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Research Article
Protective Effects of Curcumin and Ginger on Liver Cirrhosis Induced by Carbon Tetrachloride in Rats

Gamal A. Abd-Allah, Kadry A. El-Bakry, Mohamed H. Bahnasawy and El-Shymaa R. El-Khodary

Department of Zoology, Faculty of Science, Damietta University, New Damietta City, Egypt

Abstract

The aim of the present study is to investigate the protective effects of curcumin and/or ginger against carbon tetrachloride (CCl_4) induced hepatotoxicity. Therefore, 45 rats were divided into 5 groups each of 9 rats. Control group, CCl_4 group: Injected i/p with CCl_4 (0.5 mL kg^{-1} b.wt.) mixed (v/v) in olive oil, curcumin group: Injected i/p with CCl_4 and treated with curcumin (200 mg kg^{-1} b.wt.), ginger group: Injected i/p with CCl_4 and treated with ginger (100 mg kg^{-1} b.wt.) and ginger and curcumin group: Injected i/p with CCl_4 and treated with a mixture of curcumin and ginger (100 and 50 mg kg^{-1}, respectively). After 8 weeks from the start of the experiment, blood samples and liver tissues were collected for biochemical and histopathological analysis, respectively. The present study revealed that, CCl_4 elevated liver enzymes activities (ALT and AST) and increase malondialdehyde (MDA) level. On the other hand, CCl_4 decreases some biochemical parameters, such as albumin concentration, total protein concentration and decrease activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) as well as glutathione (GSH). After treatment with curcumin and ginger both of the activity of liver enzymes and level of MDA decreased significantly, but the activity of antioxidant enzymes increased. In conclusion, treatment with curcumin and/or ginger improve the antioxidant status of rats injected with CCl_4, but the combined treatment is less benefit for treatment of liver injury induced by CCl_4.

Key words: Carbon tetrachloride, hepatotoxicity, curcumin, ginger, antioxidant

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Corresponding Author: Kadry A. El-Bakry, Department of Zoology, Faculty of Science, Damietta University, New Damietta City, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The liver is a vital organ due to its various functions, such as plasma protein synthesis (Tacke *et al*., 2009), production of hormones, processing dead red blood cells, detoxification (Zhao *et al*., 2009; Yu *et al*., 2011), glucose and lipid metabolism (Liu *et al*., 2012).

Hepatic fibrosis leads to liver cirrhosis, which is characterized histologically by the formation of regeneration parenchymal nodules, separated by fibrotic septa and associated with major distortion in vascular architectural (Tsochatzis *et al*., 2014; Lee *et al*., 2015).

Carbon tetrachloride has been reported to induce acute and chronic tissue injuries and hepatotoxicity (Xu *et al*., 2010). Hepatotoxicity resulted from CCl_4 in animal model is due to the generation of lipid peroxidation, which consider an injurious agent for different body tissues (Khan *et al*., 2012). Lipid peroxidation resulted from the bio-activation of CCl_4 into trichloromethyl free radical by cytochrome P450 system in liver microsomes leading to liver injury (Srilaxmi *et al*., 2010) and alteration of the antioxidant system of the tissues, which is manifested by abnormal histopathological changes (Mohamed *et al*., 2014).

Plants are the most important source of natural antioxidants. The antioxidant activity of these plants is due to their contents of phenolic compounds (Prasad *et al*., 2010; Hu *et al*., 2014) and have the capacity to scavenge free radicals and provide antioxidant defense (Sreelatha *et al*., 2009).

Curcumin have a variety of biological properties, including antioxidant, anti-carcinogenic and anti-inflammatory properties (Noorafshan and Ashkani-Esfahani, 2013). Many studies showed that curcumin has beneficial effects in animal models of liver injury and cirrhosis (Yao *et al*., 2012).

