Hepatoprotective Activity of Polyherbal Preparation against Carbon Tetrachloride Intoxication in Rat Liver

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Abstract
The effect of polyherbal preparation (F1) on carbon tetrachloride (CCL₄)- induced acute liver damage was evaluated. The increased serum enzyme levels (aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma glutyl trans peptidase, decrease in glutathione), lipid peroxidation and a decline in weight of liver on CCL₄ induction. Increased enzyme levels, lipid peroxidation and a decline in GSH produced by CCl₄ were reversed by the polyherbal preparation in a dose response way. Results of this study revealed that polyherb afforded a significant protective action with no interaction and toxicity in the alleviation of CCL₄ induced hepatocellular injury.

Keywords: Carbon tetrachloride; GSH; polyherbal preparation (F1); hepatoprotection

1. INTRODUCTION
Polyherbal preparation of the combination of Glycyrrhiza glabra, Clerodendrum serratum and Allium cepa confined to the temperate regions of Asia. Different parts of the herbs are used in Indian traditional medicine for the treatment of a broad spectrum of ailments including Glycyrrhiza glabra widely used in gastritis (inflammation of the stomach) and ailments of the upper respiratory tract; Clerodendrum serratum used as an appetiser, digestive, stomachic, blood purifier, alleviates kapha, fever, worms, burning sensation, laxative, cephalalgia and ophthalmia; Allium cepa used as an antihelmintic, antiparasitic, diuretic and repellent, in the treatment of asthma, bronchitis, whooping cough, warts, acne, appetite loss and urinary tract disorders. All three are individually proved to be effective in hepatoprotection[1]. In this regard, we have previously evaluated a significant hepatoprotective potential of polyherbal preparation in the light of inhibition of lipid peroxidation and subsequent normalization of (GSH)- related enzymes linked with the antioxidant defence system against carbon tetrachloride (CCL₄)- evoked liver damage in rat[2]. In the present communication, we have assessed further the hepatoprotective activity of polyherbal preparation in vivo by monitoring the changes in several serum and liver as the experimental model. The parameters studied include the serum enzyme level of glutamate pyruvate transaminase (SGPT), glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase, gamma glutyl transpeptidase (GGTP), serum bilirubin, MDA (malonyl di aldehyde) (Babaie et al., 2007), liver weight, which are known to be the most frequent and satisfactory markers for evaluating hepatocellular damage[3,4].

2. MATERIALS AND METHODS
2.1. Preparation of polyherbal preparation
Fresh root barks of Glycyrrhiza glabra (300) were dried and crushed in gr inder and treated with petroleum ether (5 times the volume of drug) for 24 hours and further treated with 1:5 (drug: solvent) of 50:50 hydroalcoholic solvent for 52 hours[5]. Clerodendrum serratum 200 g while Allium cepa 600 g produces a yield of 6.66, 6.5 and 3.33%[6]. The extraction was carried out in a cold room (20± 1ºC). The homogenate was then dried on water bath till semisolid dry extract was obtained which was then shade dried.

2.2. Treatment of animals
Adult swiss albino rats, weighing between 100-150 g, were acclimatized to conditions in the laboratory (room temperature, 60-80% relative humidity, day night cycle) for 10 days prior to the commencement of the treatment, during which they received food and tap water ad libitum. Rats were then divided in several groups of 6 rats each. Initially Acute toxicity study and DPPH free radical scavenging activity were performed on rats and further evaluation of hepatoprotective activity was done through carbon tetrachloride model as follows[14,15]. The normal group was given distilled water orally at 10 mg/ Kg body weight once daily for 7 days in succession, while the treated group was administered Carbon tetrachloride after 7 days of polyherbal administration. Apart from all this the model control group was treated with only hepatotoxin. After 24 hours of toxin injection, pentobarbital sleep induction is checked so as to note out any decline in duration of pentobarbital action [7].

Group 1: Saline as single dose, followed by pentobarbitone sodium (75 mg/Kg) i.p. after 1 hour.
Group 2: Polyherb as a single oral dose at 400 mg/Kg followed by pentobarbitone after 1 hour.

Group 3: 4 doses of normal saline at 12 hrs interval after 1 hour CCl4 administered followed by pentobarbitone sodium i.p.

Group 4 doses of polyherb at 12 hours interval after 1 hour CCl4 administered followed by pentobarbitone sodium was injected i.p.

Group 5: 4 doses of standard and after 1 hour, sodium was injected i.p.

