STANDARDIZATION OF ETHANOL INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT
Liver is among the organs most susceptible to the toxic effects of ethanol. Ethanol induced liver injury is one of the widely used animal model to induce hepatotoxicity in rats. The dose and concentration of ethanol required to induce liver injury varies to a great extent. The aim of the study was to standardize the dose of ethanol to induce liver injury, for selection in future model to assess hepatoprotection of the extract. Varying doses of 40% ethanol i.e 4 gms/kg of body weight/day, 7.9 gms/kg of body weight/day and 15.78 gms/kg of body weight/day were given orally for 21 days to induce liver injury in female Sprague Dawley rats. Blood was collected terminally and serum parameters of the liver enzymes like alkaline phosphate (ALP), Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvate transaminase (SGPT) and Total Bilirubin (TB) were estimated to determine the level of liver injury. A histopathological analysis of the liver tissue was also done to assess the extent of liver injury. Elevated serum parameters indicated that different doses of 40% ethanol had resulted in liver injury, but histopathological studies revealed there was only hepatocellular degeneration which is less ideal for chronic model of hepatotoxicity. Thus there is need to increase dose of ethanol and/or duration of treatment with ethanol to induce sufficient liver damage.

Keywords
Dose, Ethanol, Hepatotoxicity, Liver.

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INTRODUCTION
In order to study the hepatoprotective effect of plant extracts or pure isolates it is necessary to induce liver toxicity in experimental models. A large number of agents are known, which on upon administration produces acute liver toxicity. Drugs such as paracetamol or isoniazid are widely used to induce liver injury, chemicals such as ethanol or CCl₄ are widely used, and also hepatotoxicity can be induced by fungal or parasitic infections like Schistosomiasis, Kodo millet and aflatoxins. The reported results for the mentioned agents of induction, of liver toxicity vary greatly in terms of the used hepatotoxins, doses, duration and route of administration. In chemical induced hepatotoxicity, ethanol induced liver damage is one of the widely used model to study hepatoprotective activity of drugs. Alcohol consumption on chronic basis is known to cause fatty infiltration, hepatitis and cirrhosis. Fat infiltration is a reversible phenomenon which occurs, when alcohol replaces fatty acids in mitochondria. Hepatitis and cirrhosis may occur upon consumption of alcohol because of enhanced lipid peroxidation reaction during the microsomal metabolism of ethanol. [1] Alcohol administration on chronic basis is known to cause accumulation of reactive oxygen species, which causes lipid peroxidation of cellular membranes and proteins and DNA oxidation resulting in hepatocyte injury. [2] It has been found that ethanol induced liver injury varies greatly between different studies, in relation to concentration, dose and duration of treatment. Ethanol concentration to induce liver damage varies from 20% to 56% (maximum), whereas duration of it varies from 3 weeks to 6 months and dose varies from 1.5 gm/kg to 12 gm/kg. [1, 3] The current study was aimed so as to select a dose of ethanol which would induce liver injury. The study plan included treatment of female Sprague Dawley rats with 40% ethanol for 21 days and induce liver damage, various biochemical parameters like alkaline phosphatase (ALP), Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvate transaminase (SGPT) and Total Bilirubin (TB) were determined to assessed extent of liver injury.

MATERIALS AND METHODS

Chemicals
Ethyl alcohol (absolute alcohol) was purchased from SD Fine Chemicals. ALP, SGPT, SGOT, TP and TB kits were purchased from CREST BIOSYSTEMS, Mumbai.

Animals
Female Sprague Dawley rats, weighing 150-200g were obtained from Glenmark Pharmaceuticals, Mahape, Navi Mumbai. Rats were maintained on 12 hr dark/light cycle in an air-conditioned room at 20±3ºC and 50-70% relative humidity. They were provided with distilled water and standard diet ad libitum throughout the investigation. The experimental protocol was approved in accordance with the guidelines provided by CPCSEA.

Experimental design
After 1 week of acclimatization, rats were randomly divided into 4 groups (n=2) as follows:
- **Group 1-Vehicle control:** Received distilled water 2ml/kg/day p.o. for 21 days
- **Group 2-4gm/kg:** Received (40%) ethanol 4gm/kg/day p.o. for 21 days [4]
- **Group 3-7.9gm/kg:** Received (40%) ethanol 8gm/kg/day p.o. for 21 days [5]
- **Group 4-15.78gm/kg:** Received (40%) ethanol 15.78gm/kg/day p.o. for 21 days [6]

Biochemical investigations:
Blood was collected terminally on 22nd day i.e 24 hrs from last dosing of ethanol administration. Serum was separated for estimation of ALP [7], SGPT [8], SGOT [8] and TB [9] levels as markers of liver injury, using standard kits.

Histopathological investigations:
Rats were sacrificed and livers were collected immediately washed with ice cold saline. Excised livers were further processed and were fixed in 10% formalin solution for histopathological study to confirm hepatotoxic effects of ethanol.

