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Standardization of Carbon Tetrachloride-Induced Hepatotoxicity In the Rat

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ABSTRACT

The aim of the present study was to optimize carbon tetrachloride (CCl_4) induced hepatotoxicity in the rat with respect to dose and time course. Female Sprague Dawley rats, weighing 150-200g were used in the present study. Hepatotoxicity was evaluated by measuring the activity of serum enzymes, alanine transaminase [ALT], aspartate transaminase [AST], alkaline phosphatase [ALP] and total bilirubin [TBIL] level. Experimental hepatotoxicity was induced by administering 1, 1.5, 2 ml/kg CCl_4 (dissolved in an equal volume of olive oil) intraperitonially (*i.p*) and observed for enzyme levels at time intervals of 0, 2, 24, 48 hours after CCl_4 challenge. Result values are analyzed by One way ANOVA followed by Dunnett's test. 1 ml/kg CCl_4 increased the levels of serum enzymes that reached a peak after 24 hr and showed moderate hepatitis which is ideal for development of acute hepatotoxicity. It is possible to select optimum dose of CCl_4 to induce hepatotoxicity by single *i.p* injection in order to study hepatoprotective activity of herb without causing death of animals.

Keywords- Carbon tetrachloride, Dose, Hepatotoxicity, Time course

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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 animal models. A large number of chemicals are known which on administration to animals like rats will produce acute liver injury. The reported results for induction of liver toxicity vary greatly in terms of the used hepatotoxins, doses, duration and route of administration. Carbon tetrachloride (CCl_4) is one of those hepatotoxins. Liver damage induced by CCl_4 is the most widely used model to study hepatoprotective activity of drugs. It is one of the powerful hepatotoxins which cause liver tissue necrosis leading to biochemical changes [1–3]. We found that acute hepatotoxicity caused by a single dose of CCl_4 varied greatly between different studies, in relation to dose and time course. Also it was already reported that subcutaneous injection does not cause any increase in serum enzymes [alkaline phosphatase (ALP), alkaline aminotransferase (ALT), alkaline aspartate (AST) and total bilirubin (TBIL)] that were selected as liver biomarkers to evaluate hepatotoxicity of CCl_4 [4, 5]. The time course of CCl_4 is not described in most published studies [6–8].

Intraperitonial route of administration was selected for this model as it seems to be the most commonly used method for carbon tetrachloride administration due to the ease of handling and rapid onset of action [9]. Thus in an effort to properly characterize the model, we have examined the dose and time course with a fixed route of administration.

MATERIALS AND METHODS

Chemicals

The kits for all biochemical estimation (ALT, AST, ALP and TBIL) were purchased from Crest Biosystems, Mumbai. CCl_4 was purchased from Research labs and olive oil used was of LR grade.

Test 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.

Induction of CCl_4 hepatotoxicity [10, 11]

After an acclimatization period of 7 days, the rats were randomized in to four groups of 6 animals each (n=6). CCl_4 treated group was treated with CCl_4 (1, 1.5, 2 ml/kg) dissolved in olive

oil (1:1) *i.p.* The control group (0 ml CCl_4/kg) was administered olive oil which was used as vehicle. Rats were fasted for 12 hrs before CCl_4 administration.

Biochemical analyses of serum enzymes

Blood was collected from retro orbital plexus at time points 0, 2, 24, 36, 48 hrs after CCl_4 administration. Serum was separated for the estimation of ALT [12], AST [13], ALP [14], TBIL [15] levels as markers of liver injury using enzymatic kits.

Histopathology studies of liver after treatment with CCl_4

Toxicity produced by CCl_4 was confirmed through histopathological studies on liver of rats. After collections of blood for biochemical estimation, rats were sacrificed and the liver was excised, cleaned of extraneous tissue, and fixed in 10% formalin and were given for histopathological study.

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.

