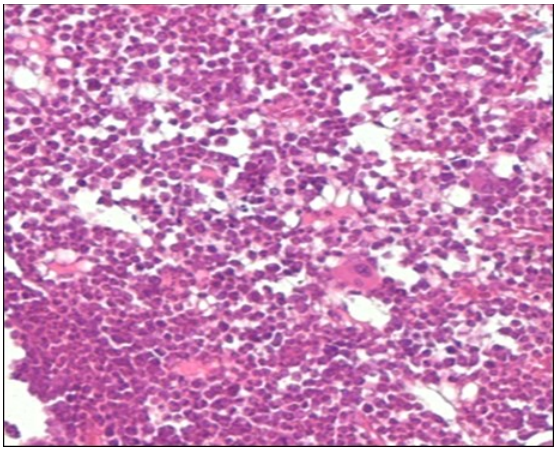


Fig. 7: Long Bone group IV: Marrow is completely packed with hemopoetic cells with presence of megakaryocytes (H & E 20X)

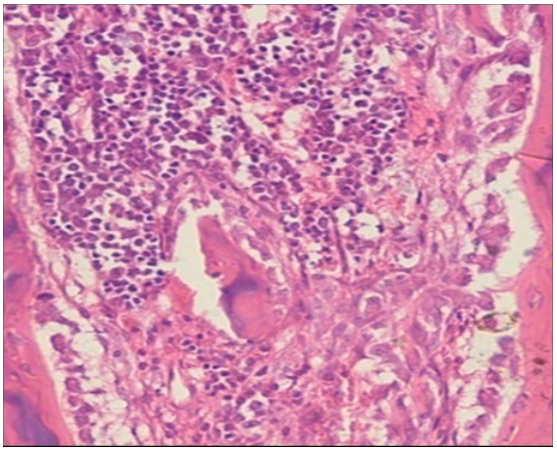


Fig. 8: Long Bone group V: Decrease in the cellularity of the myeloid and erythroid precursor cell as compared to Group III, IV and healthy control (H & E 20X)

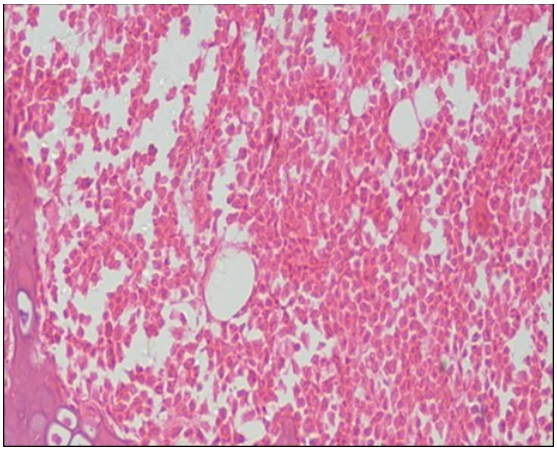


Fig. 9: Long Bone group VI: Decrease in the cellularity of the myeloid and erythroid precursor cells as compared to Group IV and healthy contol (H & E 20X)

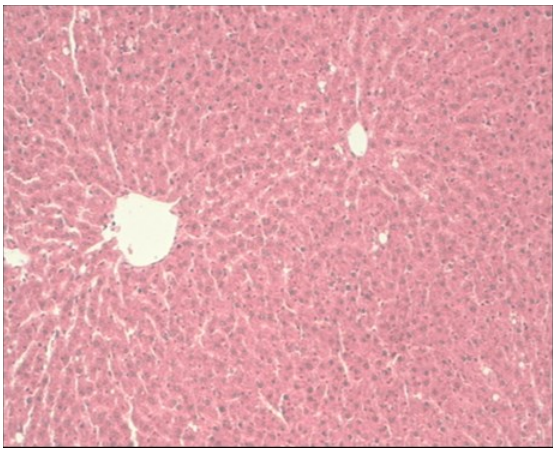


Fig. 10: Liver section Group I: Normal architecture of hepatic lobules (H& E 10X)

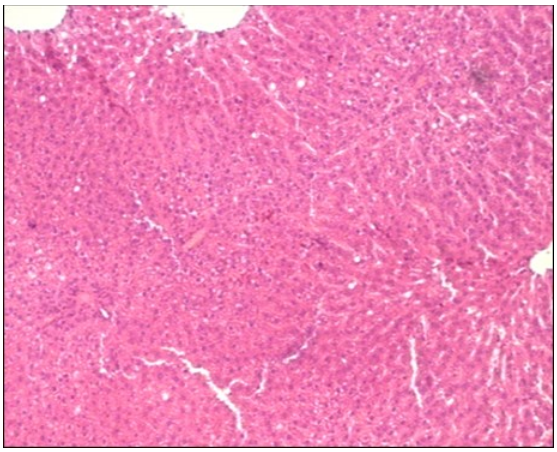


Fig. 11: Liver section Group II: Swollen hepatic cells with fatty changes in periportal and midzonal areas (H& E 10X)

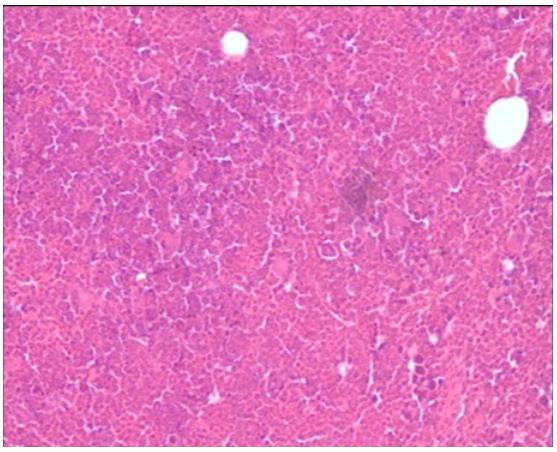


Fig. 12: Liver section Group III: Degeneration of hepatocytes with fatty changes, mild fibrocellular infiltration in the portal connective tissue (H&E 10X)

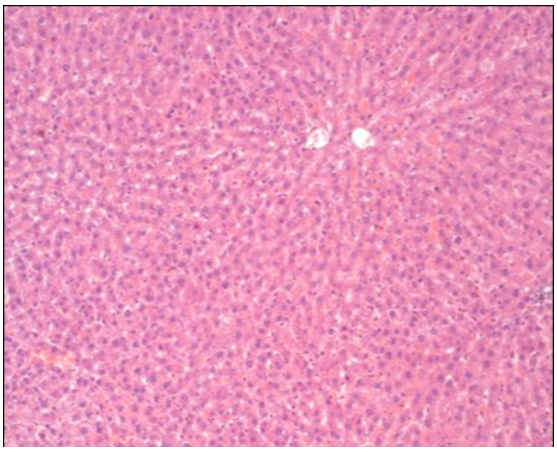


Fig. 13: Liver section Group IV: Apparent normal architecture of the hepatic lobules (H&E 10X)

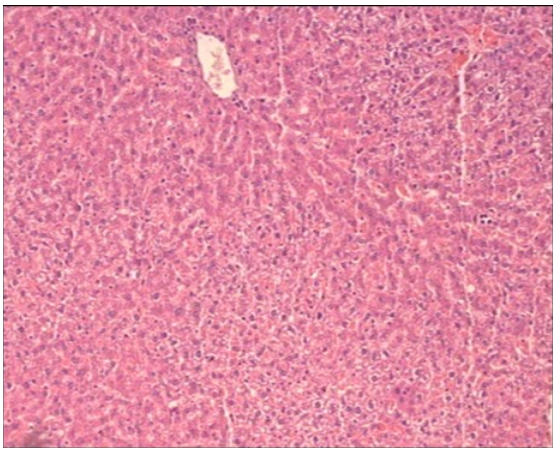


Fig. 14: Liver section Group V: Disorganized cords and degenerated hepatic cells, sinusoids dilatation and few mononuclear cells in the sinus spaces and around the portals (H&E 10X)

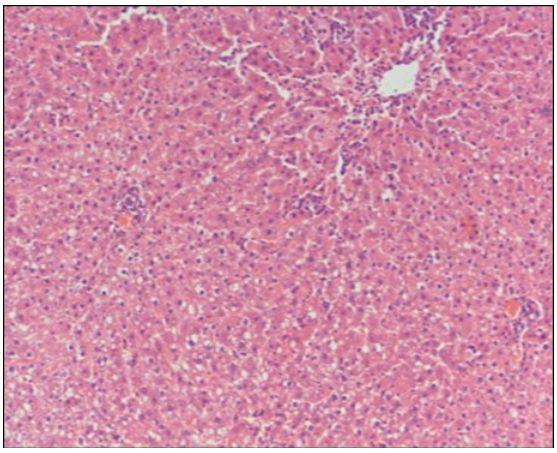


Fig. 15: Liver section Group VI: Degenerated hepatocytes and aggregates of hematopoetic cells scattered in the hepatic lobules (H&E10X)

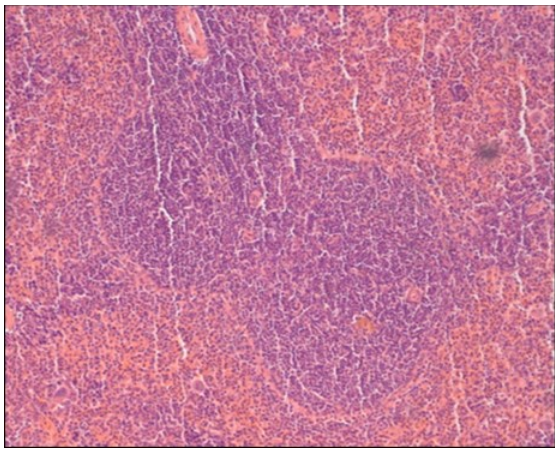


Fig. 16: Spleen section Group I: Normal architecture of white and red pulp region with normal lymphoid tissue cells or macrophages and normal splenic cords (H&E 10X)]

