



Potential protective effects of *Costus igneus* leaves alcoholic extract against CCl₄ -induced Hepatotoxicity in Rats

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Abstract: *Costus igneus* is a promising medicinal plant due to the presence of flavonoids and phenolic compounds as major contents of its constituents. This study was aimed to investigate the hepatoprotective effects of oral administration of *Costus igneus* leaves alcoholic extract (CLAE) against CCl₄-induced liver injury in rats. Forty two adult male Wistar rats were divided into six equal groups (7 for each) as follows: group(1): negative control group, group (2): positive control (CCl₄) group injected subcutaneously by a single dose of CCl₄ (2 ml/kg BW) at the last day of the experiment, group (3): rats treated with standard drug Silymarin (200 mg/kg BW) once daily for 4 weeks prior a single subcutaneous injection of CCl₄ (2 ml/kg BW.) and groups (4, 5 and 6) were orally administered CLAE at doses of (150, 300 and 600 mg/kg BW) once daily for 4 weeks prior a single subcutaneous injection of CCl₄ (2 ml/kg BW.) to induce experimental hepatotoxicity. The results showed that oral administration of CLAE in a concentrations of 600 mg/kg BW to rats for 4 weeks prior inducing hepatotoxicity by CCl₄ significantly improved total cholesterol (TC), triglycerides (TG), lipoprotein fractions, decreased the elevated serum levels of liver enzymes (alanine aminotransferase, ALT, aspartate aminotransferase, AST, alkaline phosphatase, ALP, total bilirubin and increased serum total protein when compared to the control positive group. Oxidative stress markers as antioxidant activity enzymatic (glutathione peroxidase, GPx, superoxide dismutase, SOD, catalase, CAT and non-enzymatic glutathione, GSH), also malondialdehyde (MDA) were significantly improved as compared to the control positive group. Histopathological examination of liver section of rats orally given CLAE prior inducing hepatotoxicity by CCl₄ showed alleviation of histological degeneration changes in protected groups compared to control positive group. This study concluded that, CLAE has high hypolipidemic, hepatoprotective effect and antioxidant effects in CCl₄-intoxicated rats. Hepatoprotective effect of CLAE could be due to presence of many phenolic compounds detected in this study.

Key words: *Costus igneus*, oxidative stress, antioxidant enzymes, liver functions, histopathological examination

Introduction

Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a surprising role in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways of growth, fight against disease, nutrient supply, energy provision and reproduction (Mroueh *et al.*, 2004). Liver regulates the synthesis and secretion of bile. Additionally, it allows the detoxification of various xenobiotic (Casarett and Doll's, 2008). Toxic injury occurs in the liver more often than other organs, because all ingested substances that are absorbed, first presented to the liver and then the liver is responsible for the metabolism and elimination of many substances (Tavga *et al.*, 2009). Thus, liver diseases are some of the fatal disease in the world today. Carbon tetrachloride (CCl₄) is a potent environmental hepatotoxin, has been served as a model compound for study of hepatotoxicity and the cellular mechanisms behind oxidative damage. The principle causes of CCl₄ - induced liver injury is lipid peroxidation, induced by free radical derivatives of CCl₄ (Basu, 2003 and Prasenjit *et al.*, 2006).

Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for their treatment of liver disorders. However, there is not much drugs available for the treatment of liver disorders. Therefore, many folk remedies from plant origin are tested for its potential antioxidant and hepatoprotective liver damage in experimental animal model (Koul and Kapil, 1999 and Alexenizor and Dorn, 2007)

Costus igneus, family *Costaceae*, is a herbaceous cum ornamental plant commonly known by many names including fiery *Costus*, Step ladder, Spiral flag and Insulin plant (Jose and Reddy, 2010). It is native to South and Central America. It is a member of *Costaceae* family and newly introduced plant in India from South and Central America. It is a perennial, upright, spreading plant reaching about two feet tall, with spirally arranged leaves and attractive flowers. In southern India, it usually grows as an ornamental plant (Benny, 2004). Also, *Costus igneus* commonly known as Fiery costus or Spiral flag is a species of herbaceous plant Paraskevi and Ronald (2006). *Costus igneus* (Fam: Zingiberaceae) is a tropical evergreen shrub with large, smooth, dark green leaves. 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 level Eevera *et al.*, (2010). In traditional medicine it is also used to promote longevity, Treats rash, Reduces fever, asthma, bronchitis and eliminates intestinal worms. The diverse activity of *Costus igneus* inspired us to investigate its antidiabetic activity.

Preliminary phytochemical screening of this plant extract revealed the presence of carbohydrate, protein, steroids, alkaloids, tannins, glycosides, saponins, fixed oils and flavonoids (Nandhakumar *et al.*, 2007). Studies have shown the antioxidant activity of plant flavonoids (Malomo *et al.*, 2011). Earlier studies suggests that Administration of the aqueous and ethanolic stem extract of *Costus igneus* to rats with experimentally induced urolithiasis by ethylene glycol has been found to reduce the growth of urinary stones (Manjula *et al.*, 2012). Contents of *Costus igneus* plant preparation indicate its potential to reduce the oxidative stress Shivaprakash *et al.*, 2014. The present study aimed to evaluate the hepatotoxic and antioxidant effect of CLAE on hepatotoxicity–induced by CCl₄ in rats

Materials and Methods

Materials

***Costus igneus* leaves**

Dried leaves of *Costus igneus* were purchased from a local market, Cairo, Egypt. Leaves were finely grinded using a mechanical grinder into a fine powder till used for both **phytochemical screening** and for preparation of alcohol extract.

