Liv.100 pretreatment inhibits the CCl_4 induced lipid peroxidation in rat liver – An *in vitro* study

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The protective effect of Liv.52 and Liv.100 against *in vitro* peroxidation induced by carbon tetrachloride in rat liver homogenate has been studied. Lipid peroxidation was measured in terms of thio-barbituric acid reactive substances (TBARS), and reduced glutathione was also assayed. Addition of the two polyherbal formulations, Liv.52 and Liv.100, inhibited the peroxidation effect of carbon tetrachloride in a dose and time-dependent manner. The results suggest on the antioxidant potential of Liv.52 and Liv.100. Liv.100 is a new ayurvedic formulation.

LIPID peroxidation is a natural phenomenon and occurs on exposure to oxygen[1]. Active oxygen molecules such as superoxide radicals ($O_2^{\cdot}$, $^{\cdot}OOH$) and hydroxyl radicals ($OH^{\cdot}$) play an important role in the inflammation process after intoxication by carbon tetrachloride, ethanol or carrageenan[2-5]. These radicals react with cell membranes, induce lipid peroxidation and have been implicated as important pathologic mediators in many clinical conditions[6-8]. This communication is based on the potential of the two ayurvedic formulations, Liv.52 and Liv.100. Liv.52 is commercially available in India and has been reported to be clinically effective in treating hepatotoxicity and a wide range of liver diseases[9-11]. Recent reports also suggested the protective effect of Liv.52 against radiation-induced chromosome damage[12,13] (lipid peroxidative changes) in mouse bone marrow. Therefore, an attempt was made to study the effect of the new ayurvedic formulation Liv.100 on lipid peroxidation in rat liver homogenate exposed to carbon tetrachloride challenge and compared with that of Liv.52. Liv.100 is also a product of Himalaya Drug Co, a polyherbal preparation composed of *Cichorium intybus*, *Solanum nigrum*, *Phyllanthus amarus*, *Piccorhiza curra* and *Embelica ribes* in the ratio 5 : 3.75 : 2.62 : 2 : 1. These herbs are individually reputed for their hepatoprotective nature[9,11,14,15].

Male albino rats of wistar strain (100 ± 125 g) maintained on a standard pellet diet supplied by M/s Hindustan Lever Ltd (Bangalore, India) and water *ad libitum* were used for the experiment.

Rats were sacrificed by cervical dislocation and liver was immediately excised. A homogenate (1 g, w/v) was prepared using phosphate buffer saline (PBS), pH 7.4, in cold. It was centrifuged at 2000 *g* for ten minutes. Supernatant was collected and finally suspended in PBS. It contained approximately 0.8–1 mg protein in 1 ml of suspension. This was used to perform the *in vitro* experiment.

To establish the role of Liv.52 and Liv.100 on carbon tetrachloride-induced lipid peroxidation[16], different concentrations of Liv.52 and Liv.100 were added to 3 ml of liver homogenate, mixed and incubated at 37°C for 20 min. This was followed by the addition of carbon tetrachloride (1 mM), mixed gently and further incubated at 37°C for 25 min to induce the production of TBA-reactive substances (TBARS). The control group contained buffer and different concentrations of Liv.52 and Liv.100. Lipid peroxidation was measured in terms of TBARS as described by Yagi *et al.*[17] with slight modification and reduced glutathione was measured by the method of Ellman[18]. Protein was determined by the method of Lowry *et al.*[19] using bovine serum albumin as standard.

The results presented in the paper are the mean ± SD of six animals. Level of significance has been evaluated by using Student's *t*-test.

The reaction mixture that contain carbon tetrachloride *per se* resulted in a significant increased ($P < 0.001$) formation of TBARS (Table 1). Addition of the two drugs proved their protective nature against carbon tetrachloride-induced lipid peroxidation. A significant ($P < 0.001$) dose-dependent effect was afforded by the drugs, and did not show any augment in TBA-reactive substances up to 6.6 mg/ml.

Figure 1 shows the effect of Liv.52 and Liv.100 (1 mg/ml) on reduced glutathione (GSH). In normal condition, GSH content decreased after 20 min as an outcome of auto-oxidation, but in the (1 mg/ml) drug-treated group, the rate of lowering was found to be minimum and the level was maintained up to 40 min. This effect was seen even in the presence of 0.25 mM carbon tetrachloride. The effect of the drugs was found to be appreciable in maintenance of the levels of glutathione at normal (Figure 1).

In the present study, carbon tetrachloride was used as a peroxidative agent, and has been justified to produce free radicals or deplete antioxidant defenses. Carbon tetrachloride is converted to a free radical by hepatic cytochrome P-450.

$$CCl_4 \xrightarrow{\text{Cytochrome P-450}} Cl^{\cdot} + CCl_3^{\cdot}.$$

This radical reacts with oxygen to give a perox radical $CCl_3^{\cdot} + O_2 \rightarrow CCl_3O_2$ which is a good inhibito of lipid peroxidation. Consequently, there is depletio of antioxidant, especially glutathione. Loss of glutathion causes secondary oxidative damage, which can contribut to hepatic failure.