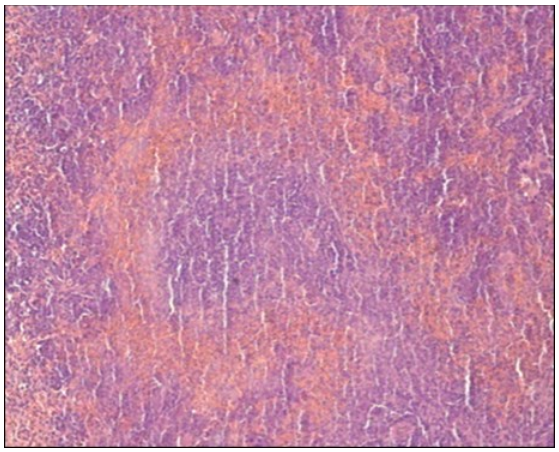


Fig. 17: Spleen section Group II: Reduced lymphoid cells in white pulp and increased number of macrophages and neurtphils in the red pulp sinuses and cords (H&E 10X)

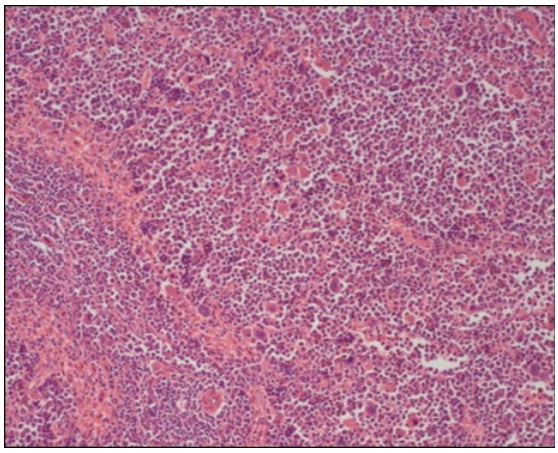


Fig. 18: Spleen section Group III: Hyperplasia of lymphoid tissue in PALS and follicles, activated macrophages and neutrophils in red pulp (H&E 10X)

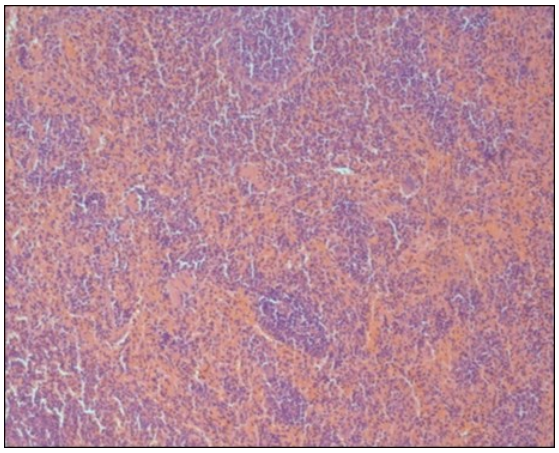


Fig. 19: Spleen section Group IV: Extramedullary hematopoesis with collection of erythroid and myloid precursors cells and plenty of megakaryocytes in red pulp and white pulp zones. The white pulp follicles showing germinal centers with presence of plasma cells (H&E 10X)

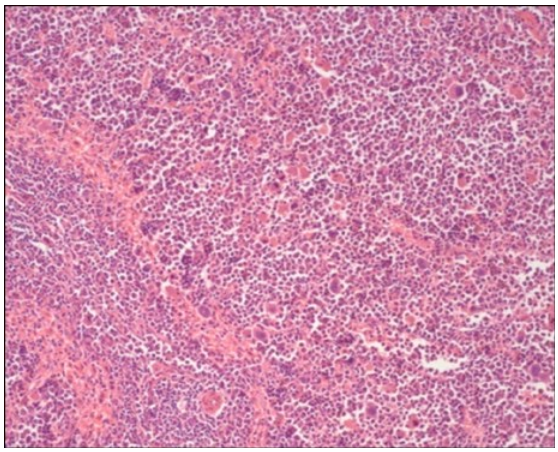


Fig. 20: Spleen section Group V: Hyperplasia of lymphoid tissue in PALS and follicles with presence of germinal center in some follicles and sinusoids are filled with RBC and moderate number of neutrophils (H&E 10X)

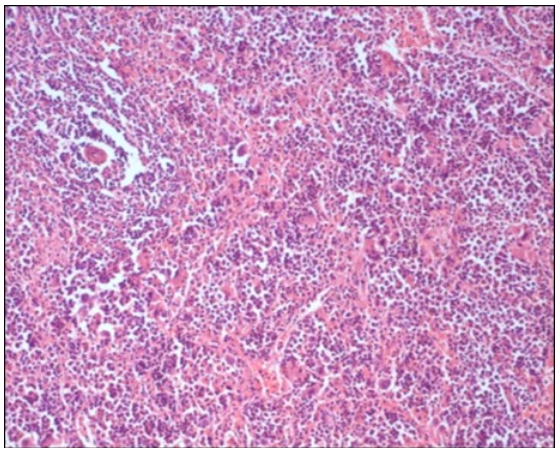


Fig. 21: Spleen section Group VI: Extramedullary hematopoetic cells in white pulp and red pulp with plenty of megakaryocytes and plasma cells and less number of neutrophils in red pulp (H&E 10X)

pulp follicles showing germinal centers with presence of plasma cells which are also visible in large numbers in red pulp areas and graded 2.5. The spleen of group Gr. V showed hyperplasia of lymphoid tissue in PALS and follicles with presence of germinal center in some follicles and sinusoids are filled with RBC and moderate number of neutrophil and graded 1. The spleen of Gr. VI showed extramedullary hematopoetic cells in white pulp and red pulp with plenty of megakaryocytes and plasma cells and less number of neutrophils in red pulp as compared to Gr. V and graded 1.5 .

Table 3. Grading of histopathological changes in bone marrow, liver and spleen tissue of aplastic pancytopenic rats received different treatments

Groups (n=6)	Group I	Group II	Group III	Group IV	Group V	Group VI
HPS grade	3	0	2	2.5	1	1.5

* Gr.I: Healthy control, Gr.II: Disease control, Gr. III: Low dose(LD) NAC, Gr.IV: High dose(HD) NAC, Gr. V: Low dose(LD) Desmopressin, Gr.VI: High dose (HD) Desmopressin

As per the scoring system Gr.IV rats scored best (2.5) with regards to bone marrow stimulation and regenerative changes in histopathogy of bone marrow, liver and spleen section followed by Gr.III (2) . Rats of disease control (Gr.II) showed poor score (0) with severe aplastic pancytopenia and degenerative changes in histopathogy of bone marrow, liver and spleen section as compared to healthy control (Gr.I).

4.2 Screening, diagnosis and therapy of aplastic pancytopenia and or hepatobiliary dysfunction (HBD) associated with canine haemoprotozoan diseases (CHDs) in RVP, IVRI

4.2.1 Screening of animals with characterstics clinical signs in RVP, IVRI.

Dogs presented to OPD III, RVP, IVRI were screened for haemoprotozoan infection based on the characteristic clinical signs suggestive of babesiosis, ehrlichiosis and hepatozoonosis during the study period from March 2021 to May 2022. Out of 4536 dogs presented during the present study period, 96 (2.1%) dogs were suspected for hemoprotozoan disesase with signs like anemia, fever, tick exposure, lymph node enlargement etc. Among those dogs, 32 (33%) dogs were found positive for hemoprotozoan disesases like *Babesia gibsoni, Babesia*

canis, Ehrlichia canis and Hepatozoan canis infection by microscopic examination. Out of 32 hemoprotozoan positive dogs, only 12 positive dogs were subjected in the present study.

4.2.2 Breed wise distribution

The breed wise predisposition of haemoprotozoan infection in the present study has been given in Fig. 23 Out of 32 hemoprotozoan positive dogs, 7 (21.8%) Labrador retriever, 5 (15.6%) Pomeranian, 11 (34.3%) German shepherd, 4 (12.5%) Rottweiler, 2 (6.25%) pug and 3 (9.3%) Mongrel were found infected.

4.2.3 Sex wise distribution

The sex wise distribution of haemoprotozoan infection in the present study has been given in Fig. 24. Out of 32 haemoprotozoan infected dogs, 19 (59%) dogs were male and 13 (40.6%) dogs were female.

4.2.4 Diagnosis of canine hemoprotozoan disease

Giemsa stained peripheral blood smears were examined microscopically for the presence haemoprotozoan infection. All vital parameters like temperature, mucus membrane, tick exposure, lymph node enlargement were recorded in the infected dogs. Blood and serum samples were collected before treatment for complete blood count analysis, oxidative stress, serum biochemistry studies. Dogs with visible signs of organ enlargement were subjected to ultrasonographic examination. Dogs found positive for hemoprotozoan infection with anemia cum thrombocytopenia were selected for study and randomly divided into two groups for different therapeutic interventions. At the end of therapy blood and serum samples were collected again for monitoring the therapeutic response.

4.2.5 Parasite wise distribution

The parasite wise distribution of hemoprotozoan infection in the present study has been given in Fig.26. Out of 32 haemoprotozoan infected dogs, 11 (37.5%) dogs were infected with *Ehrlichia canis*, 8 (25%) were suffered from *Babesia gibsoni*, 7 (21.8%) with *Babesia canis*, 2 (6.25%) with *Hepatozoan canis* and 3 (9.3%) with mixed infection of *Babesia and Ehrichia* spp.

