



Trends in
Medical Research

ISSN 1819-3587



Academic
Journals Inc.

www.academicjournals.com

Efficiency of *Cupressus sempiverens* L. and *Juniperus phoenicea* Against Carbon Tetrachloride Hepatotoxicity in Rats

¹Maha Z. Rizk, ²Mohga S. Abdallah, ²Hayat M. Sharara, ¹Sanaa A. Ali,
¹Nabaweya A. Ibrahim and ¹Magda M. Moustafa

¹Department of Medicinal Chemistry, National Research Centre,

²Department of Chemistry, Faculty of Science, Helwan University, Egypt

Abstract: The methanolic extracts of both *Cupressus sempiverens* and *Juniperus phoenicea* were investigated for their efficiency in reducing CCl₄-induced hepatotoxicity in rats. Several biochemical analyses were performed, namely, serum aminotransferases (AST and ALT), alkaline phosphatase (ALP), bilirubin (Bil), triglycerides (TG) and total cholesterol (HDL and LDL). On the other hand, liver antioxidant parameters, namely, glutathione (GSH), lipid peroxides (LP) and nitric oxide (NO) were also measured. The data obtained demonstrated that rats injected with a single toxic dose of CCl₄ and sacrificed after 24 h induced remarkable disturbances in the levels of all tested parameters. However, rats injected with the toxic agent and left for one and a half month to self recover showed moderate improvements in the serum and liver biochemical parameters. On the other hand, treatment with both extracts ameliorated the levels of the disturbed biochemical parameters, *Cupressus sempiverens* revealing a more remarkable effect. It could be concluded that the two extracts under study possess potent activities against hepatotoxicity compared to hepatic self recovery and may thus be helpful in treatment and safe recovery of liver disorders.

Key words: *Cupressus sempiverens*, *Juniperus phoenicea*, CCl₄ hepatotoxicity, liver function, antioxidants

INTRODUCTION

The liver plays an essential role in maintaining the biological equilibrium of body systems (Rajesh and Latha, 2004; Girish *et al.*, 2004). The problems associated with conventional hepatoprotective drugs in clinical trials are induced toxicity due to chronic administration of these drugs (Nursal *et al.*, 2005). Thus, it is of prime importance to develop novel hepatoprotective drugs for treatment of liver injury to replace currently used drugs of doubtful efficacy and safety and thus may reduce the risk of toxicity and maintain the therapeutic effectiveness. In this respect, there is a worldwide trend to go back to natural products in traditional medicinal plants in use for treatment of liver ailments (Shahani, 1999; Mitra *et al.*, 2000).

In the present study, we will establish the effect of two new plant extracts, namely, *Cupressus sempiverens* L. and *Juniperus phoenicea* L. not new in folk medicine but they are new in their activities on liver; in which no reports were shown in the last few years about the two extracts as hepatoprotective agents to prevent or decrease the toxicity of the liver or liver diseases in general.

Cupressus sempiverens L. Leaves and its cones play an important role in traditional medicine in which, it is used as an anti-septic, anti-rheumatic, anti-hemorrhoidal, anti-diarrheic, vasoconstrictive agents, for cough, colds, parasitic infections, inflammation and as strong hair tonic. It is used for treatment of the gastrointestinal disorders (diarrhea) and against dermatosis. The fruits of the plant are

used traditionally for curing diabetes (Said *et al.*, 2002). The pharmacological action of *Cupressus* was studied by Madar *et al.* (1995), who reported that terpenoids produced by cypress gave anti-fungal activity. In Albania, a hydro-alcoholic extract of the cones of *Cupressus sempervirens* (CSE) is used to treat a variety of disorders including hyperlipidemia in Wister rats (Karkabounas *et al.*, 2003). This plant has a highly abundant essential oil with a typical monoterpene and is considered as a radical scavenger with lipid peroxidation inhibition effects (Gianni *et al.*, 2005).

On the other hand, *Juniperus phoenicea* L. leaves were found to contain active components and owed with antiproliferative activity against a broad range of human tumors. Traditionally, juniper has been taken by mouth to treat conditions of the gastrointestinal tract, such as gas indigestion and poor appetite. Additionally, juniper has some antiseptic action that may help to eliminate gastrointestinal bacteria and parasites. Recent laboratory studies show that juniper preparations may stop or slow down the spread of some organisms that contaminate foods and it may have some effects against certain kinds of cancer, as well as juniper is also thought to slightly increase the loss of water from the body, this mild diuretic action may be useful for relieving excess water accumulation (Bayazit, 2004).

Based on the broad range of activities of both *Cupressus sempervirens* and *Juniperus phoenicea*, the present research aimed at evaluating the efficiency of the methanolic extracts of these two plants in restoring metabolic disorders induced by CCl₄ hepatotoxicity. It was previously reported that CCl₄ causes oxidative stress resulting in excessive lipid peroxidation in endoplasmic reticulum and decreased glutathione (Lee *et al.*, 2003; Shukla *et al.*, 2004), damage to plasma membrane which affects the permeabilities of mitochondria, resulting in the loss of cellular calcium sequestration and homeostasis, which can contribute heavily to subsequent cell damage (Weber *et al.*, 2003).

Acute liver injury by CCl₄ has been documented by measuring different biochemical parameters in liver and plasma (Tanaka *et al.*, 1999; David and Thomas, 2003). Serum enzyme tests, hepatic excretory tests or alterations in the chemical constituents have proved more sensitive indicators of damage (Michael *et al.*, 2000). These include disturbance in the activities of lactate dehydrogenase (LDH), aminotransferases (AST and ALT), alkaline phosphatase (ALP) and bilirubin (Weber *et al.*, 2003). Cellular leakage and loss of function and integrity of cell membrane in liver are also observed (Ilter *et al.*, 2004). Moreover, triglycerides accumulate in liver rats with CCl₄ induced chronic liver injury and cirrhosis (Ohta *et al.*, 1999). The possible curative role of these plants on the different disturbed biochemical parameters may open new areas for the use of these plants as alternative medicine in liver diseases.

