

## Protective Effects of Garlic Oil against Liver Damage Induced by Combined Administration of Ethanol and Carbon Tetrachloride in Rats

Ashraf B. Abdel-Naim<sup>a</sup>, Amani E. Khalifa<sup>a</sup>, Sherif H. Ahmed<sup>b</sup>

<sup>a</sup> Department of Pharmacology and Toxicology, Faculty of Pharmacy,  
Ain Shams University, Cairo, Egypt.

<sup>b</sup> Department of Biochemistry, Faculty of Agriculture,  
Cairo University, Cairo, Egypt.

### Abstract

Herbs are known to play a vital role in the management of various liver diseases. Garlic oil (GO) contains numerous organosulfur compounds with potential hepatoprotective effects. The present work was planned to evaluate the possible preventive role of GO on biochemical and histopathological alterations induced by combined administration of ethanol (EOH) and carbon tetrachloride (CCl<sub>4</sub>) in rat liver. Two dose levels of GO (5 or 10 mg/kg/day) were administered orally to rats for 7 consecutive days with EOH + CCl<sub>4</sub>-induced liver damage. Activity of GO against liver damage was compared with that of silymarin (25 mg/kg/day, p.o. for 7 consecutive days). Biochemical parameters including serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase ( $\gamma$ -GT), alkaline phosphatase (ALP) and bilirubin were estimated to assess the liver function. In addition, the level of total proteins, triglycerides, total cholesterol, glutathione (GSH), and thiobarbituric acid reactive substances (TBARS), in liver tissues were estimated. Liver damage was evidenced by an increase in the activity/level of AST, ALT,  $\gamma$ -GT, ALP and bilirubin in sera of rats after the combined administration of EOH and CCl<sub>4</sub> compared to normal animals. Pretreatment of rats with GO reduced the EOH + CCl<sub>4</sub>-induced elevated levels of the above indices. Similarly, GO significantly prevented the decline in total proteins and the increase in triglycerides and total cholesterol resulted after EOH + CCl<sub>4</sub> administration in rat liver homogenates. In addition, GO pretreatment restored liver GSH levels decreased due to EOH + CCl<sub>4</sub> administration. The elevation in liver TBARS level due to EOH + CCl<sub>4</sub> administration was also prevented by pretreatment with both low and high doses of GO. Histopathological examination indicated that GO exhibited an obvious preventive effect against the centrilobular necrosis and nodule formation induced by EOH + CCl<sub>4</sub> administration. In conclusion, GO exerts hepatoprotective actions against EOH + CCl<sub>4</sub>-induced toxicity in rats.

**Key Words:** garlic oil, alcohol, carbon tetrachloride, hepatotoxicity, rats

### Introduction

Liver diseases remain one of the serious health problems. The high prevalence of bilharziasis and viral hepatitis in Egypt resides behind the wide occurrence of liver disorders especially hepatic fibrosis and cirrhosis (el-Zayadi *et al.*, 1992). Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. In the absence of reliable liver protective drugs in medical practices, herbs play a role in the management of various liver disorders. A number of plants have been

documented to exhibit hepatoprotective properties (Handa *et al.*, 1986; Mehandale, 1990).

For thousands of years, garlic extracts have been used to treat infectious diseases. Contents of garlic oil, particularly E-ajoene, have shown a broad activity against several DNA and RNA viruses (Sheen *et al.*, 1999). GO has been reported to reduce serum cholesterol and triglycerides' levels (Fenwick and Hanley 1985). GO contains other organosulfur compounds including diallyl disulfide. These compounds are

