Hepatoprotective Activity df Salvia Hypoleuca Extracts Against Carbon Tetrachloride-Induced Hepatic Damage In Rats

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Summary

The aim of this work was to investigate the effects of Salvia hypoleuca extracts against carbon tetrachloride (CCl4)-induced hepatotoxicity in rats. Hepatotoxicity was induced in male Wistar rats by intraperitoneal injection of CCl4 (1 ml/kg/day for 7 days). Salvia hypoleuca ethanol extract was administered to the experimental rats (400 mg/kg/day, p.o. for 10 days). The hepatoprotective effect of extract was evaluated by the assay of liver function biochemical parameters (total bilirubin, serum protein, alanine aminotransaminase, aspartate aminotransaminase, and alkaline phosphatase activities), liver weight and histopathological studies of the liver. Results showed that the toxic effect of CCl4 was controlled significantly by restoration of the levels of serum bilirubin, protein and enzymes as compared to the normal and the standard drug silymarin- treated groups. Histology of the liver sections of the animals treated with the extract showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration, which further evidenced the hepatoprotective activity. It can be concluded that Salvia hypoleuca extract possess significant hepatoprotective activity.

Keywords: Salvia hypoleuca, Hepatoprotective activity, Liver, Rat.

Introduction

The liver plays an essential role in drug and xenobiotic metabolism and in maintaining the biological equilibrium of the organism. The role played by this organ in the removal of substances from the portal circulation makes it susceptible to a persistent attack by offending foreign (xenobiotic) compound culminating in liver dysfunction. Despite the tremendous strides in modern medicine, there are few drugs that stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cells. Herbal drugs are frequently considered to be less toxic and free from side effects than synthetic drugs. Nowadays, medicinal plants receive attention to research centers because of their special importance in safety of communities. The curative properties of medicinal plants are mainly due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (1, 2). Medicinal plants like Andrographis paniculata, Boerhaavia diffusa (3) Hibiscus rosasinensis (4), Phyllanthus amarus (5), are well known for their hepatoprotective effects. The genus Salvia, one of the most important genuses of Lamiaceae family, is widely used in flavouring and folk medicine all around the world. Fifty-eight species of this genus are documented in the Flora of Iran; 17 of them are endemic (6-8). The plants of the genus Salvia, which consist about 900 species are generally known for their multiple pharmacological effects such as analgesic and anti-inflammatory (9) hepatoprotective (10), hypoglycemic activities (11), and antiischemia (12, 13). Literature reviews indicated that the hepatoprotective activity of Salvia hypoleuca has not been clinically evaluated so far. In view of this, the present study was aimed at evaluating the hepatoprotective activity of the Salvia hypoleuca against carbon tetrachloride (CCl4)-induced hepatotoxicity in albino rats.

Materials and Method

Preparation of the extract of Salvia hypoleuca

Air dried Powdered plant was divided in to two equal parts (1 kg each); one part was macerated with ethanol (90 % v/v) in glass percolator and allowed to stand at room temperature for about 24 hours. Then the extract obtained was filtered, concentrated by rotary vacuum pump to get the solid mass. The percentage yield was 19.6 %, second part of powdered was cold macerated with distilled water then the same procedure was repeated as mentioned above. The percentage yield was 10.5%.

Animals' treatment

Albino Wistar rats weighing 150-200 g of either sex, 4 months of age were used for this study. The experimental animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3º C and 35-60% humidity). Standard pelletized feed and tap water were provided ad libitum. The Institutional Animal Ethical Committee biochemical investigations were carried out to assess liver function viz ., total bilirubin, total protein, serum transaminases and serum alkaline phosphatase. Weight of the livers was also calculated. Four groups of animals containing six each were used for the study. The animals of Group I served as the control and received the vehicle 1% w/v gum acacia. Groups II-IV received 0.25 mL of CC14 in liquid paraffin (1:1) 1 mL kg-1 body weight intraperitoneally (IP) for 7days. The standard drug Silymarin (Ranbaxy Lab. Dewas) was administered to Group III animals in the dose of 100 mg/kg/day p.o. for 10 days. While, Group IV was treated with Salvia hypoleuca in the dose of 400 mg/kg/day, p.o. (as per acute toxicity studies) for 10 days. The CCl4, silymarin and the extract were administered concomitantly to the respective groups of animals. All the animals were killed on day 11 and the blood samples were collected by cardiac puncture method into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 min and biochemical investigations were carried out to assess liver function viz ., total bilirubin, total protein, serum transaminases and serum alkaline phosphatase. Weight of the livers was also calculated.