Ginger is one of the most widely used spice in the world (Baliga *et al*., 2012) and have medicinal properties to treat cold, headaches, nausea, stomach upset, diarrhea, digestive gastrointestinal disturbances, rheumatic complaints and parasitic infections (Haniadka *et al*., 2013). Phytochemical studies have shown that the unique properties of ginger are due to the presence of phytochemicals like zingerone, shogaols, gingerols, pardols, cineole, curcumene, geranyl acetate, terphineol, terpenes, borneol, limonene, β-elemene and others (Baliga *et al*., 2011). The aim of the present study is to demonstrate and evaluate the protective role of curcumin and ginger against toxicity with CCl_4 as well as the efficacy of concomitant treatment with curcumin and ginger.

MATERIALS AND METHODS

Animals: Forty five male albino Wistar rats weighting (79-140 g) were used for the experimental study. Animals were obtained from Helwan Animal Station, Ministry of Health, Egypt. Rats were housed in the Animal House of Department of Zoology, Faculty of Science, Damietta University, New Damietta, Egypt. They were housed in plastic cages under controlled environment of air and temperature with access of water and diet.

Chemicals: Carbon tetrachloride (CCl_4) was purchased from Modern Lab Co. for chemicals, Egypt. Curcumin and ginger powdered were obtained from local market (Hyper market, New Damietta City, Damietta, Egypt).

Experimental design: Rats were divided randomly into five groups each of 9 rats as follow:

- **Normal group:** Rats were given basal diet and water for 8 weeks
- **CCl_4 group:** Rats were injected i/p with CCl_4 (0.5 mL kg^{-1} mixed (v/v)) in olive oil
- **CCl_4+Curcumin:** Rats were injected i/p with CCl_4 (0.5 mL kg^{-1} mixed (v/v)) in olive oil and given diet containing curcumin (200 mg kg^{-1}) for 8 weeks
- **CCl_4+Ginger group:** Rats were injected i/p with CCl_4 (0.5 mL kg^{-1} mixed (v/v)) in olive oil and given diet containing ginger (100 mg kg^{-1}) for 8 weeks
- **Mixed group:** Rats were injected i/p with CCl_4 (0.5 mL kg^{-1} mixed (v/v)) in olive oil and giving diet containing a mixture of curcumin and ginger (100 and 50 mg kg^{-1}, respectively) for 8 weeks

After 8th week, rats were sacrificed after being anesthetize. Blood was collected on EDTA and dry tubes for whole blood and serum preparation, respectively. Large lobe of liver of each rat was quickly removed, washed with normal saline and dried with filter paper then fixed immediately in 10% formalin for histopathological studies and the other parts were packaged and frozen at -20°C until preparation of tissue homogenates.

Preparation of homogenate: Known weight of liver (0.5 g) from the frozen parts of liver tissues was homogenized in normal physiological saline solution (0.9 NaCl) (1:9 w/v). The homogenate was centrifuged at 4°C for 5 min at 3000 rpm. The supernatant was used for estimation of the antioxidant parameters.

Physiological studies

Estimation of liver enzymes (ALT and AST): The activity of ALT and AST were assayed according to Reitman and Frankel (Reitman and Frankel, 1957).

Estimation of metabolites (albumin, total protein and creatinine): The concentration of albumin was determined by the method of Doumas *et al.* (1971), this method depend on the formation of colored complex, which is measured colorimetrically at 620 nm. Total protein was assayed according to Lowry *et al.* (1951). The reaction in this method is a result of Biuret reaction of protein with copper ion in alkaline medium and the blue complex formed is measured at 600 nm. Creatinine was measured by the method of Bartels and Böhmer (Bartels and Bohmer, 1971). Creatinine in the sample reacts with picrates in alkaline medium forming a colored complex at 500 nm.

Determination of lipid peroxidation (oxidative stress): Lipid peroxidation was determined as malondialdehyde (MDA). Its concentration was calculated using the extinction coefficient value 1.56×10^5 M^{-1} cm^{-1} and read at 532 and 600 nm by the method of Stock and Donnandy (Stocks and Dormandy, 1971).

Determination of antioxidant enzymes (SOD, CAT and GSH): Superoxide dismutase (SOD) was carried out by the method of Nishikimi *et al.* (1972), where the oxidation of NADH was mediated by superoxide radical and the following increase in absorbance, measured at 560 nm using the molar extinction coefficient of NADH (6.22×10^3 M^{-1} cm^{-1}).