known as hepatoprotective agents (Sheen *et al.*, 1999). Also, organosulfur compounds suppress inducible levels of cytochrome P450 2E1; which is responsible for the bioactivation of a wide variety of hepatotoxins and for generation of deleterious oxyradicals (Kwak *et al.*, 1995). In addition, organosulfur compounds enhance phase II enzymes such as glutathione S-transferases, UDP-glucuronyl transferase and microsomal epoxide hydrolase activities, which are essential for hepatic detoxification processes (Siess *et al.*, 1997). Furthermore, organosulfur compounds in garlic oil enhance the activity of glutathione peroxidase and superoxide dismutase; the well documented antioxidant enzymes (Banerjee *et al.*, 2001). Therefore, the present work was designed to explore the potential protective effects of GO against EOH + CCl<sub>4</sub>-induced hepatotoxicity in rats. For that purpose the activity/level of AST, ALT,  $\gamma$ -GT, ALP, and bilirubin were assessed in rat sera. Moreover, the level of total proteins, triglycerides, total cholesterol, GSH and TBARS were estimated in liver tissues. Furthermore, histopathological studies of rat liver sections were performed. The effects of GO against EOH + CCl<sub>4</sub>-induced hepatotoxicity were also compared with that of silymarin; a known hepatoprotective agent whose efficacy is clinically established in alcoholic liver disease (Wellington and Jarvis, 2001).

## Materials and Methods

### Chemicals

GO was purchased from Handa Fine Chemicals, Nottingham, UK. GSH, 5,5'-dithio-bis-(2-nitrobenzoic acid), thiobarbituric acid (TBA), 1,1,3,3-tetraethoxypropane, and trichloroacetic acid were all obtained from Sigma Chemical Co. (St. Louis, MO, USA). Silymarin was purchased from Ranbaxy, Delhi, India. All other chemicals were of the highest available commercial grade.

### Animals and Animal Treatment

Forty male Sprague-Dawley rats, weighing 200-240 g, were obtained from our animal facility (Ain Shams University, Cairo, Egypt). They were divided into 5

groups, each group consisting of 8 rats and kept under standard laboratory conditions. They had free access to a commercial pellet diet and water *ad libitum*. The room temperature was maintained at  $25 \pm 2^\circ\text{C}$ .

The dose and duration of EOH administration in combination with CCl<sub>4</sub> were standardized in a preliminary experiment. Accordingly rats were orally administered 2 ml/100 g body weight of 40% ethanol (v/v); daily for 21 consecutive days. On day 20, animals were injected subcutaneously with 0.1 ml of CCl<sub>4</sub> in olive oil (1:1) per kg body weight and were sacrificed 48 h later. Group I represented control vehicle-treated rats. In group II, rats were given 40% ethanol daily for 21 days, and on day 20 they were injected with CCl<sub>4</sub>. Animals of group III, IV and V were put to similar protocol as those of Group II. In addition they received a predetermined dose of GO (dissolved in corn oil) equivalent to 5 or 10 mg/kg/day p.o. or silymarin (25 mg/kg/day p.o.) daily from day 15 onward till day 21 (Karunakar *et al.*, 1997). The doses of GO were based on changing the recommended human daily dose into rat doses using Paget and Barnes tables (1964).

### Preparation of Samples for Biochemical Studies:

Rats were anesthetized with ether; blood was collected by intracardiac puncture and livers were collected. Then, all animals were sacrificed. Blood samples were kept for 30 min without disturbing. The samples were centrifuged for 15-20 minutes at 2000 rpm to separate sera.

### Biochemical Study

ALT and AST were determined by the method of Reitman and Frankel (1957). Serum bilirubin was estimated as described by Dangerfield and Finlayson (1953). Also, sera were used to assess ALP and  $\gamma$ -GT activity following the methods of Kind and King (1954) and Tate and Meister (1985) respectively.

Liver homogenates were used to determine the level of triglycerides and total cholesterol (Fletcher, 1968; Allain *et al.*, 1974). GSH was determined in liver homogenates following the method of Ellman (1959). Concentration of TBARS was measured in liver using the modified

method of Ohkawa *et al.* (1979). The concentration of TBARS was expressed as nmoles of malondialdehyde per mg of protein using 1,1,3,3- tetraethoxypropane as the standard. Total protein in the tissue homogenates was also estimated (Lowry *et al.*, 1951).

#### Histopathological Studies

Two rats from each group were sacrificed under light ether anesthesia and the liver samples of all groups were preserved in 10% neutral buffered formalin as described by Luna (1968). Thin sections were stained by hematoxylin and eosin stain. Representative sections were photographed under light microscope.