Rats and basal diet

Forty two mature male rats of Sprague Dawley strain weighing 200±5 g body weight were obtained from the Laboratory Animals Farm, Helwan, Egypt. Basal diet constituents (Casein, cellulose, vitamin mixture, mineral mixture and choline chloride) were purchased from El-Gomhorya Company for Trading the Drugs, Chemicals and Medical Instruments, Cairo, Egypt.

Chemicals

Carbon tetrachloride (CCl₄) was purchased from El-Gomhorya Company, Egypt in the form of 40% liquid dispensed in 1 L plastic bottles.

Silymarin tablets were purchased from Sigma Chemical Co. St. Louis, MO., USA. All chemicals were of the highest analytical grade.

All kits for biochemical analysis were purchased from Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

Methods

***Costus igneus* leaves extracts preparation**

Costus igneus leaves were powdered with a mechanical grinder to obtain a fine powder passed through sieve, 80 mesh per inch². The fine powder of leaves was packed in high quality filter paper, which was then subjected to successive extraction in a soxlet- apparatus. The methanol extract was prepared by soaking 200 g of fine powder in 1 liter of 90% ethyl alcohol with daily shaking for 5 days and kept in a refrigerator. The ethanol was evaporated using a rotatory evaporator apparatus (manufactured in Russia) attached with a vacuum pump. Twenty grams of either extract (semisolid) were suspended in 100 ml distilled water with 2 ml of Tween 80 (suspending agent) to prepare a 20% alcoholic extract (Kanchana and Nuannoi , 2012).

Preliminary phytochemical screening of *Costus igneus* leaves extract

Preliminary screening of *Costus igneus* leaves extract was performed to investigate the presence or absence of the different phytochemical constituents such as phenolic, flavonoids, tannins, saponins and alkaloids using standard procedures described by Harborne, (2007).

Preparation of the basal diet

Basal diet was prepared according to Reeves *et al.*, (1993). It consists of 20 % protein (casein), 10 % sucrose, 4 % corn oil, 0.2% chlorine chloride, 1% vitamin mixture, 3.5 % salt mixture , 5% fibers (cellulose) and the remainder was corn starch up to 100 % .

Induction of hepatotoxicity

All animals, except normal control group, will be injected subcutaneously by a single dose of CCL₄ (2 ml/kg BW) at the last day of experiment to induce acute hepatotoxicity according to the method described by Sundaresan and Subramanian (2003).

Experimental design and grouping of rats

All animals were housed at a controlled room temperature of 23±1°C, 55% humidity and under a 12 h light/12-h dark schedule. The animals were fed on basal diet and water was provided ad libitum for one week before starting of the experiment for acclimatization. After one week adaptation period, the rats were randomly distributed into 6 equal groups, of 7 rats each. Group (1): was fed on basal diet and kept as a negative control group (normal rats). Group (2): (Hepatotoxin group) Rats injected subcutaneously by a single dose of CCL₄ (2 ml/kg BW) at the last day of experiment to induce acute hepatotoxicity (Sundaresan and Subramanian, 2003). Group (3): orally given Silymarin in a dose of 200 mg/kg BW. of for 4 weeks followed by injection subcutaneously by a single dose of CCL₄ (2 ml/kg BW) at the last day of the experiment. Groups 4, 5 and 6 were orally given *Costus igneus* leaves extract in doses of 150, 300 and 600 mg/kg BW respectively towards the end of the experiment period rats were injected subcutaneously with CCl₄(2 ml/kg BW). After 24hrs of CCL₄ injection all animals were sacrificed, blood was collected to separate serum for biochemical analysis. Liver was excised out, washed in ice cold saline and small portion was fixed in 10% formalin for histopathological analysis and the other portion was frozen to homogenate.

Biochemical analysis

Triglycerides (TG), total cholesterol (TC) and high density lipoprotein cholesterol (HDL-c) were determined such as described by Trinder (1969), Allain *et al.*, (1974) and Lopes-Virella *et al.*, (1977), respectively. Low density lipoprotein cholesterol (LDL-c) concentration was calculated by using formula of Friedwald *et al.*, (1972) as follow:

$$\text{LDL-c} = \text{Total cholesterol} - (\text{HDL-c} + \text{TG}/5).$$

Activities of serum liver enzymes aspartate and alanine aminotransferases and alkaline phosphatase (AST, ALT and ALP) were chemically estimated according to Bergmeyer *et al.*, (1978). Total protein (TP), albumin, total bilirubin (TBil) were chemically determined

as described by Burtis *et al.*, (2006). Serum uric acid and serum creatinine concentrations were determined as described by Fossati *et al.*, (1980) and Husdan and Rapoport, (1968) , respectively.