MATERIALS AND METHODS

Chemicals

All chemicals used in the present study were of high analytical grade, products of Sigma (USA), Merck (Germany), BDH (England), Riedal de Ha'en (Germany), Fluka (Switzerland), Randox (United Kingdom) and Bio-diagnostic (Egypt).

The extracts of two plants were kindly supported by Pharmacognosy Department, National Research Center.

Extraction of the Powdered Leaves

The dried powdered leaves (500 g) of either *Cupressus sempervirens* L. or *Juniperus phoenicea* L. were extracted in a Soxhlet apparatus with methyl alcohol. The methanolic extract was evaporated to dryness.

The dried methanolic extract (~28 g) was dissolved in a suitable amount of hot distilled H₂O-MeOH (95: 5 v/v, 200 mL) and partitioned between ethyl acetate and methanol. Column chromatography Sephadex LH20 of the ethyl acetate and methanolic fractions was performed followed by paper chromatography.

Animals

Adult female albino rats weighting ~120 g, supplied from the animal house of National Research Center, Dokki, Egypt were used for experimental investigation. Animals were kept for two weeks to accommodate on laboratory conditions; they were kept under constant environmental and nutritional conditions given food and water all over the period of the experiment. Appropriate anaesthetic and sacrifice procedures were followed ensuring that animals did not suffer at any stage of the experiments. Anaesthetic procedures are complied according to legal ethical guidelines approved by the Ethical Committee of the Federeal Legislation and National Institute of Health Guidelines in USA. An overdose of ether was given gradually to mice and then the abdomen was opened by a mid-line incision and livers were separated.

Experimental Design

Fifty female adult albino rats were classified into five groups of ten rats each, the first untreated group served as control (Group I). The second group received a single intraperitoneal CCL₄ dose, 10 mL kg⁻¹ body weight (1:9 v/v with olive oil, 0.1 mL CCL₄ with 0.9 mL olive oil), sacrificed after 24 h and served as the cirrhotic control group (Group II) (Nishida *et al.*, 1996).

The remaining three groups were given CCL₄ as mentioned before and divided as follows:

- Group III: CCL₄-treated rats administered *Juniperus phenocia* methanolic extract (E1) (300 mg kg⁻¹ body weight) three times per weeks orally for one and half month
- Group IV: CCL₄-treated rats administered *Cupressus sempervirens* methanolic extract (E2) (300 mg kg⁻¹ body weight) three times per weeks orally for one and half month
- Group V: CCL₄ treated rats, left for one and half month to develop self recovery.

Preparation of Samples

At the end of experiment, animals of Groups I, III, IV and V were fasted for 24 h, then blood was withdrawn from sublingual vein after anesthetizing with diethyl ether. Blood was collected in a clean test tube and allowed to clot. Blood samples were centrifuged for ten minutes at 3000 rpm and then serum was separated and stored into aliquots in eppendroff tubes at -20°C to be used for biochemical analyses.

Animals were then sacrificed, liver tissue was rapidly removed and freshly used or frozen at -70°C for biochemical analyses.

Preparation of Liver Homogenate

Liver tissues were homogenized in normal physiological saline solution (0.5 N NaCl) by ratio of 1:4 W/V, the homogenate was centrifuged for 5 min at 3,000 rpm at 4°C and the supernatant was collected, stored into aliquots in eppendroff tubes and kept at -80°C. These supernatants were subjected to the following determinations:

Determination of Aminotransferases (AST and ALT) Activities in Serum

AST and ALT activities were measured according to the method of Reitman and Frankel (1957) using a diagnostic kit by monitoring the concentration of oxaloacetate or pyruvate hydrazones formed with dinitrophenyl hydrazine (DNP) in alkaline medium, using L-aspartate as substrate for AST and L-alanine as substrate for ALT. The absorbance of sample was measured against blank at wave length 540 nm.

Determination of Alkaline Phosphatase (ALP) Activity in Serum

This was an optimized standard method according to the principle of Kochmer and Moss (1976) using Randox Diagnostic kits, following the instructions of manufacturer.

Alkaline phosphatase acts upon the AMP-buffered sodium thymolphthalein mono phosphate. The addition of alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically at 590 nm against standard and blank.

Determination of Total Bilirubin in Serum

This was done according to Henry (1974) using Randox Diagnostic kit, following the instructions of manufacturer.

Bilirubin is determined by reaction with diazotised sulfanilic acid, in the presence of caffeine, which releases albumin bound bilirubin with the final production of an-azo pigment, then absorbance of sample is measured against the blank at wave length 578 nm.

Determination of Triglycerides (TG) Level in Serum

This was determined according to Fossati (1982) using Randox Diagnostic kit, following the instructions of the manufacturer. Triglycerides are determined after enzymatic hydrolysis with lipase enzyme. The indicator is a quinonemine formed from hydrogen peroxide, 4-amino phenazone and 4-chlorophenol under the catalytic influence of peroxidase. The absorbance of the sample and standard are then measured against reagent blank at wavelength 500 nm.

Determination of Total Cholesterol (TC) Level in Serum

This was done according to the procedure of Thomas (1992); using Randox diagnostic kit, following the instructions of the manufacturer. Cholesterol is determined after enzymatic hydrolysis and oxidation; the indicator quinonemine is formed from hydrogen peroxide and 4-amino antipyrine in the presence of phenol and peroxidase. The absorbance of sample and standard are measured against reagent blank at wave length 500 nm by spectrophotometer.

Determination of High and Low-Density Lipoprotein Cholesterol (HDL-C and LDL- C) Level in Serum

This was done according to the procedure of Lopes-Virella (1977); using Randox diagnostic kits (United Kingdom); following the instructions of the manufacturer. Low-density lipoproteins and very low-density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in presence of magnesium ions. After centrifugation, the cholesterol concentration in HDL (high-density lipoproteins) fraction, which remains in the supernatant, is determined as previously described.

