



Bio evaluation of the Effect of Costus speciosus Koen Extracts on Chemically Induced Jaundice in Albino Rats

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Abstract

The crude and purified extracts of *Costus speciosus* Koen were used to study the hepatoprotective/ hepatocurative activity in albino rats. Two doses of extracts viz. 100mg, 300mg of crude and 10mg, 30mg of purified extracts per kg body weight were given orally to albino rats for 7 days. On the 3rd and 6th days the animal, received CCl₄ @3ml/kg body weight and olive oil subcutaneously (50:50). Control group of animals were also maintained. On the 8th day of the experiment, blood samples were collected by direct puncture of the heart and the serum was extracted from the samples. The animals were then sacrificed by chloroform and the tissue samples (liver) were used for hepatoprotective studies. To assess the extent of hepatic damage and to monitor the degree of hepatoprotection of the extracts serum alkaline phosphatase, serum-γ-glutamyl transferase, serum glutamate pyruvate transaminase, total bilirubin were assayed. It was observed that the animals fed with the crude and purified extracts exhibited statistically highly significant hepatoprotective action in comparison to animals treated with only CCl₄ which resulted hepatic disorder.

Key words: *Costus speciosus*; Rhizome; Jaundice; Crude extract; Hepatoprotective; Albino rats; CCl₄

Introduction

Liver disease is a serious health problem. In the absence of reliable liver protective drugs in allopathic medical practice, herbs play a role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional system of medicine in India. However, we do not have satisfactory remedy for serious liver disorder; most of the herbal drugs speed up the natural healing process of liver. Recently interest in medicinal plants research increased all

over the world. It has been reported that medicinal plants, used in various traditional systems, have immune potential against various diseases [1]. More than 13,000 plants have been studied during last five years for various pharmacological properties. Indian medicinal plants belonging to about 40 families were investigated as liver protective drugs [2]. Plant drugs are widely used in the Northeast part of India for the cure of various diseases including jaundice and other liver disorders. The extract of rhizome of *Costus speciosus* Koen are widely used in N.E. region for the treatment of jaundice since long. Liver is the organ which is most susceptible to the toxic effects of carbon tetrachloride (CCl₄) due to overdoses. The present study is aimed to evaluate hepatoprotective/ hepatocurative effects of *Costus speciosus* Koen on CCl₄ induced liver damage in albino rats [3].

Materials and Methods

The rhizome of *Costus speciosus* Koen was collected by uprooting the plant from Jorhat district, Assam. The samples after cleaning were dried in shade, powdered in cutter mill and extracted with distilled methanol. The crude extract of *Costus speciosus* Koen was prepared by dissolving the cleaned, dried powder of rhizome in distilled methanol for 48 hours at room temperature. The purified extracts were prepared by loading 10g of dried crude extract in a column of silica gel 300g placed with Hexane and 400 ml of fraction-1(hexane), fraction-2(Ethyl acetate/hexane) and fraction-3(Chloroform) descended below were collected. These extracts were evaporated in rotary evaporator (Bibby, Sterilin) under reduced pressure. The viscous semi-liquid extracts obtained from rotary evaporator were dried in lyophilizer (Heto, LYO LAB3000) at -55°C. Methanol and CCl₄ were obtained from Ranbaxy Laboratories.

Sprague Dawley albino rats were procured locally and bred in Department Pharmacology and Toxicology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam. The animals were housed in clean polypropylene cages and given standard ration with ad-libitum supply of drinking water. Twenty four rats of either sex weighing between 130-160 gm were chosen and were divided into four groups containing six rats in each group [4].

The treatment protocol was planned to study the role of crude and purified extracts of *Costus speciosus* Koen in both preventive and curative aspects of CCl₄ induced hepatotoxicity.

Group I: (Vehicle control) Received tween 80 (20%) orally for seven days and on 3rd and 6th days received normal saline and olive oil subcutaneously.

Group II: (CCl₄ treated group) received tween 80 (20%) orally for seven days and on 3rd and 6th days received CCl₄ @3ml per kg body weight and olive oil subcutaneously.

Group III: Received crude extract @ 100mg and purified extract of *Costus speciosus* Koen @10mg per kg body weight along with tween 80 (20%) as vehicle orally for seven days and on 3rd and 6th days received CCl₄ @3ml per kg body weight and olive oil subcutaneously.

Group IV: Received crude extract @ 300mg and purified extract of *Costus speciosus* Koen @30mg per kg body weight along with tween 80 (20%) as vehicle orally for seven days and on 3rd and 6th days received CCl₄ @3ml per kg body weight and olive oil subcutaneously.