Lipid peroxidation and antioxidant enzymes in liver tissues

Liver homogenate was used for determination of tissue lipid peroxide (MDA), enzymatic (GPx, SOD and CAT) and non enzymatic (GSH) antioxidants. Malondialdehyde was determined according to Ohkawa *et al.*, (1979). The reduced glutathione (GSH) content in liver homogenate was determined colorimetrically by the method modified by Vaziri *et al.*, (2000). Activities of Glutathione Peroxidase (GPx), Superoxide Dismutase (SOD) and Catalase (CAT) antioxidant enzymes were determined chemically according to Paglia and Valentine (1979), Spitz and Oberley (1989) and Sinha (1972) respectively.

Histopathological examination

Liver of the scarified rats was taken and immersed in 10% formalin solution. The fixed specimens were then trimmed washed and dehydrated in ascending grades of alcohol. Specimens were then cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness and stained with Haematoxylin and Eosin stain for histopathological examination as described by Carleton, (1979).

Statistical analysis

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) for Windows, version 20 (SPSS Inc., Chicago, IL, USA). Collected data was presented as mean± standard deviation (SD). Analysis of Variance (ANOVA) test was used for determining the significances among different groups according to Dominick and Derrick, (2001). All differences were considered significant if $P \leq 0.05$.

Results

The phytochemical screening of *Costus igneus* leaves alcoholic extract (CILAE) revealed that it contains large amounts of Phenolic acids, flavonoids, and tannins moderate amounts of alkaloids Steroids and absence of the Anthraquinon, Saponin and Sterols as depicted in Table (1).

As shown in Table (2), rats injected subcutaneously with CCl₄ (2 ml/kg BW) at the end of the experimental period had significant increases (P< 0.05) in serum levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL) and decreased level of HDL-c (183.59±4.14, 120.35±2.25, 126.85±0.05 and 32.67±3.64 mg/dl, respectively) when compared to the negative control group (84.57± 4.59, 40.25±2.31, 28,.51 ±1,22 mg/dl and 48.01±2.90 mg/dl, respectively) or with stander group (86.32±1.32, 40.98 ±1.41,30.13±0.02 and 47.99 ±1.01 mg/dl, respectively). Pre-treated with Oral administration of *Costus igneus* leaves extract at (150, 300 and 600 mg/kg) to rats intoxicated with a single subcutaneous injection of CCl₄ at the last day of the experimental period caused a significant (P<0.05) decrease in the elevated serum TC, TG and LDL levels and increased serum HDL when compared to CCl₄-intoxicated group. Both of Silymarin and high dose of alcoholic extract of *Costus igneus* leaves had the best effect near to the normal group.

Table (1): Phytochemical screen of CLAE

Photochemical	Test result
Phenolics	+++
Flavonoids	+++
Tannins	+++
Alkaloids	++

The following symbol indicated the intensity of active compounds: a moderate amount (++) and large amount (+++).

Table (2): Hepatoprotective effect of oral administration of CLAE on serum levels of total cholesterol (TC), (TG), LDL-c and HDL-c in rats injected S/C with CCl₄ in the last day of the experimental period

Groups	TC (mg/dL)	TG (mg/dL)	LDL (mg/dL)	HDL (mg/dL)
Negative control (-ve)	84.57± 4.59d	40.25± 2.31d	28,.51 ±1,22d	48.01 ± 2.90a
Positive control (+ve)	183. 59± 4.14a	120.35± 2.25a	126.85±0.05a	32.67±3.64d
Silymarin + CCl ₄	86.32± 1.32d	40.98 ± 1.41d	30.13±0.02d	47.99 ±1.01a
<i>Costus igneus</i> at (150ml/kg BW)+CCl ₄	162. 82± 3.13b	105.64 ±1.11b	105.35±0.06b	36.34± 2.21c
<i>Costus igneus</i> at (300ml/kg BW)+CCl ₄	130.35± 1.17c	74.91 ±3.51c	75.16±0.06c	40 .21±1.10b
<i>Costus igneus</i> at (600ml/kg BW)+CCl ₄	89.52± 1.97d	43. 15± 2.73d	34.76. ±0.07d	46.13±2.13a

Data are presented as means ± SD, (n= 7 for each group). Values with different letters within the same column are significantly different at P< 0.05.

From data recorded in Table (3) it could be noticed that rats acutely intoxicated by a single subcutaneous injection of CCl₄ at the last day of the experimental period had significant increases (P< 0.05) in the serum activities of AST, ALT and ALP enzymes (140.22±0.21, 120.81±0.22 and 133.53±1.55 U/L, respectively) as compared to control (-ve) group (52.22±1.36, 49.04±0.36 and 59.77±8.90 U/L, respectively) or with stander group (53.45±2.44, 51.01±0.13 and 59.01±1.34). Administration of *Costus igneus* leaves extract at (150, 300 and 600 mg /kg b. wt) for four weeks before subcutaneous injection of CCl₄ induced significant decrease (P<0.05) in all the elevated serum marker levels of AST, ALT, ALP , when compared to the hepatotoxic rats (control +ve group). Intoxicated rats pretreated with the large dose (600 mg/kg BW) of *Costus igneus* leaves extract caused the highest reduction of hepatotoxicity in the elevated serum liver enzymes AST, ALT and ALP enzymes (60.52±1.78, 55.29±0.46 and 55.29±0.46 U/L, respectively) compared to CCl₄-intoxicated group (+ve) group.

