Potential Hepatoprotective Effects of Licorice Root (*Radix glycyrrhizae*) Extract against Carbon Tetrachloride-Induced Hepatotoxicity in Isolated Rat Hepatocytes

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Abstract: *Radix glycyrrhizae* is one of the native Mediterranean plants. Licorice root is a popular soft drink in Egypt. Literatures cited therapeutic effects of licorice. The present work is to evaluate the potential hepatoprotective effects of aqueous licorice root extract against the cytotoxic effects and the oxidative stress induced by carbon tetrachloride (CCl_4) in isolated primary rat hepatocytes. Hepatocytes were isolated by collagenase perfusion technique. Cytotoxicity was determined by assessing cell viability and leakage of cytosolic enzymes, such as lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Oxidative stress was assessed by determining reduced glutathione (GSH) level and lipid peroxidation as indicated by thiobarbituric acid reactive substances (TBARS) production. Exposure of isolated rat hepatocytes to CCl_4 (5mM) caused cytotoxicity and oxidative injury, manifested by loss of cell viability and significant increase in LDH, ALT and AST leakages. As well as, CCl_4 caused progressive depletion of intracellular GSH content and significant enhancement of TBARS accumulation. Preincubation of hepatocytes with either licorice (25 μM/ml) or silymarin (0.5mM) which is a known hepatoprotective agent, ameliorated the hepatotoxicity and oxidative stress induced by CCl_4, as indicated by significant improvement in cell viability, significant decrease in LDH , ALT and AST leakages, significant prevention of GSH depletion and significant decrease in TBARS formation as compared to CCl_4 alone-treated cells. The present results indicate that CCl_4 has a potential cytotoxic effect in isolated rat hepatocytes; and licorice extract possess a highly promising hepatoprotective effects against CCl_4-induced hepatotoxicity.
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1.Introduction

Liver is the key organ of metabolism and excretion. It is often exposed to a variety xenobiotics and therapeutic agents. Until today, people have not yet found an actual curative therapeutic agent for liver disorder.In fact; most of the available remedies help the healing or regeneration of the liver **(Huo *et al.*, 2011).**

Large number of xenobiotics is reported to be potentially hepatotoxic. Free radicals generated from the xenobiotic metabolism can induce lesions of the liver and react with the basic cellular constituents such as proteins, lipids, RNA and DNA **(Ajith *et al.*, 2007)**. CCL_4 is a potent environmental hepatotoxin, has been served as a model compound for study of hepatotoxicity and the cellular mechanisms behind oxidative damage and further was used to evaluate the therapeutic potential of drugs and dietary antioxidants **(Prasenjit *et al*., 2006)**.

Nowadays, many investigators have been focused for searching for the best approach in treatment of liver diseases using the effective herbal preparations. Natural products are gaining a revitalized attention in medical community and their therapeutic uses are gradually increasing. As many synthetic drugs have revealed serious side effects. Therefore, a better strategy is to look for natural substances with strong pharmacological action and less cytotoxicity. In the last few years much attention was directed to the potential health promoting properties of phenolic phytochemicals **(Kartal, 2007).**

Licorice has been used as a medicinal plant for thousands of years. The active component of licorice, glycyrrhizic acid, is hydrolyzed *in vivo* to glycyrrhetinic acid, which is responsible for most of its pharmacological properties. In ancient Chinese medicine and during Roman times, licorice was also recommended to cure sterility in women **(Davis and Morris, 1991; Armanini *et al.*, 2002)**.Derivatives of licorice root have been used in Asia to treat children with biliary atresia , a cholestatic liver disease **(Sokol *et al.*, 2003).** Licorice has held claim for therapeutic use for fevers, liver ailments, dyspepsia, gastric ulcers, sore throats, asthma, bronchitis, Addison's disease and rheumatoid arthritis and has been used as a laxative, antitussive and expectorant **(Schulz *et al.*, 1998; Wang *et al.*, 2000)**. Among its most consistent

uses are as a demulcent for the digestive system, to treat coughs, to soothe sore throats, and as a flavoring agent.

The present study was undertaken to evaluate the hepatoprotective effects of licorice aqueous extract against cytotoxicity and oxidative stress induced by carbon tetrachloride in isolated primary rat hepatocytes.

2.Materials and methods

Animals and Chemicals

Animals:

Male Sprague - Dawley rats of locally bred strains (225 - 250 g) were supplied from faculty of Veterinary Medicine, Cairo University. They were kept under good ventilation and standard hygienic conditions and allowed free access to balanced standard laboratory chow (El-Nasr Co., Abo-Zaable, Egypt) and tap water *ad libitum*.