Fig. 22: Screening of animals for hemoprotozoan diseases

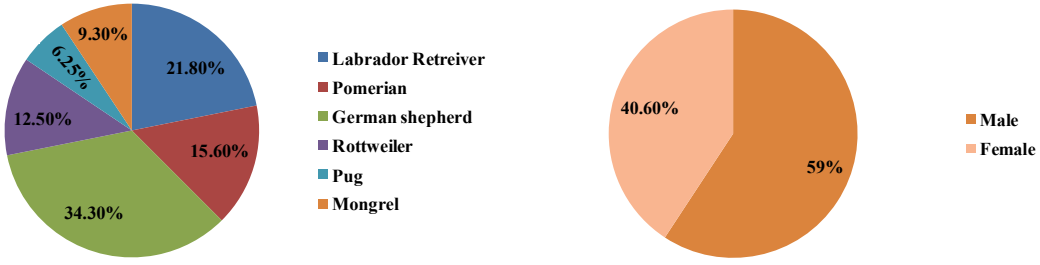


Fig. 23,24 : Breed wise and sex wise distribution of haemoprotozoan infection

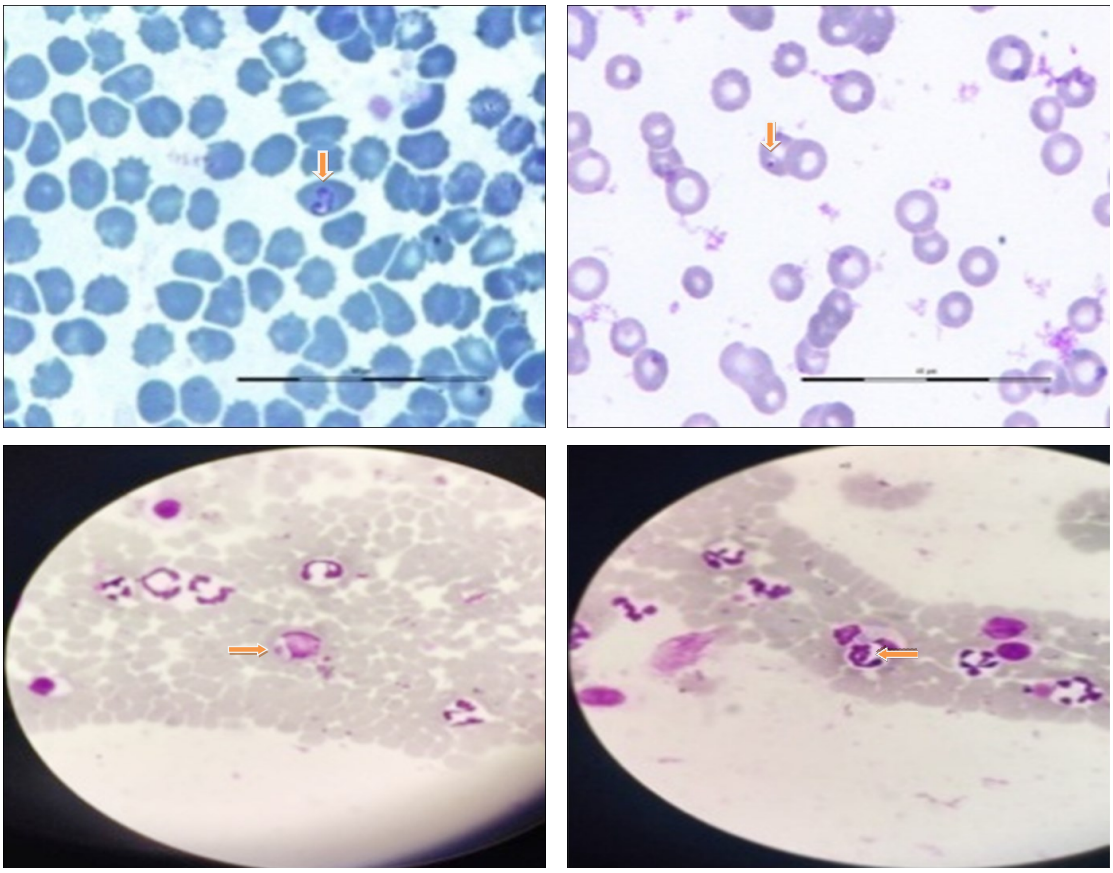


Fig. 25: Hemoparasites under microscopic examination

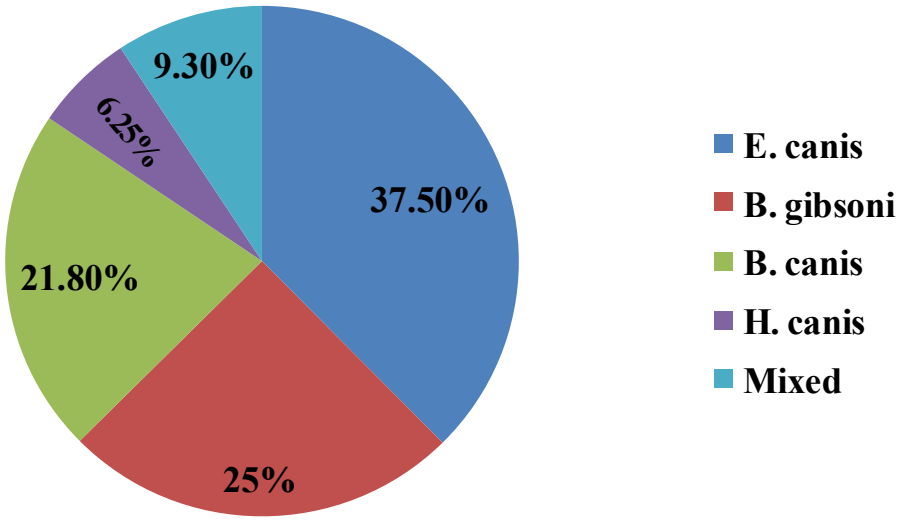


Fig. 26: Parasite wise distribution of haemoprotozoan infection

4.3 Evaluation of novel therapeutic regimen against aplastic pancytopenia and hepatobiliary dysfunction (HBD) associated with CHDs

Out of total 36 positive cases, only 12 dogs as per available technical and managemental facilities were subjected in the present study.

Groups* (n=6)	Therapeutic agent
I	Disease specific therapy+ Symptomatic supportive therapy
II	Disease specific therapy+ Symptomatic supportive therapy+ Bone marrow stimulator (*NAC @15mg/Kg B.Wt PO) for 14days

All the dogs were reexamined for giemsa stained peripheral blood smears even after 14 days of treatment. Therapeutic efficacy has been assessed based on clinical score, hematobiochemical and USG observation.

4.3.1 Haematological changes

Pre and post treatment changes in the hematological parameters have been given in table 4. Significantly ($P<0.05$) increased level of hemoglobin, packed cellular volume (PCV), total erythrocyte count (TEC) and platelets was observed in Gr I and II after 14 days of treatment as compared to day 0. The Hb level and platelet count of Gr. II was significantly ($P<0.05$) increased after 14 days of therapy as compared to Gr I. The PCV level and TEC of Gr. II was non-significantly increased after 14 days of therapy as compared to Gr I. With respect to total leukocyte count (TLC), non significantly decreased TLC was observed at the end of therapy as compared to day 0 in both treatment groups.

Critical analysis of the data revealed better efficacy in animals supplemented with NAC @15mg/Kg B.Wt with respect to Hb and platelet count.

Table 4. Mean±SE values of Complete blood count (CBC) in haemoprotozoan infected dogs received different treatments

Groups*	Hb (g/dl)		PCV (%)		RBC ($\times10^{6}$/µl)		WBC ($\times10^{3}$/µl)		Platelet (lakhs)	
	0 Day	15Day	0 Day	15Day	0Day	15Day	0Day	15Day	0Day	15Day
I	7.1 ± 0.90^{bB}	9.7 ± 0.78^{aB}	21.1 ± 2.64^{bA}	29.1 ± 2.21^{aA}	3.4 ± 0.47^{bA}	4.6 ± 0.21^{aA}	16.3 ± 9.85^{aA}	9.4 ± 0.75^{aA}	0.90 ± 0.07^{bA}	2.5 ± 0.18^{aB}
II	7.9 ± 0.77^{bA}	11.7 ± 0.76^{aA}	24.1 ± 2.94^{bA}	32.1 ± 2.47^{aA}	3.5 ± 0.34^{bA}	4.7 ± 0.36^{aA}	12.7 ± 1.63^{aA}	11.0 ± 1.48^{aA}	0.91 ± 0.12^{bA}	3.8 ± 0.23^{aA}

Mean±SE values within same column for a particular parameter (Capital letter) and in same row (small letter) bearing similar superscript do not differ at $P<0.05$

*Gr.I: Disease specific therapy treated group, Gr.II: Disease specific therapy plus NAC treated group