Table (3): Effect of oral administration of CLAE on serum liver enzyme AST, ALT and ALP in rats injected S/C with CCl₄ in the last day of the experimental period

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Negative control (-ve)	52.22±1.36e	49.04± 0.36e	59.77±8.90e
Positive control (+ve)	140.22±0.21a	120.81±0.22a	133.53±1.55a
Silymarin + CCl ₄	53.45± 2.44e	51.01±0.13de	59.01 ± 1.34 e
<i>Costus igneus</i> at (150ml/kgBW)+CCl ₄	104.23±4.76b	100.15±0.48b	102.21±21.78b
<i>Costus igneus</i> at (300ml/kg BW)+CCl ₄	79 .58±3.43 c	69.37±0.33c	80.45±14.91 c
<i>Costus igneus</i> at (600ml/kg BW)+CCl ₄	60. 52±1.78d	55.29± 0.46d	66.64 ± 1.51d

Data are presented as means ± SD, (n= 7 for each group). Values with different letters within the same column are significantly different at P< 0.05.

Table (4): Effect of oral administration of CLAE on total protein, total bilirubin and albumin in rats injected S/C with CCl₄ in the last day of the experimental period.

Groups	Total protein (g/dL)	Albumin (g/dL)	Total bilirubin (g/dL)
Negative control (-ve)	8.95 ±1.32a	4.43± 0.02a	5.53 ±1.78d
Positive control (+ve)	5.31 ± 1.2 d	2.99±0.12c	9.42 ± 1.76a
Silymarin + CCl ₄	8.66 ±1.73a	4.15±0.32b	6.17±1.94c
Costusigneus at (150ml/kg BW)+CCl ₄	7.12±11.15b	3.17±0.04b	7.99 ± 0. 83b
Costusigneus at (300ml/kg BW)+CCl ₄	7.61± 10.01b	3.35±0.04b	7.32± 0.74b
Costusigneus at (600ml/kg BW)+CCl ₄	8.17±15.51a	3.93±0.11b	6.31 ±1.45c

Data are presented as means ± SD, (n= 7 for each group). Values with different letters within the same column are significantly different at P< 0.05.

Results in Table (4) explained that a single subcutaneous injection of CCl₄ to male rats at the last day of the experimental period (control positive group) induced a significant liver damage which observed from a significant decrease (P<0.05) in both total protein (TP) and albumin and increase in the level of serum total bilirubin (5.31±1.2, 2.99±0.12 and 9.42 ± 1.76 g/dL respectively) compared with control negative group (8.95±1.32, 4.43±0.02 and 5.53±1.78 g/dL respectively). Rats orally given *Costus igneus* leaves extract (150, 300 and 600 mg/kg BW.) for 4 weeks and injected with CCl₄ subcutaneously at the last day of the experiment showed elevation in serum TP, Albumin and decrease in bilirubin level. The highest protection was observed in both intoxicated rats pre-treated with Silymarin (8.66±1.73, 4.15±0.32 and 6.17±1.94 g/dL respectively) and the group of intoxicated rats pre-treated with *Costus igneus* leaves extract in a dose of 600 mg/kg BW., (8.17±15.51, 3.93±0.11 and 6.31±1.45 g/dL respectively) compared with control positive group (+ve) (5.31±1.2, 2.99±0.12 and 9.42±1.76 g/dL respectively).

Data illustrated in Table (5) showed that rats subcutaneously injected with a single dose of CCl₄ at the last day of the experimental period (+ve) had significant decrease in antioxidant enzymes activity (glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) and also in non enzymatic (GSH) antioxidant system in liver tissue and enhanced the end product of lipid peroxidation (MDA) level in liver tissues as compared with the negative control group. Oral administration of *Costus igneus* leaves extract at (150, 300 and 600 mg/kg) or Silymarin group for four weeks and injected with a single dose of CCl₄ at the last day of the experimental period showed significant increase in both of the enzymatic (GPX), (SOD), (CAT) and non enzymatic (GSH) antioxidant systems in liver tissue, while the elevated (MDA) levels were found to be reduced back towards the normal level in pre-treated rats given the highest dose of *Costus igneus* leaves extract as well as intoxicated rats pre-treated with Silymarin. The level of antioxidant enzyme was significantly improved by administration of 600 mg/kg BW *Costus igneus* leaves extract and in Silymarin treated rats intoxicated with CCl₄ at the last day of the experimental period.

