EVALUATION OF HEPATOPROTACTIVE ACTIVITY OF RHIZOMES OF *COSTUS SPECIOSUS* (J. KONIG) SMITH

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Summary

The methanolic extract of the rhizomes of *Costus speciosus* was evaluated for hepatoprotactive activity by observing its effects on carbon tetrachloride (CCl_4) induced hepatotoxicity in liver histoarchitecture and alteration in certain biochemical parameters. The biochemical parameters studied were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin and total protein. The crude extract was administered through the intraperitonial route in two dose groups, a lower dose group receiving 50mg/kg body weight/day and a higher dose group receiving 100mg/kg body weight/day. The crude extract administration led to reversal of the altered biochemical parameters in the group receiving the higher dose. Also, significant alterations of CCl_4-induced changes in the histoarchitecture of the liver cells were observed in the same. The study was carried out using Swiss albino mice of either sex, the weight of which ranged from 20-35 gm. The results were compared with Silymarin, which was used as a standard, confirmed the presence of hepatoprotactive activity in the methanolic extract of the rhizomes of the said plant.

Key words: *Costus speciosus,* Hepatoprotactive activity, Carbon tetrachloride, Silymarin, Hepatotoxicity.

Introduction

Costus speciosus (J. Konig) Smith, (Zingiberaceae) an erect plant, up to 2.7 meters high; root stock tuberous; stem sub-woody at the base occurring in the moist and wet evergreen areas of the Indo-Malayan region and Sri Lanka. Within India it occurs from Central and Eastern Himalayas to Southern India. Rhizomes have anti-fertility, anabolic properties. Traditionally, it is indicated in the treatment of cough, fever, skin diseases, snake bite, anemia and inflammation. The juice of fresh tips of young branches is instilled in case of otitis[(1)]. It is used mixing with sugarcane juice along with other herbs to cure jaundice[(2)]. It is also used in arthritis and applied as a paste [(3)]. Tigogenin and diosgenin from rhizomes and stems have been isolated. Also, α-amyrin stearate, β-amyrin and lupeol have been isolated from its rhizomes. Isolation of palmitates has been reported from leaves. The seed fat contains palmitic acid, stearic acid, oleic acid, linoleic acid, arachidic acid, gadoleic acid, and behenic acid. Two new quinines dihydrophytilplastoquinone and its methyl derivatives along with α-tocopherolquinone have been isolated from seeds. It is also reported to isolate diosgenone, prosapogenin B of dioscin, cycloartanol, 25-en-cycloartenol and octacosanoic acid along with diosgenin from the rhizome [(4,5)]. As in India jaundice and other related liver diseases are rampant; hence it seemed interesting to see if *Costus speciosus* alone offers any hepatoprotection. The studies were undertaken to see if pre-treatment of methanolic extract of *C. speciosus* exerted any protective effect on mice exposed to carbon tetrachloride (CCl_4).

Methods

The rhizomes of *Costus speciosus* has been collected from places in and around Dibrugarh University, Assam, India during the month of October-November and identified as *Costus speciosus* (J. Konig) Smith, vide letter no. BSI/EC/Tech./2007/581 by Dr. T. M. Hynniewta of Botanical Survey of India, Eastern Circle, Shillong. A voucher specimen herbarium of the said plant has been deposited in the departmental museum of Department of Pharmaceutical Sciences, Dibrugarh University. The rhizomes were first washed thoroughly under running water to remove traces of soil particles adhered to it. Then it was subjected to shed drying for about three to four weeks to remove the resident moisture. After this the rhizomes were cut to small pieces, coarsely powdered and about 20 gm. were packed in Soxhlet apparatus and extracted with methanol for 12 hours. The extract so obtained was filtered and concentrated at low temperature using rotary vacuum evaporator and finally dried in a vacuum desiccator. The yield of the methanolic extract was used as a fine suspension in 30% Tween 80 for experimentation.

Swiss albino mice of either sex weighing between 20-35 gm obtained from the departmental animal house were used in the experimentation with due approval from the University ethical committee. They were fed on standard laboratory diet with water *ad libitum* and housed in plastic cage at room temperature and were exposed to natural day and night cycles.

The animals were divided into five groups, each groups contain six animals.

