THERAPEUTIC EVALUATION OF CAJANUS CAJAN FOR THE MANAGEMENT OF HEPATIC DISORDER IN CANINE

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Cajanus cajan (Arhar pulse) is a legume crop. The present study was designed to show the efficacy of methanol extract of leaves of *Cajanus cajan* as a hepatoprotective agent in dogs with chronic hepatic disorder. The main clinical signs observed in dogs with liver disorders are diarrhoea, constipation, vomition, anorexia, jaundice, polyuria, polydipsia, ascites, dehydration, pain on abdominal palpation and different colour of conjunctival mucous membrane like pink, congested, pale, severely pale and icteric. Levels of mean values of Hb, PCV, TEC, total protein, albumin, A/G ratio, glucose, cholesterol decreased when compared to healthy control group and parameters like AST, ALT, ALP, BUN, creatinine and TLC increased significantly than healthy control group which significantly changed after treatment with methanol extract of *Cajanus cajan* leaves along with conservative therapy.
Keywords: Antioxidant, *Cajanus cajan*, Dogs, Hepatic disorders, Jaundice, Polyuria, Polydipsia.

Hepatic affections in dog are associated with variable clinical signs and thus frequently observed in dog which poses a challenge in accurate diagnosis and treatment. Hepatic disorder in dogs, either drug induced or sequelae to infectious diseases is sometimes encountered during pet animal practice. Early and accurate identification of hepatic disorders is important to improve the long term outcome. *Cajanus cajan* (L.) Millsp (Leguminosae) also known as pigeon pea, congo pea, toor dal or Arahar dal. Leaf extract of pigeon pea has protective action against liver damage. Antioxidant properties of the leaves of pigeon pea due to their chemical constituents viz. flavonoids and stilbenes. Keeping the above facts the present study was designed to evaluate the efficacy of *Cajanus cajan* methanol extract as adjunct therapy of hepatic disorder in dogs.

Materials and Methods

Clinical, haemato-biochemical and ultrasound examinations were undertaken in the dogs presented in the Teaching Veterinary Clinical Complex for diagnosis of hepatic disorder in dogs. The dogs presented with diarrhoea, constipation, vomition, anorexia, jaundice polyuria, polydipsia, ascites, dehydration, pain on abdominal palpation and colour changes of conjunctival mucous membrane were considered as clinical signs of liver disorder and such cases were selected for further investigation. About 3 ml of blood was collected from recurrent tarsal vein into vials containing EDTA for haematology and 2 ml blood into vials containing sodium fluoride for blood glucose estimation. Another 5 ml of blood was drawn in a sterilized serum separating tube without any anticoagulant for biochemical estimation. Blood was centrifuged at 3000 rpm for 3-4 minutes to separate the plasma and stored the plasma at -20^0C for further use. Haematological parameters viz. Haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocytic count (DLC) were estimated by using standard technique. The biochemical parameters viz. Total protein, ALT, AST, ALP, albumin, globulin, A/G ratio, bilirubin, BUN, creatinine, cholesterol and glucose were estimated in semi automatic analyzer following protocols mentioned in the supplied kits. Liver disorders were also diagnosed with the help of radiography and ultrasonography. For radiographical examination the ailing dogs were restrained in dorsal recumbency for ventro-dorsal view and left/right lateral recumbency for lateral view of the abdomen. Ultrasonographical examination was carried

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out by using standard technique with the help of Mindray machine using 5MHz transducer. A total number of 18 dogs aged between 3-7 years of different signalment were screened and included in the therapeutic studies. The dogs were diagnosed as acute hepatitis and chronic hepatitis based on haematobiochemical alterations, radiographical and ultrasonographical changes. The chronic hepatic animals were divided into two groups namely Gr.C_1 & Gr.C_2 having six animals in each group. Gr.C_1 treated with Inj. Dextrose10% @ 10-15 ml/Kg BW daily intravenously for 5 days, Tab Frusemide-Spironolactone @ 0.5 mg/Kg BW daily for 14 days, Cap. Amoxicillin @ 10 mg/Kg BW thrice daily for 7 days, Followed by liver tonics, orally for 30 days, Astymin liquid @ 5ml bid for 30 days. Gr.C_2 treated similarly as Gr.C_1 along with methanol extract of *Cajanus cajan* leaves (100 mg/kg BW).