#### Statistical Analysis

Results are reported as mean  $\pm$  standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA). If the overall F-value was found statistically significant ( $p < 0.05$ ), further comparisons among groups were made according to post-Hoc Tukey's test. All statistical analyses were performed using SPSS statistical software package (SPSS<sup>®</sup> Inc., USA), version 8. Graphs were sketched using GraphPad Prism (ISI<sup>®</sup> software, USA) version 2.

## Results

Results demonstrated significant increase in the activity/level of serum AST, ALT,  $\gamma$ -GT, ALP and bilirubin associated with marked depletion in the content of total liver proteins after EOH + CCl<sub>4</sub> administration (Table 1, Fig. 1A). Treatment with the two doses of GO resulted in restoration of serum and liver parameters disturbed after EOH + CCl<sub>4</sub> administration. The lower dose of GO resulted in 21% , 36 % , 52 % ,23 % , and 21% reduction in elevated activities/level of AST, ALT,  $\gamma$ -GT, ALT, and bilirubin respectively. The higher dose of GO decreased the increased activities/level of AST, ALT,  $\gamma$ -GT, ALP, and bilirubin by 30%,56%, 72%, 33%, and 43% respectively. Results also showed that silymarin caused 41%, 49%, 77%, 31%, and 43 % reduction in the elevated activities/level of AST, ALT,  $\gamma$ -GT, ALP, and bilirubin respectively (Table I). All treatment conditions significantly increased ( $P < 0.05$ ) the depleted content of total liver proteins caused by EOH + CCl<sub>4</sub> administration ( Fig A).

**Table (I): Effects of GO on activity/level of AST, ALT,  $\gamma$ -GT, ALP, and bilirubin in sera of rats given combined administration of EOH +CCl<sub>4</sub>**

Parameters	Group I Control	Group II EOH + CCl <sub>4</sub>	Group III 5 mg GO/Kg /day + EOH + CCl <sub>4</sub>	Group IV 10 mg GO/Kg /day + EOH + CCl <sub>4</sub>	Group V 25 mg/Kg Silymarin/day + EOH + CCl <sub>4</sub>
AST (IU/L serum)	20.30 $\pm$ 0.41	39.60 $\pm$ 1.40 <sup>a</sup>	31.10 $\pm$ 1.70 <sup>a,b</sup>	27.50 $\pm$ 1.2 <sup>a,b</sup>	23.30 $\pm$ 1.20 <sup>b</sup>
ALT (IU/L serum)	22.10 $\pm$ 0.62	77.90 $\pm$ 4.60 <sup>a</sup>	49.50 $\pm$ 3.60 <sup>a,b</sup>	33.80 $\pm$ 2.1 <sup>a,b</sup>	39.60 $\pm$ 1.50 <sup>a,b</sup>
$\gamma$ -GT (IU/L serum)	3.62 $\pm$ 0.04	21.60 $\pm$ 1.10 <sup>a</sup>	10.30 $\pm$ 1.21 <sup>a,b</sup>	5.90 $\pm$ 0.40 <sup>a,b</sup>	4.80 $\pm$ 0.13 <sup>a,b</sup>
ALP (IU/L serum)	74.20 $\pm$ 5.20	135.30 $\pm$ 7.10 <sup>a</sup>	103.20 $\pm$ 5.60 <sup>a,b</sup>	89.80 $\pm$ 4.7 <sup>a,b</sup>	93.20 $\pm$ 5.30 <sup>a,b</sup>
Bilirubin (mg/dl)	0.24 $\pm$ 0.01	0.71 $\pm$ 0.02 <sup>a</sup>	0.56 $\pm$ 0.02 <sup>a,b</sup>	0.40 $\pm$ 0.02 <sup>a,b</sup>	0.40 $\pm$ 0.01 <sup>a,b</sup>

