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Hepatoprotective Effect of Costus Roots Extract against Carbon Tetrachloride (CCl_4)-Induced Liver Injury in Guinea Pigs

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Abstract: SC (*Saussurea costus*) (Asteraceae) is a traditionally known and potent plant which is well considered for its medicinal uses in different indigenous Indian systems of medicine. This study was undertaken to investigate the hepatoprotective effect of SC against liver injury induced by CCl_4 intoxication. Animals were divided into five groups. Group I, served as normal control. Group II received 2 mL CCl_4/kg b.w. diluted with olive oil, at 1:1 ratio on day 11. Groups III and IV were pre-treated orally respectively with 100 and 200 mg/kg b.w. aqueous roots extract of SC for 10 days followed by subcutaneous injection of CCl_4 (2 mL/kg b.w.), once on day 11. Group V were orally given Liv-52 (100 mg/kg b.w.) once daily for 10 days followed by subcutaneous injection of CCl_4 (2 mL/kg b.w.), once on day 11. Our results show that, the activity of serum hepatic enzymes (ALT, AST, and ALP) were significantly elevated in guinea pigs treated with CCl_4, while both the SC root extract and Liv-52 reduced significantly these enzymes activity. Also, the levels of glucose, urea and cholesterol were decreased when compared with intoxicated control. Histopathological examination of intoxicated animals showed dilation, hemorrhage, vacuolization, inflammation and necrosis indicating liver damage, while the animals received SC or Liv-52 that showed less pathological effects or normal liver when compared to animals treated with CCl_4 alone. Biochemical and histological results confirm the hepatoprotective effect of aqueous extract of SC.

Key words: *Saussurea costus*, CCl_4, hepatotoxicity, extract, Liv-52, histopathology, biochemistry.

1. Introduction

The search for new drugs and formulations that are affordable, safe, and effective against all stages of the disease is highly recommended. Plant-derived drugs have recently become of great interest owing to their versatile applications. They constitute a considerable fraction of total medicines worldwide [1, 2]. Approximately one third of adults in the Western world use alternative therapies, including herbs. Herbal medicine is as old as the history of humankind [3]. Plant extracts often contain complex mixture of many different compounds with distinct polarity, antioxidant and pro-oxidant properties [4]. In contrast to chemical drugs, herbs have sometimes been claimed to be non-toxic, because of their natural origin and long-term use as folk medicines [5]. Indeed, the effectiveness of some medicinal herbs in the treatment of disease has been validated through research and clinical studies [6, 7]. In Yemen which is one of the poorest Arabic countries in the region, liver diseases are hyper-endemic [8, 9] and the majority of the liver disease patients use plant and/or plant derived extracts, due to poverty and difficulties of accessing modern medicine.

SC (*Saussurea costus*) belonging to the family Asteraceae, is a perennial, aromatic, and medicinal plant growing in the Himalayan region between 2,500 to 3,500 m altitude [10]. It is commonly known as Costus in English and has different vernacular names

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in India like, Kuth, Kur, Kot, Kushta, etc. [11]. Dried roots of SC are commonly used in folk medicine for the treatment of various ailments and diseases such as asthma, dyspepsia, certain bronchitis, ulcer, rheumatism, cough, throat infections, tuberculosis, stomach problems and many other diseases since ancient times [12, 13]. Terpenes, anthraquinones, alkaloids and flavonoids are the main active constituents of the plant [14]. Many of these active constituents have been found to have biological activities. For example, costunolide and dehydrocostus lactone, are major components of the roots. Recently, these compounds were found to possess various biological activities such as antifungal [15], anthelmintic [16], antidiabetic, antitumor [17] antimicrobial [18], immuno-stimulant [19], antiulcer [20], anti-inflammatory [21] and antihepatotoxic [22]. Different extracts of this plant have been found to exhibit anti-ulcer, anti-inflammatory, hepatoprotective, anticancer, immunomodulatory and pesticidal activities [10]. It was reported that both costunolide and dehydrocostus lactone suppressed the HBsAg production by Hep3B cells in a dose dependent manner [10]. The *in vitro* antiviral activity of SC root against hepatitis B virus had been reported [23]. There is, however, no reported *in vivo* activity related to liver damage in guinea pigs. So, the aim of the present study was to study the hepatoprotective effects of the aqueous roots extract of SC against CCl_4-induced acute liver injury in guinea pigs.