Isolation of Hepatocytes

The hepatocytes were isolated by a collagenase two-step perfusion technique **(Berry and Friend, 1969)** with slight modifications as published by **El-Tawil and Abdel-Rahman (1997)**. The rat was anaesthetized with 100 mg ketamine/ kg, restrained, and an incision was made in the abdominal cavity to expose the portal vein. A polyethylene cannula was inserted into the portal vein and the liver was perfused in situ for 8 min with calcium-free Hank's bicarbonate buffer maintained at 37oC. The liver was then mechanically dislocated from the abdomen with the cannula in place and recirculated for 10 min in collagenase (0.67 mg/ ml) containing 5 mM calcium chloride. The isolated liver cells were filtered through four layers of cotton gauze and centrifuged for 2 min at 600 rpm. The cells were washed twice and suspended in HEPES-bicarbonate buffer (pH 7.4) containing 0.5 % bovine albumin. The isolated hepatocytes were counted in a hemocytometer, while the viability of the cells was assessed by 0.4% Trypan Blue in Krebs Hanseliet buffer without albumin **(Baur *et al.*, 1985)**. Each freshly prepared cell suspension had 90% or greater viability prior to each experiment.

Incubation and treatment of hepatocytes

Freshly isolated hepatocytes (5 X 10^6 cells/ ml) were suspended in a HEPES-bicarbonate buffer (pH 7.4) and incubated at 37oC in a shaking water bath at 30 oscillations per minute. Hepatocytes were incubated in plastic vials equipped with covers and were used for determination of CCL_4 cytotoxicity and the possible protection with Licorice extract and compared with silymarin as a known hepatoprotective agent at different incubation time intervals (30, 60, 120 min).

The concentrations of CCL_4, Licorice extract and silymarin in the incubation medium were adjusted to reach a final concentration of 5mM CCL_4 **(Dvorak *et al.*, 2003)**, 25 µM Licorice **(Gumpricht *et al.*, 2005)** and 0.5mM silymarin (**Farghali *et al.*, 2000**). Twelve replicates were used for each group. Cytotoxicity was determined by assessing of cell viability using trypan blue exclusion method, cytosolic enzymes leakage percent [lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST)], GSH content and thiobarbituric acid reactive substances (TBARS) accumulation. Control replicates were carried out simultaneously under the same conditions and at the same time intervals.

Sample preparation for enzyme leakage:

Enzymes activity (ALT), (AST) and (LDH) was monitored using Sigma–Aldrich ready made kits (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) in an aliquot of cell-free medium and compared to the total activity achieved after lysis of the cells **(Moldeus *et al.*, 1978)**. The cell-free medium was obtained by centrifugation of the aliquots at 2200 rpm's for 15 min. Lysate was obtained by addition of 1% triton X-100 and shaking for 15 min followed by centrifugation at 2200 rpm's. The leakage was expressed as percentage of total lysate activity at each time point.

Glutathione (GSH) assay

Reduced GSH levels in hepatocytes were determined by measuring total soluble-reduced sulfhydryl content. Aliquots were collected at specified time points and centrifuged with phosphate buffer saline (PBS) at 3000 g for 5 min. The obtained precipitate was mixed with 0.7 ml of 0.2% triton X-100 and 2.5% sulfosalicylic acid. Solutions were centrifuged at 3000 g for 5 min. A 0.5 ml aliquot of the acid-soluble supernatant medium was then added to 1.0 ml of 0.3 M Na_2HPO_4 solution. Spectrophotometric determinations were performed at 412 nm immediately after the addition of 0.125 ml of 5,5`-dithiobis-(2-nitrobenzoic acid) **(Beutler *et al.*, 1963).**

Lipid peroxidation assay

Lipid peroxidation was assessed by determining thiobarbituric acid reactive substances (TBARS) in hepatocyte culture media by the method of **Uchiyama and Mihara (1978).**

Data Analysis

The GRAPHPAD (ISI Software, Philadelphia, PA, USA) computer program was used to conduct regression analysis and to plot collected data. Data were expressed as means ± standard error of means (S.E). Assessment of the results was performed using one-way analysis of variance (ANOVA) procedure followed by Tukey-Kramer multiple comparison

post-tests. Statistical analyses were performed using Software GRAPHPAD INSTAT (Version 2). The 0.05 level of probability was used as the criterion for significance.

3. Results

Effects of Carbon tetrachloride (CCL_4) and Licorice on viability of isolated rat hepatocytes

Cell survival was assessed by trypan blue exclusion method after exposing isolated rat hepatocytes to CCL_4 (5mM), licorice extract (25 µM/ml), and silymarin (0.5mM). A significant progressive time dependent decrease in cell viability was observed as early as after exposure to $CC1_4$ compared to control cells. Prior incubation of hepatocytes with licorice (25 µM/ml) offered a significant protection against CCL_4. A marked protection was shown after 30, 60, and 120 min. On the other hand, concomitant incubation of the cells with silymarin and CCL_4 inhibited the decrease in the cell viability caused by CCL_4 alone (Fig.1).

