Down Regulation of Tumour Necrosis Factor Activity in Experimental Hepatitis by a Herbal Formulation, Liv.52

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ABSTRACT

A herbal hepatoprotective formulation Liv.52 down regulated the tumour necrosis factor (TNF) production in Charles Foster rats treated with CCl_4. Inhibition of TNF activity was proportional to the hepatoprotective activity.

INTRODUCTION

Hepatotoxicity by different chemicals is well know[1-3]. Liver becomes the primary target, as it is the major site for detoxification. Besides chemicals, microbes and their toxins pose another challenge to liver cells[1-3].

Hepatitis-induced liver damage is increasing with the increase in water pollution and blood tranfusion[1]. From time immemorial, physicians practicing different principles including Ayurveda have tried various plant products to alleviate somatic, psychosomatic and infectious diseases[4,5].

Mehendale[6] has reviewed the mechanism of liver damage by CCl_4. CCl_4 and other chemicals induced hepatic damage and tumor necrosis factor (TNF) was detected in serum of these animals[7]. It was hypothesized that TNF is the mediator of liver damage, and other markers of liver function are released from the liver as a result of the damage[8,9]. This cytokine, in association with other inflammatory cytokines, brought about many damaging effects to the soma[10].

Various hepatoprotective substances effective in improving hepatic function are available [11-16]. Many of these formulations are prescribed for treatment of various types of jaundice. Though their usefulness has been reported, their molecular mechanisms of action have not been elucidated[11-18]. Moreover, it is not clear whether these drugs really inhibited the TNF activity or its induction. Therefore, the present study has been undertaken to elucidate the role of a commercially available herbal preparation (Liv.52) on TNF, following administration of a hepatotoxicant (CCl_4) in rats. To the best of our knowledge, this is the first report on the mode of action of herbal hepatoprotective compounds.

MATERIAL AND METHODS

Carbon tetrachloride (CCl_4; Analar grade) was obtained from BDH. Liv.52 was obtained from The Himalaya Drug Company. L929 cells were originally obtained from NIH, USA and maintained in the laboratory in Minimum Essential Medium (MEM Earle's) with 10% foetal calf serum (Gibco). Nunclon 96 Well microtitre plates used for TNF assay were obtained from NUNC Inc. Denmark.

Charles Foster rats were bred in the animal house of the Institute and were maintained on a normal diet.

The rats (8week old) were divided into 4 groups of 6 animals each. Group A was maintained on normal diet without any CCl_4 or Liv.52 treatment. Group B was maintained similarly as above but treated with 0.5 ml of Liv.52 for one day prior to the day of experiment. The animals were also force-fed with Liv.52 just before CCl_4 challenge. Group C was maintained on normal diet and on the day of experiment the rats were force-fed with 0.5 ml of Liv.52 just before the CCl_4 challenge. Animals in Group D were maintained on normal diet and were force-fed with CCl_4 along with paraffin as vehicle on the day of experiment. After 24 hour of CCl_4 challenge, the animals were bled through cardiac puncture and the serum samples were collected. The serum samples were assayed on L929 cells for TNF activity.

TNF in serum was assayed as described using L929 cells[7-19]. The activity was measured by the cytolysis of L929 cells by experimental serum.
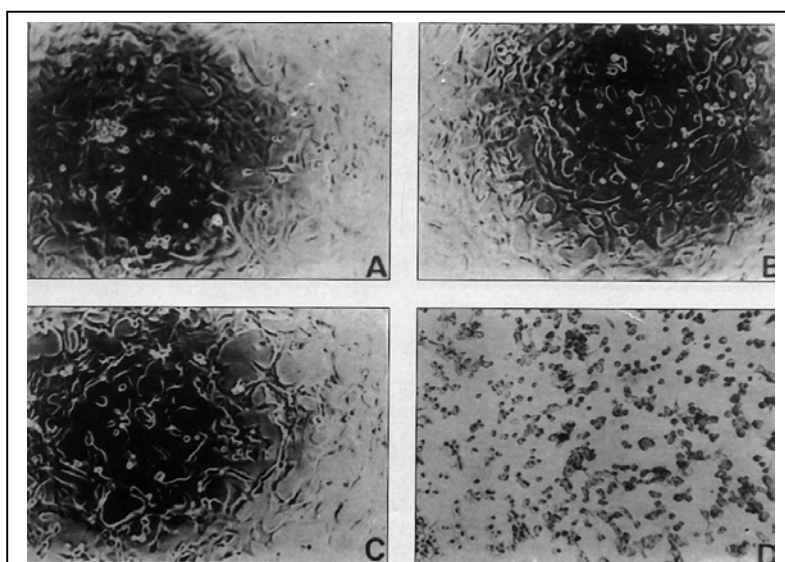
RESULTS

TNF activity (Fig. 1 A-D) — Morphology of L929 cells showed no necrosis (Group A), no TNF like activity (Group B), very little TNF like activity (Group C), and a very high amount of TNF activity (Group D) with most of the cells lysed and necrotised.

