Asian Journal of Chemistry Vol. 21, No. 2 (2009), 1377-1380

Anti Hepatotoxic Effect of Cow Urine Distillate Against Paracetamol Induced Hepatic Damage in Rats

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The effect of cow urine distillate was studied on paracetamol induced hepatic injury in albino rats. The hepatotoxicity was induced in rats by oral administration of paracetamol (2 g/kg). The effect of cow urine distillate were studied in three dose level and compared with that of standard drug silymarin (100 mg/kg, po). The cow urine distillate produced dose dependent significant ($p < 0.05$) lowering of the elevated SGOT, SGPT, ALP, GGT and total bilirubin levels when compared with the toxic control. The results were comparable with the standard drug silymarin.

Key Words: Antihepatotoxic, Cow urine distillate, Paracetamol, Silymarin.

INTRODUCTION

The revered Indian cow, *Bos indicus* known as 'Kamadhenu' in Indian scripts, is believed to be 'mobile hospital' for most of the diseases. A number of incurable diseases can be cured by regular use of medicines derived from cow. Urine of cow is elaborately described in ancient Ayurvedic scriptures like *Charaka samhita*, *Shushruta samhita*, Brahad-Wagbhatt as bitter, pungent, spicy and warm. It is an anti poisonous insecticide and a regulator for disorders like gas, acidity and couph. It promotes the power of wisdom in human beings, acts like a universal medicine and is easily digested by all[1].

The root cause of various diseases in human beings is believed to be due to shortage or accumulation of certain elements, which are already in the body. The urine of the cow contains all such elements. Hence, according to Ayurveda it is considered as a natural and universal medicine to fulfill the shortage of element or to equalize and reduce the increased elements level in the body by restoring the excretion mechanisms of the body. For patients with cancer, the urine of cow and essence of dung appear to be the alternative to chemotherapy and have no side effects[2].

Though Indian Ayurveda literature cites about the medicinal properties of cow urine, there is very little scientific evidence that supports the literature. Recently scientific attempts have been made to support the view[3].

EXPERIMENTAL

Cow urine distillate: The first voided early morning urine of female *Malenadu gidda* cows which were fed on open grass field free from pollution was collected from the local cow sheds belonging to Sri Ramachandrapura math, Hosanagara, immediately distilled and stored below 10 ºC for further use.

All chemicals and reagents used were of analytical grade and obtained from Sigma Chemical Company (St. Louis, MO, USA). The kit for the estimation of SGOT, SGPT, ALP, GGT and total bilirubin were purchased from Span Diagnostics (Surat, India). The standard drug silymarin was purchased from a local chemist shop in Mangalore, India.

Selection of dose: The survey carried out in and around Mangalore district revealed that the Ayurvedic clinicians practiced a maximum dose administration of cow urine distillate as 60 mL/d. (human dose) This dose was converted into rat dose by multiplying by a factor 0.018 × 5 *i.e.*, 60 × 0.018 × 5 which is equal to 5.4 mL/kg body weight (first dose)[4]. The second dose was selected which was twice that of first dose *i.e.* 10.8 mL/kg body weight. The third dose was selected which was 50 % of the first dose *i.e.* 2.7mL/kg body weight.

Animal treatment: Albino wistar rats of either sex (180-260 g) were obtained time to time from the laboratory of K.S. Hegde Medical Academy (KSHEMA), Deralakatte, Mangalore, India and were maintained on 12 h light/dark cycle and allowed food and water *ad libitum*. The institutional Animal Ethics committee of K.S. Hegde Medical Academy (KSHEMA), Deralakatte, Mangalore, India, approved the experimental protocol in accordance with the guidelines provided by committee for the purpose of control and supervision of Experiments on Animals (CPCSEA) with registration number KSHEMA/AEC/049/2007.

Animals were randomly assigned to six groups of six animals each. Group I received saline (10 mL/kg, po.) as normal control for seven days. Group II received a single dose of paracetamol (2 g/kg, po.) as treated control group on the day seven. Group III received silymarin (100 mg/kg, po) once daily as standard reference for 7 d followed by a single oral dose of paracetamol on 7th day. Groups IV, V, VI received cow urine distillate at the dose of 2.7 mL. 5.4 mL and 10.8 mL per kg body weight orally once daily, respectively for 7 d followed by a single oral dose of paracetamol on the 7th day. After 24 h of paracetamol treatment, blood was collected from the retro orbital plexus of all the rats and the animals were sacfrificed. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation and analyzed for SGOT, SGPT, GGT, ALP and Total bilirubin[5]. The liver was quickly dissected out, washed with saline and preserved in 10 % formalin solution for histopathological investigation.

Statistical analysis: The data were expressed as mean ± SEM and analyzed using one way analysis of variance (ANOVA), followed by Dunnet's "t" test. A probability value of $p < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

The paracetamol administration resulted in significant ($p < 0.05$) rise in SGOT, SGPT, ALP, GGT and total bilirubin levels when compared with Group I (vehicle control) (Table-1). The oral administration of cow urine distillate and silymarin attenuated the paracetamol-induced rise in SGOT, SGPT,ALP,GGT and total bilirubin significantly ($p < 0.05$) when compared with Group II (paracetamol-treated group) (Table-1).