Catalase activity was measured by the method of Aebi (1974). The rate of decomposition of H_2O_2 was measured spectrophotometrically at 240 nm. Glutathione (GSH) was assayed using dithiobis-2-nitro-benzoic acid (DTNB) according to Beutler *et al.* (1963). The color developing reaction was read at 412 nm.

Statistical analysis: Data were tabulated as Mean±SD of different treated group. The results were analyzed using student t-test taking in consideration the control results as basal value. Statements of significance are based of the probability (p) levels of ≤0.05, 0.01 and 0.001 to be considered significant, very and extremely, respectively.

RESULTS

Liver functions (ALT and AST): Table 1 showed that, the activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes were significantly increased in CCl_4 group compared with control group. After treatment with curcumin and ginger, the activity of ALT and AST were significantly improved compared with normal group. On the other hand, treatment with a mixture of curcumin and ginger, the activities of ALT and AST were significantly increased compared with normal group.

Table 1: Activity of ALT and AST enzymes of the experimental groups

Groups	ALT (U mL^{-1})	AST (U mL^{-1})
Normal	15.25±1.83	15.87±1.45
CCl_4	29.00±1.30#	31.75±3.60#
CCl_4+curcumin	17.70±1.09***	28.00±1.65*
CCl_4+ginger	24.66±1.50***	29.44±1.33#
CCl_4+curcumin+ginger	29.42±0.78#	30.71±2.28#

Values are expressed as Mean±SD of 9 rats in each group, #p<0.001 when compared to normal group, *p<0.05 when compared to CCl_4 group, ***p<0.001 when compared to CCl_4 group, ALT: Alanine aminotransferase and AST: Aspartate aminotransferase

Table 2: Concentrations of serum albumin, total protein and creatinine of experimental groups

Groups	Albumin (g dL^{-1})	Total protein (g dL^{-1})	Creatinine (g dL^{-1})
Normal	3.47±0.37	6.94±0.50	0.720±0.015
CCl_4	2.27±0.12#	4.60±0.50#	0.980±0.044#
CCl_4+curcumin	2.81±0.16**	5.55±0.32**	0.811±0.028***
CCl_4+ginger	2.94±0.15***	5.51±0.28**	0.790±0.033***
CCl_4+curcumin+ginger	2.75±0.34**	5.94±0.83	0.830±0.027***

Values are expressed as Mean±SD of 9 rats in each group, #p<0.001 when compared to normal group, *p<0.05 when compared to CCl_4 group, **p<0.01 when compared to CCl_4 group and ***p<0.001 when compared to CCl_4 group

Physiological parameters (albumin, total protein and creatinine)

Albumin concentration: Albumin concentration was significantly decreased in CCl_4 group compared with normal group. In curcumin or ginger treated groups, albumin concentration was significantly raised compared with CCl_4 group. However, a significant decrease was noticed in CCl_4 injected group treated with a mixture of curcumin and ginger compared with normal group (Table 2).

Total protein concentration: From Table 2 the concentration of serum total protein was significantly lower in CCl_4 injected group compared with normal group. After treatment of CCl_4 injected groups with curcumin and ginger, the total protein concentration significantly increased compared with CCl_4 group. No significant differences were observed in CCl_4 injected group treated with the mixture compared with normal group.

Creatinine concentration: Table 2 indicated that, there was a significant increase of serum creatinine concentration in

CCl_4 group compared with normal group. Otherwise, there was a significant decrease of creatinine concentration in CCl_4 groups treated with curcumin and ginger compared with CCl_4 injected group. In CCl_4 group, which treated with a mixture of curcumin and ginger, there was a significant increase in creatinine concentration compared with normal group.

Malondialdehyde level (MDA): The level of MDA was significantly increased in CCl_4 group compared with normal group. In CCl_4 groups treated with curcumin and ginger, a significant decrease in MDA level compared with CCl_4 group was observed. After treatment of with a mixture of curcumin and ginger, no significant differences were found compared with normal group (Fig. 1).

