HEPATOPROTECTIVE ACTIVITY OF CAULIFLOWER LEAF EXTRACT AGAINST ISONIAZID AND RIFAMPICIN INDUCED TOXICITY IN ALBINO RATS

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ABSTRACT
The most common complication of widely used antitubercular drugs like isoniazid and rifampicin was hepatotoxicity. The main purpose of the present study was to evaluate the hepatoprotective activity of cauliflower ethanolic leaf extract against a rat model of INH- RMP induced hepatotoxicity. In this study the hepatoprotective activity of ethanolic extract of cauliflower leaf (EELC) was determined by estimating the serum parameters like AST, ALT, ALP, bilirubin, cholesterol, triglycerides and total serum proteins. Preliminary phytochemical studies revealed the presence of phytoconstituents like alkaloids, flavonoids, steroids, carbohydrates, proteins, tannins, glycosides, saponins, thiols, phenols, triterpenoids and purins. Silymarin and EELC 200 and 400 mg/kg when administered to rats exhibited protection against RMP-INH induced hepatotoxicity as manifested by the reduction in toxin mediated rise in serum enzymes (AST, ALT, ALP, bilirubin, cholesterol, triglycerides) and increased total protein levels. The significantly increased percentage inhibition of DPPH and lipid peroxidation scavenging activity with EELC was noticed. The histopathological studies also revealed the preservation of liver integrity in treated groups.

INTRODUCTION
Liver, the largest organ of the body comprising 2-3% of the total adult body weight and is primarily concerned with the metabolic activity of organisms. It is the central site for the biotransformation of xenobiotic chemicals and for detoxifying the chemical substances in the blood and in this process it is exposed to high concentrations of toxicants and toxic metabolites making it susceptible to injury. Drug-induced hepatotoxicity, a leading cause of liver injury, poses an important challenge to clinicians. Isoniazid (INH) and rifampicin (RMP), the most important first line antitubercular drugs (ATD) have been used for the treatment of TB. These drugs are also used in combination with other medicines to treat co-infections, and to reduce the duration of anti-TB therapy from 18 months to 6 months [1]. ATD are the most common cause of drug-induced acute liver failure in India [2]. Despite the undefined mechanism of Isoniazid hepatotoxicity, hydrazine and acetyl hydrazine are regarded as the main toxic metabolites of Isoniazid. It is highly suggested that these two bioactive metabolites are produced by a series of enzymes including cytochrome P450 [3,4] and could induce oxidative stress to cause hepatotoxicity[5]. RMP generally co-administered with INH in the treatment of tuberculosis. Its antibacterial activity is mediated by the inhibition of bacterial RNA polymerase. Furthermore, rifampicin is considered as a powerful inducer of mixed-function oxidase that increases the hepatotoxicity of isoniazid by enhancing the

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production of toxic metabolites from acetyl hydrazine [6] and enhances hydrazine production [10] by enzyme induction. The high reactivity of hydrazine with sulphydryl groups results in glutathione (GSH) depletion within the hepatocytes leading to cell death. Many antioxidants, naturally occurring from plant sources, have been identified as free radical or active oxygen scavengers [7]. Cauliflower (Brassica oleracea. var botrytis) belongs to family brassicaceae, vegetables of Brassicaceae family are the essential sources of phenolic compounds in the human diet. They also contain derivatives of hydroxy cinnamic, caffeic, chlorogenic, ferulic, and synapic acids as well as flavonoids (kaempferol and quercetin derivatives) and anthocyanins (red cabbage) [8,9]. Brassica vegetables have been reported as good sources of antioxidants. Several epidemiological studies showed that they were associated with reduction of cancer [10]. The present study was aimed to evaluate hepatoprotective activity of Ethanolic Extract of Leaf of Cauliflower against hepatotoxic albinino rats by studying the serum enzymes (AST, ALT, ALP, DB, TB, cholesterol and triglycerides) and total protein levels.

MATERIALS AND METHODS

Male Wistar rats weighing (150-200g) were purchased from National Centre for Laboratory Animal Sciences, C/O Sri Venkateswara Enterprises, Bengaluru, India. They were housed in polypropylene cages in a controlled room temperature 22±1°C and relative humidity of 60-70%. They were kept under standard conditions of lighting 12/12 h light and dark cycle. The animals were maintained with standard pellet diet and water ad libitum [11]. The animals were acclimatized to laboratory condition for seven days before commencement of experiment. All studies were carried out using 6 animals in each group. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee and was cleared by the same before starting.