TABLE-1
EFFECT OF COW URINE DISTILLATE AND SILYMARIN ON VARIOUS BIOCHEMICAL PARAMETERS IN RATS WITH PARACETAMOL INDUCED HEPATOTOXICITY

Groups	SGOT (U/L)	SGPT (U/L)	ALP (IU/L)	GGT (IU/L)	Total bilirubin (mg/dl)
I	68.25±2.30	46.53±4.22	72.32±1.32	6.48±0.17	0.44±0.04
II	189.31±2.18[b]	127.36±2.57[b]	135.31±2.11[b]	31.18±3.28[b]	1.98±0.91[b]
III	74.38±3.13[a]	53.37±3.12[a]	76.13±1.47[a]	8.32±1.07[a]	0.51±0.02 [a]
IV	94.72±3.71[a]	75.1±1.27[a]	86.71±2.29[a]	13.98±1.91[a]	0.83±0.01[a]
V	91.03±1.21[a]	74.33±2.41[a]	85.5±1.27[a]	13.04±0.41[a]	0.79±0.03[a]
VI	84.17±1.78[a]	70.41±1.49[a]	83.81±3.11[a]	12.02±0.65[a]	0.77±0.04[a]

Values are mean + SEM, [a]$p < 0.05$ when compared with Group II, [b]$p < 0.05$ when compared with Group I. n = 6
Group I = Control group
Group II = Paracetamol treated
Group III = Paracetamol + Silymarin (100 mg/kg, po)
Group IV = Paracetamol + Cow urine distillate (2.7 mL/kg, po)
Group V = Paracetamol + Cow urine distillate (5.4 mL/kg, po)
Group VI = Paracetamol + Cow urine distillate (10.8 mL/kg, po)

Histopathological observations: The histological observations basically support the results obtained from serum enzyme assays. Histology of the liver section of normal control animals showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein. The liver sections of paracetamol intoxicated rats showed massive fatty changes, necrosis, degeneration and broad infiltration of the lymphocytes around the central vein and loss of cellular boundaries.

The histological architecture of liver sections of rats treated with cow urine distillate showed more or less normal lobular pattern with a mild degree of fatty changes, necrosis and lymphocyte infiltration almost comparable to the normal control and the sylimarin treated group (Figs. 1-4).

Hepatic cells appear to participate in a variety of enzymatic metabolic activities and paracetamol produced marked liver damage at the given doses as expected. Administration of paracetamol in larger doses produces liver necrosis after undergoing bio-activation to a toxic electrophile, N-acetyl-*p*-benzoquinone-imine (NAPQI) by

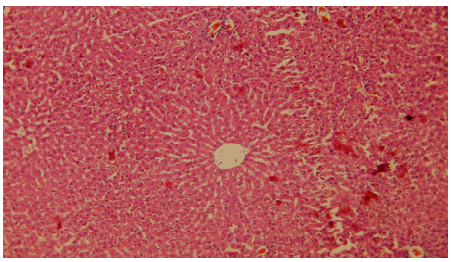

Fig. 1. Normal liver showing a normal central vein, sinusoids and cord arrangement of hepatocytes)

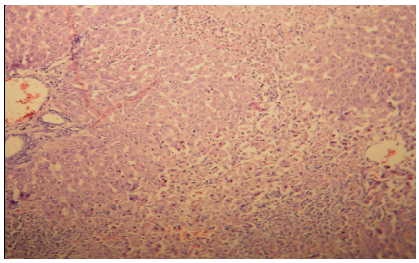

Fig. 2. Paracetamol treated liver showing marked fatty changes around portal tract as well as around central vein. Hepatocytes are with fat vacuoles and showing peripherally pushed nuclei

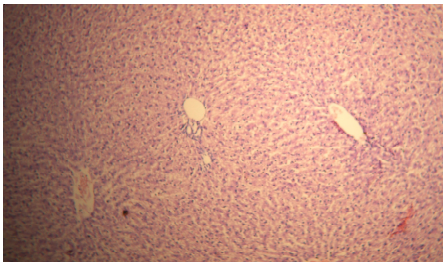

Fig. 3. Liver exposed to paracetamol and pretreated with cow urine distillate showing almost normal appearing hepatocytes. Fine fat vacuoles are seen only in occasional hepatocytes

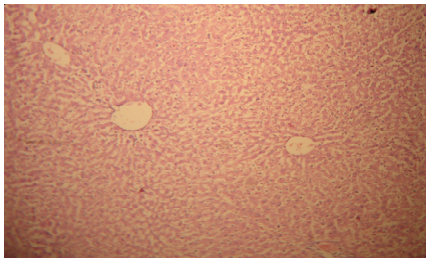

Fig. 4. Liver exposed to paracetamol and pretreated with silymarin showing normal appearing hepatocyte and no fatty change change and also no inflammation or necrosis

cytochrome P-450 monooxygenase. NAPQI binds to macromolecules and cellular proteins and also oxidizes lipids and alters homeostasis of calcium after depletion of glutathione. Pretreatment with cow urine distillate brought down the elevated levels of SGOT, SGPT, ALP, total bilirubin and GGT. These biochemical restorations may be due to the inhibitory effects on cytochrome P-450 or promotion of its glucuronidation[6].

Thus on the basis of results obtained above, it can be concluded that cow urine distillate seems to possess hepatoprotective activity. Further studies are needed to evaluate the potential usefulness of this in clinical conditions associated with liver damage.

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(*Received*: 5 February 2008; *Accepted*: 1 October 2008) AJC-6918