RESULTS AND DISCUSSION

Many of the published articles evaluating hepatoprotective activity of their herbs do not quote the appropriate reason for using the mentioned dose of CCl_4 to induce hepatotoxicity in their respective articles. Also there is a lot of controversy in the dose of CCl_4 used, the extent and time course of liver injury produced at the respective doses. The discrepancy in dose and time course of CCl_4 to induce hepatotoxicity led us to reexamine the optimum dose of CCl_4 for inducing hepatotoxicity and time course for this effect in ambient lab conditions. An attempt was carried out in order to find the suitable dose of CCl_4 to induce acute hepatotoxicity for further testing hepatoprotective activity of herbal drug of interest.

The mechanism for hepatotoxicity of CCl4 has been well documented [16, 17]. The hepatotoxicity induced by CCl_4 is due to its metabolite trichloromethyl free radicals (CCl3*), a free radical that binds to lipoprotein and leads to peroxidation of lipids of the endoplasmic reticulum which causes changes in the physical and chemical properties of cellular membranes, thus effecting their fluidity and permeability for ion exchange, resulting in leakage of enzymes in blood and finally results in swelling, cytolysis, and cell death [18]. Measurement of the activity levels of enzymes in the body fluids is a useful monitor of disease state. An obvious sign of hepatic injury is leakage of cellular enzymes into serum and their increased levels in serum. Low dose of CCl_4 (1ml/kg) caused significant increase in the activities of ALT, AST, ALP, and TBIL, at time point

of 24 hr, sufficient to study hepatoprotective activity of herb efficiently and are reliable biochemical parameters that determine functionality of liver. Following are the biochemical results in relation to different doses of CCl_4.

Serum parameters of liver function

Serum ALT, AST, ALP, and TBIL elevations were estimated as markers of liver injury over a time course (0-48 h) after different doses (0, 1, 1.5,2 ml/kg) of CCl_4 administration by *i.p.* route. In the group receiving 1 ml/kg CCl_4 no significant change in serum enzyme levels was observed within a period of 2 hr. while maximum enzyme elevations occurred at 24 h after which enzyme levels declined towards normal. For example, increased ALT (from 38.46±2.051 to 187.7±18.35), AST (96.83±4.03 to 225.30±5.2585) ALP (117.12±4.77 to 217.16±2.6734) and TBIL (0.45±0.028 to 1.33±0.0363) as indicated in Fig.1-4. In the groups receiving 1.5 and 2 ml/kg CCl_4 hepatotoxic effect was evident within 2 hr reaching its peak at 24 hr.

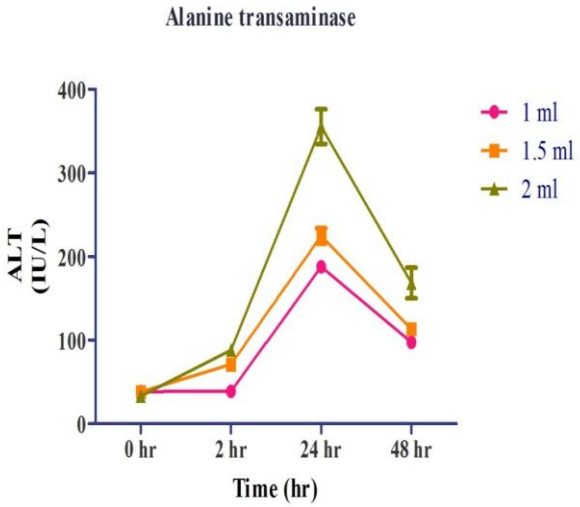

Figure1. Serum ALT (IU/L) measured over a time period of 0-48 hr after administering 1, 1.5, 2 ml CCl_4/kg in olive oil (1:1) *i.p* injection. Results are expressed as mean ±SEM (n=6) with significant differences (*p<0.01)*