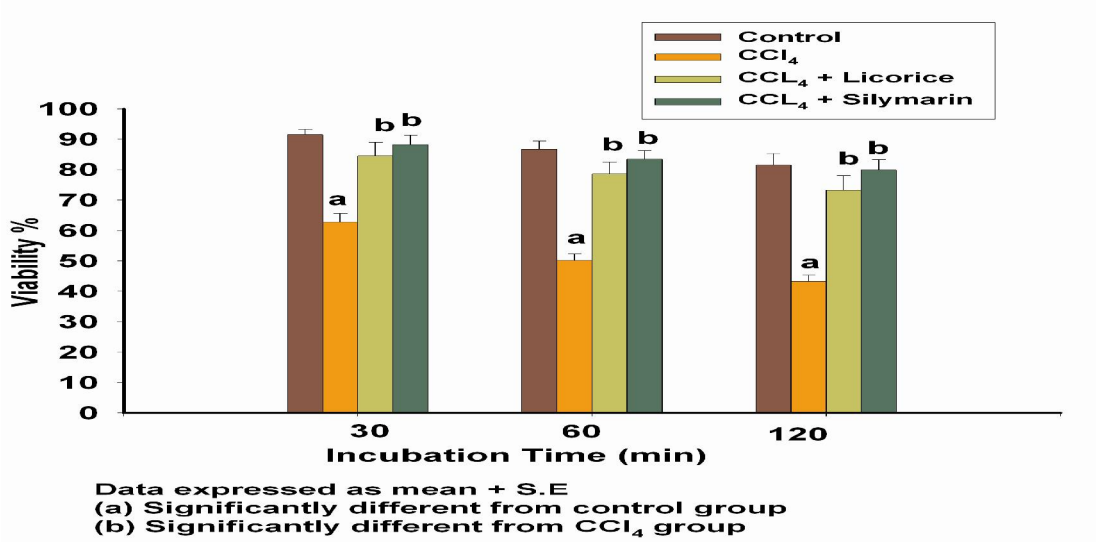

Fig (1): Effects of CCL_4 and Licorice Extract on Viability % of isolated rat hepatocytes

Effects of Carbon tetrachloride (CCL_4) and Licorice on the LDH, ALT and AST leakage% of isolated rat hepatocytes

Plasma membrane damage was assessed by monitoring LDH, ALT and AST enzyme leakages from hepatocytes exposed to CCL_4, licorice (25 µM/ml) and silymarin. Figure (2) demonstrates the time course of LDH leakage in the perfusion medium of hepatocytes treated with CCL_4 alone and those pretreated with, licorice extract or silymarin followed by CCL_4. Exposure of hepatocytes to CCL_4 resulted in a significant increase in the leakage of LDH enzyme into the culture medium as early as 30 min of incubation. Pretreatment of hepatocytes with licorice extract (25 µM/ml) or silymarin (0.5mM) ameliorated the effects of CCL_4 on LDH enzyme leakage

Figure (3) demonstrates the effect of CCL_4, licorice extract (25 µM/ml) and silymarin on ALT leakage of isolated hepatocytes. $CC1_4$ caused time dependent significant increase in the leakage of ALT in comparison to control. Pre-incubation of isolated hepatocytes with licorice (25 µM/ml) or silymarin decreased the ALT leakage after 60 and 120 min as compared to CCL_4 treated cells.

The time course of AST leakage from isolated hepatocytes is demonstrated in (Fig.4). CCL_4 caused significant time dependent increase in AST leakage in comparison to control. This increase of AST leakage% was elevated by time after 30, 60 min, and showed a significant increase at 120 min as compared to control group. Pretreatment with licorice (25 µM/ml) for 30 min before CCL_4 addition induced a significant protection against CCL_4-induced AST leakage after 60 and 120 min as compared to CCL_4 group. Also, pretreatment with silymarin showed protective effect similar to licorice extract as compared to CCL_4 group.

Effects of Carbon tetrachloride (CCL_4) and Licorice on reduced glutathione (GSH) depletion of isolated rat hepatocytes

Assessment of oxidative stress-induced by CCL_4 in isolated hepatocytes was done by measuring cellular GSH level. Figure (5) depicts the time-course effects of CCL_4 on hepatocytes glutathione content and its possible protection by either licorice extract or silymarin. CCL_4 caused significant depletion of glutathione content from isolated rat hepatocytes compared to control during the 2-h incubation period. Concomitant incubation of cells with silymarin and

CCL_4 or licorice extract and CCL_4 prevented the depletion of glutathione induced by CCL_4 exposure alone. The protection against glutathione depletion by silymarin or licorice extract in the presence of CCL_4 was almost the same at the incubation time studied.