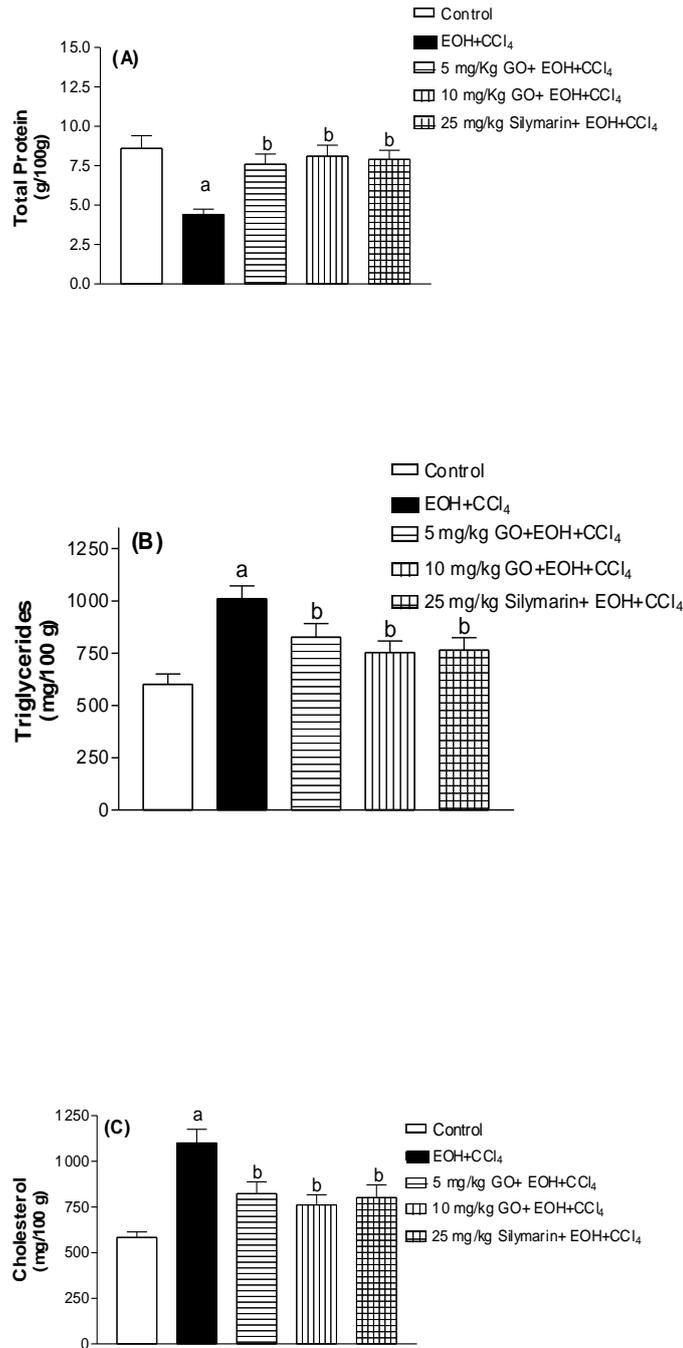
Data are presented as mean  $\pm$  SEM.

<sup>a</sup>Significantly different from corresponding control at  $P < 0.05$ .

<sup>b</sup>Significantly different from corresponding EOH + CCl<sub>4</sub> treated group at  $P < 0.05$ .

In group II, a significant increase ( $p < 0.05$ ) in the levels of triglycerides, and cholesterol in liver tissues was observed as compared to normal animals (Fig. 1B, 1C; respectively). Treatment with GO alone at both lower and higher doses (Groups III

and IV) and silymarin (Group V) gave different degrees of protection against EOH +  $\text{CCl}_4$ -induced hypertriglyceridemia (21%, 25% and 24% respectively) and hypercholesterolemia (25%, 30% and 27% respectively).