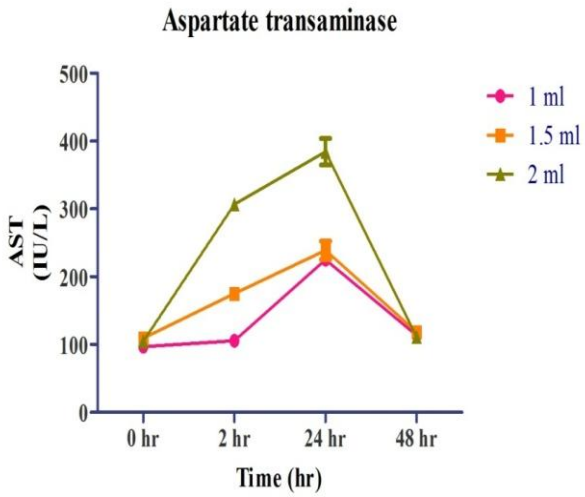

Figure2. Serum AST (IU/L) measured over a time period of 0-48 hr after administering 1, 1.5, 2 ml CCl_4/kg in olive oil (1:1) *i.p* injection. Results are expressed as mean ±SEM (n=6) with significant differences (*p<0.01)*

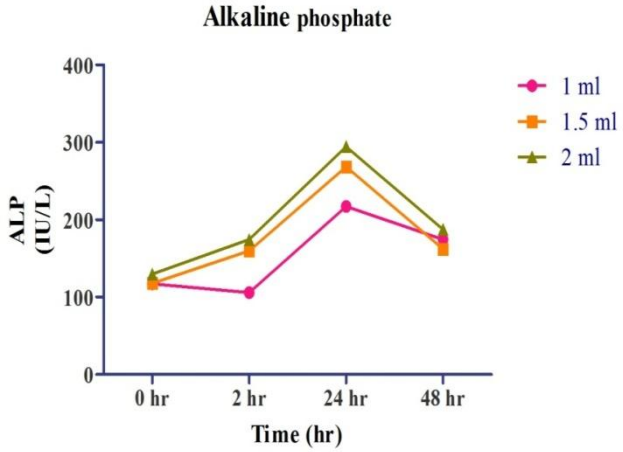

Figure3. Serum ALP (IU/L) measured over a time period of 0-48 hr after administering 1, 1.5, 2 ml CCl_4/kg in olive oil (1:1) *i.p* injection. Results are expressed as mean ±SEM (n=6) with significant differences (*p<0.01)*

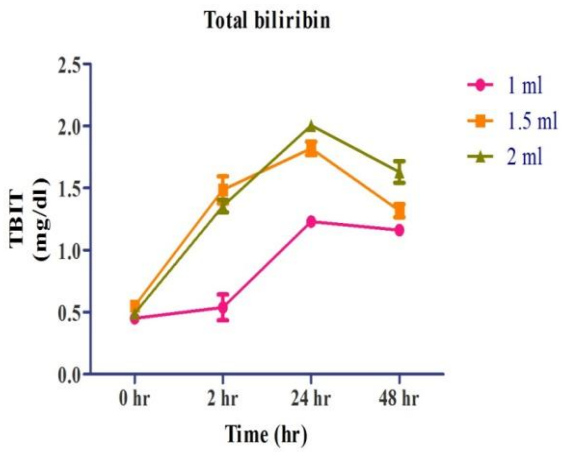

Figure4. Serum TBIL (mg/dl) measured over a time period of 0-48 hr after administering 1, 1.5, 2 ml CCl_4/kg in olive oil (1:1) *i.p* injection. Results are expressed as mean ±SEM (n=6) with significant differences (*p<0.01)*