On the 8th day of the experiment blood samples were collected by direct puncture of the heart and serum was collected from the samples. The blood serum samples were collected for analysis of biochemical parameters. The activities of serum hepatic marker enzymes, namely serum alkaline phosphatase (SAP), serum- γ -glutamyl transferase(SGPT), serum glutamate pyruvate transaminase (SGOT), total bilirubin were assayed in serum using standard kits obtained from Ranbaxy Laboratories and PDP, Spawn. The results were expressed as international units/ liter (IU/L). The statistical analysis was carried out by one way Analysis of Variance (ANOVA).

Results

In the present study , a dose of 3ml of CCl₄ per kg body weight was used to induce liver damage in the rat as indicated by a significant ($P<0.05$, $P<0.01$, $P<0.001$) elevation of serum marker enzymes viz. SAP,SGPT,SGOT and a significant($P< 0.01$, $P<0.001$) reduction of serum total bilirubin, total protein(Table1-4) . The results are in agreement with the earlier reports. Histopathological changes has been seen which confirmed the hepatic damages. Compared to normal liver tissue, carbon tetrachloride (CCl₄) treatment showed extensive centrilobular necrotic patches extending to midzone with neutrophilic collection. Central to central bridging necrosis was seen, however the liver tissue of rats fed with the purified extracts of *Costus speciosus* Koen significantly improved the necrotic symptoms (Figures 1-3).

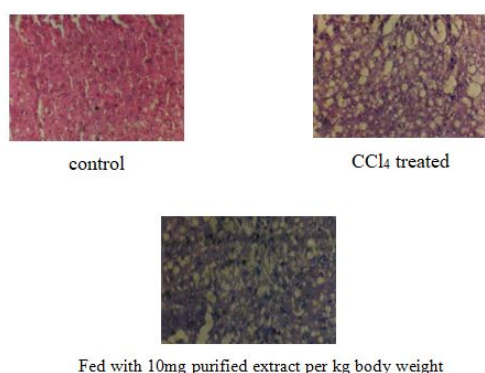
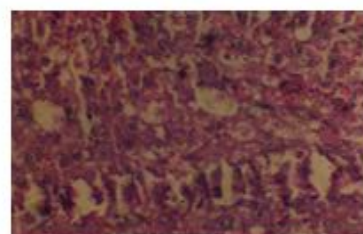


Figure1: Histopathological changes of the liver tissues of albino rats fed with purified extract of *Costus speciosus*, fraction-1 (Hexane) (Magnification: 40x2.5).



Fed with 10mg purified extract per kg body weight

Figure2: Histopathological changes of the liver tissues of albino rats fed with purified extract of *Costus speciosus*, fraction-2 (Ethyl Acetate+Hexane 1:7) (Magnification: 40x2.5).



Fed with 10mg purified extract per kg body weight

Figure3: Histopathological changes of the liver tissues of albino rats fed with purified extract of *Costus speciosus*, fraction-3 (Ethyl Acetate+Hexane 1:10) (Magnification: 40x2.5).

Both preventive and curative aspects of the liver were studied. Liver has got the capacity to regenerate. This regenerative capacity was assessed by administering 3ml CCl₄ per kg body weight orally for two days and with a vehicle for seven days. The results as shown by group II indicate that no regeneration had taken place. This group also acted as control for curative treatment. The extracts of *Costus speciosus* Koen exhibited an ability to counteract the CCl₄ induced changes in the biochemical parameters both in preventive and curative measures.

Group	SAP	SGOT	SGPT	Bilirubin
	K.A. units	U/ml	U/ml	g/dl
1. GroupI(Control)	25.77 $\pm$ 1.00	143.67 $\pm$ 7.40	69.55 $\pm$ 1.17	0.56 $\pm$ 0.06
2. GroupII(CCl ₄)	57.69 $\pm$ 0.69**	295.83 $\pm$ 4.15**	198.4 $\pm$ 5.86**	1.56 $\pm$ 0.12*
3.GroupIII(Preventive)	44.25 $\pm$ 0.57*	202.67 $\pm$ 4.94**	137.67 $\pm$ 4.64*	0.80 $\pm$ 0.12
4.GroupIV(Curative)	39.27 $\pm$ 0.37*	162.5 $\pm$ 3.40**	118.67 $\pm$ 3.09**	0.73 $\pm$ 0.03*

Table 1: Effect of crude extracts of *Costus speciosus* on biochemical parameters in albino rats.

