Hepatoprotective activity of Costus igneus against Paracetamol induced liver damage

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ABSTRACT
Costus igneus commonly known as Fiery costus or Spiral flag is a herbaceous plant. This plant is grown in America and is becoming popular in India because of its medicinal value. It is now accepted and used widely as an Ayurvedic medicinal herb. Paracetamol was used in a dose of 300mg/kg orally to induce hepatic damage. The induction of liver damage was confirmed by elevated serum enzyme levels and histopathological picture showing zonal focal necrosis. Administration of Costus igneus extract prior to acetaminophen administration effectively (P< 0.05) prevented the induction of damage by acetaminophen, which was confirmed by normal enzyme levels and lack of necrotic changes in the histopathological studies. The effect produced by 400mg/kg of Costus igneus was comparable with that produced by the standard drug silymarin.

1. INTRODUCTION
Liver diseases are posing as a major health problem around the world. Toxic chemicals, aflatoxins and water pollutants are major risk factors for liver diseases. Costus igneus commonly known as Fiery costus or Spiral flag is a species of herbaceous plant. Costus igneus (Fam: Zingiberaceae) is a tropical evergreen shrub with large, smooth, dark green leaves. This plant is grown in America and is becoming popular in India because of its medicinal value. It is now accepted and used widely as an Ayurvedic medicinal herb. In south India, the leaves of Costus igneus is used to control blood sugar levels.

Drugs and chemicals induce liver toxicity. Drugs like acetaminophen, isoniazid, rifampicin, alcohol etc. are known hepatotoxins. Acetaminophen a widely used analgesic and antipyretic drug, produces acute liver damage in high doses. Acetaminophen administration causes necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis and eosinophilic cytoplasm followed by large excessive hepatic lesion. In spite of the tremendous advances in modern medicine no effective drugs are available, which stimulate liver functions and offers protection to the liver from damage or help to regenerate hepatic cells. On this basis, we took interest in studying effect of the leaves of Costus igneus on major organ like liver.

2. MATERIALS AND METHODS
2.1. Preparation of Ethanolic Extract
The leaves of Costus igneus were collected from Puttur, Karnataka, India during June 2009. They were dried in shade. The shade dried powdered leaves (5kg) were soaked in ethanol (95%) and kept aside for four days. After four days the ethanol layer was decanted off. The process was repeated for four times. The solvent of total extract was distilled off and concentrate was evaporated on a water bath to a syrupy consistency and evaporated to dryness.

2.2. Selection of animals
Wistar albino rats weighing between 180 – 200 g were selected for the acute toxicity and hepatoprotective. The animals were stabilized for 1 week and were given standard pellet diet and water ad-libitum throughout the course of the study.

3. Acute toxicity studies
The preliminary pharmacological studies were conducted to assess the acute pharmacological effects and LD50 of the ethanolic drug extract. The acute toxicity study was carried out in adult female albino rats by “up and down” method (OECD guidelines 425). The animals were fasted overnight and next day extracts of the plant Costus igneus (suspended in 0.6 % w/v sodium CMC) were administered orally at different dose level
(100mg/kg to 2000mg/kg). Then the animals were observed continuously for three hour for general behavioral, neurological, autonomic profiles and then every 30 min for next three hour and finally death after 24 hour.

4. Selection of doses
For the assessment of hepatoprotective activity, three dose levels were chosen in such a way that, middle dose was approximately one tenth of the maximum dose during acute toxicity studies, and a low dose, which was 50% of the one tenth dose, and a high dose, which was twice that of one tenth dose (100mg/kg, 200mg/kg 400mg/kg).

5. Statistical Analysis
The data were expressed as mean ± SEM. The data of hepatoprotective activity was analyzed by one way analysis of variance (ANOVA) followed by Dunnet’s ‘t’ test. A p value less than 0.05 was considered as statistically significant.