PREPARATION OF PLANT EXTRACT

The fresh leaves of cauliflower were collected. It was defatted using petroleum ether. The marc obtained was dried and subjected to extraction by adding dried leaf powder and distilled water (1:10), heated to 50-60°C under constant stirring conditions for 1 hour and filtered. The ethanol extract was prepared by using Sohxlet's apparatus. The leaf extract of cauliflower was concentrated to dryness under reduced pressure at 40°C using standard procedures. Preliminary Phytochemical Screening: The preliminary phytochemical screening of the crude extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites using standard procedures. [12]

Acute toxicity studies:

Acute toxicity study was carried out using male Wistar rats. The rats were fasted overnight and the weight of each rat was recorded just before use. Animals were divided randomly into six groups viz; a control and five treatment groups, each group comprising of six rats. Control group received only the vehicle and each treatment group received orally the 90% ethanol extract of cauliflower leaf up to 4000 mg/kg in a graded fashion. Animals were kept under close observation for 4 hours after administering the extract, and then they were observed daily for three days for any change in general behaviour and/or other physical activities [13].

Grouping animals:

Animal are divided into 5 groups of 6 animals each. Normal control group (Group I) received only vehicle. (Group II) received only INH-RMP without any treatment. Group III received standard drug Silymarin (50 mg/kg), Group IV and group V received ethanolic leaf extract of cauliflower (200 mg/kg;400 mg/kg p.o.), once daily for 15 days. GroupIII to V receive the above mentioned drugs 45 min prior to INH-RMP treatment.

Isoniazid, Rifampicin induced hepatic damage

Hepatic damage was induced by administration of INH (50 mg/kg); RMP (100 mg/kg) orally, once daily induced hepatic injury. Drugs were given as suspension orally 45 min before standard and test drugs treatment. At 15th day 1 ml blood was collected from all animals by retro-orbital puncture for the evaluation of serum parameters like AST, ALT ALP, bilirubin, cholesterol, triglycerides and total serum protein [14].

Histopathological studies

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

Statistical analysis

Results of all parameters were expressed as mean ± standard deviation for each group. One way ANOVA followed by Dunnett test.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening:

The preliminary phytochemical screening of the ethanol extract of cauliflower leaf revealed the presence of alkaloids, carbohydrates, steroids, glycosides, tannins, proteins, saponins, flavonoids, thios, phenols, triterpenoids and purins.

Acute toxicity studies

Acute toxicity studies conducted revealed that the administration of graded doses of the crude ethanol extracts (up to a dose of 2000 mg/kg) of cauliflower leaf did not produce significant changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma and appearance of the animals. Mortality was not noticed up to 2000 mg/kg. One-tenth of this dose i.e. 200 mg/kg was selected as the therapeutic dose for the evaluation of hepatoprotective activity.

Effect of EELC on biochemical parameters:

There was an enormous increase in the liver parameters like AST (110.36±0.24 IU/L), ALT (138.20±0.16 IU/L), ALP (437.98±0.11 IU/L), DB (1.137±0.011 mg/dL), TB (0.981±0.004 mg/dL), CHO (351.40±0.39 mg/dL), TG (0.981±0.004 mg/dL), DB (0.981±0.004 mg/dL), TB (0.981±0.004 mg/dL), CHO (351.40±0.39 mg/dL), TG (0.981±0.004 mg/dL). Statistical analysis

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Table 1: Effect of EELC on biochemical parameters

