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Abstract

This study was undertaken to investigate the hepatoprotective effect of costus (*Costus igneus*) root powder and ethanolic extract against liver injury induced by CCl4 intoxication. Thirty-six white male albino rats weighing (140±10g) were used and divided into 6 groups, each group (6) rats. Rats infected of hepatic by injected with carbon tetrachloride (CCl4) 0.2 ml/100 g body weight of 40 ml/l CCl4 dissolved in paraffin oil. Liver enzymes such as serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), kidney functions (uric acid, urea and creatinine), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (LDL-c), low-density lipoprotein cholesterol (HDL-c), very low lipoprotein cholesterol (VIDL-c), oxidative enzymes such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) activity were determined. Identification of phenolic compounds of costus roots was determined using the HPLC technique. The HPLC results showed that the costus roots contained high amounts of bioactive compounds. Results also indicated that the costus roots improving all tested biochemical analyses such as serum liver enzymes, kidney functions, lipids profile and anti oxidative enzymes in rats. In conclusion, 400 mg/kg costus roots ethanolic extract recorded the best levels for protection, improvement liver enzymes, kidney functions, anti oxidative enzymes and lipids profile. Therefore, costus roots could be used in our daily drinking for liver ailment, besides its many health benefits.

Keywords: Medicinal plants, Hepatoprotective effect, Phenolic compounds and Biochemical analysis.

Introduction

The liver is one of the largest organs in the human body and the primary focus of metabolic and excretory activity. As a result, it plays an unexpected role in the body's maintenance, performance, and regulation of homeostasis. As a result, liver illnesses are among the deadliest diseases today. Modern medicine has little to offer in the way of hepatic disease relief, hence plant-based treatments are mostly used to treat liver ailments **(Tejaswi *et al.*, 2018)**. Because most drugs go through the first-pass metabolism in the liver, it is the primary target of toxicity for several chemicals. As a result, it becomes a crucial organ for researching the effects of various drugs injected into the body **(Tousson *et al.*, 2020)**. Storage, filtration, excretory, and metabolic processes are all performed by the liver. The liver is a vital organ for converting and removing toxins, and it is vulnerable to their toxicity. More than 900 medicines have been linked to liver damage, and drugs are responsible for 20-40% of all cases of fulminant hepatic failure. Hepatotoxicity can also be caused by other organic and inorganic substances used in laboratories and industries, as well as natural chemicals (such as microcystins) and some herbal medicines. Hepatic damage causes hepatocyte activities to be disrupted, resulting in plasma membrane leakage and a rise in serum enzyme levels **(Sivakrishnan and Swamivelmanickam, 2019)**.

The liver is an essential target organ for pharmacological, xenobiotic, and oxidative stress toxicity because it is a crucial organ that plays a central role in converting and removing substances and is vulnerable to their toxicity. Hepatotoxicity is the most common pathology in the world, accounting for up to 83 percent of all cases and causing the most serious health consequences Independent of the original causative agent, free radicals and reactive oxygen species are increasingly thought to play a key role in the initiation and progression of liver disorders. Carbon tetrachloride (CCl_4) is a hepatotoxic substance that is converted by the cytochrome P450 enzyme into extremely reactive metabolites such as the trichloromethyl free radical (CCl_3). and trichloromethyl peroxy radical (CCl_3O_2) **(Al-Harbi *et al.*, 2014)**. Hepatotoxicity studies typically use CCl_4 as a model

drug. The extremely reactive trichloromethyl radical is formed when CCl_4 is metabolized in the liver, and this free radical promotes auto-oxidation of the fatty acids contained in the cytoplasmic membrane phospholipids, resulting in functional and morphological alterations in the cell membrane **(Weber *et al.*, 2003)**.

Phenols, coumarins, lignans, terpenoids, carotenoids, glycosides, flavonoids, organic acids, alkaloids, and xanthenes are some of the key active ingredients found in plant-based hepatoprotective medicines or medications. Several phyto molecules have been found to have hepatoprotective properties. To solve the pharmaceutical imbalance between medicines that protect the liver and drugs that cause hepatotoxicity, more research into lead compounds that may have greater therapeutic benefits is needed **(Ahmed *et al.*, 2008)**.

Consumption of fruits and vegetables has been linked to a lower risk of chronic diseases in epidemiological studies. Hepatic dysfunction caused by hepatotoxins including acetaminophen, cadmium chloride, ethanol, carbon tetrachloride (CCl_4), and allyl alcohols is on the rise all over the world. Carbon tetrachloride is processed by cytochrome P450 in the endoplasmic reticulum of liver cells, resulting in the formation of an unstable CCl_3 radical complex, which interacts swiftly with oxygen to produce the hepatotoxic trichloromethyl peroxy radical **(Cha *et al.*, 2010)**. When compared to control animals, carbon tetrachloride intoxication induces a considerable increase in liver enzymes. This increase could be related to the release of these enzymes from the cytoplasm into the bloodstream following plasma membrane rupture and cellular injury **(Naik and Panda, 2007)**.

