Report of the Project

on

Effects of Livsee on induced hepatopathy in black Bengal goats

DEPTT. OF VETERINARY PHARMACOLOGY AND TOXICOLOGY

West Bengal University of Animal & Fishery Science

MOHANPUR CAMPUS, NADIA, WEST BENGAL

Title	:	Effects of Livsee on induced hepatopathy in black Bengal goats.
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Date of reporting	:	April, 2003.

This is to certify that the study entitled " Effects of Livsee on induced hepatopathy in black Bengal goats" was carried out West Bengal University of Animal and Fishery Science, Mohanpur, Nadia, West Bengal under the sponsorship of M/S Legend Remedies Private Ltd., Baroda. The entire study was conducted from the month of September 2002 to February 2003 by the Deptt. of Veterinary Pharmacology & Toxicology, WBUAFS under the close supervision of the following scientist with records of all facts and truthful observation.

Mandal 30/4/03.

Dr. Tapan Kumar Mandal
Reader & Head,
Principal Investigator
of the Project.

Introduction

The clinical manifestations of hepatic disease are directly attributed to alteration in the metabolic excretory, synthetic and digestive function of liver. The liver has great functional reserve and signs of hepatic failure often do not develop until 70% or more of the functional capacity of the liver is lost. Importantly, even when a major function of the hepatocellular mass has been lost in hepatic injury, recovery is possible because of the unique capacity of the liver to regenerate.

Livsee an ayurvedic preparation is extensively used by the field veterinarians as hepato protective and hepatostimulatory drug. The principal objective of this study is to find out the role of Livsee for rejuvenating the carbon tetrachloride induced liver damage.

Materials and Methods

Goats were divided into 4 equal groups each consisting of 4 animals.

a) Selection of animals: Sixteen clinically healthy black Bengal goats weighing between 7-10 kg of 1-1½ yr. of age were used.

Treatment of animals

Group I : Control

Group II : Carbontetrachloride was administered to damage the liver. Livsee was given orally twice daily.

Group III : Carbontetrachloride was administered to damage the liver but no Livsee was given.

Group IV : Livsee was administered to healthy animals twice daily orally for 23 days.

b) **Route of administration and doses:**

i) **Induction of liver damage:** Liver of goats of group II and III were damaged by subcutaneous administration of carbontetrachloride at 0.75 ml/kg with equal volume of liquid paraffin following over night fasting. Carbontetrachloride was administered on three occasions at 48 hr interval and the intensity of liver damage was assessed by performing Bromosulphophthalein clearance test, icterus index (Oser, 1979), serum aspartate (SAST) and serum alanine (SALT) aminotransferase activity (Yatazidis, 1960) and GSH level.

ii) **Rejuvenation of Liver:** Livsee was administered at 20 ml orally twice daily to each goat of group II from 120 hr post dosing of carbontetrachloride and animals of group IV received Livsee at the same dose from 1st day. Liver function test was monitored weekly to observed the intensity of rejuvenation.

c) **Housing and feeding condition:** The animals were kept individual custom made steel case (size – 48" x 48" x 36") in controlled room temperature (22±2°C) having provision of artificial light. The animals were kept in the laboratory condition for 7 days to allow for an adaptation period. The animals were fed with standard ration consisting of 2 parts of wheat husk, 1 part groundnut cake, 1 part crushed gram, 1 part crushed maize and two parts green. Water was provided *ad libitum*.

d) **Duration of Study:** The test was carried out for 28 days.

e) **Collection of samples:** Blood samples (15 ml) were collected from the jugular vein. Out of 15 ml, 10 ml of blood was taken for collection of serum and utilized for BSP, icterus index SAST, SALT test. The remaining 5 ml of blood was taken in a heparinized test tube for separation of plasma to estimate the level of GSH.

f) **Storage condition:** All samples were processed as early as possible if not, the samples were stored at -20°C.

g) **Statistical analysis:** Mean value and standard error of data were calculated by using standard formulae.