The concentration of LDL-C in supernatant (mg dL^{-1}) is determined by the equation:

$$\text{LDL} = \text{Total cholesterol} - \text{Ttriglycerides} / 5 - \text{HDL cholesterol}$$

Determination of Liver Glutathione Content

Glutathione was estimated according to the method of Moron *et al.* (1979). For estimation of glutathione, 20% tissue homogenate was diluted to final concentration of 5% homogenate using 25% TCA and centrifuged at 300 rpm for 15 min. The supernatant was added to phosphate buffer and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and vortexed together. The developed yellow color was read against blank at 412 nm within 5 min in spectrophotometer using serial concentrations of standard GSH solution (5-100 μg).

Determination of Liver Lipid Peroxides Level

Lipid peroxides expressed as malondialdehyde were estimated by thiobarbituric acid as described by Ohkawa *et al.* (1979). A working solution was prepared by mixing one volume of solution A (saturated thiobarbituric acid) with three volumes of solution B (20% trichloroacetic acid), boiled for 20 min and centrifuged. The developed color was read against blank at 535 nm and malondialdehyde concentration was calculated using extinction coefficient value of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

Determination of Nitric Oxide Level in Liver Homogenate (NO)

Nitric oxide was estimated by method of Montgomery and Dymock (1961) using biodiagnostic kit following the instructions of the manufacturer.

In acid medium and in the presence of nitrite, the formed nitrous acid diazotise sulphanilamide and the product is coupled with N-(1-naphthyl) ethylene diamine. The resulting azodye has a bright reddish-purple color, which can be measured against standard and blank at 540 nm.

Statistical Analysis

All data obtained are expressed as the mean \pm SD. Results were analyzed by a computerized statistical program. Values were compared by one-way analysis of variance and Fisher's protected least significance difference for multiple comparisons as the post hoc test. A p-value of less than 0.0001 was considered to be statistically significant (Arkin and Colton, 1992).

RESULTS

Chemical Composition of the Methanolic Extract of *Cupressus sempervirens* L. or *Juniperus phoenicea* L.

Column chromatography followed by paper chromatography were performed for identification of the different components of the methanolic extracts of both plants and revealed the presence of five major flavonoids and two phenolic compounds with varying proportions namely, cupressuflavone, amentoflavone, myricetin, quercetin, quercitrin, ρ -coumaric acid and caffeic acid.

Serum Biochemical Parameters

The data in Table 1 demonstrate that after 24 h, CCl₄ induced a remarkable elevation in AST, ALT, ALP and bilirubin in rat serum. Left to self recover for one and a half month resulted in considerable improvement in the levels of these parameters. Treatment with both extracts caused more progressive improvements, with ALP reaching normal using *Cupressus sempervirens* L. and bilirubin normalized using both extracts. The percentage of improvement was greatest in ALT, followed by bilirubin, markers of liver damage.

Table 1: Effect of *J. phoenicea* and *C. sempervirens* leaves on the levels of AST, ALT, ALP and Total Bilimbin in different CCl₄-intoxicated rat serum

Parameters	Control G ₁	CCl ₄ (24 h) G ₂	CCl ₄ -Self Recovery G ₃	Treated groups		Improvement (%)			ANOVA
				Ext ₁ G ₄	Ext ₂ G ₅	G ₃	G ₄	G ₅	
AST	127.97 \pm 7.25 (2,3,5)	287.66 \pm 23.62 (1,3,4,5)	192.11 \pm 25.02 (1,2,4,5)	147.75 \pm 29.17 (2,3)	154.44 \pm 25.58 (1,2,3)	74.6	109.3	104.10	<0.0001
ALT	137.71 \pm 25.71 (2,3)	424.23 \pm 31.51 (1,3,4,5)	174.34 \pm 23.38 (1,2,4,5)	47.26 \pm 125.59 (2,3)	141.56 \pm 10.91 (2,3)	181.4	201.1	205.27	<0.0001
(ALP)	341.15 \pm 44.39 (2,3)	903.63 \pm 134.46 (1,3,4,5)	436.02 \pm 86.51 (1,2,4,5)	353.84 \pm 37.42 (2,3)	342.56 \pm 37.16 (2,3)	161.1	161.6	164.46	<0.0001
T.Bilirubin (T.Bili.)	0.081 \pm 0.028 (2)	0.232 \pm 0.044 (1,3,4,5)	0.095 \pm 0.024 (2)	0.081 \pm 0.023 (2)	0.082 \pm 0.025 (2)	169.1	186.4	185.19	<0.0001

Data are expressed as mean \pm SD of ten rats in each group. Values of AST, ALT and ALP are expressed as U/L and for Total bilirubin as mg dL⁻¹, p is level of significance, where p<0.0001 is significant, Analysis of data is carried out by one way (ANOVA) (analysis of variance) accompanied by post hoc (LSD) (Least Significant Difference) (SPSS Computer programme). Numbers between brackets indicate that these groups are significantly correlated, Ext₁: CCl₄+ Me-OH extract of *J. phoenicea* leaves and Ext₂: CCl₄+Me-OH extract of *C. sempervirens*

Table 2: Effect of of *J. phoenicea* and *C. sempervirens* leaves on the levels of Total cholesterol (T.Chol), Triglycerides (TG), High-density lipoprotein (HDL-Chol) and Low-density lipoprotein (LDL-Chol) in different groups