Medicinal plants have been used for healing purposes throughout human history; even today, up to 80% of the world's population, the majority of whom live in developing countries, rely on traditional herbal medicine as their primary health care system. Many of the herbal drugs prescribed in traditional medicine have insufficient knowledge and have not been tested by scientific methods **(Qazi and Molvi, 2016)**. Many medicinal plants produce a variety of secondary metabolites known as phytochemical compounds, which can inhibit microbial growth in

a variety of ways, including interfering with cellular metabolic processes, disrupting cellular membranes, or modulating signal transduction or gene expression pathways **(Mohamed *et al.*, 2017)**. Several herbal plant extracts and products have been utilized to treat a variety of fatal diseases **(Tousson, 2016)**.

Costus (*Costus igneus*) was a Roman general (Falc.) Lipschitz, also known as *Saussurea lappa* C.B. Clarke, is a member of the Asteraceae family, which includes over 1000 genera and 30,000 species found around the world. India, on the other hand, has a diverse range of species **(Pandey *et al.*, 2007)**. It is commonly known as Costus in English and has different vernacular names in India like Kuth, Kur, Kot, Kushta **(Kirtikar and Basu, 2001)**. It is also distributed in Pakistan and some parts of the Himalayas **(Shah, 2006)**. *Costus igneus* is well-known in Islamic medicine, and it is mentioned in the Prophet Muhammad's Holy Ahadith (Peace be upon him) **(Ahmad *et al.*, 2009)**. It has been utilized by traditional healers in Arab countries since the era of Islamic culture under the name "Al-Kost Al-Hindi." Costus is one of these antioxidant-rich herbal plants that is widely used in various traditional systems of medicine all over the world for the treatment of several ailments such as diarrhea, tenesmus, dyspepsia, vomiting, and inflammation **(Irshad *et al.*, 2012)**. Costus roots have been tested for a variety of pharmacological actions, including antioxidant activity, anti-hepatotoxic activity, anti-diabetic, anti-fungal impact, anti-tumor, anti-inflammatory, anti-ulcer, anti-microbial, and immunostimulant effects **(Yaeesh *et al.*, 2010)**. Natural antioxidant phytochemicals appear to be a promising potential agent for preventing and protecting against oxidative stress-induced liver damage. Costus methanolic extract has been shown to protect against paracetamol-induced liver injury, like silymarin-treated liver. As a result, the study's findings will be extremely useful in treating the liver injury with naturally occurring plant extracts **(Nancy *et al.*, 2019)**. The major active ingredients of the plant are terpenes, anthraquinones, alkaloids, and flavonoids. Biological activity has been discovered in several of these active components. Costunolide and dehydrocostus lactone, for example, are important components of

the roots. These chemicals have recently been discovered to have a variety of biological functions **(Zahara *et al.*, 2014)**.

Pretreatment of rats with costus root extracts dramatically reduces CCl4-induced liver damage. As a result, costus aqueous extracts may be useful against a variety of liver-related illnesses **(Al-Duais and Al-Awthan, 2017)**.

This study was undertaken to investigate the hepatoprotective effect of costus root powder and ethanolic extract against liver injury induced by CCl4 intoxication.

Material and methods

Materials

Plant materials

- Dried roots of costus (*Costus igneus*) were purchased from a local herbalist market in Cairo City, Egypt, 2020.

Carbon tetrachloride (CCl_4)

Carbon tetrachloride (CCl_4), paraffin oil were obtained from Al-Gomhoria Company for Drugs Chemicals and Medical Supplies, Assiut, Egypt.

Costus igneus root extracts preparation

Costus igneus roots were powdered with a mechanical grinder to obtain a fine powder passed through a 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 Soxhlet- 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) was suspended in 100 ml distilled water with 2 ml of Tween 80 (suspending agent) to prepare a 20% alcoholic extract **(Kanchana and Nuannoi, 2012)**.

Chemicals and kits

Pure white crystalline cholesterol powder, saline solutions, casein, cellulose, choline chloride powder and DL-methionine powder, were obtained from Morgan Co. Cairo, Egypt. Chemical kits used in this study (TC, TG, HDL-c, ALT, AST, ALP, urea, uric acid, and creatinine) were obtained from Al-Gomhoria

Company for Drugs, Chemicals and Medical supplies, Assiut, Egypt. While GSH, CAT, SOD kits were obtained from SIGMA Chemical Co., Cairo, Egypt.

Experimental animals

A total of 36 adult normal male albino rats Sprague Dawley strain weighing 140±10 g was obtained from the animal house of the Faculty of Medicine, Assiut University.

Methods

Preparations of costus roots

To prepare the dried costus powder roots, which were ground to a fine powder using an air mill, high-speed mixture (Molunix, Al-Araby company, Benha, Egypt) then serving as powder seize was kept in polyethylene bags at freezing temperature until using.