1. Group 1- it served as the control and received the vehicle (30% tween 80 in distilled water) at a dose of 0.1ml/100g body weight/day/intraperitonially (i.p.) for 14 days.[(6)]

2. Group 2 – it served as the toxicant group, and received carbon tetrachloride (CCl_4) prepared in the vehicle, at a dose of 0.1ml/100g body weight/ twice a week/i.p. The second dose of the toxicant was given 36hrs. after administering the first dose.[6,7]
3. Group 3 – it served as test group with lower dose and received the methanolic extract at a dose of 50mg/1000g body weight/day/i.p. for 7 days. 24 hrs after the final extract dose the animals were intoxicated with CCl_4 (0.1ml/100g body weight/twice a week/i.p.) and 36-48 hours after the second CCl_4 injection the animals were sacrificed. Liver was dissected out weighed and preserved in Bouin's solution.[6,7,8]
4. Group 4 – it served as test group with higher dose and received the direct methanolic extract at a dose of 100mg/1000g body weight/day/i.p. for 7 days. 24 hrs after the final extract dose the animals were intoxicated with CCl_4 (0.1ml/100g bdoy weight/twice a week/i.p.) and 36-48 hours after the second CCl_4 injection the animals were sacrificed. Liver was dissected out and preserved in Bouin's solution.[6,7,8]
5. Group 5 – it served as the standard group and received Silymarin at a dose of 100mg/1000g body weight/day for 14 days/i.p.[9]
6. Group 6 – it served as the standard control group, received silymarin at a dose of 100mg/1000g body weight/day for 7 days/i.p followed by toxicant, CCl_4 (0.1ml/100g body weight/twice a week/i.p.) [9] .

Blood was collected by heart puncture method and allowed to stand for 20min, and then centrifuged for 15-20 minutes at 2000 rpm to separate the serum and the latter was used for biochemical estimations of parameters like Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), Bilirubin and Total protein.

All the above biochemical parameters were assayed using standard laboratory kit from Crest Biosystems; namely: Alkaline Phosphatase Kit, Bilirubin Kit, Total Protein Kit, SGOT (ASAT) Kit and SGPT (ALAT) Kit.

Statistical analysis of the data obtained was carried out by employing Student's 't' test for unpaired data using Origin 6.1, a statistical analysis software, at $P<0.05$.

Liver samples of each of the animals were dissected out and kept in Bouin's solution and preserved at a temperature of 2-3^{0}C in a refrigerator. For the performance of histopathological examination samples were prepared using Rapid process: liver samples were first cleared by washing with acetone and allowed to stand in acetone for 30 minutes followed by three washings with acetone. The samples were dehydrated by keeping them in benzene for half hour. Three such washings of each sample were done. Then paraffin was melted and liver samples were embedded into it and cut into thin ribbon using rotary microtome and strips placed in glass slides. These slides were then incubated overnight at a temperature of 37^{0}C. Then the slides were melted and washed with xylene, then with absolute alcohol, 90% and 70% alcohol for 3-5min. and finally with water for 20min. Then the slides were stained with haematoxylin and allowed to stand for 3-5 min and excess stain washed off with water. The slides were then treated with acid-alcohol (1% HCl in absolute alcohol) and washed with water and treated with eosin, after which it was fixed and observed under microscope.

Results

Serum Parameters:

It can be noted from Table-1 that the levels of all the enzyme parameters of serum i.e. AST, ALT, ALP, total protein and bilirubin were found to be significantly increased in the CCl_4 treated mice group (TX). Treatment with lower dose of methanolic extract of rhizomes of *C. speciosus* showed decreased activities of serum transaminase along with total protein and bilirubin levels.

However, the significantly increased activities of serum ALT, AST in CCl_4 treated mice groups were predominantly reversed by the higher dose of methanolic extract of rhizomes of *C. speciosus* receiving group (TB) the effects of which is comparable with silymarin; which was used as standard.

At $P<0.05$ levels it showed maximum recoupment in serum enzyme activities and bilirubin and protein levels with extract treatment at a higher dose of 100mg/kg in the current study.