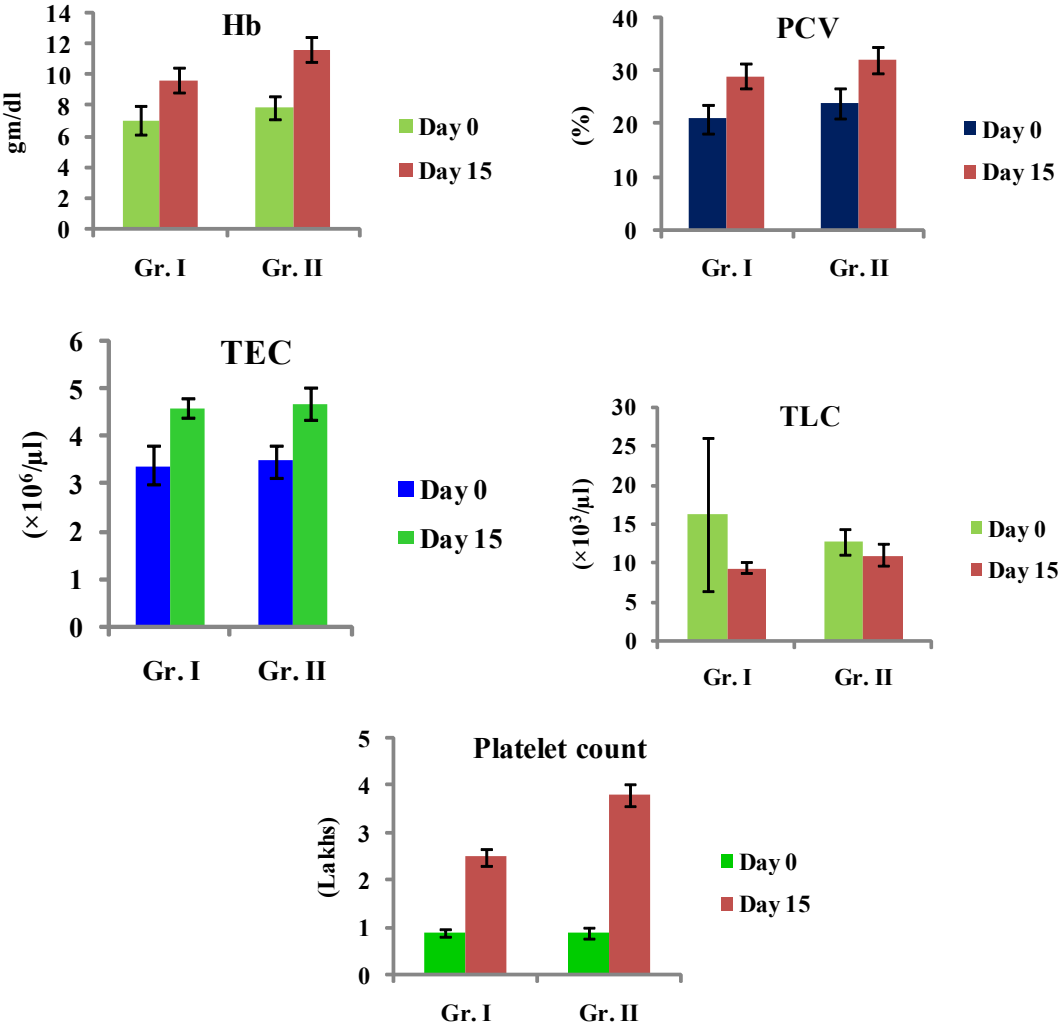


Fig. 27: Complete Blood Count changes in treated dogs

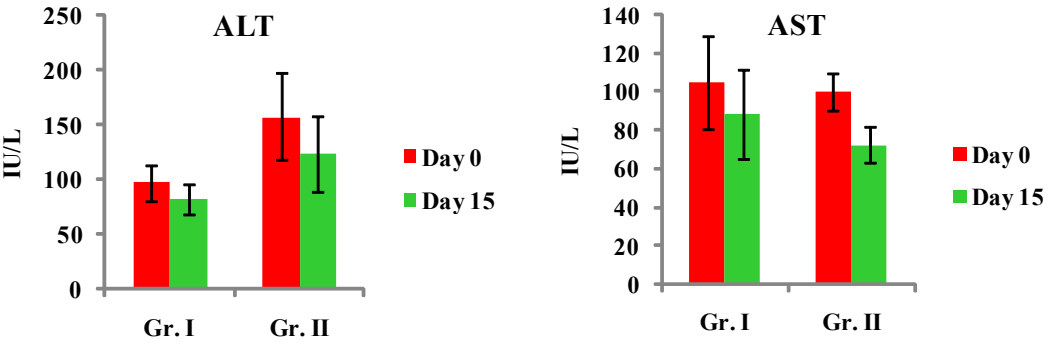


Fig. 28: Serum biochemical profile in treated dogs

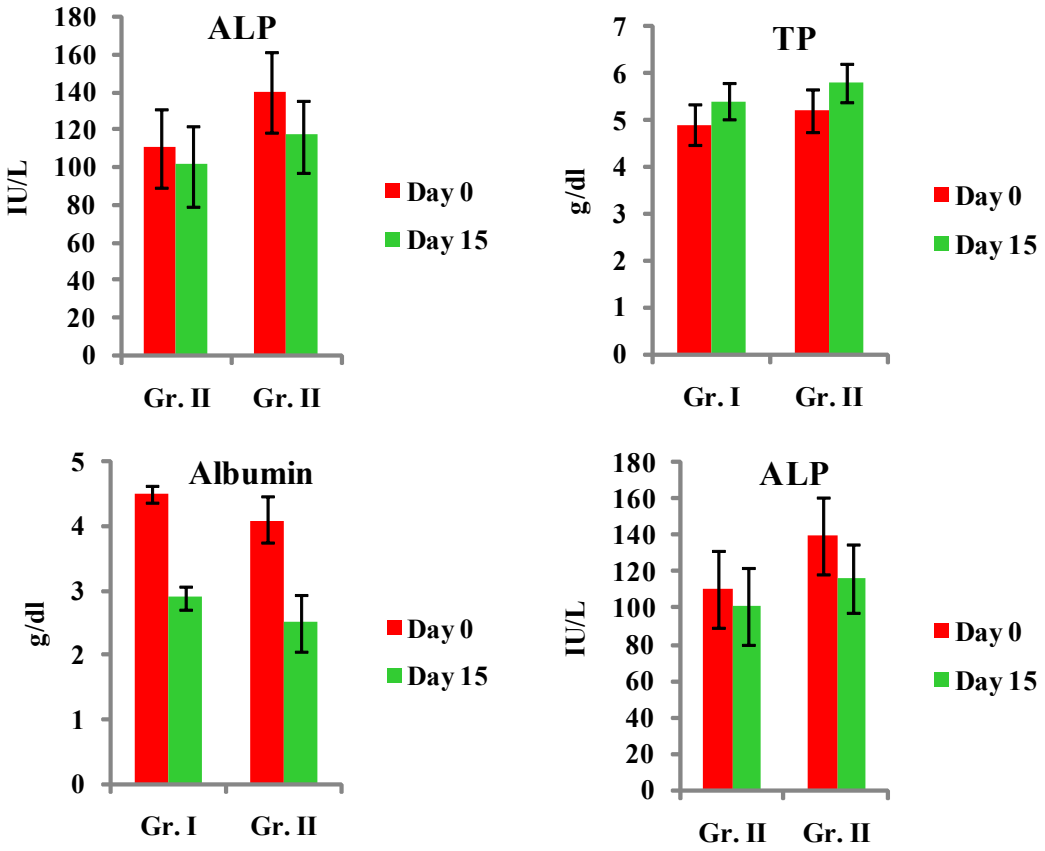


Fig. 28: Serum biochemical profile in treated dogs

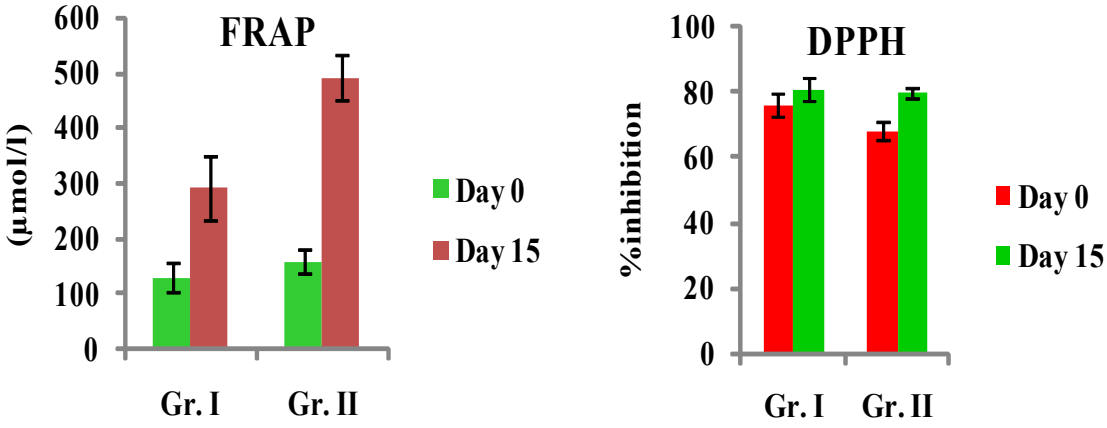


Fig. 29: Anti-oxidant changes in the treated dogs

Table 5. Mean±SE values of serum biochemical profile in haemoprotozoan infected dogs having received different treatments

Groups*	ALT (IU/L)		AST (IU/L)		ALP (IU/L)		TP (g/dl)		Albumin (g/dl)	
	0 Day	15Day	0 Day	15Day	0Day	15Day	0Day	15Day	0Day	15Day
I	97.5±16.29^{aB}	82.3±13.89^{bB}	105.4±24.24^{aA}	88.8±22.95^{bA}	110.1±21.15^{aB}	100.8±21.11^{bB}	4.9±0.43^{bA}	5.4±0.38^{aB}	2.8±0.19^{aA}	3.2±0.15^{aA}
II	157.6±39.57^{aA}	123.8±35.17^{bA}	100.5±9.84^{aA}	72.6±8.97^{bB}	139.5±21.34^{aA}	116.5±18.97^{bA}	5.2±0.46^{bA}	5.8±0.41^{aA}	2.4±0.27^{bA}	3.1±0.10^{aA}

Mean±SE values within same column for a particular parameter (Capital letter) and in same row (small letter) bearing similar superscript do not differ at $P<0.05$

*Gr.I: Disease specific therapy treated group, Gr.II: Disease specific therapy plus NAC treated group

4.3.2 Serum biochemical alterations

Pre and post treatment changes in the serum biochemical parameters have been given in table 5. Significantly ($P<0.05$) decreased level of ALT, AST and ALP was observed in the both groups at the end of trial as compared to day 0. In Gr. I and II significantly P(<0.05) increased level of total protein (TP) was observed within the groups. Non-significantly increased value of albumin was found in Gr.I whereas in Gr.II, albumin value was significantly P(<0.05) increased after 14 days of treatment as compared to day 0.

Critical analysis of the data revealed better efficacy in animals supplemented with NAC @15mg/Kg B.Wt with respect to ALT, AST, ALP and TP.

4.3.3 Anti Oxidant parameters

4.3.3.1 Ferric- Reducing Antioxidant Power Assay (FRAP) and

In this FRAP assay, antioxidant activity was assessed based on the ability to reduce ferric (III) iron to ferrous (II). The standard curve was generated using different concentration of ferrous sulphate and the result was expressed as µM ferrous ion equivalent per liter ($y= 0.0002x+ 0.0096$, $R^2=0.9917$). FRAP assay of both the groups showed significantly ($P<0.05$) increased values in serum samples at 14 days of treatment as compared to day 0 (Table 6).

Critical analysis of the data revealed better efficacy in animals supplemented with NAC @15mg/Kg B.Wt with respect to FRAP.

4.3.3.2 Determination of Free Radical Scavenging Activity by DPPH assay

DPPH is a well known radical scavenger. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. This assay is based on the theory that a hydrogen donor is an antioxidant and measures compounds that are radical scavengers. The result of this assay was expressed as % inhibition. The DPPH activity of gr. I &II was significantly ($P<0.05$) increased in serum samples after 14 days of treatment as compared to day 0 (Table-6).