Histopathological studies

Histopathological examination showed no histological change in the liver structure of normal control rats (Photo1). Hepatic intense centrilobular necrosis, vacuolization and macro vesicular fatty changes were observed in the liver sections of rats subcutaneously injected with a single dose of CCl₄ at the last day of the experimental period (control positive group) (Photo 2). Examined liver sections of rats orally given standard drug, silymarin and intoxicated with CCl₄ at the last day of the experimental period showed normal hepatocyte (Photo 3). The intoxicated animals pre-treated with different doses of *Costus igneus* leaves extract showed recovering of hepatocyte, as follow. The pre-treated with *Costus igneus* leaves extract at 150 mg/kg BW, shows a moderate number of recovered hepatocyte with a small amount of necrosis, vacuolization and macro vesicular fatty changes (Photo 4). While the 300 mg/kg BW. treated group, showed minimal inflammation and near-normal architecture possessing higher hepato-protective activity (Photo 5). The liver sections of the intoxicated animals pre-

treated with *Costus igneus* leaves extract at 600 mg/kg BW., exhibited significant liver protection against CCl₄ as evident by the presence of normal hepatic cords, absence of necrosis and fatty infiltration, supplementing the protective effect (Photo 6).

Table (5): Effect of oral administration of *Costus igneus* leaves extract on GPX, SOD, CAT, GSH and MDA in rats injected S/C with CCl₄ in the last day of the experimental period.

Groups	GSH (μ mol/dl)	MDA (μ mol/dl)	CAT (U/mg)	SOD (U/mg)	GPx (U/mg)	GSH (μ mol/dl)
Negative control (-ve)	4.72 \pm 0.02a	10.55 \pm 1.02d	74.35 \pm 0.97 a	85.22 \pm 1.25 a	53.44 \pm 3.33 a	4.72 \pm 0.02a
Positive control (+ve)	2.54 \pm 0.01d	21.99 \pm 1.01a	35.54 \pm 1.76 e	43.43 \pm 1.81 e	25.19 \pm 1.33 d	2.54 \pm 0.01d
Silymarin + CCl ₄	4.32 \pm 0.03a	13.61 \pm 1.02cd	73.15 \pm 1.01a	85.55 \pm 1.32a	50.02 \pm 2.31a	4.32 \pm 0.03a
<i>Costus igneus</i> at (150ml/kg BW)+CCl ₄	3.16 \pm 0.17bc	18.02 \pm 1.04b	35.45 \pm 2.16 d	56.18 \pm 1.43 d	42.42 \pm 1.12 c	3.16 \pm 0.17bc
<i>Costus igneus</i> at (300ml/kg BW)+CCl ₄	3.75 \pm 0.10b	17.92 \pm 1.02bc	49.01 \pm 1.36 c	68.25 \pm 1.32 c	42.33 \pm 1.16c	3.75 \pm 0.10b
<i>Costus igneus</i> at (600ml/kg BW)+CCl ₄	4.34 \pm 0.01a	15.42 \pm 1.04c	71.80 \pm 2.31ab	80.75 \pm 2.11b	45.15. \pm 4.23a b	4.34 \pm 0.01a

Data are presented as means \pm SD, (n= 7 for each group). Values with different letters within the same column are significantly different at P< 0.05.

Discussion

Hepatotoxicity implies chemical driven liver damage. Liver plays central role in transformation and clearance of most chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents, when taken in overdoses and sometime even when introduced within therapeutic ranges, may injure the organ. Chemicals that cause liver injury are called hepatotoxins (Tavga *et al.*, 2009).

The liver is the major site for the synthesis and metabolism of cholesterol, bile acids and phospholipids (Tavga *et al.*, 2009). Distinct

alterations in lipid metabolism have been reported in CCl₄-induced hepatotoxicity in rats (Khan *et al.*, 2011). In the current study the results revealed that intoxicated rats with CCl₄ resulted in significant increase in serum level of total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-c) accompanied with a significant decrease in high density lipoprotein cholesterol (HDL-c) level as compared to the negative control group. Our results in agreement with El-Habibi *et al.*, (2009) and Al-Dosari (2010) who reported that there was an increase in the levels of cholesterol, triglycerides, and free fatty acids in plasma and tissues of rats intoxicated with CCl₄ these results might be due to an increase in the synthesis of fatty acids and triglycerides from acetate which is responsible for the transport of acetate into the liver cell, resulting in increased substrate (acetate) availability or it could be due to increase the synthesis of cholesterol.

The effect of flavonoids and flavonoid rich extracts on reducing lipid levels effectively has been reported in several studies (Anila and Vijayalakshmi, 2002 and Chacko *et al.*, 2012). Silymarin, a flavonolignan has been widely used from ancient times to treat liver disorders, including acute and chronic viral hepatitis, toxin/drug-induced hepatitis and cirrhosis/alcoholic liver diseases. In the present study intoxicated rats pre-treated with silymarin significantly reduced serum total cholesterol, LDL-cholesterol and triglycerides with elevation of HDL. Our result in agreement with Vaughn (2006).