Results and Discussion

Out of 18 animals diarrhoea was observed in 12 (66.66%) animals, constipation was observed in 2(11.11%) animals and remaining 4(22.22%) animals had normal defecation status. Vomiting was seen in 7(38.89%) animals. Anorexia with weight loss was a predominant sign in 8(44.44%) animals. 6(33.33%) suffered from inappetance and 4(22.22%) dogs have normal appetite. Water intake was normal in most of the animals but there was signs of polydipsia in 3(16.67%) animals and polyuria in 2((11.11%) animals. Jaundice was seen in 6(33.33%) animals. Ascites was observed in 4(22.22%) dogs in which 1(5.55%) have acute hepatitis and 3(16.67%) were having chronic hepatitis. 3(16.67%) dogs have mild dehydration, 10(55.55%) dogs were moderately dehydrated, 3(16.67%) dogs were severely dehydrated and dehydration was not detected in rest of 2(11.11%) animals. Conjunctiva was pinkish in 2(11.11%) animals, congested in 1(5.55%) animals, pale in 5(27.78%) animals severely pale in 3(16.67%) animals and icteric in 7(38.89%) animals. Pain on abdominal palpation was observed in 8(44.44%) dogs. The clinical manifestations observed in the present investigation depicted in table-1.

Table-1: Observation of clinical signs

Clinical signs	Total no of cases	No. of positive cases	Percentage (%)
Diarrhoea	18	12	66.67
Constipation	18	2	11.11
Vomition	18	7	38.89
Anorexia	18	8	44.44
Polyuria	18	3	16.67
Polydipsia	18	2	11.11
Jaundice	18	6	33.33
Ascites	18	4	22.22
Dehydration	18	Mild dehydration- 3 Moderate dehydration -10 Severe dehydration- 3 No dehydration -2	16.67 55.56 16.67
Pain on abdominal palpation	18	8	44.44
Conjunctival mucous membrane	18	Pinkish- 2 Congested- 1 Pale- 5 Severely pale- 3 Icteric- 7	11.11 5.56 27.78 16.67 38.89

Therapeutic efficacy will be assessed based on the clinical recovery and alteration of haematobiochemical parameters.

Table-2: Clinical score in dogs of Gr. C_1 and Gr.C_2 animals after treatment

	Before treatment		1week after treatment		2weeks after treatment		3weeks after treatment		4weeks after treatment	
	Gr.C_1	Gr.C_2	Gr.C_1	Gr.C_2	Gr.C_1	Gr.C_2	Gr.C_1	Gr.C_2	Gr.C_1	Gr.C_2
Diarrhoea	+	+	-	-	-	-	-	-	-	-
Constipation	+	+	+	+	+	+	-	-	-	-
Vomition	+	+	-	-	-	-	-	-	-	-
Anorexia	+	+	-	-	-	-	-	-	-	-
Polydipsia	+	+	+	+	+	+	-	-	-	-
Polyuria	+	+	+	+	+	+	-	-	-	-
Jaundice	+	+	+	+	+	+	+	+	+	-
Ascites	+	+	+	+	+	+	-	-	-	-
Dehydration	+	+	-	-	-	-	-	-	-	-
Pain on abdominal palpation	+	+	+	+	+	+	-	-	-	-
Icteric Conjunctival mucous membrane	+	+	+	+	-	-	-	-	-	-

+ = present; - = absent.

Assessment of clinical score in dogs given in table-2. After a week of treatment animals of both the groups showed clinical improvement in terms of reduced discoloration of mucous membrane, disappearance of clinical signs like vomition, diarrhoea and regaining the appetite. In the third week of treatment the animals showed moderately pink conjunctival mucous membrane, improvement of jaundice, ascites, polyuria, polydipsia. Improvement of jaundice was seen in Gr.C_2 after four week of treatment and the animals showed total recovery and become alert and active.

Haematobiochemical studies (before treatment and after treatment) in dogs with hepatitis depicted in table-3.