Identification of phenolic compounds

HPLC analysis of extracts was performed using an Agilent 1200 chromatograph equipped with a PDA model G1315B, a Bin pump model G1312A, an auto-sampler model G1313A and a RR Zorbax Eclipse Plus C18 column (1.8 µm, 150 mm ×4.6 mm). Mobile phase A was 0.2 % formic acid in water and mobile phase B was acetonitrile. Elution was performed at 0.95 ml min-1 with the following gradient program of solvent B: 0–20 min, 5-16 %; 20–28 min, 16-40 %; 28–32 min, 40-70 %; 32-36 min, 70-99 %; 36-45 min, 99 % and 45-46, min. 99-95 %.30. The injection volume was 10 µl. Wavelengths of 280 nm (for flavan-3-oils and derivatives of benzoic acid) and 360 nm (for flavonols and derivatives of cinnamic acid) were selected for detection; quantification of the compounds was realized using calibration curves obtained by HPLC of pure standards. The HPLC method was used according to **Radovanović *et al.*, (2010)** with some modifications (elution gradient and flow rate).

The induction of liver experimental

Rats were injected subcutaneously at a dose of 0.2 ml/100 g body weight of 40 ml/l CCl4 dissolved in paraffin oil **(Diao *et al.*, 2011)**. Carbon tetrachloride was injected three times per week for 4 consecutive weeks.

Experimental design

Thirty-six adult male white albino rats, Sprague Dawley Strain, 10 weeks age, weighing (140±10g) were used in this experiment. All rats were fed on a basal diet (casein diet) prepared according to **AIN (1993)** for 7 consecutive days for adaptation. After this adaptation period, rats were divided into 6 groups, six rats per each as follows: group (1): rats fed on basal diet as a negative control. Group (2): injected by 0.2 ml/100 g body weight of 40 ml/l CCl4 dissolved in paraffin oil (**Dong *et al.*, 2005**). Carbon tetrachloride was injected three times per week for 4 consecutive weeks and used as a positive control group. Group (3): hepatotoxic injured rats fed on a basal diet containing 2% of costus roots powder. Group (4): hepatotoxic injured rats fed on a basal diet containing 4% costus roots powder. Group (5): hepatotoxic injured rats fed on 200 mg/kg B.W costus roots extract. Group (6): hepatotoxic injured rats fed on a 400 mg/kg B.W costus roots extract. During the experimental period, the experiment continued for 28 days, at the end of the experimental period each rat weight separately then, rats are slaughtered, and blood samples were collected.

Blood sampling

After fasting for 12 hours, blood samples were obtained from the hepatic portal vein at the end of each experiment. The blood samples were collected into dry clean centrifuge glass tubes and left to clot in a water bath (37°C) for 30 minutes, then centrifuged for 10 minutes at 3000 rpm to separate the serum, which was carefully aspirated and transferred into a clean cuvette tube and stored frozen in the deep freezer till analysis according to **Schermer (1967)**.

Biochemical analysis

Serum lipids profile

Serum total cholesterol was determined according to the colorimetric method described by **Thomas (1992)**. Serum triglycerides were determined by the enzymatic method using kits according to **Young and Pestaner (1975) & Fossati and Principle (1982)**. HDL-c was determined according to the method described by **Grodon and Amer (1977)**.

VLDL-c was calculated in mg/dl according to **Lee and Nieman (1996)** was using the following formula: **VLDL-c (mg/dl) = Triglycerides / 5**

LDL-c was calculated in mg/dl according to **Lee and Nieman (1996)** as follows:

LDL-c (mg/dl) = Total cholesterol – HDL-c – VLDL-c

Liver enzymes.

Determination of serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), serum alkaline phosphatase (ALP) were carried out according to the method of **Clinica Chimica Acta (1980)**; **Hafkenscheid (1979) and Moss (1982**); respectively.

Determination of enzyme activities

Determination of catalase (CAT) activity

Liver catalase (CAT) was determined by Goth's colorimetric method, in which supernatant was incubated in H2O2 substrate and the enzymatic reaction was stopped by the addition of ammonium molybdate. The intensity of the yellow complex formed by molybdate and H2O2 was measured at 405 nm according to the method described by **Goth (1991)**.

Determination of superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was determined by using a measurement method developed by **McCord and Fridovich (1969)**. This method is based on the generation of superoxide radicals produced by xanthenes and xanthenes oxidase, which react with 2-(4-iodophenyl)-3- (4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. SOD activity was expressed as units per gram protein.

Determination of glutathione peroxidase (GSH-Px) activity

Glutathione peroxidase (GSH-Px) activity was measured at standard assay conditions in 340 nm (absorbance) at 37°C according to the method developed by **Paglia and Valentine (1967)**. In this measurement, GSH-Px catalyzes the oxidation of glutathione by cumene hydro-peroxide. Measurements were performed by an auto-analyzer according to the Randox application procedure. Activities of GSH-Px were expressed as units per gram of protein.

Kidney functions

Serum urea was determined according to the enzymatic method (**Patton and Crouch, 1977)**. Serum uric acid was determined colorimetrically according to the method of **(Barham and Trinder, 1972)**. Creatinine was determined according to the kinetic method **(Henry, 1974)**.

Statistical analysis

The data were analyzed using a completely randomized factorial design **(SAS, 1988)** when a significant main effect was detected. The means were separated with the Student-Newman-Keuls test. Differences between treatments at $P \leq 0.05$ were considered significant using Costas Program. Biological results were analyzed by One Way ANOVA.

