Hepatoprotective activity of Haridradi ghrita on carbon tetrachloride-induced liver damage in rats

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Haridradi ghrita, a ghee based polyherbal formulation, (50, 100, 200 and 300 mg/kg) significantly lowered marker enzymes (SGPT, SGOT, ALP) and bilirubin in serum and liver peroxide, superoxide dismutase and catalase in liver homogenate following CCl₄ (0.7 ml/kg, ip) toxicity. The protective effect was further supported by reversal of CCl₄ induced histological changes. The results demonstrate significant hepatoprotective action of H. ghrita in CCI₄ damaged rats.

Key words: Haridradi ghrita, Hepatoprotection, Lipid peroxidation, Panchgavya

Indian medicinal plants and many herbal formulations belonging to the traditional systems of medicine like Ayurveda, have been investigated as liver protective drugs¹. Side effects and expenses associated with allopathic drugs have provoked the need for research into drugs which are without the side effects, especially those belonging to the traditional systems of medicine. Research emphasis has been directed towards herbal and herbo-mineral preparations, either single drug or in combination (polyherbal) having specific diagnostic and therapeutic principles².

Panchgavya is a term used in Ayurveda to describe the five important products obtained from bovine origin, viz. milk, curd, ghee, urine and dung. These components are used either alone or in combination with herbs for the treatment of several diseases and disorders³. Many formulations based on Panchgavya are described in ancient Ayurvedic texts. Haridradi ghrita (HG) is a ghee containing polyherbal formulation ascribed with immunostimulant and hepatoprotective activity in traditional practices. Recently HG has been reported to promote cellular and humoral immunity in rats⁴. The focus of the present study is to provide an evaluation of the hepatoprotective effect of HG. The protective effect is analysed against carbon tetrachloride (CCl₄)-induced liver toxicity in rats. Active oxygen molecules 'CCl₃ and CCl₃O₂ play an important role in the inflammation process induced due to CCl₄ intoxication⁵.

Antioxidant enzymes such as superoxide dismutase and catalase serve as a defence mechanism by converting the active molecules into non-toxic compounds thereby preventing the lipid peroxidation in body. The present article is an attempt to investigate the hepatoprotective potential of Haridradi ghrita.

Haridradi ghrita (HG), received as a gift sample from Go-Vigyan Anusandhan Kendra, Nagpur was used. The detailed composition is described elsewhere⁶. HG contains dried finely powdered herbs: Emblica officinalis (fruits, 4% w/w), Terminalia chebula (fruits, 4% w/w), Terminalia bellirica (fruits, 4% w/w), Azadirachta indica (leaves, 4% w/w), Sida cordifolia (roots, 4% w/w) and Glycyrrhiza glabra (roots, 4% w/w), uniformly mixed and blended with Ghee (76% w/w).

Reagents and assay kits for antioxidant enzymes were purchased from Sigma Chemical Co., USA. Carbon tetrachloride was obtained from E. Merck, Mumbai, India, and all other chemicals used were of analytical grade.

Animals and treatment—Male Wistar albino rats (150-250 g) were housed under standard conditions of temperature (23°C ± 1°C), 12:12 hr. L:D and fed with standard pellet diet (Gold Muho Brand, Lipton India Ltd.) and water ad libitum.

The rats were divided into 7 groups of 8 animals each. Group I served as control, receiving vehicle only (1% gum acacia solution, po). CCl₄ (0.7 ml/kg, ip) was administered to animals of the remaining six groups, every alternate day for 7 days. Group II animals received only CCl₄. Reference drug Silymarin (20 mg/kg, as 2 mg/ml solution in 1% gum acacia, po)
was administered to group III. The test formulation HG was administered to groups IV, V, VI and VII at doses 50, 100, 200 and 300 mg/kg, po in 1% gum acacia suspension respectively. HG and reference drug Silymarin were administered daily for 7 days. After completion of experimental regimen, the rats were fasted overnight and blood samples were collected by puncturing the retro-orbital plexus under light ether anaesthesia. Serum was separated by centrifugation at 3000 rpm in cold for different assays. Subsequently animals were sacrificed with an overdose of ether and liver were excised immediately for analysis.

Assessment of liver function — Serum glutamate-pyruvate-transaminase (GPT)\(^a\), glutamate-oxalate-transaminase (GOT)\(^b\), alkaline phosphatase (ALP)\(^c\) and bilirubin\(^d\) were estimated.

Histopathology — Liver lobes were removed and washed with normal saline. Small pieces of liver tissues were processed for histological analysis. After fixing in 10% formalin solution, the tissues were dehydrated with 90% ethanol, embedded in paraffin, cut into thin sliced sections (7 µm thick), stained with haematoxylin-eosin dye and observed under a light microscope, for cell necrosis, vacular degenerative changes, inflammation and fibrosis.