Effects of Carbon tetrachloride (CCL_4) and Licorice on lipid peroxide formation of isolated rat hepatocytes

Assessment of oxidative stress-induced by CCL_4 in isolated hepatocytes was also demonstrated by measuring lipid peroxidation. The effect of CCL_4, licorice extract and silymarin on lipid peroxidation, as indicated by TBARS formation, was estimated (Fig.6) The hepatotoxin CCL_4 (5mM) induced a significant increase in the level of lipid peroxidation starting at 30 min after addition of CCL_4 as indicated by elevation in TBARS level. CCL_4 treated group showed a significant increase in TBARS level within the incubation period as compared to the control group. Both licorice extract and silymarin significantly decreased the TBARS formation induced by CCL_4. This protective effect was a time dependent, reached the maximum at 120 min as compared to CCL_4 group.

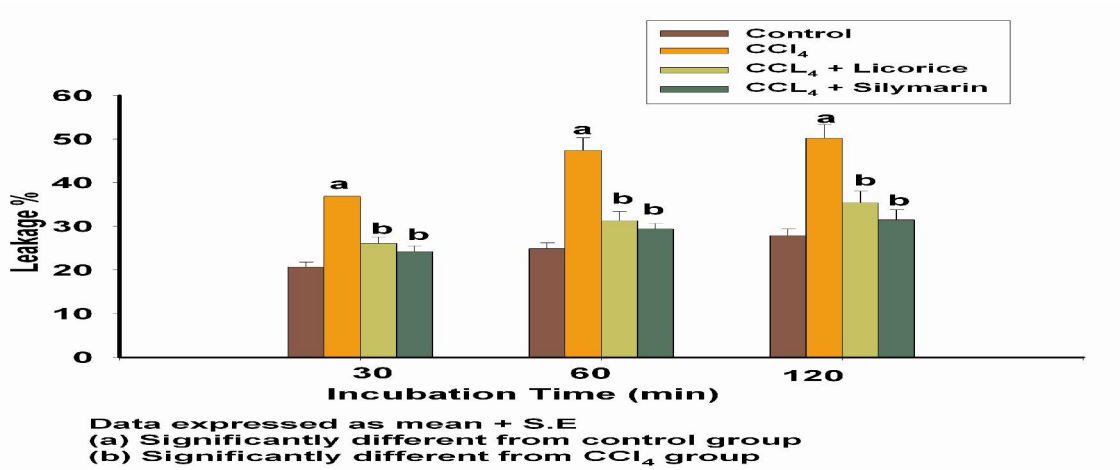

Fig (2): Effects of CCL_4 and Licorice Extract on LDH Leakage% of isolated rat hepatocytes

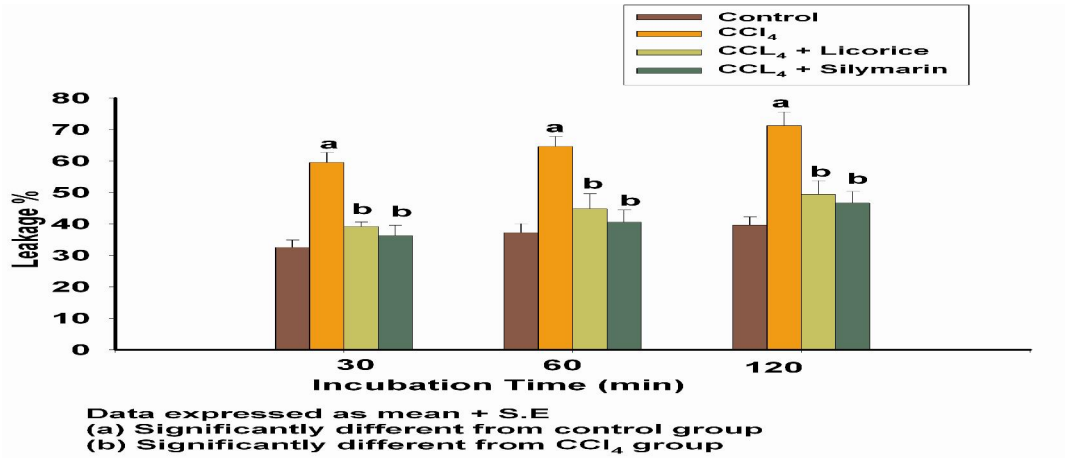

Fig (3): Effects of CCL_4 and Licorice Extract on ALT Leakage% of isolated rat hepatocytes