Results and discussion

Identification of phenolic compounds of costus roots

Data tabulated in Table (1) showed the identification of phenolic compounds of costus roots by the HPLC technique. The obtained results indicated that the highest phenolic compounds identified in costus roots were recorded for quercetin, caffeic acid and vanillin. The values were 21.50, 13.37 and 10.45 mg/100g; respectively. On the other hand, the lowest phenolic compounds identified in costus roots were recorded for chlorogenic acid, *p*-coumaric acid and ferulic acid. The values were 0.82, 1.47 and 2.40 mg/100g; respectively. These findings support **Anubha (2013)** statement that phytochemical screening revealed the existence of numerous types of phytochemicals such as alkaloids, saponins, flavonoids, steroids, tannins, and other compounds that

could be responsible for the varied pharmacological activities. The therapeutic properties of medicinal plants may be due to the presence of secondary metabolites such as phenols, flavonoids, glycosides, saponins, and alkaloids, among others.

As a standard, six phenolic acids (gallic, caffeic acid, syringic, coumaric, vanillin, and cinnamic acid) and three flavonoids (catechin, quercetin, and rutin) were found. Quercetin was the most prevalent phenolic compound in both cider and costus, ranging from 9.8 to 24.4 mg/100 g DW. Both costus and cider had the same low quantities of coumaric acid (1.1 mg/100 g DW). Vanillin was found as a phenolic component in costus and cider, with concentrations ranging from 8.3 to 12.7 mg/100 g DW. **(Basudan, 2018)**.

Costus root also contains phytoconstituents such as quercetin, protocatechuic acid, triterpenoids such as faradiol, oleanolic acid, beta-amyrin calendula diol, glycosides, sterol glycoside staraxasterol, lupeol, brain, arnidiol, erythritol, collodion, and manilla **(Wang *et al.*, 2006 and Shivaprakash *et al.*, 2014)**. The mechanisms of action of phenolic compounds could be linked to their antioxidant properties and/or their ability to scavenge free radicals through the presence of hydroxyl groups in these compounds **(Djeridane *et al.*, 2006)**.

Table (1): Identification of phenolic compounds of costus roots ND = Not detectable

Active compounds of Costus roots	Concentration mg/100 g
Gallic acid	2.60
Chlorogenic acid	0.82
Catechin	5.10
Caffeic acid	13.37
Quercetin	21.50
Ferulic acid	2.40
Vanillin	10.45
p-coumaric acid	1.47
Benzoic acid	4.57
Ellagic acid	6.15

Effect of costus roots powder and extract on liver enzymes of hepatic fibrosis rats

Data, given in Table (2), show the effect of costus roots on (ALT, AST and ALP) of hepatic fibrosis rats. The obtained results indicated that the ALT liver enzyme of the positive control rats group recorded a higher value when compared with the negative control group with a significant difference ($P \leq 0.05$). The mean values were 68.50 and 30.00 U/L; respectively. While the highest ALT liver enzyme of the treated group (hepatic fibrosis rats) was recorded for the group fed on 2% costus roots powder while the lowest value was recorded for the group fed on 400 mg/kg costus roots extract with significant difference ($P \leq 0.05$). The mean values were 56.00 and 37.00 U/L; respectively.

On the other hand, the AST liver enzyme of the positive control rats group recorded a higher value when compared with the negative control group with significant differences ($P \leq 0.05$), which were 110.0 and 50.00 U/L; respectively. While the highest AST liver enzyme of the treated group was recorded for the group fed on 2% costus roots powder but, the lowest value was recorded for the group fed on 400 mg/kg costus roots extract with a significant difference ($P \leq 0.05$). The mean values were 80.50 and 54.00 U/L; respectively.

As for ALP liver enzyme of positive control rats group recorded the higher value when compared with the negative control group with significant difference (P≤0.05), which were 90.0 and 40.00 U/L; respectively. While the highest ALP liver enzyme of the treated group was recorded for the group fed on 2% costus roots powder but, the lowest value was recorded for the group fed on 400 mg/kg costus roots extract with a significant difference (P≤0.05). The mean values were 71.00 and 46.50 U/L; respectively. These findings confirm the claim of **Tousson *et al.*, (2020)**, who found that hepatic damage is a common pathogenic hallmark in numerous liver disorders. The long-term presence of hepatic damage can lead to fibrosis, cirrhosis, and even liver cancer. As a result, treating and preventing hepatic damage is critical in the clinical management of liver illnesses.

ALT, AST, and ALP are common blood serum indicators used to diagnose liver disease. Increased levels of both ALT and AST transaminases are highly suggestive of hepatotoxicity **(Reuben, 2004)**. ALP is a very sensitive hepatobiliary damage marker. Increased levels are linked to bile duct congestion or obstruction, indicating cholestasis **(Sheehan and Haythorn, 2007)**.

The activity of blood hepatic enzymes (ALT, AST, and ALP) was dramatically increased in guinea pigs given CCl4, but the activity of these enzymes was greatly lowered by (Saussurea costus) root extract. In addition, when compared to negative controls, glucose, urea, and cholesterol levels were lower **(Al-Duais and Al-Awthan, 2017)**.