Antioxidant status: Table 3 showed that, the activity of glutathione (GSH) was extremely significantly decreased in CCl_4 group compared with normal group. However, there was a significant increase of glutathione activity in CCl_4 groups and treated with curcumin and ginger compared with CCl_4 group. In CCl_4 group treated with a mixture of curcumin and ginger, the activity of GSH was significantly decreased compared with normal group.

In CCl_4 group, there was a significant decrease in superoxide dismutase (SOD) activity compared with normal group. While in injected groups treated with curcumin and ginger, there was a significant increase in SOD activity compared with normal group. After treatment of CCl_4 injected group with a mixture of curcumin and ginger, no significant differences were observed in SOD activity compared with normal group (Table 3).

From Table 3, there was a significant reduce of catalase (CAT) activity in CCl_4 injected group compared with normal group. Statistical analysis showed that, there was a significant increase in CAT activity in CCl_4 injected groups treated with curcumin and ginger compared with CCl_4 group. No significant decrease was found of CAT activity in injected groups treated with the mixture compared with normal group.

Histopathological findings: Sections of normal rats liver showed the normal structure of the liver tissue (Fig. 2a). Liver sections of CCl_4 injected rats showed thickening in blood vessels and lymphatic infilteration in most of the portal areas are seen in all examined livers (Fig. 2b). Adding curcumin or ginger to CCl_4 injected rats showed marked improvement in

Table 3: Antioxidant activity of SOD, CAT and GSH enzymes of the experimental groups

Groups	SOD (U g^{-1})	GSH (mg mL^{-1})	CAT (U g^{-1})
Normal	302.12±13.0	0.520±0.10	302.3±12.7
CCl_4	175.30±21.0#	0.042±0.01#	174.5±20.7#
CCl_4+curcumin	220.50±24.3#	0.312±0.07***	221.0±22.8*
CCl_4+ginger	254.10±26.6***	0.266±0.09***	253.0±26.6***
CCl_4+curcumin+ginger	270.00±52.7***	0.205±0.12**	269.1±52.2***

Values are expressed as Mean±SD of 9 rats in each group, #p<0.001 when compared to normal group, *p<0.05 when compared to CCl_4 group and **p<0.01 when compared to CCl_4 group, ***p<0.001 when compared to CCl_4 group, SOD: Superoxide dismutase, GSH: Glutathione and CAT: Catalase

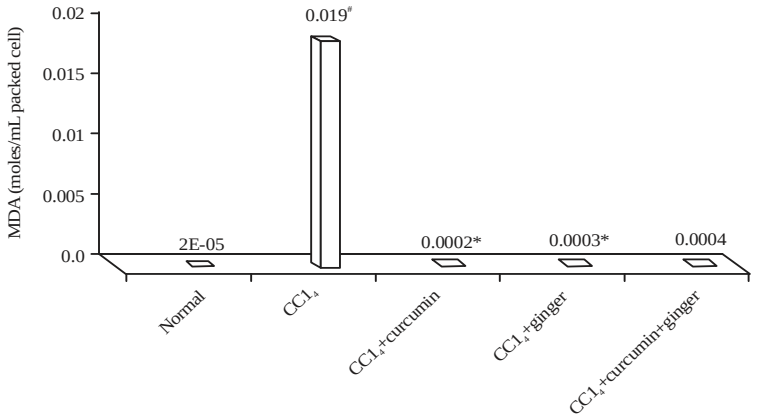

Fig. 1: Changes of malondialdehyde (MDA) level in experimental groups: Normal group, CCl_4 injected group (0.5 mL kg^{-1}), CCl_4 injected group and treated with curcumin (200 mg kg^{-1}), CCl_4 injected group and treated with ginger (100 mg kg^{-1}) and CCl_4 injected group and treated with a mixture of curcumin (100 mg kg^{-1}) and ginger (50 mg kg^{-1}). The data are expressed as Mean±SD of 9 rats in each group, #p<0.0001 when compared with normal group, *p<0.0001 when compared with CCl_4 group