In our study pretreatment of rats with *Costus igneus* leaves extract resulted in significant improvement in the tested lipid profile parameters, that could be attributed to an increase in the inhibition of intestinal absorption of cholesterol, interference with lipoprotein production increased expression of hepatic LDL receptor and their protection, leading to an increased removal of LDL-C from the blood and its increased degradation and catabolism of cholesterol from the body. All these events either individually or in combination lead to decrease in serum LDL-C levels, which reduced serum total cholesterol level during the pretreatment (Mani *et al.*, 2010 and Chacko *et al.*, 2012).

Liver injury induced by CCl₄ is the best characterized system of xenobiotic induced hepatotoxicity and is commonly used models for the screening of anti-hepatotoxic and/or hepatoprotective activities of drugs. The changes associated with CCl₄ induced liver damage are similar to that of acute viral hepatitis (Masuda, 2006). Carbon tetrachloride accumulates in hepatic parenchymal cells and metabolized by cytochrome P-450 enzyme and its metabolic product; trichloromethyl free radicals

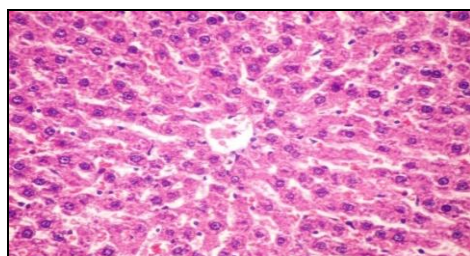


Photo (1): Liver of the negative control rat showing the normal histological structure of hepatic cells. (H and E x 400).

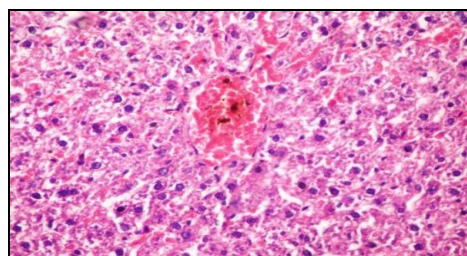


Photo. (2): Liver of intoxicated rats (non-treated) (+ve) rat showing congestion of hepatic central vein (Arrow) (H and E x 400).

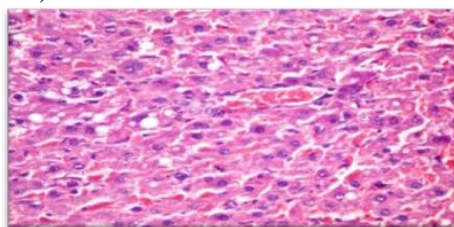


Photo (3): Liver of intoxicated rats of pretreated group with Silymarin showing normal hepatocytes (arrow mark) with central vein(H and E x 400).

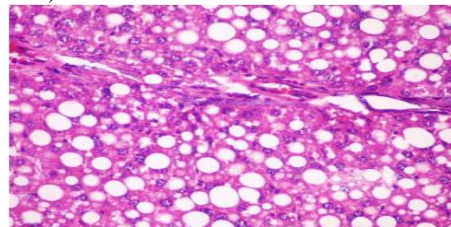


Photo (4): Liver of intoxicated rats of pretreated group with *Costus igneus* leaves alcoholic extract in a dose of 150 mg/kg showing a moderate number of recovered hepatocytes with a small amount of necrosis, vacuolization and macrovesicular fatty changes (H and E x 400).

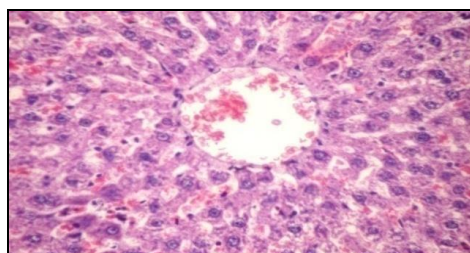


Photo (5): Liver of intoxicated rats pretreated with *Costus igneus* leaves alcoholic extract in a dose of 300 mg/kg showing minimal inflammation and near-normal architecture possessing higher hepatoprotective activity

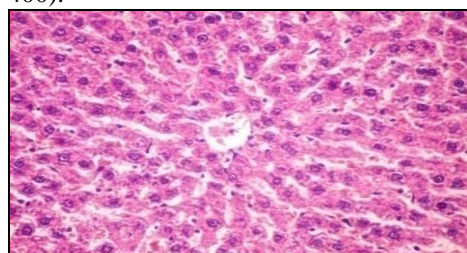


Photo (6): Liver of intoxicated rats pretreated with *Costus igneus* leaves alcoholic extract in a dose of 600 mg/kg showing Significant protection evident by the presence of normal hepatic cords, absence of necrosis and fatty infiltration, supplementing the protective effect.

(CCl₃). These free radicals are highly reactive; alkylate cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxides, leading to liver damage. Lipid peroxidation will initiate pathological changes such as depression of protein synthesis Park *et al.*, (2008).