Acute toxicity study

The acute toxicity of the ethanol extract was determined using albino mice of either sex (20-30g), those maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OECD Guideline No. 420) method of CPCSEA was adopted for the toxicity studies. No mortality was observed even at the dose of 2000mg/kg. 1/5th of LD50 dose i.e. 400mg/kg of the extract was selected for the screening of hepatoprotective activity.

Histological studies

After draining the blood, liver samples were excised, washed with normal saline and processed separately for histological observations. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. Paraffin sections were taken at 5 mm thickness, processed in alcohol-xylene series and were stained with alum hematoxylin and eosin. The sections were examined microscopically for histopathological changes.

Statistical analysis

All the values are expressed as mean ±SEM and data was analyzed by one-way ANOVA. The post hoc analysis was carried out by Dunnett's multiple comparison test to estimate the significance of difference between individual groups. $p< 0.05$ was considered statistically significant.

Results

The administration of CCl4 to the animals resulted in a marked increase in total bilirubin, serum amino transaminases (AST and ALT) and serum alkaline phosphatase activities. However, the serum total protein level was decreased. The toxic effect of CCl4 was controlled in the animals treated with Salvia hypoleuca extract by way of restoration of the levels of the liver function biochemistry similar to that of the standard drug silymarin (Table 1). Histological profile of the control animals showed normal hepatocytes (Figure 1). Group-II animals exhibited intense centrilobular necrosis, vacuolization and macrovesicular fatty change (Figure 2). The sections of liver taken from the animals treated with standard drug silymarin showed the hepatic architecture, which was similar to that of control. The animals treated with Salvia hypoleuca exhibited significant liver protection against the toxicant as evident by the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration (Figure 3).

Table 1: Effect of Salvia hypoleuca extracts on some biochemical parameters in rats subjected to CCl4 -induced hepatotoxicity.

Groups	Total Bilirubin mg/dl	Total Protein mg/dl	AST (IU/I)	ALT (IU/I)	ALP (IU/I)	Liver weight(g)
1	0.75±0.62	6.09±0.37	22.14±2.13	48.54±5.17	7.03±0.12	2.25±0.88
2	2.86±0.17*	2.97±0.99*	60.71±1.12*	96.18±1.24*	12.94±0.95*	4.68±0.17*
3	1.55±0.61*	5.11±0.86**	17.37±1.51*;	54.62±0.73*;	7.75±0.73*	3.02±0.13*
4	0.83±0.96**	6.74±0.75**	24.25±1.84*;	52.63±1.52*;	5.91±0.33*	3.43±0.43**

Values are expressed as mean±SEM. *P<0.05significant as compared to control, **P<0.05 significant as compared to CCl4 treated group.

Figure 1: Normal liver tissue from control group, H&E, 100X.

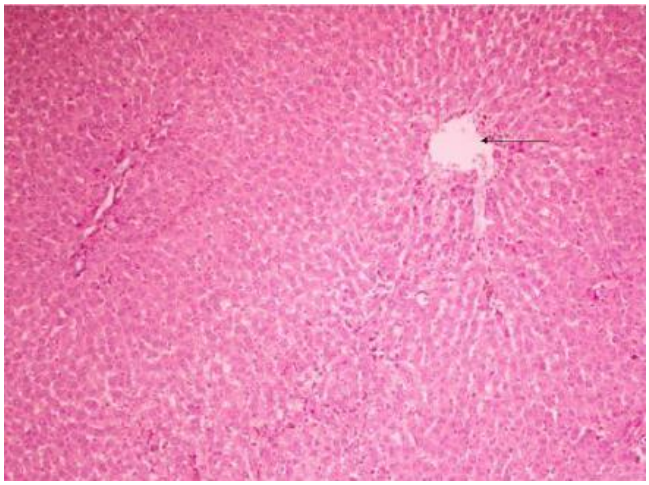


Figure 2: Liver tissue of group treated with CC14 showing periportal round cells collection and necrosis. H&E, 100X.