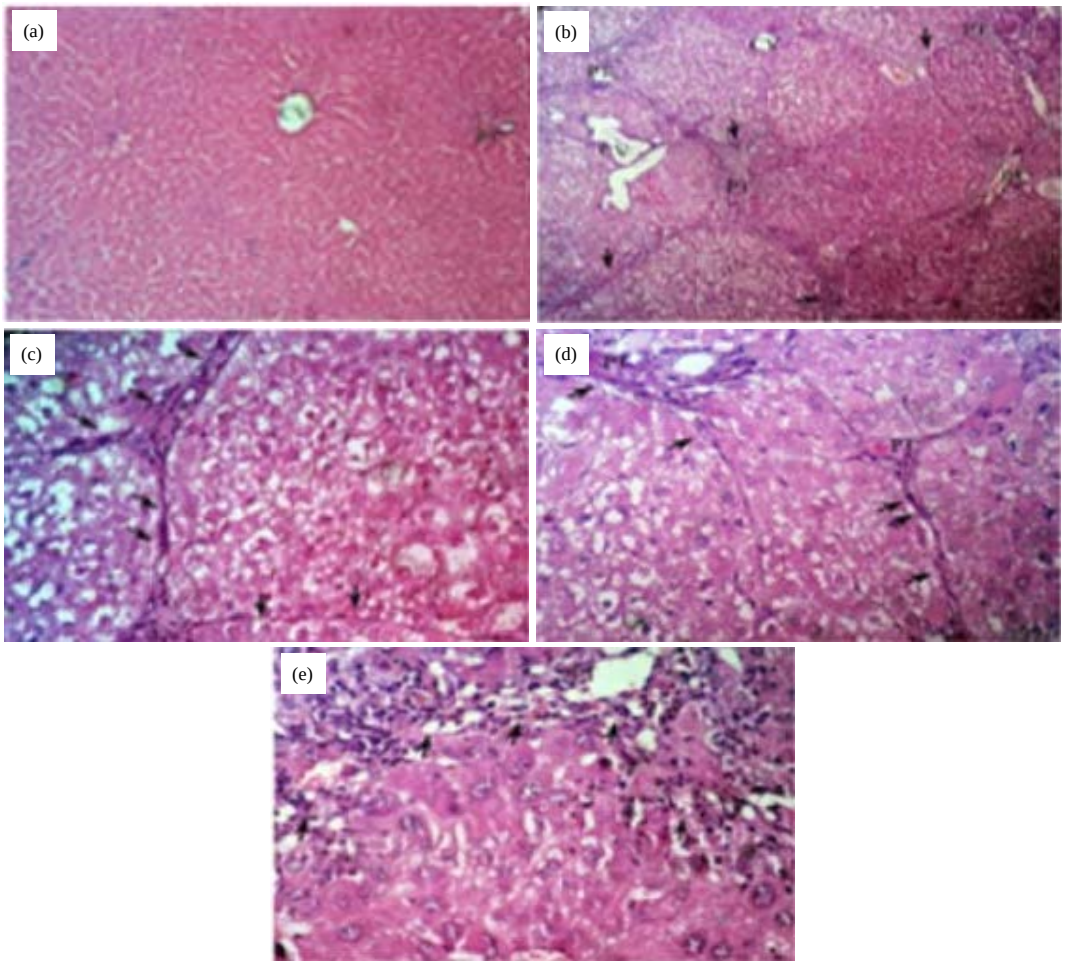

Fig. 2(a-e): Effects of curcumin and ginger on histopathological changes in rat liver in CCl_4-induced hepatic injury. Histological examination under a light microscope with haematoxylin and eosin staining, (a) Healthy liver showing normal architecture of hepatocyte, (b) Marked necroinflammatory changes and fibrosis after CCl_4 injection, (c) Liver section after adding curcumin (200 mg kg^{-1})+CCl_4 showing moderate improvement of necroinflammatory changes, (d) Moderate improvement of necroiflammatory changes after addition of ginger (100 mg kg^{-1})+CCl_4 and (e) Liver section after treatment of CCl_4 injected rats with a mixture of curcumin+ginger, CV: Central vein and PT: Portal artery

liver tissue structure as can be observed by decreased the degree of necrosis, in spite of the presence of slight thickening and fibrosis in blood vessels are shown in Fig. 2c and d, respectively. The liver sections of injected rats treated with a mixture of curcumin and ginger showed lymphatic infiltration in the portal area, degeneration of hepatocytes and thickening of blood vessels with mild fibrosis (Fig. 2e).