Table-3: Haematobiochemical studies in Gr. C_1 and Gr.C_2 animals before and after treatment

Sl. no.	Parameters	Healthy control (n=6)	Before treatment		After treatment	
			GrC_1	GrC_2	GrC_1	GrC_2
1	Hb(gm/dl)	12.76±0.17	$8.29±0.21^a$	$8.19±0.22^a$	$10.02±0.19^a$	$11.33±0.27^a$
2	TEC($x10^6$/µl)	5.79±0.05	$4.57±0.01^a$	$4.55±0.01^a$	$4.93±0.03^b$	$5.08±.06^b$
3	TLC($x10^3$/µl)	12.42±0.18	$17.33±1.33^a$	$17.38±1.23^a$	$13.51±0.15^a$	$12.61±0.11^a$
4	PCV (%)	35.66±0.66	$24±0.68^a$	$23±0.77^a$	$31.17±0.31^a$	$33.83±0.4^a$
5	DLC (%) Neutrophils	62.83±0.70	$69±0.52^a$	$69.17±0.75^a$	$65.5±0.34^a$	$64.5±0.22^a$
	Lymphocyte	33.66±0.33	$24.5±0.5^a$	$25.5±0.22^a$	$30.5±0.22^a$	$32±0.37^a$
	Basophil	0.50±0.22	$0.50±0.22^{NS}$	$0.50±0.22^{NS}$	$0.50±0.22^{NS}$	$0.50±0.22^{NS}$
	Monocyte	1.66±0.21	$4.17±0.17^a$	$3.17±0.31^a$	$2.17±0.31^a$	$1.67±0.21^a$
	Eosinophil	1.33±0.21	$1.83±0.31^{NS}$	$1.67±0.33^{NS}$	$1.33±0.33^{NS}$	$1.33±0.21^{NS}$

6	ALT(IU/L)	42.33±0.80	126.83±5.5[a]	122.67±5.54[a]	55.5±1.41[a]	45.83±0.98[a]
7	AST(IU/L)	32.33±0.49	110.33±4.6[a]	116.17±6.17[a]	36±0.82[a]	33±0.45[a]
8	ALP(IU/L)	59.83±1.30	251±8.5[a]	257±9.48[a]	63±0.77[a]	60.17±0.31[a]
9	Bilirubin (mg/dl)	0.37±0.00	2.15±.09[a]	2.09±.11[a]	0.46±.01[a]	0.38±0.00[a]
10	Direct bilirubin (mg/dl)	0.23±0.01	1.19±.04[a]	1.22±.04[a]	0.34±.02[a]	0.24±.02[a]
11	Indirect bilirubin (mg/dl)	0.14±0.01	0.96±.09[a]	0.87±0.11[a]	0.12±0.03[a]	0.14±0.02[a]
12	Total protein (g/dl)	6.36±0.01	5.2±0.02[a]	5.22±0.02[a]	5.99±0.04[b]	6.16±0.05[b]
13	Albumin (g/dl)	3.32±0.08	2.12±0.02[a]	2.15±0.02[a]	2.99±0.03[a]	3.17±0.05[a]
14	Globulin (g/dl)	3.04±0.07	3.08±0.02[NS]	3.07±0.02[NS]	3±0.04[NS]	2.99±0.06[NS]
15	A/G ratio	1.09±0.04	0.69±0.01[a]	0.7±0.01[a]	1±0.02[a]	1.06±0.03[a]
16	BUN (mg/dl)	17.00±0.57	28.52±0.61[a]	28.43±0.47[a]	20.79±0.25[a]	19.34±0.29[a]
17	Creatinine (mg/dl)	0.89±0.11	1.59±0.02[a]	1.62±0.03[a]	1.03±0.01[b]	0.97±0.02[b]
18	Glucose (mg/dl)	97.33±0.61	63.97±0.4[a]	63.3±0.6[a]	90.67±1.84[a]	91.17±1.49[a]
19	Cholesterol (mg/dl)	211.83±1.75	119.73±2.08[a]	117.73±2.74[a]	192.68±0.93[a]	204.75±0.57[a]

a= significant at 1% level ($p<0.01$), b= significant at 5% level ($p<0.05$), NS=non significant.

Haematological findings in dogs with hepatic disorder revealed anaemia which showed fall in Hb, PCV and TEC in hepatic disorder in Gr.C_1 and Gr.C_2 (pre treatment) which improved after treatment. Decreased in Hb level may be due to increased degradation of erythrocytes which was attributed by increased transit time to spleen because of reduced portal blood flow or increased fragility of erythrocytes due to elevated bile acid content as also reported by Rothuizen and Meyer (2000). Dogs with hepatic disorder showed neutrophilic leukocytosis in chronic liver disease of dogs might be due to an inflammatory response to acute phase stimulation and/or as a stress response.