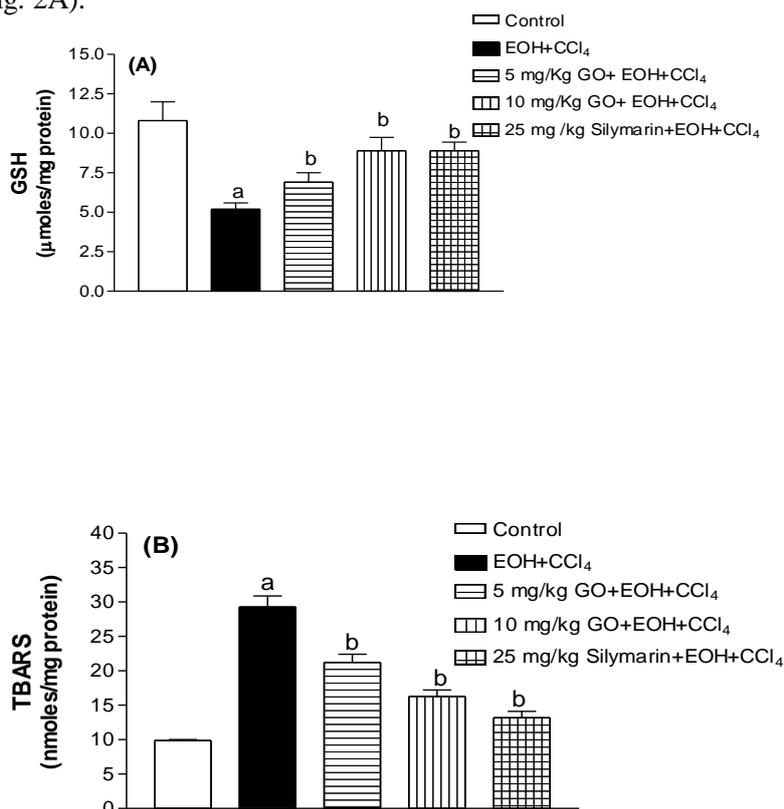
**Fig.1: Effects of GO treatment on the level of liver total proteins (A), triglycerides (B) and cholesterol (C) in rats treated with EOH +  $\text{CCl}_4$**

<sup>a</sup> Significantly different from control at  $P < 0.05$ .

<sup>b</sup> Significantly different from EOH +  $\text{CCl}_4$  treated group at  $P < 0.05$ .

Tissue GSH level was significantly decreased in animals treated with EOH + CCl<sub>4</sub> as compared to normal animals (52%). The decreased level of liver GSH due to EOH + CCl<sub>4</sub> administration was significantly ameliorated by pretreatment of rats with both the lower and higher doses of GO and with silymarin (40%, 60%, and 61% of EOH + CCl<sub>4</sub>-treated group respectively, Fig. 2A).

Combined administration of EOH + CCl<sub>4</sub> resulted in a significant increase in the levels of tissue lipid peroxidation marker; TBARS. Such increase in TBARS was inhibited by pretreatment of rats with both the lower and higher doses of GO and with silymarin (27%, 45%, and 52% of EOH + CCl<sub>4</sub>-treated group respectively, Fig. 2B).