6. Procedure for acetaminophen induced hepatotoxicity model
The experiment was carried out as according to the procedure described by E. Porchezhian et. al. Wistar rats (180-200gm) of either sex, 7-8 weeks old were used; the rats were maintained at controlled temperature, 12 hrs light/12 hrs dark conditions for 1 week before the start of the experiment. Rats were divided into six groups of six animals in each group. Group I received saline (10ml/kg, i.p.) as normal control. Group II received Paracetamol, (3gm/kg,) orally in form of suspension with 0.6% C.M.C as treated control group on seventh day. Group III received silymarin suspended in 0.6% C.M.C (100mg/kg) orally as standard reference for seven days followed by acetaminophen administration on day seven. Group IV, V, VI received 100mg, 200mg, and 400mg/kg of ethanolic extract of Costus igneus suspended in 0.6% C.M.C for seven consecutive days followed by acetaminophen administration on day seven. The rats were sacrificed 48 hour after acetaminophen administration. They were anaesthetized by pentobarbital injection and blood was collected from vena cava, and serum was separated for assessment of different enzyme activities. The biochemical parameters studied were serum glutamate-pyruvate transaminase (ALT), glutamate oxaloacetate transaminase (AST), alkaline phosphate (ALP), and total bilirubin were estimated using assay kits according to the methods described by the manufacturers. The rats were sacrificed by bleeding and liver was preserved into 10% formalin solution for histopathological investigation. The results were statistically analyzed by using one way analysis of variance (ANOVA), followed by Dunnet’s ‘t’, test.

7. Discussion
Acute toxicity studies
The alcoholic extract of plant Costus igneus was found to be safe up to 2000 mg/kg body wt. by oral route. After 24 hr animals were found well tolerated. There was no mortality and no signs of toxicity. So three dose levels i.e. 100mg/kg, 200mg/kg, and 400mg/kg body weight were selected for the present study.

Evaluation of Hepatoprotective activity
The alcoholic extract of the leaves of Costus igneus was used for the experiment. Paracetamol was used in a dose of 300mg/kg orally to induce hepatic damage. The induction of liver damage was confirmed by elevated serum enzyme levels and histopathological picture showing zonal focal necrosis. Silymarin was used as reference standard in a dose of 100mg/kg. Administration of Costus igneus extract prior to Paracetamol administration effectively (P< 0.05) prevented the induction of damage by Paracetamol, which was confirmed by normal enzyme levels and lack of necrotic changes in the histopathological studies. The effect produced by 400mg/kg of Costus igneus is comparable with that produced by the standard drug silymarin. In histopathological studies paracetamol treated group showed severe inflammation with focal necrosis . Liver tissues of rats pretreated with Costus igneus showed almost normal hepatocytes. It is concluded that alcoholic extract of Costus igneus was able to reverse the hepatotoxicity caused by Paracetamol.

ACKNOWLEDGEMENT
The author is thankful to Nitte Gulabi Shetty Memorial Institute of Pharmaceutical Sciences for providing necessary facilities to carry out this study.
*significant (P<0.05) compared to control, ** significant (P<0.05) compared to PCML group

Fig. 1: Liver tissue: Control Group

Fig. 2: Liver tissue: Paracetamol

Fig. 3: Liver tissue: Silymarin Treated

Fig. 4: Liver tissue: (400mg/kg extract)
Effect of Costus igneus on serum SGOT levels in PCML treated rats

**PCML**

- Drug 100 + PCML
- Drug 200 + PCML
- Drug 400 + PCML

**S E R U M  S G O T  U /L**

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Effect of Costus igneus on ALP levels

**PCML**

- Silymarin + PCML
- Drug 100 + PCML
- Drug 200 + PCML
- Drug 400 + PCML

**S E R U M  A L P  U /L**

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Effect of Costus igneus on total bilirubin

**PCML**

- Silymarin + PCML
- Drug 100 + PCML
- Drug 200 + PCML
- Drug 400 + PCML

**Total bilirubin**

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