Serum biochemical analysis showed elevated hepatic enzymes ALT, AST and ALP level before treatment which subsequently reduced after treatment (Table-3). Aminotransferases are commonly elevated in hepatocellular disease, hepatitis, hepatic trauma, anaemia, toxaemia. The magnitude of elevation of ALT enzyme is roughly proportional to the number of injured hepatocytes as also reported by Rothuizen and Meyer (2000). Elevation in ALP is subsequent to accelerate production stimulated by bile retention. Mean total bilirubin value in the affected dogs revealed significant elevation. Presence of hyperbilirubinaemia with equal amount of direct and indirect bilirubin as seen in icteric dogs was an indicative of hepatocellular damage. Hyperbilirubinaemia was due to disturbances of the balance between rate of production of bilirubin, metabolism and excretion of bilirubin. In the present study increased might be as a result of diminished excretion due to extensive hepatocytes damage or biliary obstruction or combination thereof. This finding is in agreement with the report of Vijayakumar *et al.* (2001). Hypoproteinemia and hypoalbuminaemia was observed in the dogs with hepatic disorder (Table-3). Low serum protein and albumins could be attributed to decreased production from liver besides anorexia and inappetance. Liver is the main site of synthesis and degradation of most of the proteins. Hyperglobulinemia in chronic liver disease as observed in present study could be due to increased synthesis of gamma globulin fraction associated with enhanced systemic immune reactivity against portal antigens or secondary to antibody production. The

present findings are in agreement with Jacob and Swan (1995). There was a significant decrease in the plasma glucose level in the present study. Significant hypoglycaemia was observed in chronic hepatitis might be due to inappetance and anorexia complemented by malabsorption from intestine. Blood urea nitrogen (BUN) increased significantly in chronic hepatitis and unsignificantly in acute hepatitis. Protein substrates produced from haemolysis require deamination and consequently lead to hyperammonaemia as also reported by Tantary *et al.* (2014). Creatinine increased significantly in dogs with chronic hepatitis as also mentioned by Elhiblu *et al.* (2015). There was significant decrease in cholesterol level. Hypocholesterolemia may be attributed to decrease in synthesis or absorption from the gut or excessive conversion of cholesterol into bile acids as also reported by Hall (1985).

Cajanus cajan posses significant free radical scavenging property which confer potential cytoprotective activity against this marker enzyme in the serum which increase in hepatic disorder. Beside this it helps to maintain the structural integrity of the hepatocellular membrane. This further buttressed the assertion that natural antioxidant molecules impact stabilisation to cell membrane depending on their degree of intensity of free radical scavenging capability. In this way methanol extract of *Cajanus cajan* leaves prolonged the viability of the cell membrane against the damage by causative factors as also reported by Sarkar *et al.* (2013).

The effect of Frusemide-Spironolactone against ascites in canine population has also been reported with successful results of Varshney and Hoque (2002) and Saravanan *et al.* (2013). Diuretics remove excess amount of water from body system resulting the relief of portal venous pressure thus helping to regain the efficacy. Amoxicillin group of antibiotic has been used by many authors as it is considered as safest and good choice of antibiotic for hepatic disorder. A liver tonic containing Ursodeoxycholic acid derived originally from bile of black beer which later commercialised as a synthetic product used to treat cholangiohepatitis and competitively replaces endogenous bile acids that accumulate in Cholestatis hepatic disorder and prevent the cell membrane damage, induction of apoptosis and necrosis of liver. It is also used as a powerful cholerectic agent to treat sludged bile and cholelithiasis as also mentioned by Pillai *et al.* (2009) and Bhardwaj *et al*. (2014). Astymin contained amino acid preparations which are known for its pathogenic and liver protective effect has been tried in dogs with hepatic disorder to assess its efficacy along with the combination of other drugs mentioned in groups. Methionine is a lipotropic agent contain in Astymin reduces fat accumulation in liver by increasing the mobilisation of hepatic lipids. Liver protection as well as abdominal fluid retention was possible through this combination therapy.

In the present investigation the drugs like Inj. Dextrose10%, Tab. Frusemide-Spironolactone, Cap. Amoxicilin, Liver tonics (herbal preparation) and (ursodeoxycholic acid), Astymin (amino acid preparation) and methanol extract of leaves of Cajanus cajan (Arahar dal) used and showed good clinical recovery in dogs with chronic hepatic disorder which were in agreement to the reports of Saravanan *et al.* (2013); Tantary *et al.* (2014) and Bhardwaj *et al.* (2014).

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