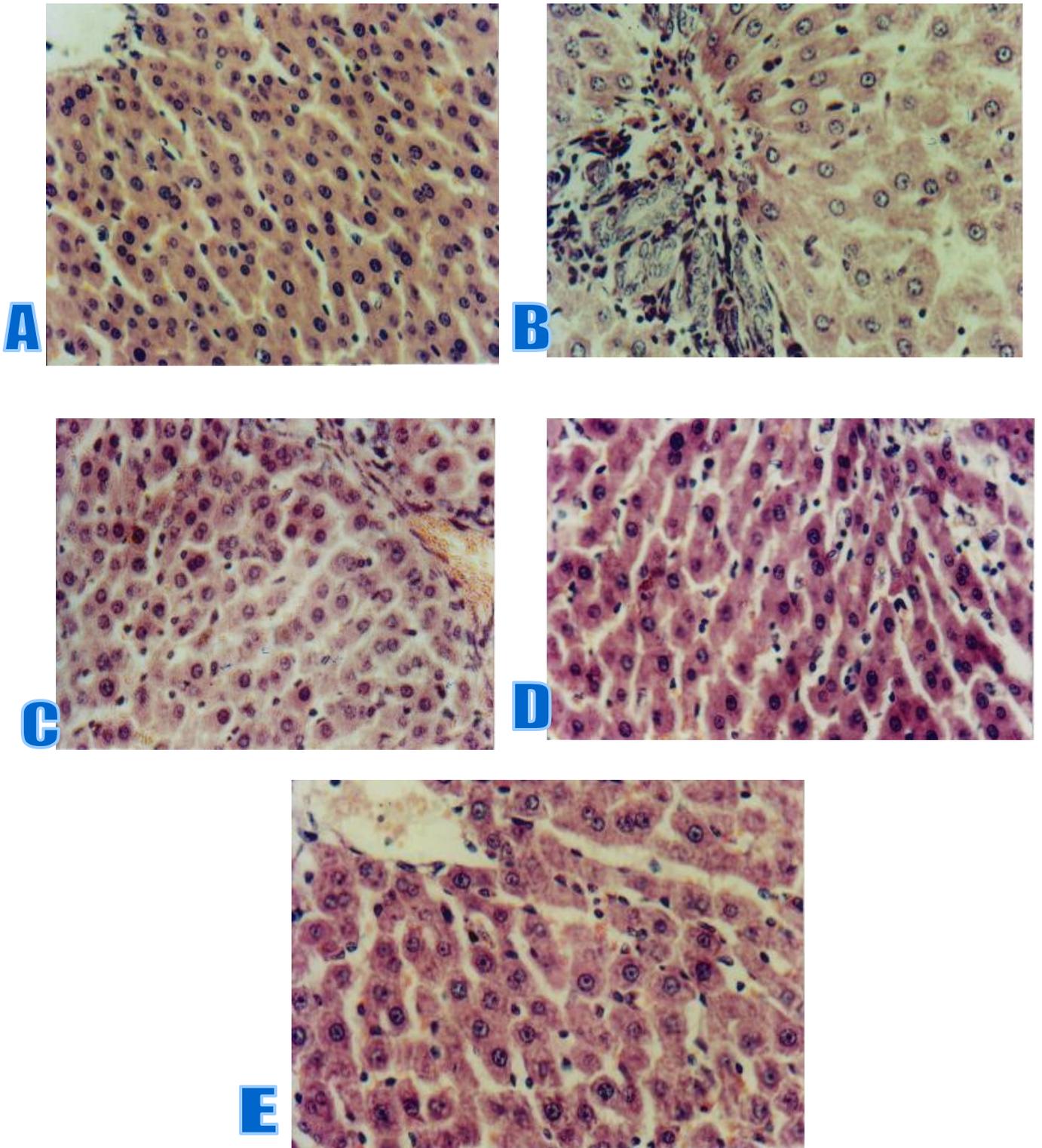
**Fig.2: Effects of GO treatment on liver GSH level (A) and lipid peroxidation (B) in rats given combined administration of EOH + CCl<sub>4</sub>**

<sup>a</sup> Significantly different from control at P< 0.05.

<sup>b</sup> Significantly different from EOH + CCl<sub>4</sub> treated group at P< 0.05.

Histopathological examination of liver sections of normal rats showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus and nucleolus and well brought-out central vein (Fig. 3A). Administration of EOH + CCl<sub>4</sub> induced centrilobular necrosis and various degenerative changes in hepatic cells surrounding central vein. Such degenerative changes were demonstrated as cloudy swelling, hydropic degeneration, necrosis

(coagulative type) with loss of nucleus and local mononuclear leucocytic inflammatory cells infiltration in between the degenerated hepatocytes with focal extravasation of red blood cells (Fig. 3B). Pretreatment of rats with both doses of GO resulted in significant protection against EOH + CCl<sub>4</sub>-induced liver damage (Fig. 3C and 3D). Pretreatment of rats with silymarin also reversed the hepatotoxicity caused by administration EOH + CCl<sub>4</sub> (Fig. 3E).



**Fig. 3: Photomicrographs of rat liver sections in all studied groups**

**A: Liver of a control; B: Liver of a rat given combined administration of EOH + CCl<sub>4</sub>; C and D: Liver of rats pretreated with 5 and 10 mg/kg GO respectively + EOH + CCl<sub>4</sub>; E: Liver of rats pretreated with 25mg/Kg silymarin + EOH + CCl<sub>4</sub>; X 160**

## Discussion

Hepatic dysfunction due to ingestion or inhalation of hepatotoxin is increasing worldwide (Loeper *et al.*, 1983; Baraona *et al.*, 1983; Ishak *et al.*, 1991). The treatment of alcoholic liver disorder is a major problem. In the present investigation we have, therefore, evaluated the protective effect of GO in rat liver damaged due to combined administration of EOH + CCl<sub>4</sub>. Such activity has been further compared with a known hepatoprotective agent; silymarin whose efficacy is clinically established in alcoholic liver disease (Wellington and Jarvis, 2001). Inclusion of CCl<sub>4</sub> in this study was made to cause hepatocellular necrosis, as alcohol administration *per se* was not able to injure the liver beyond fatty infiltration in rats possibly because of a short life span.