2. Materials and Methods

2.1 Plant Materials and Preparation of Plant Extract

Dried roots of SC were purchased from a local herbalist market in Ibb city, Yemen. The ethanol extract was prepared according to Hosbas et al.. [24] The dried roots of SC were ground by using a blender. And 50 g were taken and macerated with 70% ethanol by continuous stirring at room temperature. The plant extract was filtered by using Whatman filter paper No.1 and evaporated to dryness under reduced pressure. The aqueous extract was prepared according to Saleem et al. [25]. Plant material was cleaned, crushed and 1 kg was the powdered material that was boiled with 5 L of distilled water for 30 min and filtered through filter paper and concentrated into thick semi solid paste under reduced pressure on a rotary evaporator to obtain the aqueous extract. The extract was concentrated under reduced pressure and lyophilized. The freeze-dried material was weighed (about 30 g). The extract was soluble in normal saline and distilled water.

2.2 Phytochemical Analysis of Ethanol and Aqueous Extract

Preliminary screening of secondary metabolites such as glycosides, flavonoids, alkaloids, terpenoids, and tannins were carried out according to the common phytochemical methods described by Trease & Evans (1983) and Harborne (1973) [26, 27].

2.3 Chemicals

All the drugs and chemicals used in the study were of analytical grade. Carbon tetrachloride (CCl_4) was obtained from Merck Limited, India. Liv-52 was obtained from Himalaya Drug Company, Bangalore, India.

2.4 Experimental Animals

Experiments were conducted using adult guinea pigs (male 350-700 g), obtained from the animal house of Biology Department, Faculty of Science, Ibb University, Yemen. The animals were kept for 1 week on a commercial diet in environmentally controlled conditions (25 ± 5 °C, 55 ± 5% humidity and 12-h light-dark cycle) with free access to diet and clean drinking water ad libitum. The study received ethical approval from the University Ethics Committee of Ibb University.

2.5 Experimental Protocol

The experimental animals were allocated into five groups, each containing five animals. In group I,

(control) animals were received vehicle (olive oil) alone. In group II, animals received subcutaneous injection of 2 mL CCl_4/kg body weight (b.w.) diluted with olive oil, at 1:1 ratio on day 11. In group III, animals were orally pre-treated with 100 mg/kg b.w. of aqueous roots extract of SC for 10 days followed by subcutaneous injection of CCl_4 (2 mL/kg b.w.) on day 11. In group IV, animals were orally pre-treated with 200 mg/kg b.w. of aqueous roots extract of SC for 10 days followed by subcutaneous injection of CCl_4 (2 mL/kg b.w.) on day 11. In group V, animals were orally pre-treated with 100 mg/kg b.w. of Liv-52 for 10 days followed by subcutaneous injection of CCl_4 on day 11.

2.6 Preparation of Samples

Twenty-four hours after the administration of CCl_4, the animals of each group were anaesthetized with ether, and blood was collected directly from the portal vein. The blood sample of each animal was separated to obtain serum for biochemical analysis.

2.7 Biochemical Studies

Serum AST (aspartate aminotransferase), ALT (alanine aminotransferase) and ALP (alkaline phosphatase) were determined kinetically using Spinreact Diagnostics kits (Spain). Glucose, urea, and cholesterol were evaluated colorimetrically in blood using spectrophotometric diagnostic kits of Spinreact (Spain).

2.8 Histopathological Examination

Control and experimental animals were put under light ether anaesthesia, dissected as quickly as possible, and then livers were removed. Small pieces were fixed in 10% neutral formalin for 24 hours, then washed by the running tap water, and stored in 70% ethyl alcohol, until further processing. Blocks of about 5×5 mm size were dehydrated, cleared and embedded in paraffin wax. Paraffin sections of 5 microns thickness were cut using rotary microtome (Leica, Germany) and sections were flattened on the surface of warm water using a water bath. Slides were then dried on a hot plate for 30 minutes and stored in the incubator at 37 ºC and stained with Ehrlich's haematoxylin and counter-stained with eosin. Then mounted in Canada balsam, labeled and became ready for microscopic examination.

2.9 Statistical Analysis

Our data were analyzed using IBM SPSS statistics 20 software and the results of the biochemical estimations were reported as mean ± standard deviation S.D. Total variation, present in a set of data was estimated by one-way analysis of variance (ANOVA). Differences with a p-value < 0.05 were considered as statistically significant.

3. Results

3.1 Results of Phytochemical Analysis

The phytochemistry screening of the aqueous and ethanolic extract of *Saussurea costus* showed the presence of glycosides, flavonoids, alkaloids, tannins, triterpenoids and anthraquinones (Table 1).