DISCUSSION

Liver cirrhosis a common clinical end point of all chronic liver diseases and is characterized by tissue fibrosis and the conversion of normal liver architecture into structurally abnormal nodules (Bataller and Brenner, 2005; Iredale, 2007; Friedman, 2008).

Liver is highly susceptible to drug-induced toxicity due to its role primarily in drug metabolism. The pathophysiological mechanisms of drug-induced hepatotoxicity are still to be elucidated (Au *et al*., 2011; Gu and Manautou, 2012). Liver aminotransferases (ALT and AST) are sensitive indicators for the hepatic function and their excessive leakage into the blood circulation is usually associated with impaired hepatocellular function and designates the disruption of integrity of hepatic cell membranes (Patrick-Iwuanyanwu *et al*., 2007; Wafay *et al*., 2012).

The present study showed a significant elevation of ALT and AST enzymes activity after CCl_4 injection (0.5 mL kg^{-1}) for 8 weeks . In addition to the reduction of albumin and total protein levels. The present findings are in agreement with that of Tu *et al*. (2012) and Zhao *et al*. (2014), found that, when rats injected with CCl_4 (2 mL kg^{-1}) for 6 weeks the concentration of ALT and AST enzymes were raised. Also, Abd-Allah *et al*. (2015) stated that the activity of ALT and AST enzymes were significantly increased in rats injected with CCl_4 (0.5 mL kg^{-1}) for 4 weeks compared with normal rats. In addition to Sahreen *et al*. (2015) and Mohamed *et al*. (2014), found significant decrease in albumin and total protein levels in CCl_4 injected rats, which can be considered a useful index of the severity of cellular dysfunction in liver disease. The elevation in liver enzymes may be due to the free radical produced due to CCl_4 metabolites, which cause cellular disorders and pathological alternations (Hiraganahalli *et al*., 2012; Nazima *et al*., 2014).

The biochemical findings of the present study were confirmed by histopathological results, in which liver of CCl_4 injected rats, showed severe liver injury and fibrosis as evidence by marked necroinflammatory changes and distortion of liver architecture.

The body has an effective defense mechanism to prevent and neutralize the free radical-induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH). These enzymes constitute a mutually supportive team of defense against ROS. In CCl_4-induced hepatotoxicity, the balance between ROS production and these antioxidant defense is lost and oxidative stress results (Castro *et al*., 1974; Guerri and Grisolia, 1980; Pillai and Pillai, 2002).

In the present study, CCl_4 injected rats showed a significant increase in MDA level compared with normal rats and reduction of SOD, CAT and GSH activities. Findings of the present study are parallel with those obtained by Hismiogullari *et al*. (2015) and Abdou *et al*. (2015), observed an elevation of MDA level in CCl_4 injected rats compared with normal rats. Also, results of the present study goes with that observed by Mohamed *et al*. (2014) and Shehab *et al*. (2015), found that CCl_4 leads to a significant decrease in glutathione catalase and superoxide dismutase activities.

The elevation in MDA level may be due to an increase in the membrane lipid peroxidation. However, the reduced activities of SOD, CAT and GSH observed in the present study, indicate hepatic damage induced by CCl_4 injection. This decrease may be due to the increase production of reactive oxygen radicals that reduce the activity of these enzymes.