<table>
<thead>
<tr>
<th></th>
<th>Group-I</th>
<th>Group-II</th>
<th>Group-III</th>
<th>Group-IV</th>
<th>Group-V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ast (IU/L)</td>
<td>43.61±0.43</td>
<td>110.36±0.24**</td>
<td>40.90±0.16*</td>
<td>53.15±0.37**</td>
<td>41.86±0.13**</td>
</tr>
<tr>
<td>Alt (IU/L)</td>
<td>44.80±0.81</td>
<td>138.20±0.16**</td>
<td>44.18±1.15**</td>
<td>57.95±0.23**</td>
<td>40.75±1.16**</td>
</tr>
<tr>
<td>Alp (IU/L)</td>
<td>93.18±1.94</td>
<td>437.98±0.11**</td>
<td>156.11±0.17**</td>
<td>220.93±0.27**</td>
<td>159.00±0.31**</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.316±0.001</td>
<td>0.317±0.001</td>
<td>0.325±0.004**</td>
<td>0.446±0.007**</td>
<td>0.248±0.007**</td>
</tr>
<tr>
<td>DB</td>
<td>74.11±0.65</td>
<td>351.40±0.39**</td>
<td>89.34±1.59**</td>
<td>121.33±0.24**</td>
<td>90.06±1.16**</td>
</tr>
<tr>
<td>TB</td>
<td>181.33±0.46</td>
<td>62.25±0.32**</td>
<td>216.28±1.97**</td>
<td>241.45±0.38</td>
<td>218.25±0.84**</td>
</tr>
<tr>
<td>Tm (mg/dL)</td>
<td>8.53±0.08</td>
<td>3.33±0.13**</td>
<td>7.53±0.10**</td>
<td>5.75±0.10**</td>
<td>6.76±0.13**</td>
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<tr>
<td>Tg (mg/dL)</td>
<td>19.6±0.4</td>
<td>19.6±0.4</td>
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<tr>
<td>Tp (G/Dl)</td>
<td>19.6±0.4</td>
<td>19.6±0.4</td>
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</table>

Values are expressed as mean ± standard deviation (n=6) in each group, statistical significance (*p<0.05, **p<0.01). One way ANOVA followed by Dunnett’s test.

(Group I) received only vehicle, (Group II) received only INH-RMP without any treatment, (Group III) received standard drug Silymarin (50 mg/kg p.o.), (Group IV) and (Group V) received ethanolic leaf extract of cauliflower (200 mg/kg; 400 mg/kg p.o.) respectively.

When compared to toxicant control, EELC 200 mg/kg & 400 mg/kg treated animals have shown a dose dependent hepatoprotective activity with a significant reduction in biochemical parameters like AST (53.15±0.37 IU/L & 41.86±0.13 IU/L), ALT (57.95±0.23 IU/L & 40.75±1.16 IU/L), ALP (220.93±0.27 IU/L & 159.00±0.31 IU/L), DB (0.446±0.007 mg/dL & 0.248±0.007 mg/dL), TB (0.477±0.003 mg/dL & 0.316±0.020 mg/dL), CHO (121.33±0.24 mg/dL & 90.06±1.16 mg/dL), TG (241.45±0.38 mg/dL & 218.25±0.84 mg/dL), whereas TP levels (5.75±0.10 g/dL & 6.76±0.13 g/dL) are significantly increased.

The activity of EELC on biochemical parameters was significantly comparable to that of standard drug Silymarin. The effect of silymarin on parameters like AST (40.90±0.16 IU/L), ALT (44.18±1.15 IU/L), ALP (156.11±0.17 IU/L), DB (0.325±0.004 mg/dL),
TB (0.322±0.002 mg/dL), CHO (89.34±1.59 mg/dL), TG (216.28±1.97 mg/dL), whereas TP (7.53±0.13 g/dL).

This can be attributed to hepatic structural damage as these enzymes normally localized in the cytoplasm and released into the circulation after cellular damage has occurred [10]. Results are shown in table no 1 and fig no 1-8. Such hepatotoxic effect induced by INH–RMP administration was confirmed by histopathological findings. The pathological findings in EELC treated group depicted that the normal architecture of liver was restored and this suggests that the EELC has exhibited its protective effect on liver and successfully reversed the effect of toxicant drugs on it. Results are shown in fig no 9.

CONCLUSION

From all these findings we can conclude that the EELC has significant hepatoprotective activity as evidenced by the biochemical and histological parameters. The present findings provide scientific evidence to the ethno medicinal use of this plant genetic resource by the tribal people in treating liver disorders. The potential usefulness of the extract in clinical conditions associated with liver damage is still to be demonstrated. Further studies are needed to be carried out for the isolation of active principles responsible for hepatoprotective activity and also for the intoxication with other models such as CCL4, Theophylline etc. to prove its efficacy.

REFERENCES