RESULTS AND DISCUSSIONS

Statistical Analysis
Values are analyzed by One way ANOVA followed by Dunnett’s test when compared with vehicle control (p<0.01) expressed as mean± SEM

Mechanism of ethanol on liver damage
Ethanol related liver damage occur when it is taken chronically or in excessive amounts. Alcohol is mainly metabolized in liver through series of chemical reactions known as oxidation reactions. Most of the ethanol is metabolized in cell cytosol of the liver by the enzyme alcohol dehydrogenase which converts alcohol to a toxic intermediate substance, acetaldehyde [10] by removing two hydrogen atoms from ethanol molecule. The acetaldehyde is then further metabolized in mitochondria by enzyme, aldehyde dehydrogenase to acetate [11] by again removing hydrogen and adding oxygen. Another pathway of alcohol metabolism is via microsomal ethanol oxidizing system (MEOS). The MEOS is activated by long term heavy alcohol consumption. [12] The MEOS pathway involves the enzyme cytochrome P450 2E1 or CYP2E1 that strips hydrogen away from alcohol to produce acetaldehyde. [13] Induction of CYP2E1 by ethanol may be a significant contributor to alcohol induced liver disease. Ethanol is also metabolized by catalase in the peroxisomes of the liver and other cells metabolizes small amount of ethanol when sufficient hydrogen peroxide (H2O2) is available- without requiring NAD as a cofactor. All three of these means of metabolizing ethanol result in acetaldehyde.
Normally body deploys molecules called antioxidant to clear free radicals from liver. But in case of consumption of heavy alcohol, it not only produces free radicals but also depletes the liver antioxidant enzymes, creating an imbalance between oxygen radicals and antioxidants. When oxidative stress is chronic it contributes to necrosis and liver fibrosis. In addition to its direct effects on the liver, oxidative stress also appears to activate autoimmune reactions that further cause liver damage. [14] High ethanol and fat consumption along with low proteins and carbohydrate consumption helps to turn fatty liver into alcoholic liver cirrhosis. Ethanol also increases release of arachidonic acid from cell membranes, increasing oxidative stress. High levels of NADH in mitochondria can cause an increase in number of superoxide free radicals leaked from oxidative phosphorylation leading to formation of hydroxyl radicals, lipid peroxidation and damage to mitochondrial DNA. And also in ethanol damaged livers there is decrease in activity of S-Adenosyl Methionine (SAM) synthetase, the enzyme that synthesizes SAM from methionine. SAM is principal methylating agent in the human body-important for synthesis of proteins and nucleic acids. Deficiency of SAM results in membrane damage, which further worsens liver damage. SAM provides a source of cysteine for GSH production. [15, 16]

**Effect on biochemical parameters**

Biochemical serum parameters like ALP, SGPT, SGOT and TB levels were found to be elevated in dose dependent manner. Hepatic damage can be assessed by level of released cytosolic transaminases including SGPT and SGOT in circulation [17]. A high level of SGOT indicates liver damage that may be due to viral hepatitis as well as cardiac infarction or muscle injury. SGPT catalyzes the conversion of alanine to pyruvate and glutamate, and is released in similar manner. Therefore SGPT is more reliable as it is more specific to liver and thus a better parameter to detect liver injury. Alkaline phosphate is excreted normally via bile by liver. Prolong destruction of the hepatic cells results in more hepatic releases to exacerbate hepatic dysfunction and also causes elevation in the serum levels of ALP and TB [18]. Ethanol induced hepatotoxicity causes rise in serum parameters SGOT, SGPT, ALP and TB. [3]

The effect of various doses of 40% ethanol on biochemical parameters is describe in Table 1 and represented in graphical form in Figure 1, Figure 2, Figure 3 and Figure 4.

**Table 1: Effect of administration of different concentrations of ethanol on ALP, SGOT, SGPT and TB levels.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALP (IU/L)</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>TB (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (Vehicle Control)</td>
<td>48.90±5.385</td>
<td>127.8±8.6</td>
<td>32.52±3.09</td>
<td>0.205±0.025</td>
</tr>
<tr>
<td>Group 2 Ethanol (4gm/kg)</td>
<td>93.99±4.335</td>
<td>176±10.82</td>
<td>59.05±3.815</td>
<td>0.415±0.055</td>
</tr>
<tr>
<td>Group 3 Ethanol (7.9gm/kg)</td>
<td>128.5±3.65</td>
<td>199±5.72</td>
<td>84.99±2.925</td>
<td>0.625±0.035</td>
</tr>
<tr>
<td>Group 4 Ethanol (15.78gm/kg)</td>
<td>167.5±5.98</td>
<td>253.4±3.565</td>
<td>99.77±3.745</td>
<td>0.795±0.065</td>
</tr>
</tbody>
</table>

In each group n=2 animals. Values are expressed as ± SEM. One way ANOVA followed by Dunnett’s test when compared with Vehicle Control.

**Figure 1: Effect of administration of different concentrations of ethanol on ALP levels**
Histopathological investigations
The histopathological evaluation of ethanol induced toxicity in all groups was examined and is shown is figures (5-8). The vehicle control group showed no abnormalities, where as in group 2, group 3 and group 4 there were signs of only hepatocellular degeneration.
and congestion. There was no perivenular fibrosis and steatosis which should have been observed in the groups receiving different doses of 40% ethanol.

Figure-5: Vehicle control

Figure-6: 40% Ethanol 4 gm/kg/day showing minimum histopathological changes

Figure-7: 40% Ethanol 7.9 gm/kg/day showing minimum histopathological changes
Figure-8: 40% Ethanol 15.78 gm/kg/day showing minimum histopathological changes

CONCLUSION
Animals treated with different doses of 40% ethanol showed rise in serum parameters, but the histological report did not show any signs of liver damage. Hence there is need to increases the dose and/or duration of treatment with ethanol to induce sufficient liver damage. The finding in the present study failed to report a fix dose of ethanol which can induce liver damage in 21 days. Hence there is a need to identify the dose of ethanol which can induce sufficient liver damage.

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REFERENCES