Serum ALT, AST and ALP are reliable markers of liver function (Friedman *et al.*, 1996). Indeed, they were significantly increased in EOH + CCl<sub>4</sub>-treated group. On the other hand, in animals pretreated with GO (Groups III and IV), the increased activity of ALT, ALP and AST had decreased significantly. This suggests that the hepatoprotective action might be due to GO effects against cellular leakage and protection of the integrity of the cell membrane in rat liver. Similarly, the estimation of  $\gamma$ -GT levels is a valuable indicator for liver disease (Nemesanszky, 1996) as it is a membrane bound enzyme (Chander *et al.*, 1994). A number of hepatotoxic drugs and chemicals are known to increase  $\gamma$ -GT activity (Kim *et al.*, 1977). In the present study, the  $\gamma$ -GT activity was elevated in EOH + CCl<sub>4</sub>-treated rats indicating a severe damage to hepatocytes' membrane. Oral administration of GO to EOH + CCl<sub>4</sub>-treated rats showed reduction in  $\gamma$ -GT activity. This could reflect a possible membrane stabilizing activity of GO. In harmony with these results, GO significantly protected against the rise in serum bilirubin induced by the toxic chemical insult to rats.

The alcoholic liver injury appears to be generated by the effects of ethanol metabolism and the toxic effects of

acetaldehyde, which may be mediated, by acetaldehyde altered proteins (Zimmerman, 1986; Ishak *et al.*, 1991). Alcohol intake is known to produce hypercholesterolemia, hyperlipidemia and hypertriglyceridemia (Baraona and Lieber 1979). As a result of lipid accumulation, the liver cells become fibrotic leading to impaired liver function. Enhanced lipid peroxidation has been reported in hyperlipidemia which is also induced by ethanol (Loeper *et al.*, 1983). Ethanol increases triglycerides and cholesterol levels thus inducing imbalance in lipid metabolism in liver and this could explain the reason for the increase in lipid peroxidation in these organs. In our study, assessing liver levels of tissue total proteins, triglycerides and cholesterol in liver tissues confirmed the deleterious effects of EOH + CCl<sub>4</sub> on these indices. However, GO treatment (especially in the high dose) was effective in preventing alterations in the above mentioned indices. It is worth mentioning that the effect of GO was comparable to that of silymarin.

The GSH system is an important endogenous antioxidant that is found particularly in high concentration in the liver and is known to have key functions in cellular protective mechanisms. GSH becomes readily oxidized to GSSG while interacting with free radicals. Excessive production of free radicals results in oxidative stress, which leads to damage of macromolecules (Sinclair *et al.*, 1990). Thus, reduced GSH is essential to maintain structural and functional integrity of the cell. Human data strongly indicate a correlation between liver diseases and compromised oxidative status. Subnormal plasma concentrations of GSH were observed in cirrhotic patients (Chawla *et al.*, 1984; Shigesawa *et al.*, 1992). A four- to eight-fold decrease in plasma GSH was observed in cirrhotic patients (Loguercio *et al.*, 1992). A significant decrease in cysteine in severe cirrhosis was also observed. Altomare and collaborators (1988) reported that GSH levels decrease in alcoholic and non-alcoholic liver diseases. In the present study, the observed decrease

in liver GSH in EOH + CCl<sub>4</sub>-treated rats may be due to non-enzymatic interaction of GSH with excessive free radicals generated by the toxic insult in rat liver (Knecht *et al.*, 1995). Alternatively, it may be due to enhanced substrate utilization by glutathione peroxidase (Hiraishi *et al.*, 1999). In general, the demonstrated protective antioxidant properties of GO can be attributed to its content of the organosulfur compounds (Fenwick and Hanley, 1985). These ingredients have been shown to possess antioxidant activity and to protect against experimentally induced liver damage (Wu *et al.*, 2001). In fact, there is a direct correlation between GSH depletion and enhanced lipid peroxidation (Lieber, 1997). This was also observed in our study, as depletion of liver GSH was accompanied by significant elevation in liver TBARS. Our findings also revealed a significant reduction in liver TBARS level in the GO-treated groups (Groups III and IV). It is therefore concluded that GO could have obvious antiradical effects.

