Patho-physiological studies on the Reverse Effect of Curcumin (Curcuma longa, Zingiberaceae) and Ursofalk (Ursodeoxycholic acid) against the Toxicity of Carbon Tetrachloride on Albino Rats

Muhammad MA Salman*, Randa and Abdel-Rahman
Department of Zoology, South Valley University, Qena, Egypt

Abstract

This study, was established five groups of albino rats to determine the therapeutic effect of Curcuma longa and ursofalk against the toxicity of CCl₄ in liver and kidney. Group (1) was received orally NaCl 0.9% and used as a normal group. Group (2) was injected intraperitoneal (i.p.) with CCl₄ (1 ml/kg), 3 times weekly, for 2 weeks. Group (3) was given orally Ursofalk (100 mg/kg per body weight), group (4) was given orally Curcuma longa (100 mg/kg body weight) and group (5) was given the same doses of Ursofalk plus Curcuma longa for 30 days respectively, post-injected intraperitoneal (i.p.) with CCl₄ (1 ml/kg) 3 times weekly, for 2 weeks. Two blood samples were collected, for hematological and biochemical parameters. Specimens from liver and kidney were collected for histopathological examination. Group (2) revealed a highly significant decrease in total RBCs, platelets, Hb and PCV, serum uric acid, albumin, glucose, HDL-cholesterol, besides catalase, GSH, SOD activities in liver tissue, while WBCs, serum ALT, AST, ALP, γ-GT, creatinine, urea, cholesterol, triglycerides and LDL-cholesterol levels, besides Malondialdehyde and Nitric Oxide levels in liver tissue showed a highly significant increase. Meanwhile, groups (3, 4 and 5) displayed reverse effect in all parameters and return to normal. The histological results displayed inflammation with necrosis and degenerative changes in group (2), while remain groups showed mild changes particularly in group (5). It could be concluded that CCl₄ induced destruction in liver and kidney, which showed a clear improvement by using of Ursofalk and Curcuma longa as treatment.

Keywords: Albino rats; Carbon tetrachloride; Ursodeoxycholic acid; Curcuma longa; Patho-physiological; Antioxidant enzymes

Introduction

Carbon tetrachloride (CCl₄) is considered as one of the environmental pollutants which mainly caused hepatotoxic effect; it is most widely used for experimental induction of hepatic cirrhosis [1] CCl₄ induces liver necrosis, fibrosis, cirrhosis and acute tubular necrosis in the kidney [2]. In addition, CCl₄ also alters the antioxidant profile of the liver including the antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GSH) [3]. Thus, CCl₄ was chosen in this study as model for investigating radical-induced damage and its prevention in albino rats.

Ursodeoxycholic acid (UDCA) is normally present in human bile even in a low concentration of only about 3% of total bile acids; it has been used in Chinese traditional medicine for the treatment of liver diseases [4]. A number of clinical and experimental data have been obtained on a beneficial effect of UDCA in noncholestatic liver injury, UDCA prevents damaging liver mitochondrial function and structure in chronic alcohol intoxication [5], improves biochemical parameters and physical properties of liver plasma membranes [6].

Plants have played a significant role in maintaining human health and improving the quality of human life. Some herbal extracts are known to prevent the oxidative damages in different organs by altering the levels of cytochrome P-450 through their antioxidant properties [7]. Curcuma longa is one of the most common medicinal plants; it is a perennial herb and member of the Zingiberaceae (ginger) family cultivated extensively in Asia, India, China, and other countries with a tropical climate. It is widely used as a food additive and coloring agent [8]. It has been used as a traditional remedy for the treatment of inflammation and other pharmacological effects [9]. It acts as a free-radical scavenger or blocker to inhibit peroxidation of membrane lipids [8,10].

The aim of the work was, to elucidate the reverse effect of Curcumin (Curcuma longa, Zingiberaceae) and Ursofalk (Ursodeoxycholic acid) against the toxicity of carbon tetrachloride on albino rats.