Parameters	Treated groups									
	Control G ₁	CCl ₄ (24 h) G ₂	CCl ₄ -Self Recovery G ₃	Ext ₁		Ext ₂		Improvement (%)		ANOVA
				G ₄	G ₅	G ₃	G ₄	G ₅		
Total Cholesterol (T.Chol)	140.924± 23.973 (2,3)	236.967± 34.72 (1,3,4,5)	169.38± 23.44 (1,2,5)	154.08± 11.21 (2)	143.35± 18.16 (2,3)	47.96	58.81	66.43	<0.0001	
Serum Triglycerides (TG)	107.844± 8.22 (2,3,4)	342.93± 56.73 (1,3,4,5)	178.23± 19.56 (1,2,4,5)	137.198± 20.77 (1,2,3)	119.08± 12.36 (2,3)	152.73	190.7	207.5	<0.0001	
High-density lipoprotein (HDL-chol)	18.36± 2.77 (2,3,4)	37.72± 3.17 (1,3,4,5)	27.21± 1.62 (1,2,4,5)	20.83± 2.515 (1,2,3,5)	18.496± 1.8 (2,3,4)	57.24	94.57	104.7	<0.0001	
LDL-density lipoprotein (LDL-chol)	100.998± 22.36 (2)	130.792± 38.16 (1,4,5)	112.082± 16.42 NS	105.81± 14.01 (2)	101.14± 25.12 (2)	18.53	24.74	29.36	<0.037	

Data are expressed as means±SD of ten rats in each group. Values of Cholesterol, Triglycerides, High-density lipoprotein and low-density lipoprotein are expressed as mg dL⁻¹, p is level of significance, where p<0.0001 is significant, Analysis of data is carried out by one way (ANOVA) (analysis of variance) accompanied by post hoc (LSD) (Least Significant Difference) (SPSS computer programme). Numbers between brackets indicate that these groups are significantly correlated. Ext₁ means CCl₄+Me-OH extract of *J. phoenicea* leaves and Ext₂ means CCl₄+Me-OH extract of *C. sempervirens*, NS means non significant correlation with other groups

Table 3: Effect of *J. phoenicea* and *C. sempervirens* leaves on the levels of Lipid peroxides (LPO), Glutathione (GSH) and Nitric oxide(NO) in different groups of CCl₄-intoxicated rat livers

Parameters	Treated groups									
	Control G ₁	CCl ₄ Toxicated G ₂	CCl ₄ -Self Recovery G ₃	Ext ₁		Ext ₂		Improvement (%)		ANOVA
				G ₄	G ₅	G ₃	G ₄	G ₅		
Lipid peroxide (LPO)	1.492± 0.363 (2)	4.44± 1.56 (1,3,4,5)	2.12± 0.80 (2)	1.77± 0.43 (2)	1.71± 0.79 (2)	155.49	178.95	182.97	<0.0001	
Glutathione (GSH)	31.65± 5.26 (2,3,4,5)	14.12± 2.30 (1,4,5)	17.62± 2.94 (1,4,5)	26.05± 5.43 (1,2,3)	24.72± 5.91 (1,2,3)	11.06	37.7	33.49	<0.0001	
Nitric oxide (NO)	26.36± 3.38 (2,3)	11.28± 2.44 (1,3,4,5)	20.69± 4.6 (1,2)	23.13± 4.74 (2)	23.96± 3.71 (2)	35.7	44.96	46.1	<0.0001	

Data are expressed as means±SD of ten rats in each group. Values of Lipid peroxide are expressed as μM/mg protein, glutathione expressed as μg mg⁻¹ protein and Nitric Oxide is Expressed as μm L⁻¹, p is level of significance, where p<0.0001 is significant, Analysis of data is carried out by one way (ANOVA) (analysis of variance) accompanied by post hoc (LSD) (Least Significant Difference) (SPSS computer programme). Numbers between brackets indicate that these groups are significantly correlated, Ext₁ means CCl₄+Me-OH extract of *J. phoenicea* leaves and Ext₂ means CCl₄+Me-OH extract of *C. sempervirens*

From Table 2 a moderate improvement was recorded for the self-recovered groups, while a highly significant improvement was shown for the treated groups, greatly approaching normal levels, *Cupressus sempervirens* L. showing a more normalizing effect. It should be pointed out that the highest level of improvement was recorded for triglycerides followed by high density lipoproteins while the least improvement was shown in the low density lipoprotein.

Liver Antioxidant Status

As shown in Table 3, 24 h CCl₄ intoxication caused a drastic increase in lipid peroxides, concomitant with a decline in both glutathione and nitric oxide levels. Improvement in these parameters was recorded, the test extracts revealing more progressive significant values, especially in case of lipid peroxides.

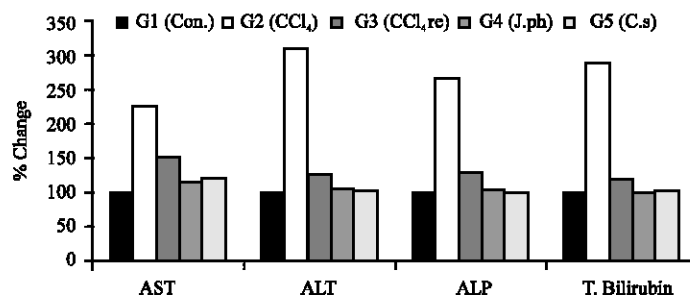


Fig. 1: Diagrammatic representation illustrating the percentage change of AST, ALT, ALP and total bilirubin in rats serum of different groups as compared to control

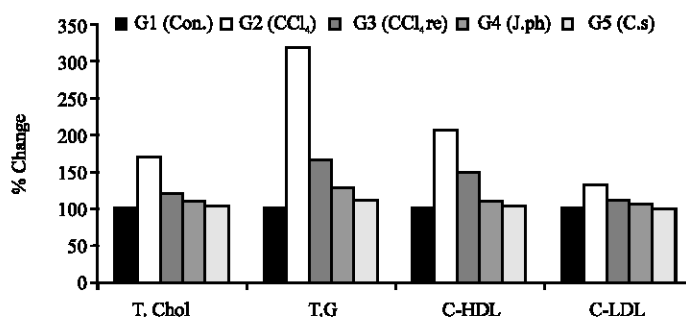


Fig. 2: Diagrammatic representation illustrating the percentage change of T. Chol, TG, C-HDL and C-LDL in rat serum of different groups as compared to control