Assessment of liver function can be performed by determining the activity of serum enzymes AST, ALT and ALP, originally present in high concentrations in the cytoplasm. When there is hepatic injury, these enzymes leak into the blood stream inconformity with the extent of liver damage (Krasteva *et al.*, 2007 and Xu *et al.*, 2007). Total bilirubin (TBil) and total protein (TP) levels on other hand are related to the function of hepatic cell (Ismet *et al.*, 2013). In the present study, the hepatotoxicity of CCl₄ in rats was confirmed by a significant elevation of AST, ALT, ALP and total bilirubin. In addition, CCl₄ intoxication produced a significant reduction in plasma total protein level. This may be due to release of these enzymes from the cytoplasm into the blood rapidly after cellular damage and a reduction in hepatic protein synthesis. Liu *et al.*, (2013) reported that elevation of AST, ALT and ALP in response to CCl₄ could be attributed to hepatic structural damage because these enzymes are normally localized to the cytoplasm and are released into the circulation after cellular damage has occurred.

Histopathological observations of the liver of CCl₄- administered rats showed revealed presence of hepatic intense centrilobular, necrosis, vacuolization and macro vesicular fatty changes. These results were in harmony with the previous data reported by (Zalatnai *et al.*, 1991 and Candasamy, 2010).

The presence of phyto-constituents such as flavonoids like Quercetin, protocatechuic acid, triterpinoids like faradiol, oleanolic acid, beta-amyrincalenduladiol, glycosides, sterol glycosidestaraxasterol, lupeol, brein, arnidiol, erythrodiol, coflodiol and manilladiol, carotenoids like lycopene, beta –carotene, flavoxanthin, terpenoids and steroids are responsible for the hypatoprotective and antioxidant effect (Defeduis *et al.*, (2003), Kishimoto *et al.*, 2005, Wang *et al.*, (2006) and Shivaprakash *et al.*, 2014. The mechanisms of action of phenolic compounds might be related to their antioxidant effect and / or due to their ability to scavenge the free radicals by hydroxyl groups present in these compounds (Djeridane *et al.*, 2006).

The results of the present study showed that, intoxicated rats pretreated with *Costus igneus* leaves extracts had an effective improvement in liver function and afforded a protection against CCl₄ induced hepatocyte toxicity. This was manifested by decreasing in elevated enzymes leakage of (ALT, AST, and ALP) and total bilirubin level and increasing total protein and albumin as compared with, CCl₄-intoxicated group. The hepatoprotective effect of *Costus igneus* leaves extract may be attributed to a significant free radical scavenging and antioxidant activity as mentioned by Jayasri *et al.*, 2009 who mentioned that *Costus igneus* leaves extract is rich in a variety of bioactive metabolites including flavonoids and terpenoids. These bioactive ingredients have potent activities for scavenger the Superoxide radicals (O₂⁻) and hydroxyl radicals (·HO) resulted from CCl₄ metabolites. As well as the reference drug Silymarin significantly reduced the elevation in the activities of these enzymes (Luper 1998, Conjeevaram and Lok 2003 and Comelli *et al.*, 2007 and Majumdar and Parihar (2012).

In the present study, a significant elevation of malondialdehyde (MDA) (lipid peroxidation product) accompanied by lower activities of the antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) had been observed in the liver tissue of CCl₄-treated group compared to control negative group. The decreased activities of the antioxidative enzymes probably occurred as a result of free radical production and the excessive use of these enzymes. These results were in accordance with the result previously reported by baravalia *et al.*, (2011). Evidence of lipid peroxidation by increased MDA level is one of the primary means of associated oxidative processes with an overall decrease in cellular function (Rosenblat *et al.*, 2006 and Majumdar and Parihar 2012). The increase in malondialdehyde (MDA) levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals (Thangakrishnakumari *et al.*, 2012).

With regard to the hepatoprotective effect of *Costus igneus* leaves extract when orally administered to rats at (150, 300 and 600 mg /kg b. wt) for 4 weeks before a single subcutaneous injection of CCl₄ at the last day of the experimental period on MDA, GSH, CAT, SOD and GPx, the results revealed that pre treatment with *Costus igneus* leaves extract significantly reduced MDA and increased levels of GSH, accompanied

by increase activities of the antioxidant enzymes, CAT, SOD, GPx compared to control positive group. These findings might be due to antioxidant activity of *Costus igneus* leaves. These result was confirmed by Herold *et al.*, 2003 and Victorrajmohan *et al.*, 2005 and Jayasri *et al.*, 2009 who mentioned that, raised levels of GSH have been reported to elicit a protective response against the toxic manifestations of chemicals, particularly those involving oxidative stress. Marles *et al.*, 1995, Anaga *et al.*, 2004 and Fonseca *et al.*, 2011 reported that, oral administration of *Costus igneus* leaves extract inhibited superoxide generation in macrophages in rats. *Costus igneus* leaves significantly increased both of catalase (CTA) and Glutathione reductase levels in blood and liver, whereas glutathione peroxidase was found to be decreased. Alcoholic and water extracts of *Costus igneus* leaves, containing flavonoids, showed antioxidant activity based on analysis of plasma and urine malondialdehyde (MDA) and urine isoprostane concentrations as demonstrated by Han *et al.*, 2006 and Kripa *et al.*, 2011.