Materials and Methods

Chemicals

- CCl₄ was obtained from El-Nasr Pharmaceutical Chemical Company.
- Ursofalk capsules (Ursodeoxycholic acid 250 mg per capsule) was purchased from local pharmacy in Egypt.
- Curcuma longa fresh rhizomes were purchased from local market, powdered and left in 95% (v/v) ethanol for 48 hours using Soxhlet apparatus. The extract was filtered and concentrated to dark yellow residue on a rotary evaporator [11].

Animals

Fifty adult males albino rats of age range (2.5-3 months) and weight about (230-280 g), where maintained in animal house in cages (10 rats/cage), in air conditioned room, they fed on adequate stable commercial

*Corresponding author: Muhammad MA Salman, Faculty of Science, Department of Zoology, South Valley University, Qena, Egypt, Tel: 201159600729; E-mail: Salman2_2014@yahoo.com

Received September 25, 2015; Accepted August 11, 2016; Published August 19, 2016

Citation: Salman MMA, Randa, Rahman A (2016) Patho-physiological studies on the Reverse Effect of Curcumin (Curcuma longa, Zingiberaceae) and Ursofalk (Ursodeoxycholic acid) against the Toxicity of Carbon Tetrachloride on Albino Rats. J Liver 5: 200 doi: 10.4172/2167-0889.1000200

Copyright: © 2016 Salman MMA, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
balanced diet and examined daily for two weeks, before starting the experiment.

**Experimental design**

The experimental animals were classified into five groups, each containing 10 rats.

- **Group 1**: The rats were received orally NaCl 0.9% as (normal group).
- **Group 2**: The rats were injected i.p. with CCl4 (1 ml/kg per b.wt.), 3 times weekly, for 2 weeks used as (control group).
- **Group 3**: The rats were injected i.p. with CCl4 (1 ml/kg per b.wt.), 3 times weekly for 2 weeks, followed by oral administrated doses of Ursosfalk drug (100 mg/kg per body weight) daily for 30 days.
- **Group 4**: The rats were injected i.p. with CCl4 (1 ml/kg per b.wt.), 3 times weekly for 2 weeks, followed by oral administrated of Curcuma longa (100 mg/kg b.wt.) daily for 30 days.
- **Group 5**: The rats were injected i.p. with CCl4 (1 ml/kg per b.wt.), 3 times weekly for 2 weeks, followed by orally administrated with Ursosfalk drug (100 mg/kg per b.wt.) plus Curcuma longa (100 mg/kg body weight) daily for 30 days.

The doses used in this study were chosen based on previous researches, CCl4 [12], ursofalk drug [13] and curcuma longa [11]. All experimental animals were sacrificed at the end of the experiment. The blood, serum, besides liver tissue were collected from the experimental groups for hematological and biochemical analysis, respectively. Specimens from liver and kidneys were collected from the sacrificed animals for histopathological examinations.

**Haematological analysis**

Complete blood picture red blood cells count (RBCs), leukocytes count (WBCs), total hemoglobin, platelets count and hematocrit assays done by Automated Hematology Analyzer (Diff3) Mek-6410/Mek-6420.

**Biochemical analysis in serum**

Alanine aminotranferase (ALT) and aspartate aminotransferase (AST) [14], alkaline phosphatase (ALP) [15], albumin [16], γ-Glutamyl transferase (γ-GT), creatinine [17], urea [18], uric acid [19], glucose [20], cholesterol [21], triglycerides [22], HDL-Cholesterol [23] and LDL-Cholesterol [24], were analyzed according to the reported methods.

**Biochemical analysis of liver tissue homogenate**

Reduced glutathione (GSH) [25], Superoxidedismutase (SOD) [26], Catalase (CAT) [27], Malondialdehyde (MDA) [28] and nitric oxide [29] were analyzed as described in the reported methods. All mentionned kits were bought from bio-diagnostic co. Giza, Egypt.