2.3. Assessment of liver function

Twenty four hours after the i.m. injection of the hepatotoxin, the rats from each group were anaesthesized with ether, and blood was collected from retro-orbital route. It was centrifuged at 2000 g for 10 minutes to separate the serum and assayed for activities of serum enzymes. SGOT, SGPT were determined by the method of Reitman and Frankel as described by Bergmeyer and Bernt (1974). Alkaline phosphatase was estimated according to Kind and King (1954) [10]. Serum bilirubin was estimated according to Malloy and Evelyn (1937) [11]. Thiobarbaturic acid reactive species determination and the weight of liver were estimated with GSH, GGTP and Lipid peroxidation concentration as per Ohkawa (1979) [12, 13].

2.4 Statistical analysis

Results are expressed as mean "S.D.". Student’s t-test was used to assess statistical significance between the mean values and control.

2.5 Standardization

The polyherbal preparation was standardized using UV fluorescence, Ash value determination and Sodium content estimation of the sample.

3. RESULTS

Rats treated with a single dose of CCl4 developed significant hepatic damage as observed by elevated hepatospecific enzymes as well as serve alterations in different liver parameters. Activities of SGOT, SGPT, ALP, in serum were found to be roused in CCl4 intoxicated as compared to control animals. It has been established that CCl4 is accumulated in hepatic parenchymal cells and metabolically activated by cytochrome P-450 dependent monoxygenase to form trichloromethyl free radical (CCl3·) which alkylates cellular proteins (including cytochrome P-450) and other macromolecules with a simultaneous attack on polysaturated fatty acids in the presence of oxygen which produces lipid peroxides leading to liver damage (Recknagel et al., 1976) [17]. For many years now, hepatotoxic compounds such as CCl4 are known to cause marked elevation in serum transaminases. Our present study elicited a significant increase in the activites of SGOT, SGPT, LDH, Alkaline Phosphatase, Sorbitol and Glutamate Dehydrogenase, within 24 hr of exposure of the rat to a single dose of CCl4 indicating considerable heptocellular injury [7].

F1could have successfully hindered the path of cyp action that’s why there occurred the fall in enzyme levels, protection of lysosomes from rupturing and maintained the liver lysosomes integrity, improved cytochrome P 450 level, protein synthesis leading to sleep prolongation with pentobarbital (Mandal et al., 1993) [20]. Also standardization of polyherbal was done.

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<th>Table 1: Evaluation of Hepatoprotection against Carbon tetrachloride induced toxicity</th>
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<td>Groups</td>
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<tr>
<td>I (Control)</td>
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<td>II (Herb single)</td>
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<td>III (4 doses of saline/Model control)</td>
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<td>IV (4 doses of herb)</td>
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<td>V (4 doses of standard)</td>
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* P<0.05; ** P<0.01; *** P<0.001; Dunnett’s t test against the respective control. Values in + SEM of 5 animals.
Photomicrographs of liver sections after CCl₄ induced hepatotoxicity.

A= Normal control
B= mild congestion with acute inflammation of periphery;
C= Hydrophil and ballooning degeneration of hepatocyte, mild congestion;
D= Mild congestion, kupffer cell hyperploid with chronic periportal inflammation and;
E= Architecture presented with hydropic and ballooning degeneration and fibrosis.

4. DISCUSSION
Results have clearly shown that various biochemical changes, produced in serum and liver were reversed by polyherbal treatment at 200 and 400 mg/Kg dose.
The probable effect of protective effect on the alteration of hepatocellular metabolism could be by protection through minimization of deleterious effect of peroxy radicals/ accelerated detoxification/ excretion, and thereby can be ranked as hepatoprotective agent. Pretreatment with F1 attenuated the increased enzyme activities produced by CCL₄ a subsequent recovery towards normalization of these enzymes strongly suggest
the possibility of being able to condition the hepatocytes so as to cause accelerated regeneration of parenchyma cells, thus protecting against membrane fragility decreasing the leakage of marker enzymes in the circulation. Stabilisation of serum bilirubin levels through administration of the extract is further a clear indication for an improvement of the functional status of liver cells [19]. However which of these components are responsible for hepatoprotection will be seen in future course of experiments. Thus all of these observations tend to conclude that F1 behaves as a hepatoprotective agent against Carbon tetrachloride induced toxicity and proves to be beneficial as compared with the standard (Silymarin). Thus the polyherb prepared was safe, comparatively effective and so was standardized for further use.

REFERENCES