Table (2) Effect of costus roots powder and extract on liver enzymes of hepatic fibrosis rats

Parameters / Groups	ALT (U/L)	Change of control (%)	AST (U/L)	Change of control (%)	ALP (U/L)	Change of control (%)
Control group (-)	30.00^{f} ±1.48	--	50.00^{f} ±0.10	--	40.00^{f} ±2.00	--
Control group (+)	68.50^{a} ±2.40	1.28	110.0^{a} ±0.13	1.20	90.00^{a} ±2.23	1.25
Hepatic fibrosis rats with 2% costus roots powder	56.00^{b} ±0.14	0.87	80.50^{b} ±0.11	0.61	71.00^{b} ±2.54	0.78
Hepatic fibrosis rats with 4% costus roots powder	48.00^{c} ±0.10	0.60	73.50^{c} ±0.12	0.47	64.00^{c} ±1.58	0.60
Hepatic fibrosis rats with 200 mg/kg costus roots extract	46.50^{d} ±0.12	0.55	61.50^{d} ±0.16	0.23	56.00^{d} ±1.14	0.40
Hepatic fibrosis rats with 400 mg/kg costus roots extract	37.00^{e} ±0.13	0.23	54.00^{e} ±0.14	0.08	46.50^{e} ±1.31	0.16
LSD (P≤0.05)	**2.150**	--	**3.252**	--	**2.120**	--

Each value represents as ± standard deviation (n=3)

Means in the same column with different superscript letters are significantly different at (P≤0.05).

Effects of costus roots powder and extract on enzymes activity (GSH, SOD and CAT) level of hepatic fibrosis rats

Data in Table (3) show the effect of costus roots and extract on enzyme activity (GSH, SOD and CAT) level of hepatic fibrosis rats. It is clear to mention that the higher glutathione (GSH-Px) level was recorded for the negative control group, while the lower level was recorded for the positive control group with a significant difference (P≤0.05). The mean values were 234.00 and 121.20 Ug-1protein; respectively. The highest mean value of glutathione for treated groups (hepatic fibrosis rats) was recorded for 400 mg/kg costus roots extract while, the lowest value was recorded for 2% costus roots powder. The mean values were 214.50 and 169.50 Ug-1protein and showed a significant difference when compared with the positive control group.

In the case of SOD enzymes, results indicated that the mean value for the negative control group was significantly higher than the positive control group, which was 25.50 and 14.60 Ug-1protein; respectively. The highest mean value of SOD enzyme for treated groups (hepatic fibrosis rats) was recorded for 400 mg/kg costus roots extract while, the lowest value was recorded for 2% costus

roots powder. The mean values were 21.63 and 17.70 Ug-1protein and showed a significant difference when compared with the positive control group.

On the other hand, results of CAT enzymes showed that the mean value for the negative control group was significantly higher than the positive control group, which was 207.00 and 112.50 Ug-1protein; respectively. The highest mean value of CAT enzyme for treated groups (hepatic fibrosis rats) was recorded for 400 mg/kg costus roots extract while, the lowest value was recorded for 2% costus roots powder. The mean values were 176.20 and 164.10 Ug-1protein and showed a significant difference when compared with the positive control group.

These findings support **(Evans and Halliwell, 2001)**; findings that antioxidant defenses are made up of low molecular mass antioxidants like vitamin C and vitamin E, as well as enzymes like SOD, CAT, and GSH-px. Their function is to act as a coordinated and balanced system to protect tissues and body fluids from damage caused by ROS/RNS/RCS, whether produced physiologically or in response to inflammation infection, or disease.

To protect the body from free radical damage, catalase collaborates with superoxide dismutase. Catalases are among the most powerful enzymes found in cells, capable of converting millions of hydrogen peroxide molecules each second. SOD turns the harmful superoxide radicals into hydrogen peroxide, which is then converted to water and oxygen by catalase **(Kula *et al.*, 2000)**.

At the expense of glutathione, glutathione peroxidase (GSH-px) catalyzes the reduction of hydrogen peroxide to water and oxygen (GSH). As a result, higher GSH-px activity means more oxidized glutathione is reduced to GSH. ALA is thought to be responsible for glutathione recycling **(Joachim *et al.*, 2011)**.

On MDA, GSH, CAT, SOD, and GPx, the hepatoprotective efficacy of *Costus igneus* extract when orally supplied to rats at (150, 300, and 600 mg/kg b. wt) for 4 weeks prior to a single subcutaneous injection of CCl4 on the last day of the study period, In comparison to the control positive group, pretreatment with *Costus igneus* extract dramatically reduced MDA and

elevated GSH levels, as well as increased antioxidant enzyme activity, CAT, SOD, and GPx. These results could be attributable to the antioxidant activity of *Costus igneus* leaves, according to **El-Masry (2017)**. Increased levels of GSH have been shown to evoke a protective response against the toxic manifestations of chemicals, particularly those involving oxidative stress, according to **Jayasri *et al.*, (2009)**. In the blood and liver, *Costus igneus* considerably elevated catalase and glutathione reductase, but glutathione peroxidase was found to be lowered **(Fonseca *et al.*, 2011)**.