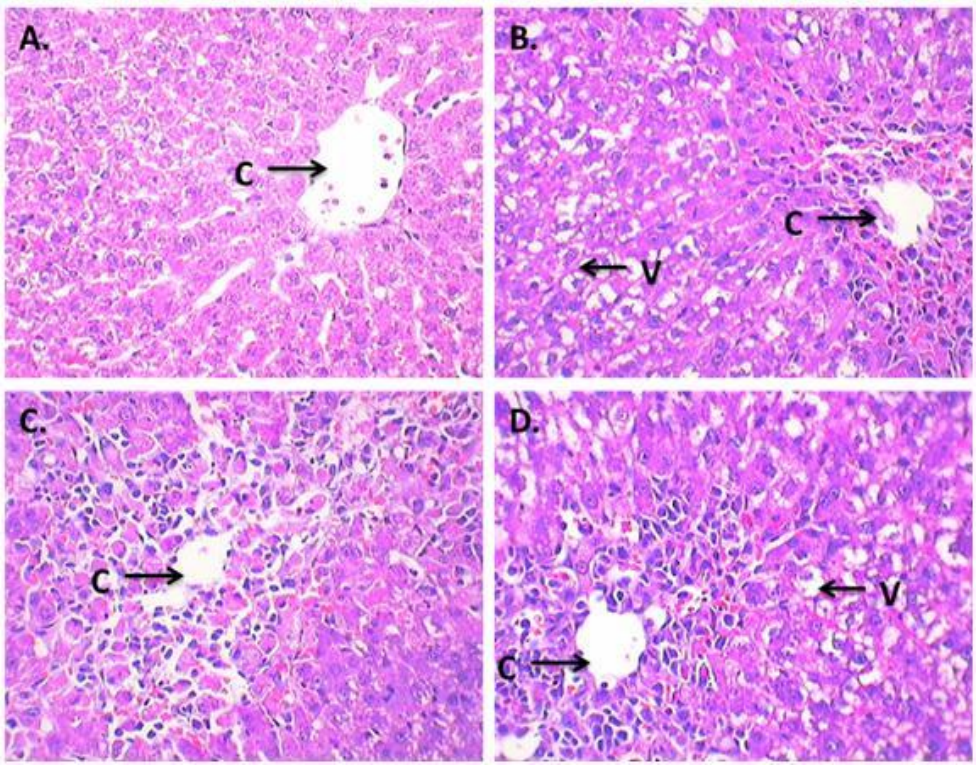

Figure5. Representative of liver histopathology after CCl_4 treatment. Histology of liver A. B. C. D. represents Control group, 1, 1.5, 2 ml / kg CCl_4 treatment respectively after 48 hours. Key: C central vein, V: Vacuoation. Magnification 40 x

Histopathology

Chemically induced hepatic injury for experimental studies should be severe enough to modify hepatic functions. In histopathology study, the section of liver of animal from control showed normal histology. Figure 5.depicts dose-related injury as observed by histopathology. When compared to normal hepatocytes of control group, 1 ml/kg of CCl_4 dose showed hepatocellular degeneration, congestion and centrilobular hepatitis. An increase in dose (1.5 ml/kg CCl_4) showed dose dependent hepatocellular damage whereas still higher dose (2 ml CCl_4 /kg) showed centrilobular necrosis.

1 ml/kg CCl_4 dose was found to be optimum or lowest and suitable dose as it caused acute hepatitis that had close resembles with viral hepatitis clinically, biochemically and histologically[19,20]. The maximum effect of which was seen at 24 hr after CCl_4 injection and then values slowly normalized. Group receiving 1 ml/kg CCl_4 dose showed moderate hepatitis which was considered ideal for model development of acute hepatitis.

Thus, in evaluation of hepatoprotective activity of herb using CCl_4 induced hepatototxicity model, the blood was thus withdrawn at 24 hr after CCl_4 administration to study protective effect of herbal extract of interest to its maximum. However, in Published studies, mostly the dose range for CCl_4 to achieve a significant increase in biochemical markers of hepatotoxicity was reported to be 1-2.5 ml/kg in rats [21–23].

CONCLUSION.

It is possible to select optimum dose of CCl_4 to set CCl_4 induced hepatotoxicity model for the purpose of preliminary investigation of herbal drug in liver disorders.

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