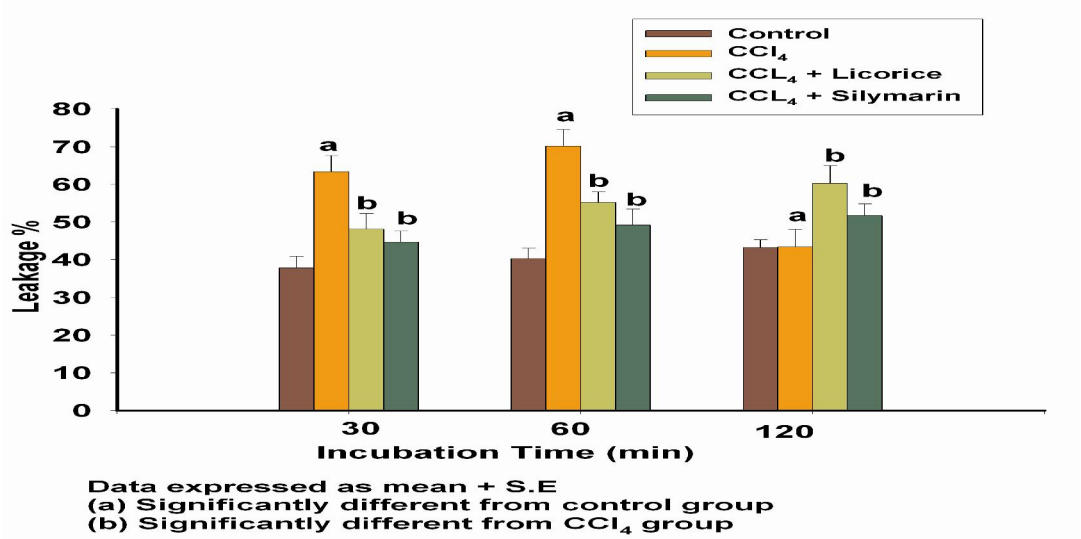

Fig (4): Effects of CCL_4 and Licorice Extract on AST Leakage% of isolated rat hepatocytes

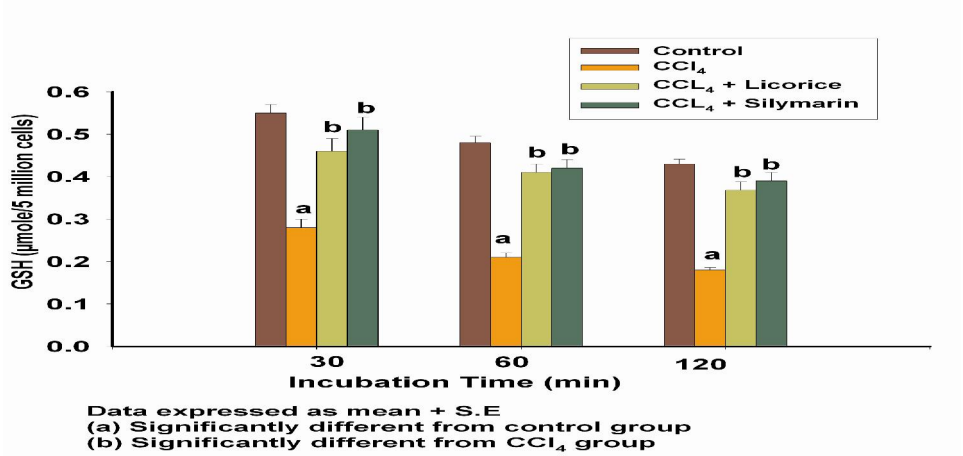

Fig (5): Effects of CCL_4 and Licorice Extract on GSH content of isolated rat hepatocytes