Table (3) Effect of costus roots powder and extract on enzymes activity level of hepatic fibrosis rats

Parameters / Groups	GSH (Ug-^{1}protein)	Change of control (%)	SOD (Ug-^{1}protein)	Change of control (%)	CAT (Ug-^{1}protein)	Change of control (%)
Control group (-)	234.0^{a} ±2.50	--	25.50^{a} ±0.05	--	207.0^{a} ±1.60	--
Control group (+)	121.20^{f} ±1.32	-0.48	14.60^{e} ±0.11	-0.43	112.5^{e} ±0.13	-0.46
Hepatic fibrosis rats with 2% costus roots powder	169.50^{e} ±1.10	-0.28	17.70^{d} ±0.03	-0.31	164.1^{d} ±0.40	-0.21
Hepatic fibrosis rats with 4% costus roots powder	185.50^{c} ±1.12	-0.21	20.00^{c} ±0.16	-0.22	172.0^{c} ±0.50	-0.17
Hepatic fibrosis rats with 200 mg/kg costus roots extract	179.50^{d} ±1.14	-0.23	18.60^{d} ±0.14	-0.27	169.9^{c} ±0.35	-0.18
Hepatic fibrosis rats with 400 mg/kg costus roots extract	214.50^{b} ±1.13	-0.08	21.63^{b} ±0.02	-0.15	176.2^{b} ±0.25	-0.15
LSD (P≤0.05)	**4.480**	**--**	**1.195**	**--**	**3.960**	**--**

Each value represents as ± standard deviation (n=3)

Means in the same column with different superscript letters are significantly different at (P≤0.05).

Effects of costus roots powder and extract on serum total cholesterol and triglycerides of hepatic fibrosis rats

The effect of costus roots powder and extract on total cholesterol and triglycerides of hepatic fibrosis rats are shown in Table (4). It is clear to notice that the total cholesterol levels of the positive control group recorded a higher value when compared with the negative control group with a significant difference at (P≤0.05). The mean values were 137.00 and 85.0 mg/dl; respectively. In the case of treated groups (hepatic fibrosis rats),

the lowest total cholesterol levels were recorded for the group fed on 400 mg/kg costus roots extract, while the highest value was recorded for 2% costus roots powder with a significant difference at (P≤0.05). The mean values were 92.0 and 120.0 mg/dl; respectively.

On the other hand, the triglyceride of the positive control group recorded a higher value when compared with the negative control group with a significant difference at (P≤0.05). The mean values were 105.0 and 64.0 mg/dl; respectively. As for treated groups (hepatic fibrosis rats), the lowest triglyceride was recorded for the group fed on 400 mg/kg costus roots extract, while the highest value was recorded for 2% costus roots powder with a significant difference at (P≤0.05). The mean values were 75.50 and 90.50 mg/dl; respectively. Changes in the levels of main lipids such as cholesterol and triacylglycerol could provide useful information on the predisposition of the heart of animals to atherosclerosis and its related coronary heart disease, according to **Yakubu *et al.*, (2008)**. The considerable decrease in triacylglycerol may be linked to impaired lipolysis, although the decrease in HDL-C at all dosages examined may not be therapeutically helpful to the animals because of the rate at which plasma cholesterol is transported to the liver will be reduced as well.

Twenty-seven rabbits were given a 2mg/kg body weight dose of *Saussurea costus* extract, which had a hypolipidemic effect. Significant reductions in serum cholesterol and triglycerides were discovered **(Madhavi *et al.*, 2012)**.

In diabetic rats, costunolide (a hypolipidemic medication derived from *Costus speciosus*) at a dose of 20 mg/kg body weight dramatically reduced plasma levels of total cholesterol, TG, and LDL **(Eliza *et al.*, 2009)**. The presence of phytochemicals, particularly flavonoids and other phenolic substances, may explain the hypocholesterolemic activity of *Costus* speciosus rhizomes **(Jha *et al.*, 2010)**, which have been reported as free radical scavengers. Indian Costus aqueous extracts could be employed as hypolipidemic and antioxidant therapies **(Hegazy *et al.*, 2020)**.

Table (4): Effect of costus roots powder and extract on total cholesterol and triglycerides of hepatic fibrosis rats

Parameters / Groups	Total cholesterol (TC) mg/dl	Change of control (%)	Triglycerides (TG) mg/dl	Change of control (%)
Control group (-)	85.00 ± 0.20f	--	64.00 ± 0.10e	--
Control group (+)	137.00 ± 1.50a	0.61	105.00 ±1.60a	0.64
Hepatic fibrosis rats with 2% costus roots powder	120.00 ± 0.10b	0.41	90.50 ± 1.30b	0.41
Hepatic fibrosis rats with 4% costus roots powder	109.00 ± 0.30c	0.28	85.00 ± 0.50c	0.33
Hepatic fibrosis rats with 200 mg/kg costus roots extract	99.00 ± 0.40d	0.16	83.00± 0.30c	0.30
Hepatic fibrosis rats with 400 mg/kg costus roots extract	92.00 ± 0.10e	0.08	75.50± 0.20d	0.18
LSD (P≤ 0.05)	**3.230**	**--**	**2.740**	**--**

Each value represents as ± standard deviation (n=3)

Means in the same column with different superscript letters are significantly different at (P≤0.05).