Figure 1 (A-D): TNF activity as evident by lysis of L929 cells by 1.25% serum from animals from group A to D, respectively.

Histomorphometric studies (Fig. 2 A-D) — Normal histology of the rat liver showing sinusoidal architecture of hepatocytes having no sign of necrosis or de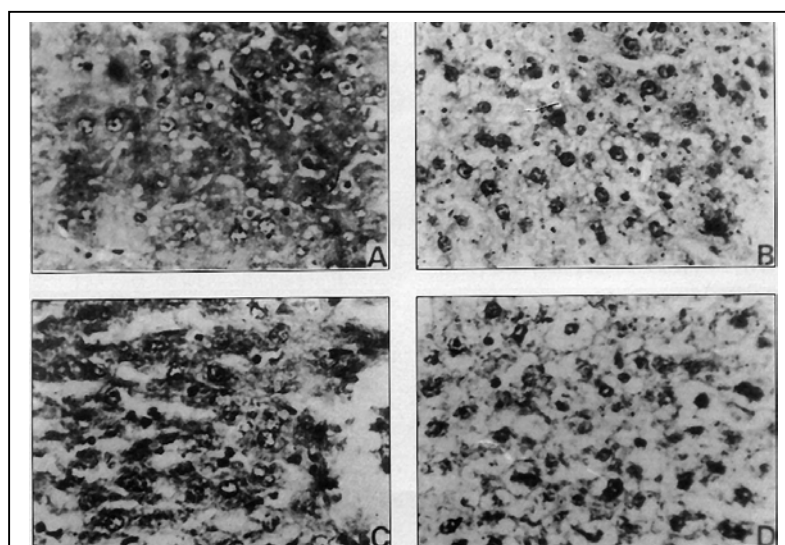generation is shown in Fig. 2A. Liver sections from rats of groups B-D show microfatty changes with dense collection of lymphoid cells (Fig. 2B) suggesting evidence of very little necrosis or degeneration; peripheral lymphoid aggregates with fatty changes around central vein (Fig. 2C) showing microvesicular fatty changes and piecemeal necrosis, degeneration of hepatocytes focal area of necrosis and fatty changes (Fig. 2D).

DISCUSSION

Infection and inflammation induce

Figure 2 (A-D): Histology of liver section from animals from group A to D, respectively

a series of co-ordinated biochemical changes in the liver; most of the changes are part of the acute phase response[20]. The changes included increased hepatic synthesis of acute phase proteins and decreased synthesis of "negative acute phase reactants" like albumin[21]. The role of these proteins is not well understood, but it is suggested they may be involved in the protective action against toxic substances produced during infection and inflammation.

Some of these acute phase proteins are antioxidants and protease inhibitors. Different cytokine binding proteins have been found in the acute phase proteins. There are also changes in the hepatic biochemistry with respect to ability to destroy or metabolize drugs[22]. The relative roles of different cytokines in these functions are not known. The liver is both source and target of TNF[23-26]. It was originally identified as a product of activated macrophages (Kupffer cells)[26].

The results of the present study clearly indicate that the animals pretreated with Liv.52 or simultaneously treated with CCl_4 produce lesser amount of TNF but the quantum of reduction was observed to increase with the duration of pretreatment.

With the present limited data, it is very difficult to postulate the mechanism whether it was really an inhibition of induction or blockage of TNF activity. However, by comparing the activity it can be postulated that the reduction of TNF activity is due to its lesser induction. If it were a blockage or inhibition, then serum from Group C rats would have similar activity as that of group B. The lesser induction of TNF may be due to either the refractile nature of TNF producing cells or inactivation of the active radical produced from CCL_4 by the ingredients present in Liv.52. It has long been suggested that hepatoxicity of CCl_4 is through the formation of free radical CCl_3, phosgene and CCl_3O_2 etc. which interacts with the lipids of the hepatocellular membrane. It is still not known whether these products are actually involved in TNF induction. The herbal products may be acting as scavengers of these toxic materials and thus the TNF triggering molecules get neutralized. The other possible pathway for this down regulation may be through the selective deactivation of cytochrome P450 system which activates CCl_4 to phosgene, $*CCl_3$ etc. It is still not clear whether these herbal products could also minimize the liver damage by CCl_4 in animals pre-exposed to chlordecone. It has been clearly shown that the recovery from liver injury requires hepatocellular regeneration within a short span of 6 hour of exposure but in case of some chemicals the regeneration process is arrested and the ordinarily sublethal dose of CCl_4 becomes lethal[27-29]. According to Bang et al.[30] prostaglandin E_2 analogues counter the effect of CCl_4-induced necrosis, thus highlighting the importance of herbal products in modulating the activity of immune effector molecules (here TNF). Further studies with other herbal products are needed to see these kinds of modulation in pre- and post-exposure conditions. The herbal drugs do bring about alleviation of the symptoms of liver damage as evident by the assay of biochemical markers and histopathological studies[11,14-18]. These drugs may directly or indirectly affect the production of TNF from Kupffer cells.

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