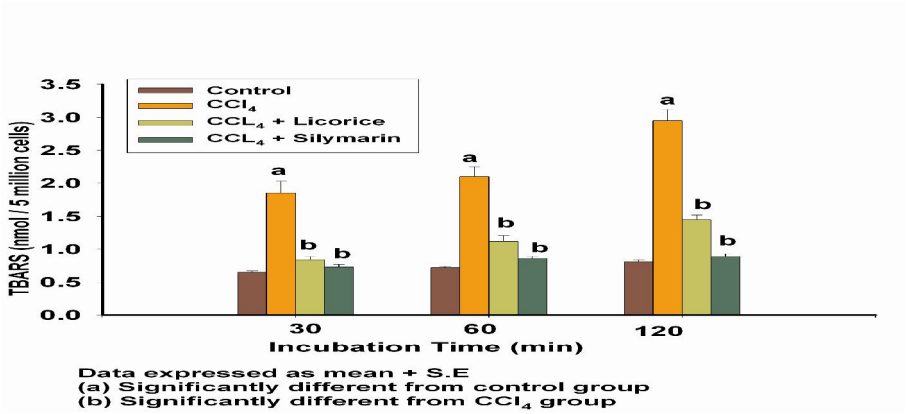

Fig (6): Effects CCL_4 and Licorice Extract on TBARS of isolated rat hepatocytes

4. Discussion

Acute and chronic liver diseases constitute a global concern, and the medical treatments for these diseases are often difficult to handle and have limited efficacy. Therefore, there has been considerable interest in the role of complementary and alternative medicines for the treatment of liver diseases **(Seeff *et al.*, 2001)**. Developing therapeutically effective

agents from natural products may reduce the risk of toxicity when the drug is used clinically **(Lee *et al.*, 2007)**.

Recently, there is a growing interest in the interaction between pharmacology and nutrition science. Pharmaceuticals are generally developed to treat, cure or prevent disease and the primary goal of nutrition is to maintain or even improve health. This does not imply that there is no role for nutrition in preventing or curing disease **(Georgiou *et al.*, 2001).**

Many naturally occurring plant constituents have been reported to protect against liver disease in experimental animals. Examples of these plants are *Silybum marianum* (milk thistle), *Allium sativa* (garlic), and *Picrorhiza kurroa* **(Luper, 1998)**. In view of this, the present study was undertaken to evaluate the hepatoprotective effects of licorice aqueous extract against cytotoxicity and oxidative stress induced by carbon tetrachloride in isolated primary rat hepatocytes.

Licorice, the root of the *Glycyrrhiza glabra* L. (Fabaceae) plant species, has been used medicinally for more than 4000 years **(Aoki *et al.*, 2005)**. The genus glycyrrhiza consists of approximately 30 species, of which six species produce a sweet saponin glycyrrhizic acid (GA) **(Fukai *et al.*, 2003)**. Licorice is one of the most widely used herbal drugs around the world, being present in most pharmacopoeias of eastern and western countries **(Biondi *et al.*, 2005)**. These medicinal plants are used as flavorings, sweeteners and herbal medicine, and also for improving health, detoxification and cures for injury **(Cherng *et al.*, 2006)**. They have been traditionally used for respiratory, gastrointestinal, cardiovascular, genitourinary, eye, and skin disorders, and for their antiviral effects **(Zhang and Ye, 2009)**. GA, the most studied active constituent of Licorice, is a sweet-tasting material. The constituent is 50 times sweeter than sugar, and is widely used as a sweetening additive in the food industry, baked goods, ice cream and soft drinks **(Acharya *et al.*, 1993)**. In many countries, GA is used as a major therapeutic agent to treat chronic viral hepatitis and allergic dermatitis **(Tanahashi *et al.*, 2002)**. It is also known to have antiinflammation **(Fujisawa *et al.*, 2000)**, antiulcer, antihepatotoxic **(Ito *et al.*, 1997)**, and antivirus activities **(Cinatl *et al.*, 2003; Fu *et al.*, 2005)**.

Oxidative stress plays an important role in hepatic injury and in initiating liver fibrogenesis through production of ROS. Hepatocytes' necrosis and apoptosis appear following lipids, proteins and DNA oxidation, followed by amplifying inflammatory response and initiating fibrogenesis. ROS stimulates both Kupffer and inflammatory cells in releasing profibrogenic mediators, which in turn stimulates HSCs proliferation which amplifies the production of ECM **(Galli *et al.*, 2000).**

Carbon tetrachloride (CCl_4) is frequently used to induce liver fibrosis in animal models **(Neubauer *et al.*, 1998)**. Treatment with CCl_4 generates free radicals that trigger a cascade of events that result in hepatic fibrosis, mimicking the oxidative stress that has a fibrogenic effect on HSC **(Poli, 2000; Reeves and Friedman, 2002; Huang *et al.*, 2003)**. In fact, reactive oxygen species may cause tissue injury through activation of the precursors of MMPs (proMMPs) **(Okamoto *et al.*, 2001; Huang *et al.*, 2003)**. Although no successful therapeutic approach to this pathogenetic mechanism in liver disease has been developed, antioxidants therapies have shown to achieve some positive effects **(Wasser *et al.*, 2001; Guo *et al.*, 2002; de Freitas *et al.*, 2003).**

In the present study, CCl_4 induced its toxicity which was indicated by a significant decrease in the viability of isolated rat hepatocytes, and a significant increase in the leakage of intracellular enzymes (LDH, ALT, and AST) into the incubation medium as compared with the control group, reflecting the cell membrane integrity. These results are in agreement with many reports of **Kim, (1995), Mahran *et al.* (1996), Wu *et al.* (1997), Du *et al.* (2000), and Dvorak *et al.* (2003).**

The decrease in hepatocytes viability% and the increase in leakage% of intracellular enzymes after CCl4 exposure was a time dependant **Berger *et al.* (1986) and Farrel (1994**).