Homogenate preparation — The liver samples were homogenized in 0.1 M phosphate buffer solution (pH 7.4, 4°C) to give a 10% homogenate in cold. Protein concentration of liver tissue was measured by the method of Lowry et al.\(^e\) using bovine serum albumin as a standard.

Lipid peroxidation — The inhibitory effect on lipid peroxidation was assessed by measuring thioarbituric acid reactive substances (TBARS). The quantitative measurement of lipid peroxides was performed in the liver homogenate according to the procedure described by Ohkawa.\(^f\) The level of lipid peroxides (LPO) was expressed as nmol malonaldehyde (MDA)/mg protein using molar extinction coefficient of 1.56 × 10^5.

Enzyme assays — Activity of antioxidant enzymes, viz. superoxide dismutase (SOD) and catalase (CAT) was assayed in liver homogenate of experimental animals. SOD was assayed according to the method described by Marklund.\(^g\) The results are expressed as units/mg protein, where one unit of enzyme is expressed as the amount of enzyme inhibiting the rate of pyrogallol peroxidation by 50%. CAT was assayed by the method of Aebi.\(^h\) The breakdown of H_2O_2 by the enzyme was measured at 240 nm. Enzyme activity was calculated using molar extinction coefficient of H_2O_2 (0.07) and the results were expressed as nM H_2O_2 decomposed/min/mg protein.

Statistical analysis — The data were analysed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer Multiple Comparisons Test. P values <0.05 were considered significant.

Liver injuries induced by CCI_4 are commonly used as model for the screening of hepatoprotective drugs.\(^i\) Raised serum enzyme levels\(^j\) in intoxicated rats can be attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and are released into circulation after cellular damage.\(^k\) The results of the serum biochemical changes observed in the present study are depicted in Table 1. The estimated values of serum GPT, GOT and ALP in the control (group I) rats were found to be raised significantly (P<0.05) by 149.79, 105.5 and 32.57% respectively after administration of CCI_4. Treatment of animals with the test formulation HG was administered to groups IV, V, VI and VII at 50, 100, 200 and 300 mg/kg/day, po returned the serum GPT, GOT and ALP values to near normal, which were significantly lower than only CCI_4 treated group and were close to Silymarin treated animals. Oral administration of HG seems to reverse the hepatic cell damage in a dose-dependent manner providing significant protection with a dose 300 mg/kg/day.

Histological observations of the liver Sections (Fig. 1) are in agreement with the serum biochemical changes. Damage caused by CCI_4 was reversed due to HG treatment at 300 mg/kg/day dose. The observations are comparable to those with Silymarin (20 mg/kg/day) treatment. After HG administration, the necrosed hepatocytes were rarely seen although cloudy swelling was evident. Few hepatocytes revealed vacular degenerative changes. Dose dependent reversal of CCI_4 damage is evident from histological observations.

It is well known that CCl_4 induced hepatic damage is due to the free trichloromethyl radical (·CCl_3). Free radical induced oxidative stress has been implicated in disorders, resulting usually from deficient antioxidant defences. Potential hepatoprotective agents therefore includes either free radical scavenging property or agents which are capable of augmenting the activity of antioxidant enzymes (SOD, CAT etc.).\(^m\) CCl_4 treatment significantly increased the lipid peroxidation level in the liver. The increased TBARS of liver indicated enhanced lipid peroxidation due to tissue injury.\(^n\) Significant restoration of LPO was observed in HG (300 mg/kg/day) supplemented rats as compared...
to CCl₄ treatments. The HG treatment also restored the depleted SOD and CAT levels. The results shown in Table 2 demonstrate that HG supports the antioxidant defence mechanisms by increasing the activity of enzymes like SOD and CAT. There is a direct co-relation between the enhanced SOD and CAT levels and the reduced LPO levels and vice versa. Ingredients of HG like Emblica officinalis are reported to have hepatoprotective activity. From the overall results obtained we can conclude that HG possesses beneficial action against liver damage induced by CCl₄. The hepatoprotective activity of Haridradi ghrita may possibly be due to its antioxidant property, acting as scavenger of free radicals like hydroxyl radicals and superoxides. Patients with liver disease may manifest different syndromes in different phases. It must be noted, however, that the possible mechanisms of protection.
Photomicrographs of rat liver sections showing histopathological changes. (a) Vehicle (b) CCl₄ (0.7 ml/kg) (c) Silymarin (20 mg/kg) (d) Haridradi ghruta (300 mg/kg) × 100.

of HG are rather speculative at this stage and more investigations are needed to identify the active components and elucidate their modes of hepatoprotection. The study however rationalizes the traditional use of HG in liver diseases. A protective mechanism not specific to injury induced by CCl₄ might be responsible for the hepatoprotective action of HG. Experiments are in progress to study such mechanisms. In conclusion, this study has demonstrated the utility of the test formulation as a hepatoprotective drug.

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References