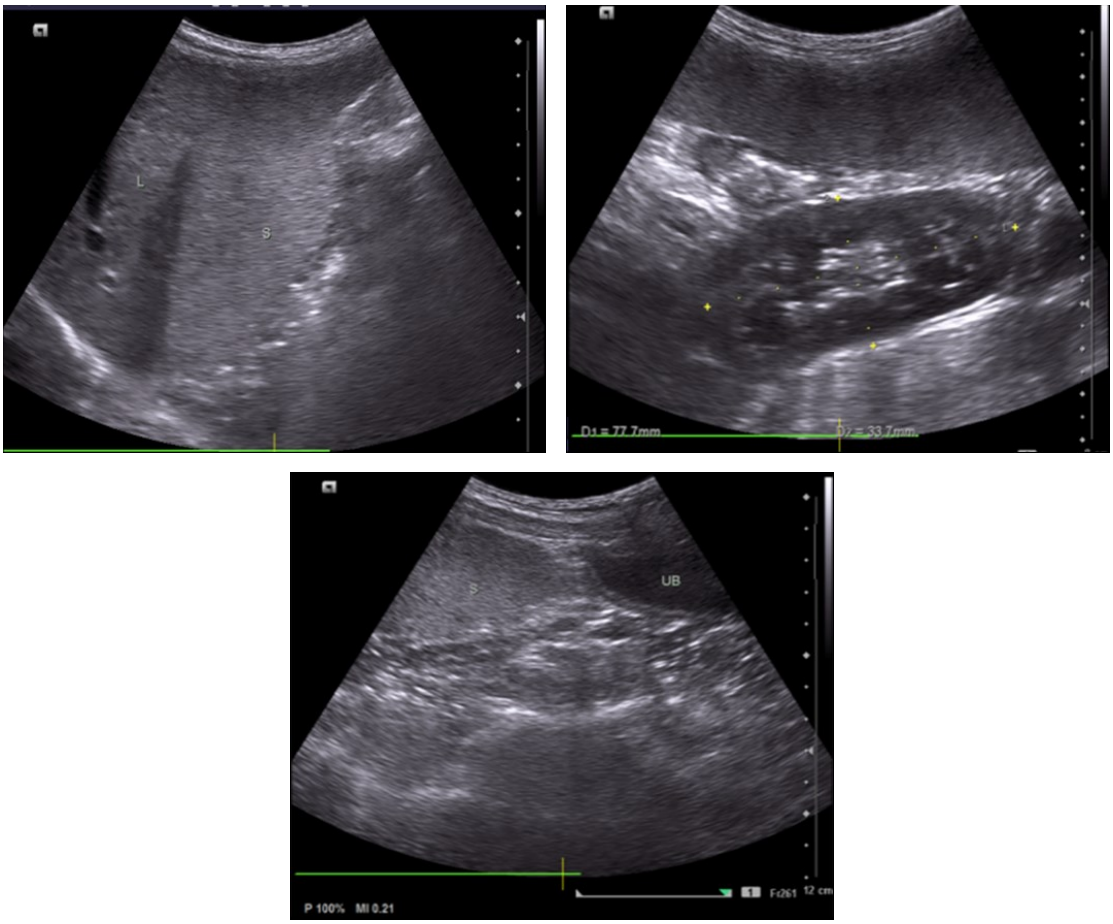


Fig. 30: Enlarged liver and spleen in a dog, suffering from hemoprotozoan infection, as viewed from the ventral sagittal section through a left subcostal window (a); enlarged spleen in a dog suffering from hemoprotozoan infection as viewed in the left lateral sagittal section (b); enlarged spleen in a dog having hemoprotozoan infection with tail portion of the spleen reaching up to the urinary bladder base, as viewed in the left lateral sagittal section (c)

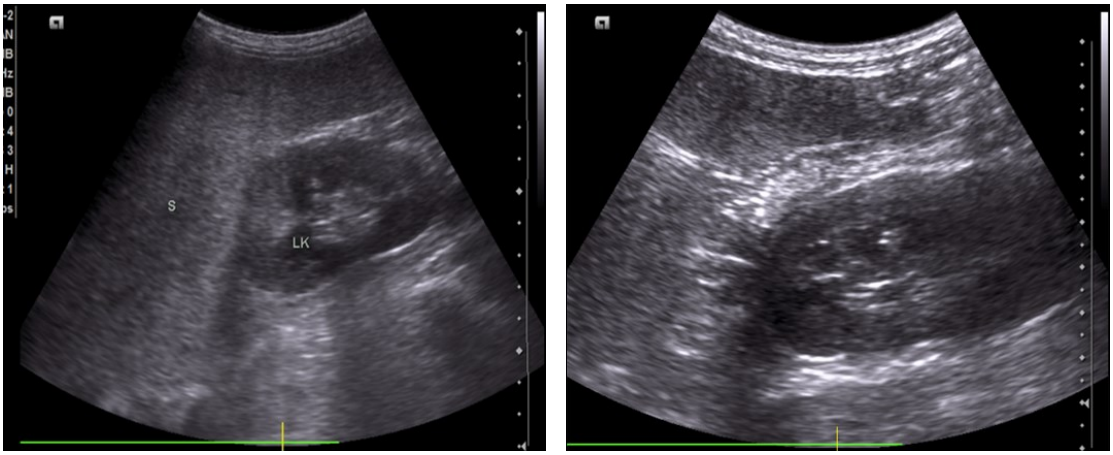


Fig. 31: Enlarged spleen in a dog, suffering from hemoprotozoan disease, as viewed from the ventral sagittal section through a left subcostal window on the day of presentation

Critical analysis of the data revealed better efficacy in animals supplemented with NAC @15mg/Kg B.Wt with respect to DPPH.

Table 6: Anti-oxidant changes in the treated dogs (mean± SE)

Group	FRAP (µmole/l)		DPPH (%inhibition)	
	0 day	15 day	0 day	15 day
I	129.3 ± 26.59^{Ab}	293.3 ± 57.8^{Ba}	76 ± 3.4^{Aa}	81 ± 3.5^{Ab}
II	158.5 ± 22.3^{Ab}	491.8 ± 42.3^{Aa}	68.3 ± 2.8^{Ba}	79.8 ± 1.6^{Bb}

Mean±SE values within same column for a particular parameter (Capital letter) and in same row (small letter) bearing similar superscript do not differ at $P<0.05$
*Gr.I: Disease specific therapy treated group, Gr.II: Disease specific therapy plus NAC treated group

4.3.4 Ultrasonography

In the present study, 12 hemoprotozoan infected dogs were subjected to ultrasonography based on physical examination. Out of 12 dogs, 5 dogs showed marked splenomegaly and 4 dogs showed marked hepatomegaly, whereas the 3 dogs showed normal architecture of liver and spleen. In the dogs showing splenomegaly, spleen was found to occupy all of the lateral abdominal wall. In one case, the tail portion of the spleen formed a flexure at the level of left kidney and extended to the right abdominal area. Tail portion was found extending upto the base of urinary bladder in some cases. In cases of splenomegaly, splenic parenchyma was hyperechoic to renal cortex. No focal lesions were observed in any case. Liver was enlarged with caudal borders of the lobes extending beyond the xiphoid and costal arch, bilaterally. Hepatic parenchyma was hypoechoic to spleen.

All the available mean hematological data has been plotted below in the table 7 as per the methods of human patient Child - Pugh score (Durand and Valla, 2008) with slight modifications in ailing dogs.

Table 7: Prognostic score card

Mean values	Prognostic Markers			
	Gr. I		Gr. II	
	0 day	15 day	0 day	15 day
Hb (12-18gm/dl)	7.1(1)	9.7(2)	7.9(1)	11.7(3)
TEC count (5-8× 10^6/ µl)	3.4(2)	4.6(3)	3.5(2)	4.7(3)
TLC count (5-14× 10^3/ µl)	16.3(1)	9.4(3)	12.7(3)	11.0(3)
Platelet count (1.5-4 lakhs)	0.9(2)	2.5(2)	0.9(2)	3.8(3)
Reticulocyte Count (60-80,000/µl)	<30,000(1)	46,000(2)	<30,000(1)	66,000(3)
Total score	7= Grade C (1^+)	12= Grade B (2^+)	9= Grade C (1^+)	15= Grade A (3^+)
Grading	**Poor Prognosis**	**Moderate Prognosis**	**Poor Prognosis**	**Good Pognosis**

4.3.5 Prognostic score card

For prognostic score card ranking, Hb, TEC, TLC, Platelet count and Reticulocyte count were taken into consideration and assigned score in the scale of (+)1 to (+)3, wherein (+)3 indicated good prognosis within average value of 13-15, similarly average value of 10-12 indicated (+)2 moderate prognosis and average value of 7-9 indicated (+)1 poor prognosis.

Aplastic pancytopenia: Response to the treatment was assessed by measuring Hb, TEC, TLC, platelet count and reticulocyte count and the result revealed that the Gr. II dogs treated with disease specific, symptomatic therapy along with N- acetylcysteine @ 15 mg/Kg B.wt revealed favourable clinical recovery as compared to Gr. I. dogs without supplementation.

Discussion

5 DISCUSSION

5.1 Experimental study of bone marrow (BM) stimulating property of N-acetylcysteine and Desmopressin in rats

5.1.1 Induction of aplastic pancytopenia by bone marrow suppression in rat

Bone marrow(BM) is one of the vital part of the body, responsible for preservation of immunity and other homeostatic functions by the production of red blood cells (RBCs), hemoglobin (Hb), white blood cells (granulocytes and agranulocytes), and platelets (Raj and Gothandam., 2015). Myelosuppression or myelotoxicity more often predisposes individuals to life-threatening conditions, such as neutropenia, thrombocytopenia, septicemia, and multi-organ failure (Richardson *et al*., 2016). In parvoviral enteritis, anemia is associated with cytotoxic effect of virus on hematopoietic proginator cells that subsequently lead to erythroid hypoplasia (Nandi and Kumar, 2010; Grimes and Fry., 2015). Myelosuppression is the most common side effect of chemotherapy. Cyclophosphamide (CP) is the commonly used myelotoxic anticancer drug which causes severe oxidative stress, inflammation and alters the hematopoietic activity of bone marrow by producing reactive oxygen species (ROS) and inflammatory cytokines (Iqubal *et al*., 2018). The other possibility behind CP-induced myelosuppression is the effect of CP or its metabolites on the DNA of bone marrow cells. In the present study, single dose of cyclophosphamide @37.5mg/Kg B.Wt SC route was effective in inducing bone marrow suppression cum aplastic pancytopenia in Gr. II rats receiving CP @37.5mg/Kg B.Wt SC single dose. It was in agreement with the study of Ashif *et al.,* (2020) who recorded significantly ($P<0.05$) reduced level of Hb, RBC, WBC, granulocyte, lymphocyte, monocyte, and platelets after single dose of cyclophosphamide @200 mg/kg b.wt I/V in rats. In the present study,

induction of aplastic pancytopenia with single S/C dosing of CP @37.5mg/Kg B.Wt revealed better and effective experimental modal to induce aplastic pancytopenia in rats with reduction of hemoglobin, packed cell volume, total erythrocyte count and platelet count of 5%, 15%, 3% and 3.5% respectively. Likewise cyclophosphamide mediated immunosuppressive model was induced in BALB/c mice (Zhang *et al.,* 2020).