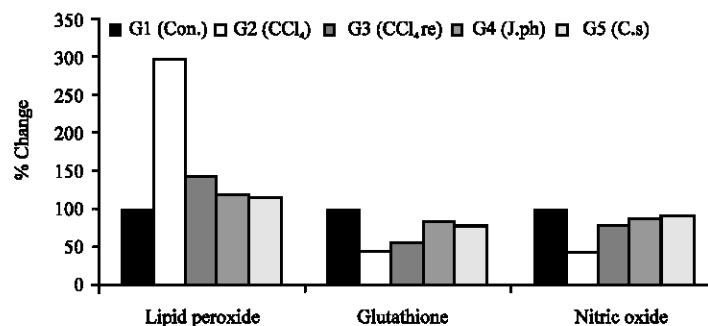


Fig. 3: Diagrammatic representation illustrating the percentage change of Lipid Peroxide, Glutathione and nitric oxide in rat serum of different groups as compared to control

Figure 1-3 shows the percentage changes between control and different treated and untreated rat groups. It is obvious that treatment with the natural extracts was more efficient in normalizing the different serum and liver biochemical parameters compared to control.

DISCUSSION

Fibrosis is the main complication of the many known chronic liver diseases (Singh *et al.*, 2005). Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequences and there is an ever-increasing need of an agent, which could protect it from such damage.

It should be pointed out that treatment of liver diseases by various synthetic drugs is costly and has side effects. An alternative to the use of chemically synthesized drugs for the treatment of liver disorders is the use of natural plant extracts. This trend has been used by traditional medical practitioners for centuries. The potency of the extracts will open new areas for the development of safe and cheap hepatoprotective drugs from natural wealth for treatment of a wide range of liver diseases (Giulia *et al.*, 1999).

In the present study rats treated with a single dose of CCl₄ developed significant hepatic damage as observed from elevated serum levels of hepatic specific enzymes as well as several alterations in different liver parameters. 24 h CCl₄-intoxicated rats showed an increase in the activities of serum ALT and AST enzymes indicating necrosis and cholestasis. The increase in these enzymes agreed with previous studies on the hepatotoxic effect of CCl₄ on liver functions (Christelle *et al.*, 2006; Ohta *et al.*, 2006).

Regarding serum ALP activity, a significant increase in its level in CCl₄-intoxicated rats was shown compared to the control value. The increase in this enzyme under the effect of CCl₄ was consistent with previous studies (Hung *et al.*, 2006). Such raised level may be due to a mechanical obstruction of bile ducts. Failure to excrete the enzyme through the relatively narrower bile passages results in its accumulation and increase of the enzyme level in plasma (Scholz *et al.*, 1989).

One of the normal functions of the liver is to excrete the breakdown product of hemoglobin, namely bilirubin, into the bile. It is well known that necrotizing agents like CCl₄ produce sufficient injury to hepatic parenchyma and cause elevation in bilirubin content in plasma (I.P.C.S., 1999). These effects were confirmed by present results since CCl₄-intoxicated rats showed an increase in serum total bilirubin level when compared to their corresponding control values.

After oral administration of Me-OH extract of *Cupressus sempervirens* and *Juniperus phoenicea* L. leaves, a pronounced improvement in AST, ALT, ALP and bilirubin was observed. This is supported by the view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (Thabrew *et al.*, 1987). Also, the results are in agreement with the previous results on the effect of herbal products to suppress the activity of ALP in the serum of rats and stabilize biliary dysfunction during chronic injury with CCl₄ (Girish *et al.*, 2004). These findings support previous studies dealing with oral administration of *Juniperus phoenicea* L leaves which showed significant anti-hepatotoxic properties (Giulia *et al.*, 1999). No previous reports to support our results are yet recorded for the anti-hepatotoxic properties of *Cupressus sempervirens* but in this study we demonstrate total protection from necrosis and cholestasis induced by CCl₄ administration mediated by *Cupressus sempervirens*.

Treatment of rats with CCl₄ causes centrilobular necrosis, which results in the accumulation of fat in liver and kidney. Fat from the peripheral adipose tissue is translocated to the liver and kidney leading to its accumulation during toxicity (Devrshi *et al.*, 1986). These findings are revealed in the present study where CCl₄-intoxicated rats showed a significant increase in the levels of serum total cholesterol, triglycerides, HDL and LDL levels. These findings are in agreement with previous reports indicating that total cholesterol and triglycerides increase in CCl₄-induced fatty liver (Torres-Duran *et al.*, 1998). This increase and accumulation of TG in liver of rats is due to impairment of TG secretion into the circulation.

Oral administration of Me-OH extract of *Juniperus phoenicea* L. or *Cupressus sempervirens* leaves caused an improvement in the levels of serum lipid parameters compared to healthy rats. These findings are supported with previous studies on the protective nature of herbal products in restoration of CCl₄-evoked changes in lipid profile of serum and tissues (Rajesh *et al.*, 2004). In a related study, Karkabounas *et al.* (2003), showed that administration of *Cupressus sempervirens* extract (CEA) induces an important lipid-lowering effect in Wistar rats.

The involvement of free radicals in the pathogenesis of liver injury has been investigated for many years in a few well-defined experimental systems (Poli, 1993). Elevated concentrations of lipid peroxides may disturb relations between protective and aggressive factors at the tissue and molecular level leading to hepatic damage (Singh *et al.*, 2005).

Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this type of damage (Venukumar and Latha, 2002).

Antioxidant action has been reported to play a crucial role in the hepatoprotective capacity of many plants, such as *Curcuma longa*, *Ganoderma formosanum*, *Solanum nigrum*, *Boehmeria nivea* and *Spirulina maxima* (Selvam *et al.*, 1995; Lin *et al.*, 1995; Sarwat *et al.*, 1995; Lin *et al.*, 1998; Torres-Duran *et al.*, 1999).