Results and Discussion

Icterus index:

The mean values with S.E. of icterus index of group I, II, III and IV goats have been presented in table 1. The values of group II goats except 28th day were significantly higher than that of control (table 1). The peak value was obtained at 5 day and thereafter the value started to decrease and return to base level on 28th day of Livsee treatment. Patra (1991) reported that carbontetrachloride induced higher icterus index unit returned to the base level after 15 days in goats when treated with Livol(R), polyherbal liver stimulant for consecutive 8 days. Nandi (1993) also reported the same after 14 day of Livol(R) treatment. Livsee is also a polyherbal drug has some delayed time of response on liver which might be due to difference in composition as well as dose level along with intensity of liver damage. The values of carbontetrachloride treated goats of group III at different times have been shown in Table 1. The icterus index values of group III goats were significantly increased from the control values at respective period. This indicated that carbontetrachloride at the recommended dose level damaged the liver.

The highest icterus value was observed at 5 day of carbontetrachloride administration. Patra (1991) reported that icterus index of carbontetrachloride treated goats started to increase from 24 hr onward with a peak level at 144 hr but in this experiment the value was high compared to the reported value of Patra (1991) may indicate more damage of liver. Similar significant increase of serum icterus index value was also reported in carbontetrachloride induced hepatotoxicity by Nandi (1993). Further goats of group III were treated with 20 ml of water orally from 5 day onward. Water did not modify the carbontetrachloride induced higher icterus index values and accordingly it has no protection effect on the damaged liver. Livsee did not alter Icterus index value from '0' hr to 28 days period in healthy goats (Table 1).

Bromosulpophthalein clearance test:

The mean value of BSP clearance (t½) of goats have been presented in Table 2.The values in group II and III goats at 2,4,5, 7 and 14 days were significantly higher compared to control goats. Similar results were also reported by Nandi (1993) in goats. In this experiment carbontetrachloride increases the t½ value above 5 min from '0' hr onward of administration. In all the groups (gr II, and gr III) where carbontetrachloride was administered the peak value in serum reached on 5 day and thereafter the value started to decrease (Table 2). Only in case of goats treated with Livsee (group II), the t½ value returned to the base level on 28th day. But t½ value of BSP was not altered in healthy animals treated with Livsee.

Serum transaminase activity:

Aspartate transaminase:

The mean value with S.E. of AST activity in goats have been presented in Table 3. The activity of AST started to increase from 2 day, peaked at 5 day and gradual decrease from 7 days in group II and III treated with carbontetrachloride. The AST activity was at base level on 28 day post treatment of Livsee. Livsee decreased the AST activity in healthy goats. Increase of serum AST activity after carbontetrachloride administration has also observed by Pradhan (1991) and Nandi (1993) in goats.

Alanine transaminase:

The mean value of with S.E. with ALT activity in group I, II, III, & IV goats has been incorporated in Table 4.Like AST activity carbontetrachloride treated goat showed significant increase in enzyme activity from 2 day onwards. Goats in all the groups of II and III showed a peak increase of serum enzyme activity on 5 day, thereafter the activity started to decline in serum of goats of group II and III but the activity reached the base level only in goats treated with Livsee (group II). Nandi (1993) reported similar trend of the increase of enzyme activity which returned to base level on 15th day following a polyherbal drug, Livol® administration.

Plasma glutathione level:

Mean values with SE of plasma GSH level in goats of different groups have been summarized in Table 5. It would be evident from Table 5 that carbontetrachloride decreased the GSH level from 2 day; maximum decrease level was observed on 5 day followed by gradual increase in level. The level returned to base level in livsee treated animal

(gr II). But the GSH level was increased in healthy animals treated with Livsee.

Histopathology:

Histopathology of liver section in animals of group III showed fatty degeneration while the intensity of degeneration was lower in livsee treated goats (gr II) (Fig.1 & 2).

Therefore the parameters so far studied like icterus index, half-life of BSP, AST, ALT and plasma glutathione level suggested that carbonteterachloride produced hepatopathy of goats. Livsee treatment almost brought all the altered values of biochemical parameters to base level and regenerated the damaged tissue of liver

Reference

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Oser, B.L. (1979) Hawk's Physiological Chemistry 14th edn. Tata McGraw-Hill Publishing Company Ltd., New Delhi.

Table 1. Serum icterus index (unit) of goat.