Curcumin exhibits a wide range of anti-fibrotic properties, including anti-epithelial-mesenchymal transition, anti-apoptosis, anti oxidation and anti-inflammation properties (Zhou *et al*., 2014). Curcumin treatment (200 mg kg^{-1}) for CCl_4 injected rats resulted in reduction of liver enzymes activity (ALT and AST) and an improvement of albumin and total protein levels. Present study results in agree with Fu *et al*. (2008), Yao *et al*. (2012) and Tu *et al*. (2012), demonstrated that curcumin significantly protect rats liver from injury by reducing activities of ALT and AST. Also, Zhao *et al*. (2014) found that low levels of albumin and total protein induced by CCl_4 increased remarkably after treatment with high and low doses of curcumin.

In consistence with the reduction of liver enzymes activities, curcumin also reduced the MDA level and elevated the activity of antioxidant enzymes (SOD, CAT and GSH). These results in agree with Hismiogullari *et al*. (2015), found that, activity of antioxidant enzymes has improved in CCl_4 injected rats after administration of curcumin. From these results, hypothesizing that curcumin might protect the liver from CCl_4 injury by attenuating oxidative stress suppressing inflammation.

Ginger possess hepatoprotective effects against the toxic effects of diverse class of xenobiotic (Shati and Elsaid, 2009). Ginger rhizome has 6-gingerol and 6-shogaol, which have a high antioxidant activity (Saeid *et al*., 2010).

The present study showed that, CCl_4 injected rats and treated with ginger (100 mg kg^{-1}) was effective in reducing the liver injury induced by CCl_4 leading to the reduction of ALT and AST activities and an improvement of both albumin and total protein levels. Findings of the present study in agree with Abdel-Azeem *et al*. (2013) reported that ginger (100 mg kg^{-1}) was effective in reducing liver enzymes activities of rat hepatotoxicity induced by acetoaminophen. In addition, Patrick-Iwuanyanwu *et al*. (2007) reported that injection with CCl_4 and fed on ginger, increase levels of albumin and total protein. On the other hand, treatment with ginger leads to a decrease in the level of MDA and increase activity of antioxidant enzymes. These results goes with Motawi *et al*. (2011) stated that treatment of liver fibrosis in rats with selected extracts of ginger significantly decreased MDA level, but significantly increased SOD and GSH activity, the same as in the present study. In addition, Bardi *et al*. (2013) and Ali *et al*. (2014) stated that, ginger improves antioxidant enzymes activity of rats injected with thioacetamide.

The histopathological results of the present study revealed that treatment of CCl_4 injected rats with curcumin and ginger showed moderate improvement of

necroinflammatory changes and fibrosis. These results clarified that treatment with curcumin and ginger, attenuated the severity of inflammation and necrosis induced by CCl_4, which might be due to their antioxidant effects.

On contrary, treatment of CCl_4 injected rats with the mixture of curcumin and ginger do not completely ameliorative the hepatotoxicity of CCl_4. Such foundation need further studies to modulate the doses, which can be completely improved the hepatotoxicity.

The present study, showed significant increase in level of creatinine of CCl_4 non-treated rats as compared with normal rats. In CCl_4 injected rats and treated with curcumin or ginger, there is a significant reduction in creatinine level. Raskovic *et al*. (2015) and Taj *et al*. (2014), reported a significant elevation of creatinine level in CCl_4 intoxicated rats. Also, the results of the present study compatible with Hismiogullari *et al*. (2015) stated that, treatment of CCl_4 injected rats with curcumin suppressed the increase in serum creatinine and blood urea nitrogen. Also, El-kott *et al*. (2015), found that treatment of CCl_4 injected rats with ginger improve kidney function by decreasing creatinine and urea levels.

CONCLUSION

The results of the present study demonstrated that curcumin protects the liver of rats from CCl_4-induced injury by suppressing hepatic inflammation and attenuating hepatic oxidative stress. Ginger has the ability to down regulate free radicals elevation, improve liver and ameliorate hepatic marker enzymes. Curcumin is more effective in improving the studied parameters. Therefore, further studies for ascertaining the best effective dose for the mitigation of liver diseases are needed. Also, from the present study, it can be concluded that, treatment with a mixture of curcumin and ginger is not effective than treatment with each of them.

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