Table-1. Analysis of Different Serum Parameters with respect to Each Group

Treatment	AST[f] (U/ml)	ALT[g] (U/ml)	ALP[h] (K.A. unit)	Total Protein (g/dl)	Bilirubin (mg/dl)
Vehicle[a] (control)	60.833±10.963[i]	32.42±5.348	1.177±0.209	5.144±0.864	5.133±0.401
TA[b]+CCl_4[c]	96.375±17.775	61.875±11.786	2.552±0.368	8.471±0.5	8.909±0.788
TB[d]+CCl_4	65.958±5.06	36.875±3.734	1.785±0.076	6.272±0.514	5.006±0.415
Silymarin[e] (standard)	58.515±4.827	28.333±5.095	1.44±0.091	7.972±0.765	5.631±0.732
Silymarin+CCl_4	65.583±10.831	43.583±3.131	0.719±0.283	7.174±0.645	6.477±2.092
CCl_4 (toxicant)	178.333±6.621	144.583±9.524	5.055±0.315	14.026±1.138	15.666±2.028

Number of animals per group (n) = 6

[a] Vehicle, 3% tween 80; dose 0.1ml/100g/day/i.p. for 14 days

[b] TA, test drug lower dose, methanol direct extract; dose 50mg/kg/day/i.p. for 7days, 24hrs after the final dose the animals were intoxicated with CCl_4 (0.1ml/100g/twice a week/i.p.) till 14th day; 36-48hrs after the 2nd CCl_4 administration the animals were sacrificed.

[c] CCl_4 , toxicant, carbon tetrachloride; dose 0.1ml/100g/twice a week/i.p. till 14th day; 36-48hrs after the 2nd CCl_4 administration the animals were sacrificed.

[d] TB, test drug higher dose, methanol direct extract; dose 100mg/kg/day/i.p. for 7days, 24hrs after the final dose the animals were intoxicated with CCl_4 (0.1ml/100g/twice a week/i.p.) till 14th day; 36-48hrs after the 2nd CCl_4 administration the animals were sacrificed.

[e] Silymarin, dose 100mg/kg/day/i.p. for 14 days;

[f] AST , Aspartate amino transferase

[g] ALT, Alanine amino transferase

[h] ALP, Alkaline phosphatase

[i] Values represent the mean ± standard error of six animals in each group

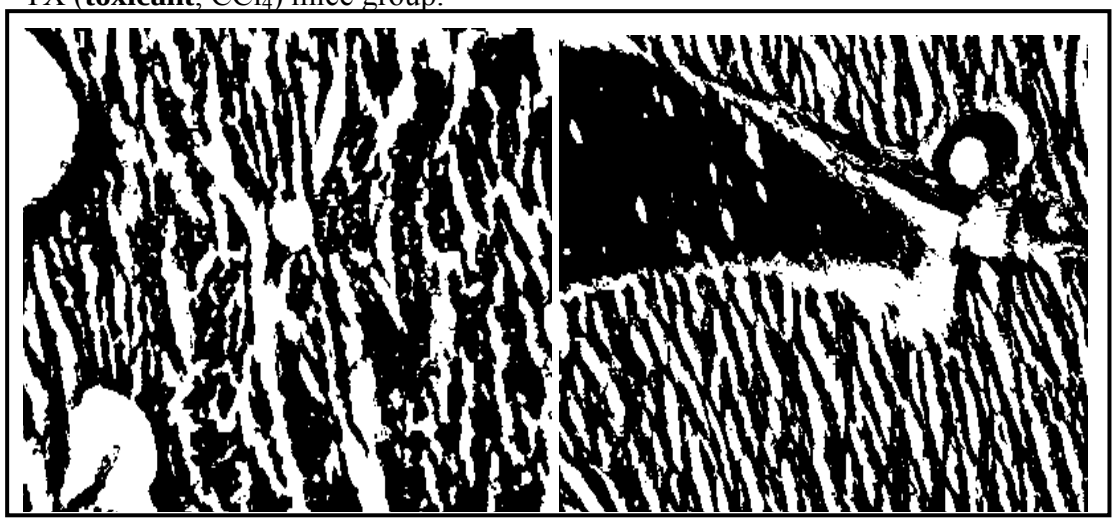
Histopathological changes:

Liver of mice treated with CCl_4 showed extensive signs of degeneration. Vacuolization of hepatocytes were very common, peripheral fibrosis, centrilobular necrosis and fatty degenerations were also observed (figure1).