In addition, the data of the present study showed a marked depletion of GSH, which reflected the redox status of hepatocytes, and a significant increase in lipid peroxidation which reflected the extent of lipid peroxidation of the isolated rat hepatocytes after CCl_4 exposure. CCl_4 is a well-known hepatotoxic agent. CCl_4 toxicity was reported to be associated with depletion of GSH where the trichloromethyl free radicals can react with compounds containing sulfhydryl groups such as GSH and protein thiols leading to membrane lipid peroxidation and finally cell necrosis **(Recknagel *et al.*, 1989).** Moreover, hepatic GSH level was decreased after CCl_4 addition due to the reaction of GSH with CCl4 derived free radicals in the hepatocytes **(Connor *et al.*, 1990; Nishida *et al.*, 1998)**. The depletion of GSH in liver is a well-known concomitant of CCl_4 toxicity **(Kim *et al.*, 1999, Dvorak *et al.*, 2003)**.

Also, increased lipid peroxidation, as evidenced by elevated levels of thiobarbituric acid reactive substances (TBARS) in isolated rat hepatocytes, was demonstrated in the present study after CCl_4 exposure. These results are in harmony with those of other investigators who reported the association between CCl_4 toxicity and lipid peroxidation **(Kim,**

1995; Kim *et al.*, 1999). The membrane lipids are susceptible to oxidation because of their association in the cell membrane with enzymatic and non-enzymatic systems capable of generating free-radical species. The oxidation of unsaturated fatty acids in biological membranes leads to a reduction in membrane fluidity and disruption of membrane structure and function **(Slater and Cheesman, 1987)**.

The increase in the leakage of intracellular enzymes (LDH, ALT, and AST), loss of cell viability, depletion of GSH content and lipid peroxidation indicated that the model had been successfully built. It was more important to confirm whether there is any difference between the treatment with or without licorice extract under the damage of CCl_4.Since free radicals play such an important role in CCl_4-induced hepatotoxicity, it seems logical that compounds that neutralize such radicals may have an hepatoprotective effect. Indeed, various natural products have been reported to protect against CCl_4-induced hepatotoxicity **(Hsiao *et al.*, 2003)**.

Silymarin (0.5mM) exerted marked protective effect against CCl4 toxicity which was indicated by increasing the viability of hepatocytes when compared to CCl4 treated group. Also, silymarin significantly decreased the leakage of intracellular enzymes to the medium, the depletion of GSH, and decreased lipid peroxidation as it was previously reported by others **Chrungoo *et al.* (1997) and Tasaduq *et al.* (2003).**

The results showed that Pre-incubation of isolated rat hepatocytes with licorice extract (25μM/ml) afforded a protection against CCl_4-induced hepatocyte toxicity, as evidenced by an increase in the viability%, decrease in the leakage% of LDH, ALT and AST, suppression of lipid peroxidation as well as by maintenance of intracellular level of GSH. The hepatoprotective effect of licorice extract against oxidative stress induced by CCl_4 attributed to its antioxidant and free radical scavenging properties which have been demonstrated in various studies using licorice extract itself and the main active constituents of licorice extract

The main components of licorice root are the triterpene, saponins, glycyrrhizin/glyccyrrhizic acid and glycyrrhetic acid. Glycyrrhizic acid (GA) or Glycyrrhizin (GL) exhibits a number of pharmacological effects including anti-inflammatory and is used in hepatoprotective formulations. Pre-treatment with GA has been reported to show protective action against carbon tetrachloride (CCl_4)-induced liver injury in rats **(Wang *et al.*, 1993)**. Glycyrrhizin is a major active constituent isolated from licorice that scavenges reactive oxygen species (ROS) and has an anti-inflammatory action **(Gumpricht *et al.*, 2005; Yoshida *et al.*, 2006).**

Suzuki *et al.* (1977) reported that the principal triterpene component of licorice root, glycyrrhizin (GL), benefits patients with chronic hepatitis C infection. Derivatives of licorice root have been used in Asia to treat children with biliary atresia **(Sokol *et al.*, 2003)**, a cholestatic liver disease, although no clinical trials have been reported. Increasing evidence supports the hypothesis that GL, or its hydrolyzed metabolite 18β-glycyrretinic acid, protects against several models of oxidant-mediated toxicity, including exposure to CCl_4 **(Jeong *et al.*, 2002),** t-butyl hydroperoxide **(*Kinjo et al.*, 2003)**, and ischemia-reperfusion injury **(Nagai *et al.*, 1991).**