The reason for having employed two different cor centrations of CCl_4 (1 mM and 0.25 mM) for the assa

Table 1. Protective effect of Liv.52 and Liv.100 on carbon tetrachloride (1 mM)-induced lipid peroxidation in rat liver

Drug concentration (mg/ml)	0	0.8	1.6	3.3	6.6
Liv.52					
Without CCl_4 'TBARS' nM/100 mg protein	117.08 ± 4.71	116.99 ± 4.20	116.83 ± 3.74	116.69 ± 3.94	116.37 ± 3.62
With CCl_4 'TBARS' nM/100 mg protein	231.85 ± 4.65*	222.90 ± 3.11*	171.97 ± 3.32*	158.47 ± 2.98*	127.36 ± 3.05*
Liv.100					
Without CCl_4 'TBARS' nM/100 mg protein	117.11 ± 4.69	116.78 ± 2.26	116.51 ± 2.35	116.35 ± 2.02	116.24 ± 2.08
With CCl_4 'TBARS' nM/100 mg protein	231.91 ± 4.58*	189.37 ± 1.97*	143.20 ± 2.60*	125.16 ± 2.23*	116.30 ± 2.01*

Values are mean ± SD of 6 animals in each group.
*$P < 0.001$.

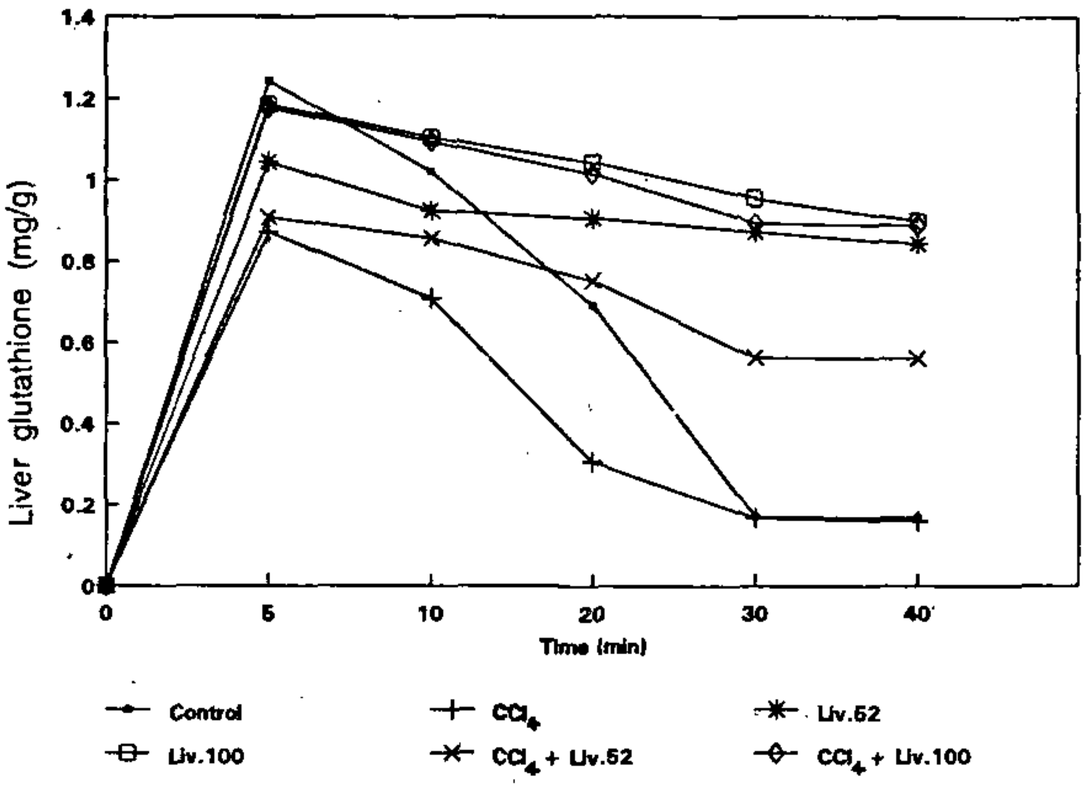

Figure 1. Effect of carbon tetrachloride (0.25 mM), and Liv.52 and Liv.100 (1 mg/ml) on the changes in reduced glutathione content in rat liver. Each point represents mean value of six animals.

of lipid peroxidation (TBARS) and reduced glutathione, is that at higher doses of 1 mM concentration, it released detectable amounts of TBARS but reduced the GSH level (almost to zero). This showed that the oxidation of GSH is followed by lipid peroxidation and therefore, quantitation of reduced GSH would be more sensitive test than TBARS formed.

Carbon tetrachloride-induced peroxidation of lipid in liver homogenate was found to be suppressed by the addition of Liv.52 and Liv.100. The effect was almost completely, inhibited by Liv.100 when compared to Liv.52.