In the present study, the elevated level of liver MDA as end product of lipid peroxidation observed in CCl₄-treated rats indicates excessive formation of free radicals and activation of LPO system resulting in hepatic damage. These findings are in accordance with previous studies on the effect of CCl₄ in increasing the level of lipid peroxides (Hung *et al.*, 2006; Ohta *et al.*, 2006).

The significant decline in the concentration of the level of LPO in the liver tissue of CCl₄ + Me-OH extract of *Juniperus phoenicea* L. or *Cupressus sempervirens* administered rats indicates an anti-lipid peroxidative effect of the two extracts. These results prove the previous studies on *Cupressus sempervirens* that it possesses an essential oil with a typical monoterpene with effective radical scavenging activities, also this oil has a lipid peroxidation inhibition effect (Gianni *et al.*, 2005). On the other hand, *Juniperus phoenicea* L. has antioxidant properties due to its content of flavonoid and phenolic compounds (Ibrahim and Risk, 2005).

Glutathione is one of the endogenous antioxidants of nonenzymatic molecules (Nursal *et al.*, 2005) and its depletion in mammalian cells causes cell damage, ultimately.

Results on glutathione content CCl₄-intoxicated liver rats revealed a highly significant reduction. In agreement with these data, several authors reported that oxidative stress causes a decrease in glutathione content (Venukumar and Lata, 2002; Hung *et al.*, 2006; Ohta *et al.*, 2006). The reduced activity of this primary defensive enzyme could be due to a direct and greater involvement of ROS in the pathogenesis of cirrhosis of liver disturbing the pro-oxidant vs. antioxidant ratio, thereby precipitating in the hepatic damage (Singh *et al.*, 2005).

Treatment of cirrhotic rats with both natural extracts caused a significant elevation in the level of GSH although *Juniperus phoenicea* L. showed a more remarkable improvement than *Cupressus sempervirens*. These improvements with both natural extracts revealed the antioxidant effect of *Juniperus phoenicea* L. and *Cupressus sempervirens* (Ibrahim and Risk, 2005; Gianni *et al.*, 2005). Explanations of the possible mechanism underlying the hepatoprotective properties of medicinal plants include the prevention of GSH depletion and destruction of free radical (Rizk, 1998).

Although it was previously reported that NO increases with hepatic injury (Chen *et al.*, 2004), data obtained in the present study reveal a remarkable decrease in its level following CCl₄ administration. Wen and Fung (2000) reported that NO in liver tissue decreases significantly with increasing amount of CCl₄ used and in time course after a fixed (0.1 mL kg⁻¹) dosage of CCl₄. This reduction may be due to one or both of these two reasons (i) consumption in terminating lipid peroxidation (ii) depletion of co-substrate NADPH caused by interaction with CCl₄. In addition, as toxicity is a complex process involving a variety of cell types and many soluble mediators, the contribution of each of these factors must be taken into account when considering the role of nitric oxide as a determinant of tissue injury (Laskin *et al.*, 2001). Furthermore, Muriel (1998) stated that NO protects the liver against oxidative injury and that its inhibition is parallel with increase in lipid peroxidation and other markers of liver injury.

Treatment of cirrhotic rats with both natural extracts caused a significant elevation in the level of NO although *Cupressus sempervirens* showed a more remarkable improvement than *Juniperus phoenicea* L. These improvements with both natural extracts revealed antioxidant effect of *Juniperus phoenicea* L. and *Cupressus sempervirens* (Gianni *et al.*, 2005). The NO signaling pathway has, in recent years, become a target for new drug development. The high level of flavonoids, catechins, tannins and other polyphenolic compounds present in herbal products is believed to contribute to their beneficial health effects. Some of these compounds induce NO formation from the endothelial cells to improve circulation and some suppress the induction of inducible NOS in inflammation and infection. (Achike and Kwan, 2003).

On the other hand, when cirrhotic rats were kept for one and a half month without any treatment, there is a significant decrease in the activities of AST, ALT and ALP enzymes, in serum bilirubin, cholesterol and triglycerides. Also hepatic lipid peroxides were declined and glutathione elevated. These findings are in agreement with previous reports about the reversibility of cirrhosis or fibrosis upon discontinuation of CCl₄ (Iredale *et al.*, 1998). But metabolic activities were not regained as when treated with *Juniperus phoenicea* or *Cupressus sempervirens*. This indicates that it is preferred to start early treatment rather than left to the defense system in our body to fight this disease.

Previous studies were also reported on the possibility of the herbal products to stabilize rat liver dysfunction during chronic injury with CCl₄ and ameliorate biochemical levels (Rajesh and Latha, 2004).

It could be concluded that this research study has highlighted that both *Juniperus phoenicea* or *Cupressus sempervirens* can be used efficiently for reducing severe liver disorders due to their potent antioxidant and antihepatotoxic activities. The use of these plants is coincident with the global current approach dealing with the use of natural wealth as an alternative tool for disease cure rather than the classical synthetic drugs. Meanwhile studies are in progress to cover the efficiency of both plants against renal toxicity as well as their effect on histological and histopathological analyses.