The biochemical results of our study were confirmed by histopathological findings, which seen in liver sections. The histological findings of liver of pre treated rats with *Costus igneus* leaves extract showed almost completely normal structure with mild fibroblastic proliferation and sporadic cell necrosis. Oval cell hyperplasia in the portal area was very clear and necrosis was more reduced than CCl₄-intoxicated rats fed on basal diet, thus may be explained by antioxidant activity of *Costus igneus* leaves that may be attributed to its constituents of phytochemical Sathuvan *et al.*, 2012. These histological findings agreed with the study of Odoh *et al.*, 2010 who reported that the oxidative damage to tissue and their cellular component can be prevented by certain antioxidant metabolites present in plants.

Conclusion

Costus igneus leaves effectively improved liver functions and protected against liver tissues damage induced by toxic substances. *Costus igneus* leaves have protective effect against the loss of antioxidant activities as result of oxidative process caused by CCl₄ injection due to its phytochemicals compounds (phenolic and flavonoids). This protective activity of *Costus igneus* leaves suggests that regular consumption of it or food containing phenolic and flavonoids may protect against liver disease and imbalanced antioxidant. Thus, the possibility that *Costus igneus* leaves reduce the risk of liver disease and

oxidation process remains open and further studies are needed to confirm the importance of it in the prevention of liver disease.

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التأثيرات الوقائية المحتملة للمستخلص الكحولي لأوراق نبات القسط الهندي تجاه التسمم الكبدي المستحث برابع كلوريد الكربون في الفئران

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الملخص العربي:

أجريت الدراسة الحالية بهدف إستكشاف التأثيرات الوقائية المحتملة للمستخلص الكحولي لأوراق نبات القسط الهندي تجاه التسمم الكبدي المستحث برابع كلوريد الكربون في الفئران. تم إجراء التجربة على عدد ٤٢ فأر قسمت إلى ٦ مجموعات كل منها ٧ فئران، تركت إحداها مجموعة ضابطة سالبة تغذت على الغذاء الأساسي فقط والمجموعه الثانية تركت كمجموعه ضابطه موجبة حيث تم حقنها تحت الجلد برابع كلوريد الكربون بجرعة ٢ مل/كيلو جرام من وزن الجسم في اليوم الأخير يوم من التجربة لاحداث التسمم الكبدي وتغذت على الغذاء اللاساسي فقط ايضا (مجموعه مصابة وغير معالجه) والمجموعه الثالثه تم حقنها فمويا بدواء السليمارين المعالج للتسمم الكبدي لمدة اربع اسابيع ثم حقنت أسفل الجلد برابع كلوريد الكربون بجرعة ٢ مل/كيلو جرام من وزن الجسم اخر يوم من التجربة لاحداث التسمم الكبدي وتركت (مجموعه مصابة معالجه بدواء ضابط) وتم إعطاء فئران المجموعات الرابعة والخامسة والسادسة عن طريق الفم المستخلص الكحولي للقسط الهندي بجرعات ١٥٠ و ٣٠٠ و ٦٠٠ ملجم/كجم من وزن الجسم على التوالي يوميا لمدة اربعة اسابيع ثم حقنت أسفل الجلد برابع كلوريد الكربون بجرعة ٢ مل/كيلو جرام من وزن الجسم اخر يوم من التجربة لاحداث التسمم الكبدي وفي نهاية فترة التجربة تم سحب عينات دم لإجراء التحليلات البيوكيميائية في المصل وأخذ الكبد للفحص الهستوباثولوجي. ولقد أظهرت النتائج أن تناول المستخلص الكحولي للقسط الهندي عن طريق الفم بتركيز ٦٠٠ ملجم/كجم من وزن الجسم الفئران لمدة ٤ أسابيع قبل إحداث تسمم الكبد عن طريق CCl4 أدى إلى تحسن ملحوظ في الكوليسترول الكلي (TC)، الدهون الثلاثية (TG)، وليبيدات الدم، وانخفاض مستويات انزيمات الكبد (ALP-ALT-AST)، البيليروبين الكلي و البروتين الكلي في الدم وكذلك تحسنت مؤشرات الإجهاد التأكسدي مثل الجلوتاثيون المختزل (GSH) والمالوندهيد (MDA) والجلوتاثيون بيروكسيداز (GPx) والسوبر اوكسيد ديسميوتيز (SOD) والكتاليز (CAT) بالمقارنة مع المجموعة الضابطة الموجبة. كما أظهر فحص أنسجة الكبد تحسن ملحوظ في التغيرات النسيجية مقارنة بالمجموعة الضابطة الموجبة. وتوصى الدراسة بأن المستخلص الكحولي للقسط الهندي له تأثير خافض للدهون وله دور وقائي للكبد وكذلك له دور فعال كمضاد للاكسدة ضد التسمم الكبدي المحدث برابع كلوريد الكربون. وقد يرجع تأثير المستخلص الكحولي للقسط الهندي إلى احتوائه على المركبات الفينولية والتي تم تحديدها في الدراسة.

الكلمات المفتاحية: القسط الهندي، الإجهاد التأكسدي، الانزيمات المضادة للأكسدة، وظائف الكبد، الفحص الهستوباثولوجي.