Days	Control (Gr I)	Carbontetrachloride induced liver damage		Healthy treated with Livsee (Gr IV)
		Treated with Livsee (Gr II)	Without Livsee (Gr III)	
0	4.64±0.10	4.45±0.40	4.30±0.70	4.60±0.15
2	4.70±0.09	8.74±0.70	8.75±0.52	4.25±0.45
4	4.69±0.25	13.78±1.28	14.66±1.25	4.50±0.50
5	4.62±0.48	20.14±2.26	20.50±2.50	4.75±0.48
7	4.65±0.50	19.51±1.75	19.65±1.25	4.55±0.65
14	4.58±0.32	10.83±1.10	14.75±1.31	4.65±0.35
28	4.60±0.35	5.30±0.80	9.62±1.00	4.67±0.54

Table 2. Bromosulphophthalein clearance (t½ values, min) from serum of goat

Days	Control (Gr I)	Carbontetrachloride induced liver damage		Healthy treated with Livsee (Gr IV)
		Treated with Livsee (Gr II)	Without Livsee (Gr III)	
0	3.32±0.36	3.05±0.15	3.07±0.11	3.25±0.42
2	3.24±0.25	5.92±0.60	8.49±0.95	3.20±0.50
4	3.25±0.30	14.06±1.04	14.25±1.32	3.15±0.41
5	3.20±0.34	21.11±2.23	20.80±2.25	3.22±0.52
7	3.22±0.32	19.14±2.32	18.90±2.41	3.54±0.25
14	3.21±0.27	12.22±1.50	14.97±1.32	3.33±0.38
28	3.17±0.31	3.07±0.45	6.11±0.60	3.24±0.44

Table 3. Serum AST activity (unit/ml) in goats.

Days	Control (Gr I)	Carbontetrachloride induced liver damage		Healthy treated with Livsee (Gr IV)
		Treated with Livsee (Gr II)	Without Livsee (Gr III)	
0	340.00±11.67	341.50±12.57	331.67±17.88	335.50±15.50
2	342.33±12.23	570.25±15.28	589.67±22.44	350.25±12.55
4	341.00±10.33	663.33±21.10	663.35±27.50	324.00±16.00
5	344.00±11.50	925.25±12.58	925.57±28.00	330.00±15.95
7	343.33±12.56	828.50±25.20	830.50±26.22	340.50±12.65
14	344.67±12.50	316.33±25.42	626.55±28.20	355.25±11.75
28	345.00±11.60	325.50±16.49	435.00±17.86	347.00±12.07

Table 4. Serum ALT activity (µg pyruvic acid/ml/hr) of goats.

Days	Control (Gr I)	Carbontetrachloride induced liver damage		Healthy treated with Livsee (Gr IV)
		Treated with Livsee (Gr II)	Without Livsee (Gr III)	
0	92.00±7.83	85.33±7.73	86.55±6.68	79.75±7.20
2	82.67±6.38	133.35±15.05	143.67±24.67	81.50±6.50
4	98.67±5.64	158.65±19.98	176.35±16.94	75.55±8.62
5	101.6514.76	213.67±23.01	235.30±29.17	70.00±5.50
7	93.00±7.26	183.50±23.38	204.25±22.29	71.66±6.20
14	95.25±8.50	75.50±9.28	179.50±22.22	68.50±5.75
28	84.67±6.71	80.25±8.50	140.65±14.73	67.20±5.60

Table 5. Plasma glutathione (reduced) level (n mol/ml) of goats.

Days	Control (Gr I)	Carbontetrachloride induced liver damage		Healthy treated with Livsee (Gr IV)
		Treated with Livsee (Gr II)	Without Livsee (Gr III)	
0	245.35±26.94	255.67±13.56	250.33±26.80	241.45±20.50
2	256.33±22.41	238.50±23.33	238.35±22.25	242.50±20.25
4	244.00±16.57	267.50±21.71	226.75±21.76	257.25±19.75
5	240.50±17.45	206.75±22.76	207.33±22.46	265.00±21.50
7	244.67±17.82	208.65±22.00	215.67±23.47	270.00±22.50
14	247.33±18.14	249.33±21.39	223.67±24.55	276.59±25.75
28	248.50±17.49	254.50±21.45	238.50±16.83	280.50±24.75