The present results show the dose and time-dependent protective response afforded by Liv.52 and Liv.100 against carbon tetrachloride-induced lipid peroxidation. These results suggest that the drugs act at the lipid peroxide scavenging system through enzyme control or otherwise, by enhanced supply of reduced glutathione[20] that suppressed the deleterious process.

However, the findings indicate that the formulations Liv.52 and Liv.100, contain certain substances capable of preventing lipid peroxidation. Polyphenolic constituents[14,21] of the herbs in the above ayurvedic formulations may be considered to be antioxidants able to react with numerous radicals in cell-free systems, including oxygen and hydroxylic ($OH^{\bullet}$) radicals forming more stable and less reactive compounds[22,23].

To conclude, it is hoped that the beneficial antioxidant nature of Liv.52 and Liv.100 may be applied clinically in the future only after considerable *in vivo* trial with the drugs.

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Isotopic compositions of boron in sediments and their implications

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The abundance and isotopic compositions of boron in sediments from the salt lakes of Qaidam Basin, China have been determined by thermal ionization mass spectrometry of cesium borate. The results show large variations in the isotopic compositions of boron. The observed variations of $\delta^{11}B$, ranging from – 25.2‰ to + 26.08‰ (relative to NBS SRM 951) indicate both an enrichment and depletion in the lighter isotope (^{10}B) in the lake sediments. The lower $\delta^{11}B$ values of sediments are mostly closer to the boron isotopic compositions of minerals. Such low $\delta^{11}B$ values are attributed to the presence of borates, ulexite and other carbonate minerals in sediments of the salt lakes of Qaidam Basin.

BORON is one of the major elements in seawater[1] and is significantly involved in marine biogeochemical cycles[2,3]. Recent studies have shown that the isotopes of boron act as excellent tracers of geochemical processes[4–6]. The differences in the behaviour of its two stable isotopes, ^{10}B and ^{11}B, result in the isotopic fractionation of boron during its biogeochemical cycling[7] and the extent of this fractionation provides insights into the various processes involved.

Although the isotopic studies of boron are being carried out for the past 2–3 decades, the difficulties involved in the accurate measurement of boron isotopic ratios led to a slow progress in this area. However, the recent advancements in the analytical techniques[8,9] have provided impetus to studies on isotope geochemistry of boron. The isotopic measurements by these techniques have shown wide variations in boron isotopic ratios in nature[10] and indicated distinct differences in the isotopic compositions of boron between the marine, non-marine and terrestrial samples and between seawater, sediments and the upper oceanic crust[11,12].

The hypersaline environments experiencing intense evaporation are excellent sites for isotopic studies of elements as considerable isotopic fractionation occurs during evaporation and precipitation in the brine[13]. The present study is based on the boron isotopic measurements carried out in sediments of the salt lakes of Qaidam Basin, China, from October to December, 1992 to understand their variation and their geochemical implication.

The salt lakes of Qaidam Basin are situated to the north of the Kunlun Tangula mountains in the northwestern part of Qinghai Plateau at an altitude of over 2600 m above sea level. This terrestrial basin (Figure 1), evolved during the Jurassic period[14], is typically a continental potassium-bearing basin with an area of 12×10^4 km^2. It is surrounded by the Altun mountains in the northeast and Kunlun mountains in the south. The streams draining from these snow-capped mountains terminate in a series of saline lakes at the margin of the basin.

The basin experiences a typical continental climate with difference in day and night temperatures, reaching as high as 30°C. The climate is arid and cold with an annual precipitation less than 50 mm and annual evaporation far exceeds precipitation. Due to the low air temperatures, high aridity and strong winds, the waters in these lakes have turned into brines.

Sediments were collected from 22 salt lakes in the Qaidam Basin (Figure 1) during 7 July–10 August, 1992. After collection, the brine was drained out and the sediments were oven dried at 60°C. The dried sediments were finely powdered and sieved through a standard 100 mesh. A known quantity of the sieved sample was treated with a minimum quantity of dilute HCl (< 0.1 N). The acid-washed sediments were filtered and the filtrate was preserved for isotopic measurements. The residue was then washed with distilled water and again filtered. The filtrates were mixed together and made up to 50 ml. Boron contents in the filtrates were determined through mannitol-alkalimetry titration[15]. A known quantity of the filtrate was used for the extraction of boron by ion-exchange for isotopic measurements[16].

A mixture of weakly basic anion exchange resin in HCO_3^- form and a strongly acidic cation exchange resin in the H^+ form was poured into a special glass column. The boron-containing solutions were slightly acidified with HCl before passing through the column. After shaking for 7–10 min, boron was eluted with 60 ml high-purity water. The yield of boron recovered from each sample was more than 98%. After elution, the $^{11}B/^{10}B$ ratio was measured by a mass spectrometer, model VG 354, based on thermal ionization mass spectrometry of cesium borate using graphite as a substrate