The histopathological findings demonstrated that combined administration of EOH + CCl<sub>4</sub> induced centrilobular necrosis and various degenerative changes in hepatic cells surrounding central vein. Treatment with GO as well as silymarin could obviously mitigate the histopathological changes. In conclusion, the results of the present investigation suggest that GO possesses a remarkable hepatoprotective activity against EOH + CCl<sub>4</sub>-induced toxic insult.

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## التأثيرات الواقية لزيت الثوم ضد تلف الكبد المحدث بالإيثانول مع رابع كلوريد الكربون فى الجرذان

أشرف بهى الدين عبد النعيم<sup>أ</sup> - أمانى إمام محمد خليفة<sup>أ</sup> - شريف حلمى أحمد<sup>ب</sup>

<sup>أ</sup> قسم الأدوية والسموم - كلية الصيدلة - جامعة عين شمس  
<sup>ب</sup> قسم الكيمياء الحيويه - كلية الزراعة - جامعة القاهرة

تم تصميم هذا البحث لدراسة التأثيرات الواقية لزيت الثوم ضد تدمير خلايا الكبد بعد تعرضها للإيثانول مع رابع كلوريد الكربون فى الجرذان بإعطاء زيت الثوم عن طريق الفم فى جرعتين مقدارهما 5 مجم/كجم 10 مجم/كجم (كل جرعة فى مجموعة منفصلة) ومقارنة هذا التأثير بالتأثير الناتج عن إعطاء مادة السليمارين فى جرعة مقدارها 25 مجم/كجم بالفم كمادة معروفة وقياسية لحماية الكبد . ولقد تم تقسيم الحيوانات إلى 5 مجموعات الأولى منها هى المجموعة الضابطة و فى الثانية تم إعطاء كل حيوان 2مل لكل 100 جم من وزن الجسم من الإيثانول لمدة 21 يوم وفى اليوم العشرين تم حقنها تحت الجلد برابع كلوريد الكربون (0.1مل) . وقد عولجت المجموعات الثالثة والرابعة بنفس الطريقة كما فى الثانية وزاد على ذلك إعطاء إحداهما 5 مجم/كجم بالفم (الثالثة) والأخرى 10 مجم/كجم بالفم (الرابعة) من زيت الثوم بداية من الأسبوع الثالث ولمدة أسبوع واحد حتى نهاية التجربة أما المجموعة الخامسة فهى تمثل المجموعة المعالجة بمادة السليمارين (25 مجم/كجم بالفم ) بدلاً من زيت الثوم فى المجموعتين الثالثة والرابعة . وبقياس مؤشرات تلف الكبد فى كل المجموعات والتى تتمثل فى زيادة مستوى أنزيمات الأسبارتات أمينوترانسفيريز والأنين أمينوترانسفيراز وجاما جلوتاميل ترانسبيبتيديز بالإضافة إلى أنزيم الكالين فوسفاتاز ومادة الصفراء فى الدم وأيضاً زيادة محتوى الكبد من الدهون المؤكسدة والترايجلسريدات والكلوستيرول مع نقص ملحوظ فى كل من محتوى الكبد من البروتينات ومن مادة الجلوتاثيون المضادة للأكسدة . هذا بالإضافة الى التلف النسيجي للكبد الذى تم توضيحه بصبغ وفحص أنسجة الكبد مجهرياً . وجد أن الإيثانول مع رابع كلوريد الكربون يؤدى إلى التغيرات السالف ذكرها وأن زيت الثوم بجرعته يؤدى إلى تعديل ملحوظ فى مستويات تلك المؤشرات مما يوفر الحماية لخلايا الكبد فى الجرذان ضد التلف الناجم عن تعرضها للإيثانول مع رابع كلوريد الكربون .