5.1.2 *In vivo* study in rat to assess bone marrow stimulating properties of N-acetylcysteine and Desmopressin

N-Acetylcysteine was used for the management of anemia and oxidative stress in hemodialysis patients and it was considered as a promising antioxidant therapy (Stel *et al*.,2000; Hsu *et al*., 2010).. Desmopressin is an analog of the antidiuretic hormone (ADH) that interacts with type 2 vasopressin receptors of endothelial cells and thereby inducing the release of von willebrand factor (VWF) and associated factor VIII which is hemostatically effective in mild hemophilia and Von willebrand type 1 diseases (Mannucci *et al*.,1977). Furthermore clinical studies showed desmopressin was effective in treating platelet disorder/hemostasis in thrombocytopenia associated with BM failure cases. However, some findings supported the efficacy of desmopressin in platelet disorders (Castaman *et al*., 1997; Noris *et al*., 1998). The current study was designed to explore the myeloprotective and hematopoietic potential of N-acetylcysteine and desmopressin in CP-induced myelotoxic wister rats in terms of bone marrow cellularity, total blood count and liver profile and found NAC @50mg/Kg B.wt orally daily depicted better efficacy in relation to bone marrow cellularity and total blood count by 10 days of therapy .

5.1.3 Parameters of Study

5.1.3.1 Haematological parameters

Emeka *et al*. (2015) demonstrated, myeloprotective activity of crude methanolic leaf extract of *Cassia occidentalis* in cyclophosphamide-induced bone marrow suppression in terms of increased hemoglobin production and leucocytosis in wistar rats. Similar changes were observed with nerolidol for myeloprotection and amelioration of hematological toxicity on cyclophosphamide-induced bone marrow suppression in swiss albino mice (Asif *et al*.,

2020). Khazaei *et al*. (2020) also revealed protective potential of royal jelly for normalizing the number of platelets in thrombocytopenic rats caused by chemotherapeutic agents (CP). Similar findings were observed in the current study with oral administration of high dose (50mg/Kg) NAC in terms of improved hematological parameters in cyclophosphamide induced bone marrow suppression. The current study showed significantly ($P<0.05$) increased haematological parameters *viz* Hb, PCV, TEC and Platelet count of Gr. IV (NAC 50mg/Kg) as compared to Gr. II (Disease control). Significant increase in the haematological parameters of Gr. IV (NAC@50 mg/kg) could be attributed to its protective and antioxidant effect on bone marrow cells. Significant ($P<0.05$) reduction was observed in the haematological parameters of Gr. II (Disease control) and this could be due to oxidative stress and direct effect of toxin and its metabolites on the DNA of bone marrow cells (Ashif *et al.* 2020).

5.1.3.2 Serum biochemical profile

Cyclophosphamide causes liver damage, indicated by increase in ALT, AST and ALP levels in serum (Mohammad *et al*.,2014). In the present study, significant ($P<0.05$) increased levels of ALT, AST and ALP in serum of Gr. II rats were noticed which was in agreement with findings of Mohammad *et al*. (2014). Administration of aqueous carob extract exhibited protection against hepatic tissue damage induced by the CP by decreasing oxidative stress (Ibrahim *et al*.,2017). Similarly in our study, Gr. IV rats treated with high dose (50mg/Kg) NAC showed improvement in the liver biomarkers. This effect may be due to free radical scvanging activity of NAC.

5.1.3.3 Histopathology of decalcified bone

Sections of rat bone marrow in healthy control group (Gr.I) revealed normal histological picture, the stromal elements form an extensive, tightly packed network in which 80% or more hematopoietic cells are embedded. Mature myeloid cells and megakaryocytes were present in intertrabecular spaces. Histological findings of cyclophosphamide induced rats in the current study revealed reduction in hematopoeitic count with increase in adipose tissue. These findings were in accordance with the Ashif *et al*. (2020) and Samah *et al.* (2021) findings who also reported that bone marrow was markedly hypo-cellular, with the distortion

of the myeloid and erythroid tissues and more empty spaces in the cyclophosphamide treated group. Treatment with high dose NAC (50mg/Kg) significantly ($p<0.05$) reversed these histological aberrations of bone marrow towards normalcy which exhibited myeloprotective activity, as illustrated in Figure 7.

Histopathology of liver

Rat liver of healthy control group (Gr. I) in the present study failed to reveal any specific pathological changes and liver section revealed normalcy of hepatic cords, well organized hepatocytes without any area of fatty changes. Hepatic damage produced by cyclophosphamide in Gr. II was characterized by swollen hepatic cells with fatty degeneration in periportal and midzonal areas, engorged sinusoids around central veins, mild fibrocellular reaction in periportal connective tissue and necrotic hepatic cells at places. This was in accordance with Hutheyfa *et al*. (2020) who defined fatty changes in the hepatocytes with diffuse kupffer cells proliferation and dilated portal vein. Rats of Gr. IV treated with NAC (50mg/kg) showed regenerative changes like completely packed bone marrow with hemopoetic cells and the presence of megakaryocytes. This hepatoprotective effect may be due to antioxidant activity of NAC. Similar hepatoprotective effect was reported by Zhaobin *et al*. (2015) who concluded that NAC treatment significantly reduced the necrotic area in the liver tissue.

Histopathology of spleen

Spleen can perform compensatory hematopoiesis in the milieu of impaired bone marrow function to restore the hematological process (Han *et al*., 2018). Sections of spleen in healthy control group (Gr.I) in the current study revealed normal architecture of white and red pulp region with normal lymphoid tissue cells or macrophages and normal splenic cords. Cyclophosphamide administration showed reduction of the diameter and lymphoid cells in white pulp and increased number of macrophages and neutophils in the red pulp sinuses and cords in Gr. II rats. This was in accordance with Fatemeh *et al*. (2019) who noticed disorganization in splenic structures such as hemosiderin deposition and reduction of the diameter of white pulp. These alterations were significantly improved in NAC(50mg/kg) treated group (Gr. IV). Significantly increased diameter of white pulps, extramedullary hematopoesis with

collection of erythroid precursors cells, myloid precursors cells and plenty of megakaryocytes in red pulp and white pulp zones were noticed in GR, IV rats (NAC @50mg/kg). Additionally the white pulp follicles also showed germinal centers with presence of plasma cells which were also visible in large numbers in red pulp areas indicating that NAC (50mg/kg) treatment promoted the recovery of this damage after cyclophosphamide administration.

5.2 Screening, diagnosis and therapy of aplastic pancytopenia and or hepatobiliary dysfunction (HBD) associated with canine haemoprotozoan diseases (CHDs) in RVP, IVRI

5.2.1 Screening of dogs for hemoprotozoan disease in RVP, IVRI

In the present study, 96 cases were suspected for hemoprotozoan disease, out of which 32 were found positive for various haemoprotozoan infection by microscopic examination. In line with this observation, Godara *et al.*(2010) reported prevalence rate of haemoprotozoan infection in dogs was 16.39%, out of which 13.1% were Babesia spp. and 4.9% were *Ehrlichia canis* in semi-arid region of Jaipur (Rajasthan). Similarlly prevalence rate of *Babesia gibsoni* was reported (0.1%) in Chennai, (9%) in Uttarpardesh (UP) and 22% in Assam (Sundar *et al*. 2004, Chaudhuri, 2006). One more study at Assam revealed prevalence rate of (47.16%) of *Babesia gibsoni* in canines (Bhattacharjee., 2013). In India very less studies interrogating the prevalence of canine ehrlichiosis using conventional examination of stained blood smears and Samarandi *et al*.(2003) have reported prevalences of 18.9% in Nagpur (n = 238), Juyal *et al*. (1994) reported prevalences of 0.35% (n = 752) in Punjab and Mallapur, (2002) reported prevalences of 55% in stray dogs in Maharashtra.

5.2.2 Breed wise distribution

In the present study, maximum prevalance of haemoprotozoan infection was found in German shepherd breed (34.3%) followed by Labrador reteriver (21.8%) and Pomerian (15.6%). These differences may be due to the canine population and breed preference of owners in present geographical location The prevalance of breed wise distribution of haemoprotzoans disease in our study was in contrary with the observation of Raguvaran *et al.* (2016) who reported frequent infections was found in Labrador breed of the dog.

5.2.3 Sex wise distribution

Out of 32 haemoprotozoan positive dogs, 19 (59%) were male and 13 (40.6%) were female in current study. Highest prevalence of haemoprotozoan infection was recorded in male which may be due to larger male population in the study area.

5.2.4 Diagnosis of canine hemoprotozoan disease

Light microscopic evaluation of blood smears is gold standard tool for acute infection with high parasitemia. PCR methods have been reported to diagnose hemoprotozoan infection with high sensitivity and specificity especially in ehrlichiosis (Birkenheuer *et al*. 2003; Inokuma *et al*. 2004). In the present study, 12 dogs were found positive for hemoprotozoan infection by light microscopic examination of giemsa stained peripheral blood smear.

5.2.5 Parasite wise distribution

Out of 32 haemoprotozoan infected dogs, 12 (37.5%) dogs were positive for *Ehrlichia canis*, 8 (25%) were positive for *Babesia gibsoni*, 7 (21.5%) positive for *Babesia canis*, 2 (6.25%) were positive for *Hepatozoan canis* and 3 (9.3%) were positive for mixed infection of *Babesia and Ehrichia* spp of present study. High prevalence of *Ehrichia canis* infection may be due to larger population of *Rhipicephalus sanguineus* ticks in the study area.