Administration of extract at dose of 100mg/kg showed nearly well maintained histoarchitecture (figure-2). There were no perinuclear vacuolation in the hepatocytes and were comparable to silymarin standard (figure-3). Nuclei of hepatocytes were also well maintained.

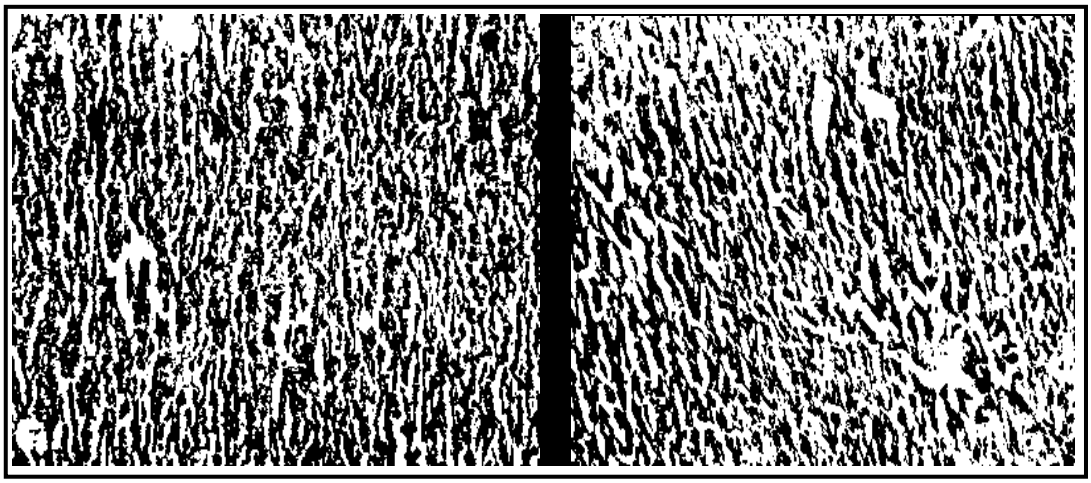
The lower dose group getting 50mg/kg dose also showed less hepatocellular damage, in some areas localized fatty changes along with diffused cloudy swellings are seen (figure 4).

Figure-1 Photomicrograph (20x, Leica photomicroscope-DM 1000) of section of liver from TX (**toxicant**, CCl_4) mice group.



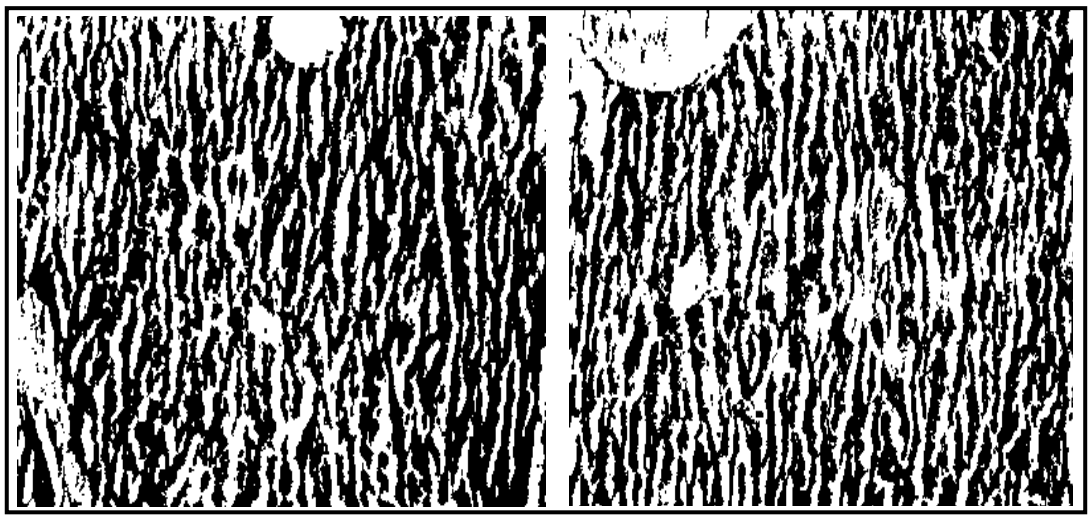
Periportal fibrosis, acute inflammatory change, centrilobular necrosis, vacuolization of cells is observed.