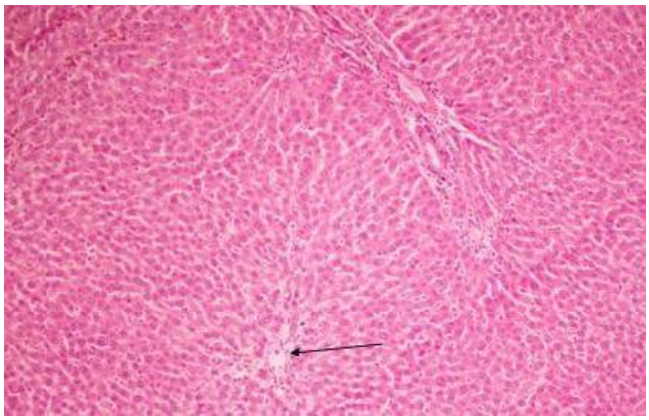
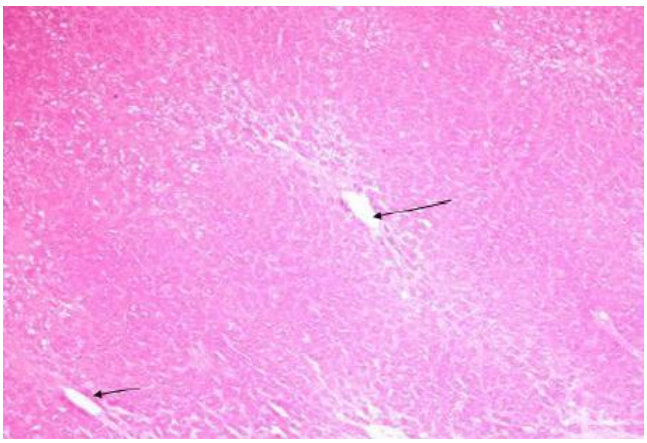


Figure 3: Liver tissue of group treated with Salvia hypoleuca showing normal histology. H&E, 100 X.



Discussion

Carbon tetrachloride has been used as a tool to induce hepatotoxicity in experimental animals. This toxic chemical caused peroxidative degradation in adipose tissue resulting in fatty infiltration of the hepatocytes. The increase in transaminases and alkaline phosphatase was a clear indication of cellular leakage and loss of functional integrity of cell membrane (14). The increase in the level of serum bilirubin reflected the severity of jaundice (15). Carbon tetrachloride, which is an intrinsic hepato toxin, was used to induce hepatic damage in this study since it has previously been shown to exert its toxic effects on the liver (16). Administration of CCl4 causes severe injury in rats` livers. This damage is recognized by an increase in serum levels of the hepatic enzymes SGOT and SGPT, which are indices of liver cell damage (17). The administration of extract showed significant hepatoprotective activity, which was comparable with the standard drug silymarin. It is known that SGOT can be found in the liver, cardiac muscle, kidney, brain, pancreas, lungs, skeletal muscle, leukocytes and erythrocytes (18). Whereas SGPT is present in highest concentration in the liver. In tissues, SGPT occurs in two locations, the cytosol and mitochondria (19). SGPT appears to be a more sensitive and specific parameter of acute hepatocellular damage than SGOT (15). Therefore, the possible hepatoprotective mechanism of the extracts of Salvia hypoleuca on the CCl4-induced liver injuries may be through the following actions; inhibition of the cytochrome P-450 activity, prevention of the process of lipid peroxidation, stabilization of the hepatocellular membrane and enhancing the protein synthesis (20). Administration of extract showed significant hepatoprotective activity, which was comparable with the standard drug silymarin. On the basis of the obtained results in this study, it can be concluded that Salvia hypoleuca extracts have preventive effect against CCl4-induced hepatocellular damage in rats. The present liver-protective effect of extract is presumably due to its contents of sulphurated, phenolic and terpenoid compounds. The effect of these compounds could be through preventing the accumulation of excessive free radicals and protecting the liver against CCl4 intoxication. The protection of liver by extract against CCl4-induced toxicity might be related to glutathione-mediated detoxification.

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