5.3 Evaluation of novel therapeutic regimen against aplastic pancytopenia and hepatobiliary dysfunction (HBD) associated with CHDs

Critical analysis of the present in vivo experimental study in rat model revealed better efficacy with NAC @ 15 mg/Kg B.wt over and above desmopressin @ 15 µg/Kg B.wt with respect to clinical and hematobiochemical improvement. During clinical study for therapeutic regimen against aplastic pancytopenia and hepatobiliary dysfunction (HBD) associated with hemoprotozoan disease in canines. The dose obtained in rat model has been converted by the formula of Reagan- Saw *et al*. (2007) with slight modifications which revealed calculated dose of dog is of 15 mg/Kg B.wt.

5.3.1 Haematological changes in the hemoprotozoan infected dogs

Hemoprotozoal diseases results in the disturbance of iron metabolism and subsequently leading to anaemia (Jitin *et al.,*2015). Giger (2005) opinioned that low values of Hb, TEC and PCV in the hemoprotozoan infection may be attributed to deficiency of iron, destruction of red blood corpuscles and/or excess amont of blood loss and reduced erythropoietic activity. Anemic changes were clearly evident in hemoprotozoan positive dogs (Warner and Harrus 2000; Aquino *et al*. 2002; Abenga *et al*. (2005). In the present study, significantly ($P<0.05$) increased level of hemoglobin, packed cellular volume (PCV), total erythrocyte count (TEC) and platelets was observed in Gr I and Gr. II after 14 days of treatment as compared to day 0. Significantly ($P<0.05$) increased Hb and platelets values were observed in Gr. II as compared to Gr.I. Non significant increased TEC , PCV and TLC values were observed in Gr. II dogs as compared to Gr.I. Post treatment hematological values in Gr. II dogs (NAC @15 mg/kg bwt) were closer to the normal/ reference values. Potent antioxidant efficacy of NAC has been revealed by (Zhaobin *et al*.,2015; Gaykwad *et al*., 2017; Ashraff *et al*., 2020 and Kyung *et al*., 2020). The present study depicted potent antioxidant activity with NAC @ 15 mg/Kg B.wt for regeneration of the hepatocytes in the dogs with HBD which in turn might improved post treated hematopoietic values.

5.3.2 Serum biochemical changes in canine hemoprotozoan infection

Anemia observed in dogs affected with hemoprotozoan infection is considered as one of the factors causing hypoxia and hypoxic liver injury, which can result in elevated levels of ALT, AST, and ALP (Wojciech *et al.,*2011). Similar trend was noticed in both the treated groups of dog on day 0. N-acetylcysteine (NAC) was found to be useful in fulminant hepatic failure associated with paracetamol overdose (Kaeys *et al*.,1991). NAC improved hepatic haemodynamics in patients with fulminant hepatic failure and these effects are mediated by cyclic 3, 5- guanosine monophosphate (cGMP) (Harrison *et al*. 1991 and 1996). NAC increased the concentrations of hepatic ATP and GSH by ameliorating the oxidative stress in obstructive jaundice (Gaku *et al*., 2000). In the present study, significantly ($P<0.05$) decreased level of ALT, AST and ALP was more pronounced in NAC supplemented dogs (Gr.II) at the

end of trial which denotes its potent antioxidant property for regeneration of the hepatocytes in the dogs with HBD which in turn might improved post treated serum biochemical and liver profile values.

5.3.3 Anti-oxidant changes

The imbalance of oxidants and antioxidants molecules in the animal body may leads to many pathological condition.(Paltrinieri *et al.*,2010). Dogs infected with hemoparasites showed increased oxidative markers like Nitric oxide·and advanced oxidation protein products (Baldisseras *et al*.,2015). In the present study, total antioxidant assay was performed by measuring FRAP and DPPH level which showed significant ($P<0.05$) increased FRAP and DPPH level at 14 day of treatment as compared to day 0 in both the groups. However, these levels were significantly ($P<0.05$) higher in Gr. II (NAC @ 50mg/kg) as compared to Gr. I . This result may be due to its protective and antioxidant effect of N-acetylcysteine. The present study depicted potent antioxidant activity with NAC @ 15 mg/Kg B.wt for regeneration of the hepatocytes in the dogs with HBD which in turn might improved post treated antioxidant values.

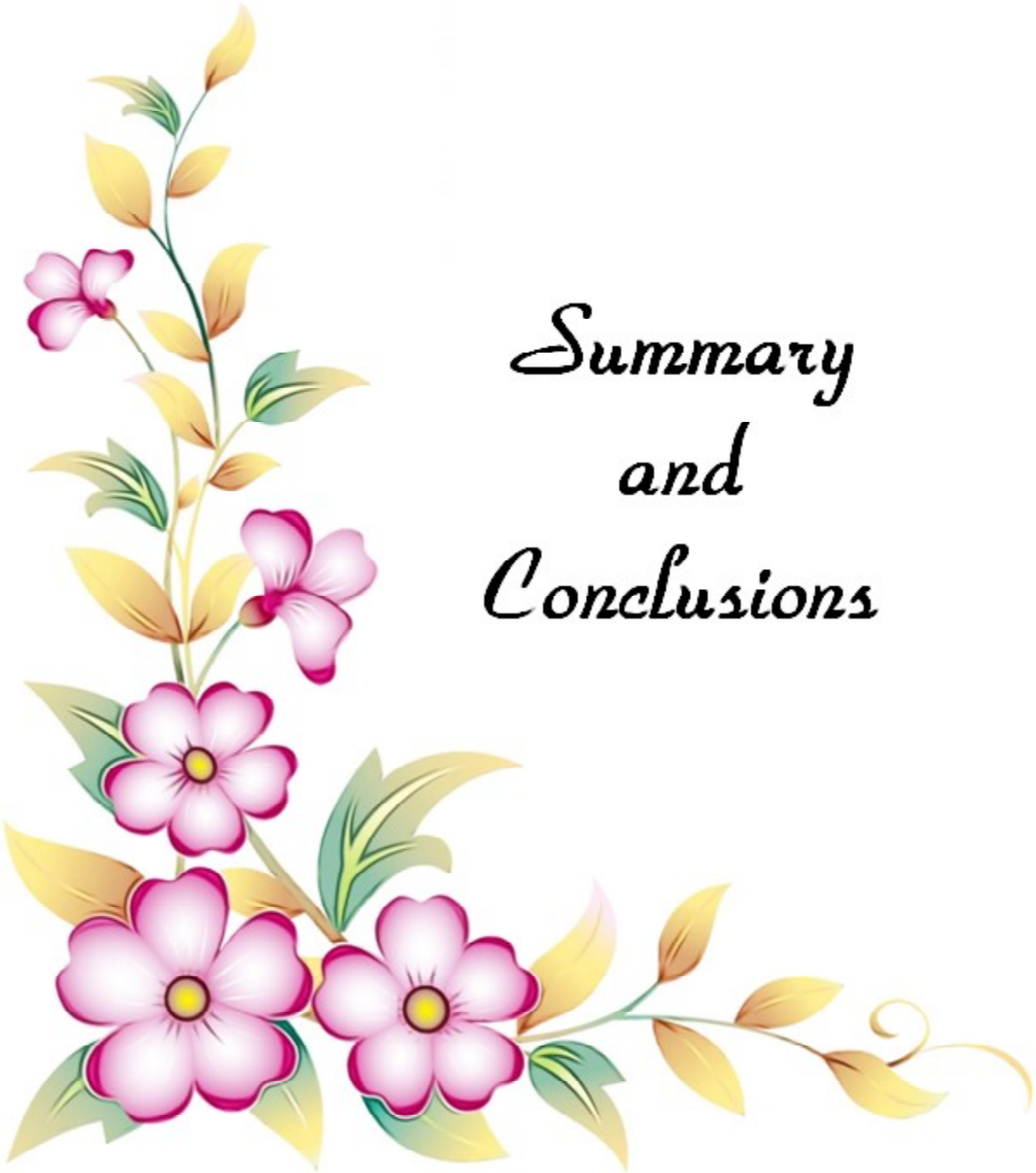
5.3.4 Ultrasonography

In present study, 12 cases were subjected for ultrasonographic examination. Out of 12 hemoprotozoan positive cases, 5 dogs showed splenomegaly and 4 dogs showed hepatomegaly. However other three hemoprotozoan positive dogs showed normal hepatic and splenic parenchyma. An enlarged spleen characterized by diffuse hypoechoic heterogeneous parenchyma was noticed in 5 hemoprotozoan affected dogs. It was in agreement with the Kumar *et al* (2004) and Fraga *et al* (2011). The spleenomegaly in the cases of hemoprotozoan infection has been attributed to acute splenitis, hemolytic anemia and toxemia (Nyland *et al.* 2002). In the present study, dogs infected with hemoprotozoan infection showed high incidence of diffuse hypoechoic hepatomegaly. This may be due to reticuloendothelial hyperplasia, acute inflammation and passive congestion (Irwin and Hutchinson 1991). This was also in agreement with the Kumar *et al* (2004) and Fraga *et al* (2011).

5.3.5 Prognostic score card

Critical evaluation of hematological parameters in both the treated groups revealed that the Gr. II dogs treated with N- acetylcysteine @ 15 mg/Kg B.wt receiving disease specific therapy, symptomatic therapy revealed favourable clinical recovery as compared to Gr. I. Prompt therapeutic interventions is very essential in treating aplastic pancytopenia in hemoparasitic diseases. Otherwise the animal may develop critical anemia which warrents blood transfusions. As like human beings, well established blood banks not widely prevelenat in canine. If at all, it is available it may not be affordable to everyone.

The present study revealed NAC @ 15 mg/Kg B.wt orally for 14 days could halter the progression of critical anemia and thrombocytopenia along with disease specific therapy and supportive therapy in dogs with compromised liver due to hemoprotozoan disease.