3.2 Biochemical Results

Table 2 shows that, CCl_4 intoxication causes a significant (p < 0.05) elevation of AST, ALT, and ALP by 2.45, 1.94 and 1.72 times respectively when compared to control animals. On one hand, the pretreatment of animals with 200 mg/kg b.w. of SC aqueous roots extract for ten days, significantly (p < 0.05) decreases the levels of AST, ALT, and ALP by 1.58, 1.51 and 1.42 times respectively compared to CCl_4 group alone. The pretreatment of animals with 100 mg/kg b.w. of SC aqueous roots extract also was insignificantly decreases AST, ALT, and ALP by 1.27, 1.26 and 1.23 times respectively compared to CCl_4 group animals (Table 2). Similar results were obtained with Liv-52 + CCl_4 group. Table 2 shows that, there was significant (p < 0.05) decrease of AST, ALT, and ALP in Liv-52 + CCl_4 group by 1.84, 1.57 and 1.44

Table 1 Phytochemical analysis of aqueous and ethanol extract of *Saussurea costus.*

TEST	RESULTS	
	Aqueous extract	Ethanol extract
Glycosides	+	+
Flavonoids	+	+
Alkaloids	+	+
Tannins	+	+
Terpenoids	-	+

(+) Present and (-) Absent.

Table 2 Effect of aqueous roots extract of SC on the activity of plasma liver enzymes (AST, ALT, ALP) in guinea pigs treated with CCl_4.

Analyte (U/L)	Groups				
	Control	CCL_4	100SC + CCL_4	200SC + CCL_4	Liv-52 + CCL_4
AST	42.4 ± 6.11	104.01 ± 13.84*	81.60 ± 9.76	65.80 ± 9.25[b]	56.6 ± 8.38[b]
ALT	52.20 ± 5.40	101.1 ± 10.77*	80.20 ± 10.2	67.1 ± 6.48[b]	64.2 ± 6.53[b]
ALP	53.4 ± 8.08	91.8 ± 11.73*	74.6 ± 7.92	64.6 ± 7.86 [b]	63.6 ± 6.80[b]

Each value represents the mean ± S.D., n = 5. Values marked with asterisks (*) differ significantly from control value: $p < 0.05$, those marked with letter (b) differ significantly from CCl_4 group: $p < 0.05$.

Table 3 Effect of roots extract of SC on plasma glucose, urea and cholesterol in guinea pigs treated with CCl_4.

Analyte (mmol/L)	Groups				
	Control	CCL_4	100SC + CCL_4	200SC + CCL_4	Liv-52 + CCL_4
Glucose	6.16 ± 0.62	9.12 ± 0.64*	7.44 ± 0.69	6.57 ± 0.68 [b]	5.91 ± 0.53[b]
Urea	8.93 ± 1.73	15.17 ± 1.32*	12.41 ± 1.63	10.07 ± 1.74[b]	9.85 ± 1.42 [b]
Cholesterol	0.50 ± 0.10	1.16 ± 0.16*	0.87 ± 0.11	0.76 ± 0.14 [b]	0.70 ± 0.12[b]

Each value represents the mean ± S.D., n = 5. Values marked with asterisks (*) differ significantly from control value: $p < 0.05$, those marked with letter (b) differ significantly from CCl_4 group: $p < 0.05$.

times respectively compared with CCl_4 group. On the other hand, the treatment of animals with CCl_4 alone induced a significant increase ($p < 0.05$) in glucose, urea, and cholesterol by 1.48, 1.70 and 2.32 times respectively compared to control animals (Table 3). In addition, orally pretreatment of animals with 200 mg/kg SC or Liv-52 reduces significantly ($p < 0.05$) the levels of glucose, urea, and cholesterol by (1.39 & 1.54), (1.51 & 1.54) and (1.53 & 1.66) times respectively in both groups with respect to CCl_4 group alone. The levels of glucose, urea, and cholesterol also were decreased in 100 mg/kg SC group compared to CCl_4 group Table 3, but this decrease was insignificant ($p > 0.05$).