*= $P<0.05$ and **= $P< 0.01$ SAP= Serum alkaline phosphatase, SGOT= Serum- γ -Glutamyl Transferase, SGPT= Serum Glutamate Pyruvate Transaminase

Group	SAP	SGOT	SGPT	Bilirubin	Total protein
	K.A units	U/ml	U/ml	g/dl	g/dl
1.GroupI(Control)	37.98±3.12.	43.61±4.14	34.26±3.41	0.42±0.04	7.31±0.41
2.GroupII(CCl4)	90.56±4.68**	290.13±22.74**	82.06±3.26**	1.35±0.08**	4.22±0.43**
3.GroupIII(Preventive)	74.42±3.16*	230.46±18.35	51.54±3.83**	0.93±0.02**	5.11±0.17
4.GroupIV(Curative)	76.34±2.94*	229.08±18.28	50.50±2.23**	0.85±0.04**	5.08±0.15

Table 2: Effect of purified fraction-1 (Hexane) of crude extracts of *Costus speciosus* on biochemical parameters in albino rats.

*=P<0.05 and **= P< 0.01

Group	SAP K.A units	SGOT U/ml	SGPT U/ml	Bilirubin g/dl	Total protein g/dl
1.GroupI(Control)	37.98±3.12.	43.61±4.14	34.26±3.41	0.42±0.04	7.31±0.41
2.GroupII(CCl4)	90.56±4.68**	290.13±22.74**	82.06±3.26**	1.35±0.08**	4.22±0.43**
3.GroupIII(Preventive)	58.18±4.33**	161.67±8.33**	42.0±7.34**	0.93±0.02**	5.15±0.09
1.GroupI(Control)	55.32±1.97**	212.33±8.84**	35.0±5.41**	0.85±0.04**	5.01±0.21

Table 3: Effect of the purified fraction-2 (Ethyl Acetate/Hexane, 1:10) of crude extracts of *Costus speciosus* on biochemical parameters in albino rats.

Group	SAP K.A units	SGOT U/ml	SGPT U/ml	Bilirubin g/dl	Total protein g/dl
1.GroupI(Control)	37.98±3.12.	43.61±4.14	34.26±3.41	0.42±0.04	7.31±0.41
2.GroupII(CCl4)	90.56±4.68**	290.13±22.74**	82.06±3.26**	1.35±0.08**	4.22±0.43**
3.GroupIII(Preventive)	74.80±4.07*	232.33±6.58	39.67±6.81**	0.83±0.05**	4.42±0.13
1.GroupI(Control)	71.61±4.67*	248.00±2.58**	38.33±6.23**	0.86±0.03**	4.68±0.10

Table 4: Effect of the purified fraction-3(Ethyl Acetate/Hexane: 1:7) of crude extracts of *Costus speciosus* on biochemical parameters in albino rats.

Discussion

Significant hepatoprotective efficacy has been reported earlier in various plants. Liver cells participate in a variety of metabolic activities and thus contain a host of enzymes. In severe acute liver damage serum transaminases level parallel to those of organs indicating that both cellular and mitochondrial membranes have been damaged. It is reported that large doses of CCl4 results in cell lysis and cytoplasmic hepatic enzymes are released into blood circulation. Many fold increase of enzyme leakage as demonstrated by increased level serum enzymes ALT and AST has been noted indicating liver cell damage by CCl4. It has also been supported by other workers [6]. Since membrane integrity is linked with intracellular metabolic states, disturbance in later results in membrane lesion with concomitant increase in enzyme leakage giving rise to hypoxia and membrane hypermeability.

The extracts of *C.speciosus* Koen is able to prevent hepatic injury/liver necrosis in preventive group and enhanced regeneration in curative groups. This was evidenced by lowering the activity of Serum marker enzymes viz.Serum alkaline phosphatase, Serum-γ-Glutamyl Transferase, Serum Glutamate Pyruvate Transaminase and a

significant reduction of serum total bilirubin. Elevation of activities of these enzymes in serum indicated membrane damage [14]. SAP and SGOT are better index of liver injury [7]. More than 50% reduction of activities of these enzymes indicated recovery of hepatocytes in spite of insult due to hepatotoxin by CCl4. Hepatotoxicity might be due to lipid peroxidation, depletion of glutathione/cytochrome P-450, an altered immunological system induced by various chemical agents or direct damage to the cell [8] .

It can be concluded that the significant antihepatotoxic activity shown by extracts of *C. speciosus* Koen may due to its inhibitory effect on serum enzymes or lipid peroxidation. The plant extract may interfere with cytochrome P-450 and ultimately hinder the formation of hepatotoxic CCl4 free radical and exert its hepatoprotective action. The extract may also have antioxidant property which inhibited the deleterious effect of free radicals generated by CCl4 influencing the membrane rigidity by prevention or inhibition of membrane peroxidation [18]. Thus present study indicated that the *C. speciosus* Koen protect the liver damage against CCl4 induced hepatotoxicity.

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