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 (CCl4). Hepatotoxicity was induced by a 1:1 (v/v) mixture of CCl4 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; CCl4; 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].

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equal to 5.4 mL/kg body weight (first dose). 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. Group I received saline (10 mL/kg, po.) for 7 days as a normal control group. Group II received a single dose of CCl₄/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 CCl₄/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 CCl₄/olive oil (1:1, 5 mL/kg, i.p.) on day 7. Twenty-four hours after CCl₄ 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, ALP, GGT, and total bilirubin levels.

**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**

CCl₄ 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 CCl₄ (P<0.05, Table 1).

<p>| TABLE 1 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (IU/L)</th>
<th>GGT (IU/L)</th>
<th>Total Bilirubin (mg/dL)</th>
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</thead>
<tbody>
<tr>
<td>I</td>
<td>68.25±2.30</td>
<td>46.53±4.22</td>
<td>72.32±1.32</td>
<td>6.48±0.17</td>
<td>0.44±0.04</td>
</tr>
<tr>
<td>II</td>
<td>210.48±3.14</td>
<td>142.71±2.17</td>
<td>131.51±2.36</td>
<td>28.59±1.87</td>
<td>2.13±0.81</td>
</tr>
<tr>
<td>III</td>
<td>72.14±1.33</td>
<td>51.55±1.42</td>
<td>78.48±2.15</td>
<td>7.98±0.97</td>
<td>0.53±0.02</td>
</tr>
<tr>
<td>IV</td>
<td>111.18±3.59</td>
<td>89.98±4.32</td>
<td>112.43±1.79</td>
<td>19.53±0.84</td>
<td>0.86±0.03</td>
</tr>
<tr>
<td>V</td>
<td>101.41±4.32</td>
<td>85.71±4.10</td>
<td>107.76±1.87</td>
<td>17.51±0.37</td>
<td>0.78±0.02</td>
</tr>
<tr>
<td>VI</td>
<td>98.79±3.41</td>
<td>80.97±3.11</td>
<td>101.32±2.48</td>
<td>16.53±0.31</td>
<td>0.67±0.05</td>
</tr>
</tbody>
</table>

Note: *P<0.05 vs group II; †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 CCl₄-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 CCl₄ and pretreated with cow urine distillate showing almost normal hepatocytes.

**FIG. 4.** Liver exposed to CCl₄ 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 (CCl₃) by liver microsomes during the metabolism of CCl₄. Free radicals are highly reactive and bind covalently to cell molecules, leading to cell necrosis. Due to the enzymatic activation by CCl₃ free radicals, elevation of SGOT, SGPT, ALP, GGT, and total bilirubin levels occurs, which was evidenced by the elevation of the above markers (P<0.05) in CCl₄ 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 CCl₃ 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. Further study is needed to evaluate its potential usefulness in clinical conditions associated with liver damage.

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**REFERENCES**


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