Summary and Conclusions

6 SUMMARY AND CONCLUSIONS

The study was conducted to evaluate bone marrow stimulating property of N-acetylcysteine and Desmopressin for therapeutic management of aplastic pancytopenia associated with canine hemoprotozoan diseases (CHD). It was conducted in three distinct phases viz Experimental study of bone marrow stimulating property of N-acetylcysteine and Desmopressin in rats, screening, diagnosis and therapy of aplastic pancytopenia and or hepatobiliary dysfunction (HBD) associated with CHDs in RVP, IVRI and to Evolve novel therapeutic regimen against aplastic pancytopenia and HBD associated with CHDs.

Firstly, pilot study was conducted to establish the experimental bone marrow suppression in rat model. In pilot study, it was concluded that single dose of cyclophosphamide (CP) @37.5mg/Kg B.Wt SC route used for induction bone marrow suppression which leads to aplastic pancytopenia.

Experimental study of bone marrow stimulating property of N-acetylcysteine (NAC) & Desmopressin in rats was studied in phase I. Rats were divided randomly into six groups having six rats (n=6) in each group. Bone marrow suppression was induced in rats by administering single dose of cyclophosphamide @37.5mg/Kg B.Wt S/C route. Significantly ($p<0.05$) decreased values of Hb, PCV, TEC, and platelet by the end of experiment in all the groups except Gr. I rats. Among the treated groups, hematological parameters (Hb, TEC, PCV) were significantly ($P<0.05$) increased on day 15 in Gr. IV as compared to Gr. II. But these values were lesser than Gr. I (healthy control). The serum biochemical parameters (ALT, AST, ALP and TP) revealed better efficacy in (Gr. IV) rats supplemented with NAC @50mg/Kg B.Wt with respect to ALT and TP.

Histopathology of bone marrow, liver, spleen sections and their respective HPS scoring system were done. Rats of Gr. IV (2.5) received NAC (50mg/kg b.wt) revealed best effect with regard to regenerative changes in bone marrow, liver and spleen. Rats of disease control Gr. II (1) scored least revealing degenerative changes as compared to healthy control Gr. I (3) with a maximum score.

In phase II study, dogs with aplastic pancytopenia and or hepatobiliary dysfunction (HBD) associated with canine haemoprotozoan diseases (CHDs) were selected for study in RVP, IVRI. During the study period ,total of 4436 dogs were presented at OPD of Referral Veterinary Polyclinic, IVRI, of which 96 dogs were suspected for hemoprotozoan infection of which 32 dogs were found positive for hemoprotozoan infection by microscopic examination. Hemoprotozoan infection was found more in males (59%) than female dogs. German shepherd breed was affected more (34.3%) followed by Labrado reteriver. More number of *Ehrlichia canis* (37.5%) cases were noticed in dogs.

Two different treatment strategies were evaluated against hemoprotozoan infected dogs. In Ist group, dogs treated with disease specific therapy, symptomatic supportive therapy. In the IInd group, dogs treated with NAC @ 15 mg/Kg PO q12h, disease specific therapy, symptomatic supportive therapy revealed favourable clinical recovery as compared to Gr. I.

Phase III study was conducted to evolve noval therapeutic regimen against aplastic pancytopenia and HBD associated in CHDs. The parameters under evaluation included hemetao-biochemical changes, Ferric-Reducing Antioxidant Power Assay (FRAP) and determination of Free Radical Scavenging Activity by DPPH assay. With regards to Hb and platelet count, Gr. II dogs supplemented with NAC @ 15 mg/kg B.wt PO bid showed significantly ($P<0.05$) increased values after 14 days of therapy as compared to Gr I. The serum biochemical parameters (ALT, AST, ALP and TP) revealed better improvement in Gr. II dogs. Ultrasonography examination revealed enlarged liver and spleen in a dog, suffering from hemoprotozoan infection. Antioxidant assay revealed better and improved antioxidant effect in Gr. II dogs supplemented with NAC and recorded more value of FRAP and DPPH.

Prognostic score card was developed as per the methods of Durand and Valla, (2008) with slight modifications. Groups were assigned score between (+)1 to (+)3 on the basis of various hematological parameters. Based on the developed score card, Gr. II dogs treated with NAC @ 15 mg/Kg PO q12h + disease specific therapy + symptomatic supportive therapy had good prognosis as compared to Gr. I

Conclusion

- Established Aplastic Pancytopenia model in RAT by single S/C inj of Cyclophosphamide @37.5mg/Kg bwt by 4th day of induction which was confirmed by reduced Hemoglobin, PCV, TEC & Platelet Count.
- NAC @ 50mg/Kg B.wt PO for 10 days depicted better efficacy to combat Aplastic Pancytopenia over & above of Desmopressin which was confirmed by Hb, PCV, TEC, Platelet count, Serum Biochemical & Histopathological Findings.
- During the study period 32 dogs confirmed for CHD with Aplastic Pancytopenia and HBD of which male German shepherd revealed higher occurrence of *E. canis*.
- NAC (a potent antioxidant) @ 15 mg/Kg bwt PO for 14 days depicted better regeneration property of hepatocytes in the CHD which was confirmed by Hematopoietic, Serum Biochemical, Liver Profile & Oxidative Stress Markers.
- NAC @ 15 mg/Kg B.wt PO for 14 days could halter the progression of critical anemia and thrombocytopenia in combination with disease specific therapy & supportive therapy in dogs with compromised liver due to hemoprotozoan diseases.
- Modified Prognostic Score Card revealed GOOD INDICATOR OF PROGNOSIS in dogs with Pancytopenia & compromised liver due to CHD.


Mini Abstract

7 MINI ABSTRACT

The present study was conducted to evaluate bone marrow stimulating property of N-acetylcysteine and Desmopressin for therapeutic management of aplastic pancytopenia associated with canine hemoprotozoan diseases (CHD). Bone marrow suppression was induced in rats by administering single dose of cyclophosphamide @37.5mg/Kg B.Wt SC. Bone marrow (BM) stimulating property of N-acetylcysteine (NAC) against cyclophosphamide induced bone marrow suppression depicted rats received NAC (50mg/kg b.wt) (Gr. IV) showed marked improvement in hematobiochemical indices and also marked regenerative changes in histopathological studies as compared to other treatment groups. The modified scoring system of HPS reaffirmed regenerative changes in the rats of Gr. IV received NAC (50mg/kg b.wt). During the study period, total of 4436 dogs were presented at OPD of Referral Veterinary Polyclinic, IVRI, out of which 96 dogs were suspected for hemoprotozoan disease. Among the 96 dogs, 32 dogs were found positive for hemoprotozoan infection by microscopic examination. Most commonly affected breed was german shepherd (GSD) (34.3%) and more number of cases of *Ehrlichia canis* (37.5%) . Out of 32 hemoprotozoan infected dogs, 19 (59%) dogs were male and 11 (40.3%) dogs were female. Therapeutic efficacy was assessed based on clinical score, hematobiochemical and USG observation. With respect to Hb, platelet count, ALT, AST, ALP, TP and antioxidant assay, critical analysis of the data revealed better efficacy in animals supplemented with NAC @15mg/Kg B.Wt. Based on the developed prognostic score card, Gr. II dogs treated with NAC @ 15 mg/Kg PO q12h, disease specific therapy, symptomatic supportive therapy had good prognosis as compared to Gr. I.

लघु सारांश

8 लघु सारांश

वर्तमान अध्ययन कैनाइन हेमोप्रोटोजोअन रोगों (सीउचडी) से जुड़े अप्लास्टिक पैन्टीटोपेनिया के चिकित्सीय प्रबंधन के लिए एन–एसिटाइलसिस्टीन और डेस्मोप्रेसिन की अस्थि मज्जा उत्तेजक संपत्ति का मूल्यांकन करने के लिए आयोजित किया गया था। चूहों में साइक्लोफॉमाइड @37.5 मिलीग्राम/किलोग्राम बॉडी वेट एकल खुराक चमड़े के नीचे देकर अस्थि मज्जा दमन को प्रेरित किया गया था। एनएसी (50 मिलीग्राम/किलोग्राम बीडब्ल्यूटी) (Gr. IV) प्राप्त करने वाले चूहों ने हेमेटोबायोकेमिकल इंडेक्स में उल्लेखनीय सुधार दिखाया और अन्य उपचार समूहों की तुलना में हिस्टोपैथोलॉजिकल अध्ययनों में पुनर्योजी परिवर्तनों को भी चिन्हित किया। यह (एनएसी) एसिटाइलसिस्टीन की साइक्लोफॉस्फेमाइड–प्रेरित अस्थि मज्जा दमन के खिलाफ अस्थि मज्जा (बीएम) को उत्तेजित करने की क्षमता के कारण है। एचपीएस की संशोधित स्कोरिंग प्रणाली ने जीआर के चूहों में पुनर्योजी परिवर्तनों की पुष्टि की। अध्ययन अवधि के दौरान, रेफरल पशु चिकित्सा पॉलीक्लिनिक, आईवीआरआई की ओपीडी में कुल 4436 कुत्तों को प्रस्तुत किया गया, जिनमें से 96 कुत्तों को हीमोप्रोटोओअन संक्रमण के लिए सकारात्मक पाए गए। सबसे अधिक प्रभावित नस्ल जर्मन शेफर्ड (जीएसडी) (34.3%) और एर्लिचिया कैनिस (37.5%) के अधिक मामले थे। 32 हेमोप्रोटोजोअन संक्रमित कुत्तों में से 19(59%) कुत्ते नर थे और 11 (40.3%) कुत्ते मादा थे। चिकित्सीय प्रभाव का मूल्यांकन नैदानिक स्कोर, हेमटोबायोकेमिकल और यूएसजी अवलोकन के आधार पर किया गया था। एचबी, प्लेटलेट काउंट, एएलटी, एएसटी, एएलपी, टीपी और एंटीऑक्सिडेंट परख के संबंध में, डेटा के महत्वपूर्ण विश्लेषण से एनएसी @15 मिलीग्राम/किग्रा बी. डब्ल्यूटी के साथ पूरक जानवरों में बेहतर प्रभावकारिता का पता चला। विकसित भविष्य सूचक स्कोर कार्ड के आधार पर, समूह, एनएसी @15 मिलीग्राम/किग्रा पीओ q12h, रोग विशिष्ट चिकित्सा, रोगसूचक सहायक चिकित्सा के साथ इलाज किए गए Gr. II कुत्तों में Gr. I की तुलना में अच्छा रोग का निदान था।

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