Data tabulated in Table (5) show the effect of costus roots powder and extract on the serum lipids profile of hepatic fibrosis rats. The results indicated that the HDL-c of the negative control rats group recorded a higher value when compared with the positive control group with a significant difference at (P≤0.05). The mean values were 48.00 and 31.50 mg/dl; respectively. In the case of treated groups (hepatic fibrosis rats), the highest HDL-c was recorded for the group fed on 400 mg/kg costus roots extract but, the lowest value was recorded for the group fed on 2.0% costus roots powder with a significant difference at (P≤0.05). The mean values were 45.00 and 37.50 mg/dl; respectively.

On the other hand, the LDL-c of the positive control rats group recorded the highest value when compared with the negative control group with a significant difference at (P≤0.05). The mean values were 84.50 and 24.20 mg/dl; respectively. As for treated groups (hepatic fibrosis rats), the highest LDL-c of the treated group was recorded for the group fed on 2% costus roots powder

but, the lowest value was recorded for the group fed on 400 mg/kg costus roots extract with a significant difference at (P≤0.05). The mean values were 64.30 and 31.90 mg/dl; respectively.

In the case of VLDL-c, the positive control rats group recorded the highest value when compared with the negative control group with a significant difference at (P≤0.05). The mean values were 21.00 and 12.80 mg/dl; respectively. While the highest VLDL-c of the treated group was recorded for the group fed on 2% costus roots powder but, the lowest value was recorded for the group fed on 400 mg/kg costus roots extract with a significant difference at (P≤0.05). The mean values were 18.10 and 15.10 mg/dl; respectively.

When compared to the control positive group, all rats poisoned by CCl4 and given Phyllanthus and costus aqueous extract at both doses showed a significant rise in (HDL-c) and a significant decrease in low and very low-density lipoprotein (LDL-c and VLDL-c) **(Naga and Bakr, 2015)**.

Our findings back up those of **Jayasri *et al.*, (2009)**, who looked at the antioxidant activity of the plant costus and found that its leaves and rhizomes had high antioxidant activity. Their findings suggested that the costus plant could be employed as a natural antioxidant source.

Table (5): Effect of costus roots powder and extract on lipids profile of hepatic fibrosis rats

Groups	Parameters					
	(HDL-c) (mg/dl)	Change of control(%)	(LDL-c) (mg/dl)	Change of control (%)	(VLDL-c) (mg/dl)	Change of control(%)
Control group (-)	48.00^{a} ±1.50	--	24.20^{f} ± 0.10	--	12.80^{d} ± 0.11	--
Control group (+)	31.50^{e} ±0.10	-0.34	84.50^{a} ± 1.55	2.49	21.00^{a} ± 1.45	0.64
Hepatic fibrosis rats with 2 % costus roots powder	37.50^{d} ±0.50	-0.22	64.30^{b} ±1.40	1.66	18.10^{b} ± 1.40	0.41
Hepatic fibrosis rats with 4 % costus roots powder	39.50^{c} ±1.20	-0.18	52.50^{c} ±1.13	1.17	17.00^{b} ± 0.30	0.33
Hepatic fibrosis rats with 200 mg/kg costus roots extract	40.40^{c} ±1.40	-0.16	42.00^{d} ±1.23	0.74	16.60^{c} ±0.20	0.30
Hepatic fibrosis rats with 400 mg/kg costus roots extract	45.00^{b} ±1.30	-0.06	31.90^{e} ±1.10	0.32	15.10^{c} ±1.10	0.18
LSD (P≤ 0.05)	**2.850**	**--**	**3.116**	**--**	**1.830**	**--**

Each value represents mean of three replicates± standard deviation. Means in the same column with different superscript letters are significantly different at P≤0.05.

Effect of costus roots powder and extract on kidney functions of hepatic fibrosis rats:

Data presented in Table (6) show the effect of costus roots powder and extract on kidney functions (urea, uric acid and creatinine) of hepatic fibrosis rats. The obtained results indicated that the urea level of the positive control rats group recorded a higher value when compared with the negative control group with significant difference (P≤0.05). The mean values were 60.00 and 27.00 mg/dl; respectively. While the highest urea level of the treated group was recorded for the group fed on 2% costus roots powder but, the lowest value was recorded for the group fed on 400 mg/kg costus roots extract with a significant difference (P≤0.05). The mean values were 50.60 and 31.50 mg/dl; respectively.

On the other hand, the uric acid level of the positive control rats group recorded a higher value when compared with the negative control group with a significant difference (P≤0.05). The mean values were 3.10 and 1.00 mg/dl; respectively. While the highest uric acid level of the treated group was recorded for the group fed on 2% costus roots powder but, the lowest value was recorded for the group fed on 400 mg/kg costus roots extract with

a significant difference (P≤0.05). The mean values were 1.80 and 1.20 mg/dl; respectively.

In the case of creatinine, the level of the positive control rats group recorded a higher value when compared with the negative control group with a significant difference (P≤0.05). The mean values were 1.75 and 0.73 mg/dl; respectively. While the highest creatinine level of the treated group was recorded for the group fed on 2% costus roots powder but, the lowest value was recorded for the group fed on 400 mg/kg costus roots extract with significant difference (P≤0.05). The mean values were 1.65 and 0.84 mg/dl; respectively. **Huang *et al.*, (2008)** found that lipid peroxidation and a reduction in antioxidant status may be implicated in the chain of events leading to methotrexate (MTX)-induced kidney injury in similar experiments. Increased serum creatinine and urea levels may also indicate renal failure and the activation of apoptotic cell markers like PARP, which also possibly contribute to MTX-caused kidney injury.