REFERENCES

- Achike, F.I. and C.Y. Kwan, 2003. Nitric oxide, human diseases and the herbal products that affect the nitric oxide signaling pathway. *Clin. Exp. Pharmacol. Physiol.*, 30: 605-615.
- Arkin, H. and R.R. Colton, 1992. *Table for Statisticians*. 2nd Edn., Barnes and Noble, Inc. New York USA., pp: 115.
- Bayazit, V., 2004. Cytotoxic effects of some animal and vegetable extracts and some chemicals on liver and colon carcinoma and myosarcoma. *Saudi Med. J.*, 25: 1329-1332.
- Chen, J.H., G.L. Tipoe, E.C. Liong, H.S. So, K.M. Leung, W.M. Tom, P.C. Fung and AA. Nanji, 2004. Green tea polyphenols prevent toxin-induced hepatotoxicity in mice by down-regulating inducible nitric oxide-derived prooxidants. *Am. J. Clin. Nutr.*, 80: 742-751.
- Christelle, G., L. S'ebastien, C. Chantal, D. Evelyne, B. Paulette, B. Charles and Alexis, 2006. Hepatic fibrosis and cirrhosis: The (myo) fibroblastic cell subpopulations involved. *Intl. J. Biochem. Cell Biol.*, 38: 135-151.
- David, Z. and D.B. Thomas, 2003. *Hepatology: A Textbook of Liver Disease*. 4th Edn., David Zakim, (Ed.), Philadelphia: Saunders.
- Devrshi, P., A. Kanase, R. Kanase, S. Mane, S. Patil and A.T. Varuthe, 1986. Effect of mandurbhasma on lipolytic activities of liver, kidney and adipose tissue of albino rat during CCl₄-induced hepatic injury. *Bioscience*, 10: 227-234.
- Fossati, P., 1982. Colorimetric determination of triglycerides. *Principle Clin. Chem.*, 28: 2077.
- Gianni, S., M. Silvia, M. Marivaittoria, S. Martina, M. Stefano, R. Matteo and B. Renato, 2005. Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals, antimicrobial in foods. *J. Food Chem.*, 91: 621-632.
- Girish, S., G. Achliya Sudhir, K. Wadodkar Aviash and Dorle, 2004. Evaluation of hepatoprotective effect of *Amalkadi ghrita* against carbon tetrachloride-induced hepatic damage in rats. *J. Ethnopharmacol.*, 90: 229-232.
- Guilia, DC., M. Nicola, A. Angelo, Izzo and C. Francesco, 1999. Flavonoids: Old and new aspects of class of natural therapeutic drugs. *Life Sci.*, 65: 337-353.
- Heury, R.J., 1974. *Clinical Chemistry Principles and Techniques*. Harper, H. and K. Row (Eds.), 2nd Edn., Academic Press N.Y., pp: 545.
- Hung, M.Y., T.Y. Fu, P.H. Shih, C.P. Lee and G.C. Yen, 2006. Du-Zhong (*Eucommia ulmoides* Oliv.) leaves inhibit CCl₄-induced hepatic damage in rats. *Food Chem. Toxicol.*, 44: 1424-31. Epub.

- IPCS, 1999. Environmental health criteria (208). Carbon tetrachloride. World Health Organization, Geneva, pp: 50.
- Ibrahim, N.A. and M. Risk, 2005. Phytochemical investigation and hepatoprotective activity of *Juniperus phoenicea* leaves growing in Egypt. Bull. Fac. Pharm. Cairo Univ., 43: 175-183.
- Ilter, K., C. Neriman, P. Hidir, S. Dilara, O. Murat and S. Mustafa, 2004. Protective effects of caffeic acid phenethyl ester (CAPE) on carbon tetrachloride-induced hepatotoxicity in rats. Acta Histochemical., 106: 289-297.
- Iredale, J.P., R.C. Benyon, J. Pickering, M. McCullen, M. Northrop and S. Pawley, 1998. Mechanisms of spontaneous resolution of rat liver fibrosis. Hepatic stellate cell apoptosis and reduced hepatic expression of metalloproteinase inhibitors. J. Clin. Investigat., 102: 538-549.
- Karkabounas, S., D. Kiortsis, J. Zelovitis, P. Skafida, C. Demetzos, M. Malamas, M. Elisaf and A. Evangelou, 2003. Effects of *Cupressus sempervirens* cone extract on lipid profile in Wistar rats. J. Atherosclerosis Supplements, 3: 137-138.
- Kochmar, J.F. and D.W. Moss, 1976. Fundamentals of Clinical Chemistry. Tietz, N.W. (Ed.), W.B. Saunders and Company, Philadelphia, PA: pp: 604.
- Laskin, J.D., D.E. Heck, C.R. Gardner and D.L. Laskin, 2001. Prooxidant and antioxidant functions of nitric oxide in liver toxicity. Antioxid. Redox Signal, 3: 261-271.
- Lee, T.Y., G.J. Wang, J.H. Chiu and H.C. Lin, 2003. Long-term administration of *Salvia miltiorrhiza* ameliorates carbon tetrachloride-induced hepatic fibrosis in rats. J. Pharm. Pharmacol., 55: 1561-1568.
- Lin, C.C., Ming-Hong Yen, Tsae-Shiuan Lo and Jer-Min Lin, 1998. Evaluation of the *nivea* var. *tenacissima*. J. hepatoprotective and antioxidant activity of *Boehmeria nivea* var. *nivea* and *B. Ethnopharmacology*, 60: 9-17.
- Lin, J.M., C.C. Lin, M.F. Chen, T. Ujiie and A. Takada, 1995. Radical scavenger and antihepatotoxic activity of *Ganoderma formosanum*, *Ganoderma lucidum* and *Ganoderma neo japonicum*. J. Ethnopharmacol., 47: 33-41.
- Lopes-Virella, M.F., 1977. Colorimetric determination of low and high density lipoproteins. Clin. Chem., 23: 882.
- Madar, Z., E. Hugo, C. Miriam, R. Joseph, S. Zvi and S. Abraham, 1995. Antifungal terpenoids produced by Cypress after infection by *Diplodia pinea* f. sp. *Cupressi*. J. Phytochem., 38: 351-354.
- Michael, I., L.P. Petia, M.S. Randle, M.G. Joanna and M.B. Yucesoy, 2000. Immunotoxicology: Role of inflammation in chemical-induced hepatotoxicity. Intl. J. Immunopharmacol., 22: 1143-1147.
- Mitra, S.K., S.J. Seshadri, M.V. Venkataranganna, S. Gopumadhavan, U. Venkatesh Udupa and D.N.K. Sarma, 2000. Effect of HD-03-a herbal formulation in galactosamine-induced hepatopathy in rats. Indian J. Physiol. Pharmacol., 44: 82-86.
- Montgomery, H.A.C. and J.F. Dymock, 1961. Colorimetric determination on nitric oxide. Analyst, 86: 414.
- Moron, M.S., J. Depierre and B. Mannervik, 1979. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochem. Biophys. Acta, 582: 67-78.
- Muriel, P., 1998. No protection of rat liver from lipid peroxidation, collagen accumulation and liver damage induced by carbon tetrachloride. Biochem. Pharmacol., 56: 773-779.
- Nishida, K., Y. Ohta, M. Kongo and I. Ishiguro, 1996. Response of endogenous reduced glutathione through hepatic glutathione redoxcycle to enhancement of hepatic lipid peroxidation with the development of acute liver injury in mice intoxicated with carbon tetrachloride. Res. Commun. Mol. Pathol. Pharmacol., 93: 198-218.
- Nursal, G., K. Levent, O. Xehirli, E. Feriha S.V. Serap, K. Meral and M. Goksel, 2005. Long-term administration of aqueous garlic extract (AGE) alleviates liver fibrosis and oxidative damage induced by biliary obstruction in rats. Life Sci., 76: 2593-2606.
- Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.