Treatment of concanavalin A (Con A)-treated mice with glycyrrhizin suppressed the increases in AST and ALT, cell infiltration and the degeneration of hepatocytes in the liver of these mice, which is due partly to the modulation of hepatic iNOS induction and of degeneration of hepatocytes **(Tsuruoka *et al.*, 2009).**

Prophylactic administration of aqueous suspension of powdered *Glycyrrhiza glabra* roots at three different doses for 7 days to mice could provide appreciable protection against acetaminophen challenge on 8^{th} day in sublethal experiments **(Sharma and Rathore, 2011).** Licorice constituents stabilize integrity of hepatic lysosomes and mitochondria **(Gumpricht *et al.*, 2005; Wu *et al.*, 2008)**.

Glycyrrhiza glabra could attenuate peroxynitrite induced renal oxidative damage through inhibition of protein nitration **(Yokozawa *et al.*, 2005).** Antioxidant capacity of licorice is used to treat kidney or urinary system based on oxygen radical absorbance capacity method **(Wajcikowski *et al.*, 2007).**

Moreover, *Glycyrrhiza glabra* and lipoic acid could prevent gentamycin induced nephrotoxicity **(Desai *et al.*, 2004).** glycrrhizin could prevent lead acetate induced hepatic oxidative stress and hyperproliferative activity in wistar rats. Pretreatment of rats orally with glycrrhizin decreased hepatic microsomal lipid peroxidation and increase in the level of GSH content and lowered DNA synthesis **(Rahman *et al.*, 2004).**

In addition, 18-betaglycyrrhetinic acid (the major active metabolite of licorice) could prevent CCl_4-induced liver injury in mice by inhibiting depletion of hepatic GSH. This component of licorice also showed antioxidant effect upon $FeCl_2$-ascorbate induced lipid peroxidation in mice liver homogenate and upon superoxide radical scavenging activity **(Jeong *et al.*, 2002).**

Glycyrrhizin was reported to have protective effect against CCl_4-induced liver injury by diminishing free radical toxic properties and inducing heme oxygenase-1 and down regulating proinflammatory mediators in mice **(Lee *et al.*, 2007).**

Lin et al. reported that a three-day pretreatment with either glycyrrhizin or glycyrrhetinic acid exhibited protective effect on retrorsine-induced liver damage in rats **(Lin *et al.*, 1999).**

Several hypotheses have been put forward to account for the hepatic protection offered by these compounds including stimulation of cytochrome P-450 and glutathione S-transferase activities **(Chan *et al.*, 2003)** or their activity as an antioxidant through glutathione preservation **(Jeong *et al.*, 2002).**

Nose et al reported that the oral administration of 18 beta-GA at 1, 24, and 48 h before D-galactosamine treatment significantly reduced the increase of serum transaminase activities 24 h after galactosamine treatment **(Nose *et al.*, 1994).**

Glycyrrhizin can reduce the mortality of acetaminophen overdosed mice, attenuate the development of acetaminophen-induced hepatotoxicity in mice, and reduce the number and area of γ-GT positive foci, thus protecting liver function and preventing hepatocellular carcinoma from occurring **(Wan *et al.*, 2009).**

In addition, Glycyrrhizin produced by the licorice plant is an anti-inflammatory that has been used in the treatment of patients with chronic hepatitis B and C **(Iino *et al.*, 2001; Miyake *et al.*, 2002; Matsui *et al.*, 2006; Yoshida *et al.*, 2007).** Furthermore, glycyrrhizin significantly prevented increased serum ALT levels and I/R- induced liver injury in rats **(Nagai *et al.*, 1992; Mabuchi *et al.*, 2009; Ogiku *et al.*, 2011).**

The components of licorice plant (triterpene, saponins, glycyrrhizic acid) in single or in combination with other components present in the licorice extract might be responsible for the hepatoprotective effect licorice extract **(Huo *et al.*, 2011).**

Based on the experimental results reported here, we hypothesize that licorice extract may play an important role in medicine by scavenging free radicals, stimulating activities of antioxidant enzymes, subsequently protecting the liver against CCl4-induced damage. We supposed that the components (triterpene, saponins, glyccyrrhizic acid) in single or in combination with other components present in the licorice extract might be responsible for its hepatoprotective properties. In conclusion, CCL4 has a potential cytotoxic effect in isolated rat hepatocytes and exposing hepatocytes to licorice extract possess a highly promising hepatoprotective effects against CCL4-induced hepatotoxicity. Licorice extracts significantly improved cell survival and played an essential role to maintain the cellular membranes integrity against CCL4 Hepatotoxicity.

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