3.3 Histopathological Results

Histopathological results of livers of guinea pigs intoxicated with CCl_4 in presence or absence of SC roots extract were depicted in Fig. 1. Microphotograph (A) showed normal hepatic lobular architecture with central veins and radiating hepatics cords and central rounded nuclei. Blood sinusoidal space appeared between the liver cords were normal. However, animals treated with CCl_4 showed various pathological alterations in liver of guinea pigs. These alterations were characterized by dilation of central vein with marked hemorrhage in some sections. Moreover, cytoplasmic vacuolization, dilation of blood sinusoids, and necrosis of hepatocytes were observed in the liver sections of CCl_4 group when compared to control group (Microphotograph B). On the other hand, liver of guinea pigs pretreated with 100 mg/kg costus extract plus CCl_4 showed marked vacuolar degeneration in the hepatocytes especially in the regions surrounding the

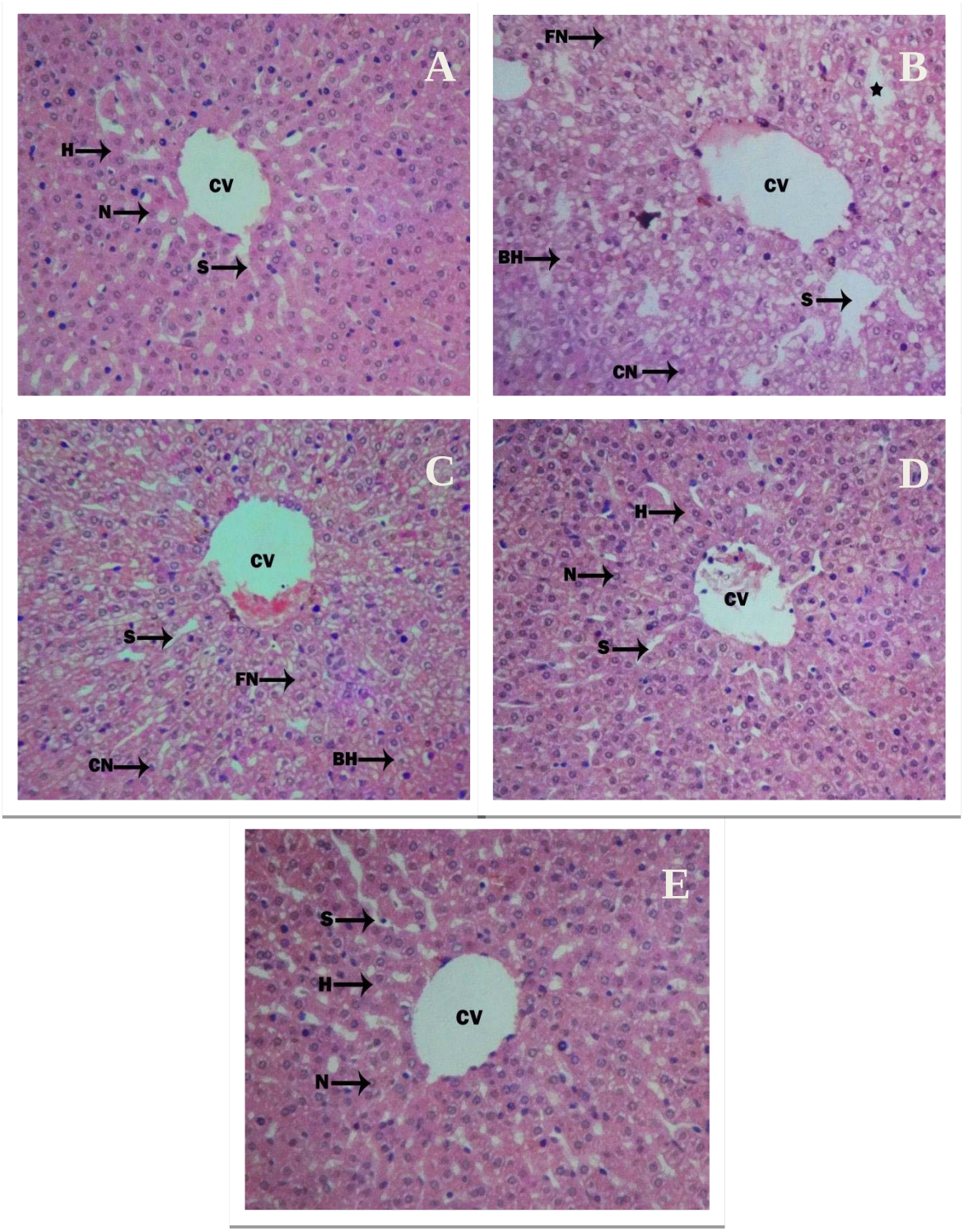

Fig. 1 Representative microphotograph (40× magnification, H&E staining).(A) Control group, showed normal polyhedral hepatocyte (H) and sinusoidal spaces (S). (B) Toxin group, showed disturbed architecture, dilated central vein (CV), marked vacuolated or BH (ballooning hepatocyte), loss of cell adhesion and many apoptotic cells (star) with condensed (CN) or FN (fragmented nucleus). (C, D) Treatment groups, showed improved architecture, less marked cellular swelling and apoptosis with increasing dose of treatment when compared to control and Liv-52 group (E).

