Attenuation of Carbon Tetrachloride-Induced Hepatotoxicity by Cow Urine Distillate in Rats

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Objective To study the carbon tetrachloride–induced hepatoprotective activity in cow urine. **Methods** Effect of cow urine distillate on liver function was studied *in vivo* in rats intoxicated with carbon tetrachloride (CCl_4). Hepatotoxicity was induced by a 1:1 (v/v) mixture of CCl_4 in olive oil (5 mL/kg i.p). Protective effect of cow urine distillate (in three dose levels) and standard drug Silymarin (100 mg/kg, p.o) on liver function were studied in intoxicated rats. Parameters in the study included liver function tests and histological observations. **Results** The cow urine distillate decreased the levels of SGOT, SGPT, ALP, GGT, and total bilirubin in a dose-dependent manner ($P<0.05$) as sylimarin. **Conclusion** The observed protective effects of cow urine distillate on liver function might be due to the presence of antioxidants in cow urine.

Key words: Hepatotoxicity; Cow urine distillate; CCl_4; Silymarin

INTRODUCTION

The revered Indian cow, *Bos indicus* known as “Kamadhenu” in Indian scripts, is believed to be a “mobile hospital” for treatment of many diseases. A number of diseases can be cured by use of medicines derived from the cow. Urine of cow is elaborately described in ancient Ayurvedic scriptures such as Charaka samhita, Shushruta samhita, and Brahad-Wagbhatt as bitter, pungent, spicy, and warm. Cow urine, used as an insecticide or as a regulator for various disorders like gas, acidity, and cough, promotes the power of wisdom in human beings, acts like a universal medicine and is easily digested by all[1].

It is believed that the root cause of various diseases in human beings is due to shortage or accumulation of certain elements in the body. Cow urine contains all such elements. Hence, according to Ayurveda, it is considered a natural and universal medicine to fulfill the shortage of such element or to equalize and reduce the increased element level in the body by restoring the excretion mechanisms of the body. Though Indian Ayurvedic literature cites the medicinal properties of cow urine, there is very little scientific evidence that supports the literature. Hence the present study was undertaken to support the view[2].

MATERIALS AND METHODS

Cow Urine Distillate

The early morning first voiding urine of *Bos indicus* fed on open grass field was collected from the local cow sheds belonging to Sri Ramachandrapura math, Hosanagara, immediately distilled at 100 ℃ with temperature controlled distillation apparatus and stored below 10 ℃ for further use.

Chemicals

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

Selection of Dose

For evaluation of hepatoprotective activity of cow urine distillate, three dose levels were selected. The rat dose was calculated from human dose (60 mL per day), multiplied by a factor 0.018×5, which is

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equal to 5.4 mL/kg body weight (first dose)[3]. The second dose selected was twice that of the first dose, namely 10.8 mL/kg body weight. The third dose selected was 50% of the first dose, namely 2.7 mL/kg body weight.

Animal Treatment

Male Albino Wistar rats, weighing 180-260 g, were obtained from the Laboratory of K.S Hegde Medical Academy (KSHEMA; Deralakatte, Mangalore, India), maintained in 12 h light/dark cycle with free access to food and water. The institutional Animal Ethics Committee of KSHEMA (Deralakatte, Mangalore, India) approved the experimental protocol in accordance with the guidelines for the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with a registration number of KSHEMA/AEC/049/2007.

Animals were randomly assigned to six groups (n=6). Group I received saline (10 mL/kg, po.) for 7 days as a normal control group. Group II received a single dose of CCl_4 /olive oil (1:1, 5 mL/kg, i.p.) as a treatment control group on day 7. Group III received silymarin (100 mg/kg, po) , once daily for 7 days, as a standard reference group followed by a single dose of CCl_4 /olive oil (1:1, 5 mL/kg, i.p.) on day 7. Groups IV-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 for 7 days followed by a single dose of CCl_4 /olive oil (1:1, 5 mL/kg, i.p.) on day 7. Twenty-four hours after CCl_4 treatment, blood was withdrawn from the retro orbital plexus of all rats. Blood samples were allowed to clot for 45 minutes at room temperature. Serum was separated by centrifugation and analyzed for SGOT, SGPT, GGT, ALP, and total bilirubin levels[4]. Two animals from each group were sacrificed on the day of blood withdrawal. Liver was quickly isolated, washed with cold saline, fixed in 10% neutral buffered formalin, dehydrated in graded alcohol and embedded in paraffin wax. Sections (5-µm thick) were stained with haematoxyline and eosin (H&E) and subjected to microscopic examination.

Statistical Analysis

The data were expressed as mean±SE and analyzed using one way analysis of variance (ANOVA), followed by Dunnet's t-test. $P<0.05$ was considered statistically significant.