The separated components lupeol and stigmasterol in aqueous and ethanolic stem extracts of C. igneus were found to considerably reduce the increased level of calcium oxalate ions in treated albino Wistar rats. In albino Wistar rats, *C. igneus* stems extract inhibited the development of stone (CaOx crystals) caused by ethylene glycol **(Manjula *et al.*, 2012)**.

The aqueous extract of Costus spiralis has also been found to prevent the formation of urinary stones in rats with experimentally generated urolithiasis **(Viel *et al.*, 1999)**.

Antioxaluric and anticalciuric actions of lupeol, a triterpene substance derived from C. nurvala, were seen in rats suffering from hydroxyproline-induced hyperoxaluria. By regulating oxidative stress and maintaining membrane integrity, lupeol and lupeol linoleate are beneficial in relieving renal abnormalities in hyperoxaluric rats **(Sudhahar *et al.*, 2008)**.

Table (6): Effect of costus roots powder and extract on kidney functions of hepatic fibrosis rats

Parameters / Groups	Urea mg/dl	Change of control (%)	Uric acid mg/dl	Change of control (%)	Creatinine mg/dl	Change of control (%)
Control group (-)	27.00^{f} ±1.10	--	1.00^{c} ± 0.30	--	0.73 $\pm 0.10^{a}$	--
Control group (+)	60.00^{a} ±0.20	1.22	3.10^{a} ± 0.10	2.10	1.75 $\pm 0.13^{a}$	1.40
Hepatic fibrosis rats with 2 % costus roots powder	50.60^{b} ± 0.20	0.87	1.80^{b} ± 0.40	0.8	1.65 $\pm 0.11^{a}$	1.26
Hepatic fibrosis rats with 4 % costus roots powder	40.35^{c} ± 0.30	0.49	1.50^{b} ± 1.50	0.5	1.60^{a} ±0.13	1.19
Hepatic fibrosis rats with 200 mg/kg costus roots extract	34.90^{d} ± 0.50	0.29	1.45^{b} ± 1.40	0.45	0.92 ±.016a	0.26
Hepatic fibrosis rats with 400 mg/kg costus roots extract	31.50^{e} ± 0.40	0.17	1.20^{c} ± 1.30	0.20	0.84 $\pm .011^{a}$	0.15
LSD (P≤ 0.05)	**2.050**	--	**0.510**	--	**0.834**	--

Each value represents as ± standard deviation (n=3)

Means in the same column with different superscript letters are significantly different at (P≤0.05).

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التأثير الوقائي لجذور القسط الهندي على اضطرابات الكبد المستحث برابع كلوريد الكربون في الفئران

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الملخص العربى

أجريت هذه الدراسة للتحقيق في التأثير الوقائي للكبد لجذور القسط الهندي على الفئران المصابة بخلل في الكبد بواسطة رابع كلوريد الكربون حيث تم استخدام ستة وثلاثين من ذكور الفئران الألبينو التي تزن (١٤٠ ± ١٠ جرام) وقد قسمت إلى ٦ مجموعات، كل مجموعة بها (٦) فئران .تم اصابة الفئران بتليف كبدي عن طريق حقنها بواسطة رابع كلوريد الكربون (0,2 مل / ١٠٠ جم) من وزن الجسم مذاب في زيت البارافين بنسبة ٤٠ مل / لتر. وتم تقدير كلا من أنزيمات الكبد (ALT, AST and ALP) ووظائف الكلى (uric acid, urea and creatinine) والكوليسترول الكلى (TC) والجليسريدات الثلاثية (TG) وصورة دهون الدم ونشاط الانزيمات المضادة للأكسدة مثل الجلوتاثيون أوكسيديز، سوبر أوكسيد ديسميوتيز ، الكتاليز. كذلك تم التعرف على المركبات الفينولية فى جذور القسط الهندي بجهاز الكروماتوجرافى عالى الاداء. أظهرت نتائج جهاز الكروماتوجرافى الغازي عالى الأداء أن جذور القسط الهندي تحتوي على كميات عالية من المركبات النشطة بيولوجيا. كما أشارت النتائج إلى أن جذور القسط الهندي تعمل على تحسين جميع التحاليل البيوكيميائية المختبرة مثل إنزيمات الكبد في الدم، ووظائف الكلى، والإنزيمات المؤكسدة، وصورة دهون الدم في الفئران. حيث سجل المستخلص الإيثانولي 400 ملجم / كجم من جذور القسط الهندي أفضل المستويات لوقاية وتحسين انزيمات الكبد ووظائف الكلى والإنزيمات المضادة للأكسدة وصورة دهون الدم. لذلك يمكن استخدام جذور القسط في المشروبات اليومية، علاوة على أن لها العديد من الفوائد الصحية.

الكلمات الدالة: النباتات الطبية ـ التأثير الوقائي للكبد ـ المركبات النشطة كيميائيا ـ التحاليل الكيميائية الحيوية.