central vein associated with congestion and hemorrhage (Microphotograph C). While, the liver tissue of guinea pigs pretreated with 200 mg/kg costus extract or 100 mg/kg Liv-52 relieve the histopathological alterations induced by CCl_4 alone (Microphotograph D & E). However, little vacuolar degeneration was detected in the hepatocytes surrounding the dilated central vein and some ballooning hepatocytes with condensed and fragmented nuclei in 200 mg/kg costus extract (Microphotograph D). Whereas, the Liv-52 treated group showed little or no histopathological alterations (Microphotograph E).

4. Discussions

In Yemen like other developing countries, the knowledge of plant-derived antioxidant for disease management could reduce the cost of health care. The antioxidants are used to protect us from the oxidative stress as a result of exposure to pollutant that enhanced the production of ROS (reactive oxygen species). The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, or externally supplied through foods and/or supplements [28]. The liver is major organ responsible for the metabolism of drugs and toxic chemicals and therefore is the primary target organ for nearly all toxic chemicals [29, 30]. CCl_4 is a frequently used model substance for hepatotoxicity studies [31]. It has been well established that CCl_4 is metabolized in the liver to the highly reactive trichloromethyl radical and this free radical leads to auto-oxidation of the fatty acids present in the cytoplasmic membrane phospholipids and causes functional and morphological changes in the cell membrane [32]. The results of the present study show that, CCl_4 intoxication causes a significant elevation of liver enzymes when compared to control animals. This elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage [33].

These results were in consistence with previous investigations [34, 35]. The rise in the enzyme AST is usually accompanied by an elevation in the levels of ALT, which plays a vital role in the conversion of amino acids to ketoacids [36].

Natural antioxidants play a major role in reducing the oxidative stress by scavenging the excess free radicals [37]. *Saussurea costus* is considered to be one of the antioxidant-rich medicinal plants. Many authors have reported that the roots of this plant possess various biological activities, including cortisol lowering effect [38], anti-infammatory [39, 40], anticancer [41, 42], antiviral [23], antimicrobial [43, 44], antifungal [15], antioxidant [45, 46], antidiabetic [47], antiulcer [20] and hepatoprotective effects [38]. Liv-52 is widely used in treatment of liver diseases of varying origins. It enhances tocopherol levels, which inhibits lipid peroxidation; scavenges free radicals [48]. In the present study, Liv-52 caused a significant decrease in serum enzymes activity induced by CCl_4 in guinea pigs. These results were in agreement with previous investigation [49, 50].

In addition, CCl_4 treatment induced significant elevation of glucose, urea and cholesterol. So, pretreatment of animals with SC and/or Liv-52 reduces significantly the levels of these parameters suggesting their hepatoprotective potential [51]. According to Bavarva and Narasimhacharya [52], *Costus speciosus* root extract possesses anti-hyperglycemic, antihyperlipidemic and antioxidative effects, which may prove to be of clinical importance in the management of diabetes and its complications. It was reported that, the ethanolic extract of *Costus* species showed significant reduction in glucose, urea, total cholesterol in blood of Albino rats [53]. The elevation of glucose level in the present study could be attributed to destruction of hepatocytes induced by CCl_4 intoxication [54] or decreasing of glycogen contents in hepatocytes [55].

Histopathologically, the protective effectiveness of SC and Liv-52 in the prevention of CCl_4-inuced liver

damage was observed. In CCl_4 group, hepatocellular necrosis and cytoplasmic vacuolization were marked in the histopathological examination of all sections. However, in the SC and Liv-52 groups little or mild histopathological alterations were observed. Thus, the finding of this study shows that the administration of an aqueous roots extract of SC appeared to protect the liver of guinea pigs from CCl_4-induced acute oxidative stress possibly through antioxidant activities [56]. It has been established that reactive oxygen species are involved in inflammation [40], and the protective action of SC roots extract against CCl_4-induced hepatic damage could involve mechanisms related to scavenging activity of ROS produced by CCl_4. The present investigation is important in presenting data suggesting considerable promise for the SC as a protective agent in CCl_4-induced damage on guinea pig liver.

5. Conclusion

In conclusion, our results indicated that the pretreatment of guinea pigs with SC roots extracts ameliorate CCl4-induced liver damage significantly. Hence the aqueous extracts of SC might be effective against different illness associated with liver. Our data also warrant further investigation of this extract as a potential treatment in other models of hepatic disorder.

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