- Ohta, Y., D. Sahashi, E. Sasaki and I. Ishiguro, 1999. Alleviation of carbon tetrachloride-induced chronic liver injury and related dysfunction by L-tryptophan in rats. *Ann. Clin. Biochem.*, 36: 504-510.
- Ohta, Y., Y. Imai, T. Matura, A. Kitagawa and K. Yamada, 2006. Preventive effect of neutropenia on carbon tetrachloride-induced hepatotoxicity in rats. *J. Applied Toxicol.*, 26: 178-186.
- Poli, G., 1993. Liver Damage due to free radicals. *Br. Med. Bull.*, 49: 604-620.
- Rajesh, M.G. and M.S. Latha, 2004. Preliminary evaluation of the antihepatotoxic activity of Kamilari, a polyherbal formulation. *J. Ethnopharmacol.*, 91: 99-104.
- Reitman, S. and S. Frankel, 1957. A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.*, 28: 56-63.
- Rizk, M., 1998. Protective effect of *Curcuma longa* against oxidative stress in *Schistosoma mansoni* infected mice livers. *Egypt. J. Bilh.*, 21: 1-8.
- Said, O., S. Khalil and H. Fulder, 2002. Ethnopharmacological survey of medicinal plants in Israel, the Golan heights and the West Bank region. *J. Ethnopharmacol.*, 83: 251-265.
- Sarwat, S., P. Shahid, I. Mohammed and A. Mohammed, 1995. Crude extracts of hepatoprotective plants, *Solanum nigrum* and *Cichorium intybus* inhibit free radical-mediated DNA Damage. *J. Ethnopharmacol.*, 45: 189-192.
- Scholz, R., K. Graham, E. Gumprecht and C. Redely, 1989. Mechanism of interaction of vit. E and glutathione in the protection against membrane lipid peroxidation. *Ann. N.Y. Acad. Sci.*, 570: 514.
- Selvam, R., S. Lalitha, R. Gayathri and N. Angayarkanni, 1995. The antioxidant activity of turmeric (*Curcuma longa*). *J. Ethnopharmacol.*, 47: 59-67.
- Shahani, S., 1999. Evaluation of hepatoprotective efficacy of APCL-A polyherbal formulation *in vivo* in rats. *Indian Drugs*, 36: 628-631.
- Shnkla, S., M. Bhadauria and A. Jadon, 2004. Effect of propolis extract on acute carbon tetrachloride induced hepatotoxicity. *Indian J. Exp. Biol.*, 42: 993-997.
- Singh, B., A.K. Saxena, K.B. Chandan, K.K. Anand, O.P. Suri, R. Suri, Singh, R.K. Singh, A.K. Tripathi, G. Cornelissen, O. Schwartzkopff, K. Otsuka and F. Halberg, 2005. Chronomics of circulating plasma lipid peroxides and anti-oxidant enzymes and other related molecules in cirrhosis of liver. *Biomed. Pharmacother.*, 59: 229-235.
- Tanaka, N., K. Tanaka, Y. Nagashima, M. Kondo and H. Sekihara, 1999. Nitric oxide increases arterial blood flow in rats with carbon tetrachloride-induced acute hepatic injury. *Gastroenterology*, 117: 173-180.
- Thabrew, M.I., P.D.T.M. Joice and W. Rajatissa, 1987. A comparative study of the efficacy of *Pavetta indica* and *Osbekia octandra* in the treatment of liver dysfunction. *Planta Medica*, 53: 239-241.
- Thomass, L., 1992. Colorimetric Determination of Tcholesterol. *Labor and Diagnosis: Clinical Chemistry*. Thomas, L. (Ed.), Frankfurt: TH Books Verlagsgesellschaft, 5th Edn., pp: 327.
- Torres-Duran, P.V., R. Miranda-Zamora, M.C. Paredes-Carbajal, D. Mascher, J. BléCastillo, J.C. Da'zZagoya and M.A. Juarez-oropeza, 1998. *Spirulina maxima* prevents induction of fatty liver by carbon tetrachloride in the rat. *Biochem. Mol. Biol. Intl.*, 44: 787-793.
- Torres-Duran, P.V., Z. Miranda and M.C. Paredes-Carbajal, 1999. Studies on the preventive effect of *Spirulina maxima* on fatty liver development induced by carbon tetrachloride in the rat. *J. Ethnopharmacol.*, 64: 141-147.
- Venukumar, M.R. and M.S. Latha, 2002. Antioxidant Activity of *Curculigo Orchioides* in carbon tetrachloride induced hepatopathy in rats. *Indian J. Clin. Biochem.*, 17: 80-87.
- Weber, L.W., M. Boll and A. Stampfl, 2003. Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. *Crit. Rev. Toxicol.*, 33: 105-136.
- Wen, Z. and P.G.W. Fung, 2000. The roles played by crucial Free Radicals like lipid free radicals, Nitric oxide and enzymes NOS and NADPH in CCl₄-induced acute liver injury of mice. *Free Radic. Biol. Med.*, 29: 870-880.