RESULTS

CCl_4 administration significantly increased the levels of SGOT, SGPT, ALP, GGT, and total bilirubin ($P<0.05$, Table 1). Oral administration of cow urine distillate and silymarin significantly attenuated the levels of SGOT, SGPT, ALP, GGT, and total bilirubin induced by CCl_4 ($P<0.05$, Table 1).

TABLE 1

Effect of Cow Urine Distillate and Silimarin on Biochemical Parameters in Rats with Carbon Tetrachloride-induced Hepatotoxicity (mean ± SE)

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	210.48±3.14[b]	142.71±2.17[b]	131.51±2.36[b]	28.59±1.87[b]	2.13±0.81[b]
III	72.14±1.33[a]	51.55±1.42[a]	78.48±2.15[a]	7.98±0.97[a]	0.53±0.02[a]
IV	111.18±3.59[a]	89.98±4.32[a]	112.43±1.79[a]	19.53±0.84[a]	0.86±0.03[a]
V	101.41±4.32[a]	85.71±4.10[a]	107.76±1.87[a]	17.51±0.37[a]	0.78±0.02[a]
VI	98.79±3.41[a]	80.97±3.11[a]	101.32±2.48[a]	16.53±0.31[a]	0.67±0.05[a]

Note. [a]$P<0.05$ *vs* group II; [b]$P<0.05$ *vs* group I. (n=6). Group I: normal control group; group II: carbon tetrachloride treatment group; group III: silymarin treatment group; groups IV-VI: cow urine distillate treatment groups (2.7 mL/kg body weight, 5.4 mL/kg body weight, 10.8 mL/kg body weight).

Histological Observations

Histological observations basically supported the results of serum enzyme assays. Histology of the liver tissue sections from normal control animals showed normal hepatic cells with well-preserved cytoplasm, prominent nuclei and nucleoli, and well brought out central vein. The liver tissue sections from CCl_4-intoxicated rats showed massive fatty changes, necrosis, degeneration, and broad infiltration of lymphocytes around the central vein and loss of cellular boundaries.

The histological architecture of liver sections from rats treated with cow urine distillate showed a

more or less normal lobular pattern with a mild degree of fatty changes, necrosis, and lymphocyte infiltration, almost comparable to the normal control and sylimarin-treated groups (Figs. 1-4).

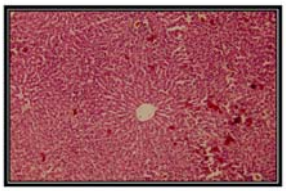

FIG. 1. Normal liver showing a normal central vein, sinusoids, and cord (arrangement of hepatocytes).

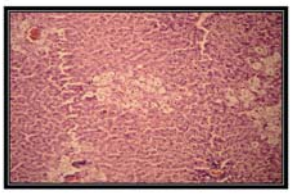

FIG. 2. Carbon tetrachloride-treated liver showing marked fatty changes around the portal tract and central vein.

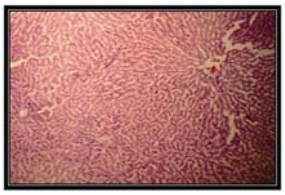

FIG. 3. Liver exposed to CCl_4 and pretreated with cow urine distillate showing almost normal hepatocytes.

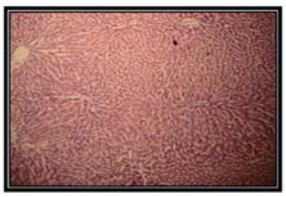

FIG. 4. Liver exposed to CCl_4 and pretreated with silymarin showing normal hepatocytes and no fatty changes in hepatocytes.

DISCUSSION

The mechanism of liver injury is through the production of toxic trichloromethyl free radicals ($CCl_3^{\cdot}$) by liver microsomes during the metabolism of CCl_4[5]. Free radicals are highly reactive and bind covalently to cell molecules, leading to cell necrosis. Due to the enzymatic activation by CCl_3 free radicals, elevation of SGOT, SGPT, ALP, GGT, and total bilirubin levels occurs[6-7], which was evidenced by the elevation of the above markers (P<0.05) in CCl_4 treated animals as observed in our study. Pretreatment with cow urine distillate reduced the elevated enzyme levels in a dose-dependent manner, indicating that it interferes with the action of $CCl_3^{\cdot}$ free-radicals produced. Since hepatoprotective properties of cow urine distillate were found in this study, the possible mechanism of cow urine distillate by which the liver function is protected may be attributed to its antioxidant property contributed mainly by volatile fatty acids and free radical scavenging[7]. Further study is needed to evaluate its potential usefulness in clinical conditions associated with liver damage.

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