Figure-2. Photomicrograph (10x, Leica photomicroscope-DM 1000) of section of liver from **TB** (test group receiving higher dose) mice group.



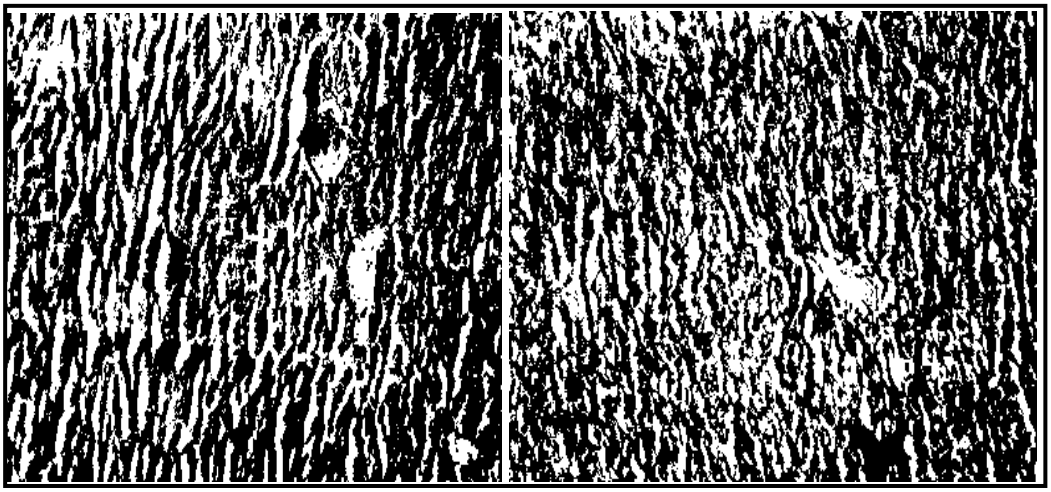
Liver cells mostly normal with localized areas of sub capsular inflammation are seen.

Figure-3. Photomicrograph (10x, Leica photomicroscope-DM 1000) of section of liver from **SC** (Silymarin + CCl_4) mice group



Mostly normal hepatocytes are seen with very less fatty change along with areas of regeneration.

Figure-4. Photomicrograph (10x, Leica photomicroscope-DM 1000) of section of liver from **TA** (lower dose group) mice group



No significant hepatocellular damage, localized fatty changes seen in some areas along with diffused cloudy swelling.

Discussion

Raised activity of serum transaminases in intoxicated mice as found in the present study can be attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and are released into the circulation after cellular damage [10].

Many fold increase of enzyme leakage as demonstrated by an increased level of serum enzymes ALT, AST and ALP was noted indicating liver damage by CCl_4 [11]. The methanolic extract of the rhizomes of *Costus speciosus* has notably prevented the leakage of these enzymes and restoring the activity of enzymatic variables.

The response to silymarin and methanolic extract of the rhizomes of *Costus speciosus* is comparable in most parameters and the differences observed are largely quantitative. Furthermore, the reversal in activities of hepatic enzymes like ALT was higher in higher dose receiving group than with silymarin. The increased levels of other parameters in serum were significantly reversed by silymarin and the effects were comparable with the group receiving the higher dose i.e. 100mg/kg body weight of the ethanolic extract of the rhizomes of *Costus speciosus*.

Histopathological studies demonstrated degenerative lesion, vacuolation, periportal fibrosis, fatty degeneration, sub-capsular inflammation in the hepatocytes induced by CCl_4. These findings were further supported by earlier reports [12, 13] showing degeneration in hepatocytes and hepatic chords. Focal and periportal area degeneration, localized acute inflammatory changes, cloudy swelling were seen. Significant normalization in the histoarchitecture was seen with the higher dose administration at 100mg/kg, however close to normal with periportal area degeneration along with localized cloudy swelling was observed, in lower dose mice group receiving dose at 50mg/kg. The observations were based upon comparison with the standard drug i.e. silymarin.

In summery it may be concluded that, both the doses of test drug selected for the study aids in reversal of hepatic enzymes in case of CCl_4 induced hepatotoxicity, thereby offering hepatoprotection. However it is noteworthy that the higher dose of test drug extract offers more significant reversal effects which are comparable with that of silymarin in most cases.

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