**Preparation of tissue homogenate**

Liver tissues were homogenized as following:

- a) Prior to dissection, perfuse tissue with phosphate buffered saline (PBS) solution pH=7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots.
- b) Homogenize the tissue in 10-15 ml cold buffer per gram tissue in pastel hemogenizer.
- c) Centrifuge at 4000 rpm for 15 min at 4ºC.
- d) Remove the supernatant for biochemical assay

**Histopathological examination**

Specimens from liver and kidneys were collected from the sacrificed animals. The tissues were kept in 10% neutral buffered formalin and processed by paraffin embedding technique, sectioned at 5 µ thickness and stained with hematoxylin and eosin [30].

**Statistical analysis**

The results expressed as means ± S.E. and made by one-way analysis of variance (ANOVA) using Graph Pad Prism 03n software. Statistical significance was set at p<0.05.

**Results**

**Haematological results**

As shown in Table 1 RBCs count, PCV value and platelets count were highly significant decreased (p<0.01) and Hb concentration was significantly decreased (p<0.05) while WBCs count was highly significantly increased (p<0.01) in control group when compared with normal group. RBCs count, Hb concentration, PCV value and Platelets count were significantly increased in rats treated with ursofalk (group3), increased in rats treated with curcuma longa (group 4) and highly significantly increased in rats treated with ursofalk drug+curcuma longa (group 5), while WBCs count was significantly decreased in groups (3 and 4) and highly significantly decreased (group 5) when compared with control group.

**Biochemical results**

The activities of serum ALT, AST, ALP and γ-GT were highly significantly increased when serum albumin was significantly decreased in control group when compared with normal group as shown in Table 2. A significant decrease of ALT, AST, ALP and γ-GT showed in (group curcuma longa) and highly significantly increased in rats treated with ursofalk drug+curcuma longa (group 5), while WBCs count was significantly decreased in groups (3 and 4) and highly significantly decreased (group 5) when compared with control group.

**Table 1**: Effect of daily oral administration doses of Ursosfalk drug (100 mg/kg body weight), Curcuma Longa (100 mg/kg body weight) and Curcuma longa+Ursosfalk drug after 30 days of treatment on complete blood count (RBCs, WBCs, Hb, platelets and PCV) of Albino rats, injected with CCl4 (1 ml/kg. b. w.).
3), the same parameters decreased in (group4). ALT, AST, ALP showed a highly significant decrease while γ-GT showed a significant decrease in (group5) when compared with control group. Serum albumin was significantly increased in (group3), increased in (group 4) and highly significant increase in (group 5) when compared with control group.

As shown in Table 3 the concentration of serum creatinine and urea in control group were highly significantly increased, while serum uric acid concentration was significantly decreased when compared with normal group. Creatinine and urea concentration showed a significant decrease (group 3), decreased (group 4) and highly significant decrease in (group5) while uric acid concentration recorded a significant increase in (group3), increased in (group 4) and highly significant increase in (group 5) when compared with control group.

Serum glucose level as shown in Table 3 recorded a significant decrease in control group when compared with normal group. A significant increase showed in glucose level (group3), increased (group 4) and a highly significant increase (group 5) when compared with control group.

As shown in Table 4 serum cholesterol, triglycerides and LDL-cholesterol were highly significantly increased while serum HDL-cholesterol was significantly decreased in control group when compared with normal group. A significant decrease in serum cholesterol, triglycerides and LDL-cholesterol and a significant increase in serum HDL-cholesterol were recorded in groups (3, 4 and 5) when compared with control group.

Liver homogenate biochemical results

The results recorded in Table 5 revealed that the activities of liver GSH and CAT were highly significantly decreased and the activity of liver SOD was significantly decreased while activities of liver MDA and NO were highly significant increased in control group when compared with normal group. The activities of liver GSH, SOD and CAT were significantly increased (group 3), increased in (group 4) and highly significantly increased in (group5) when compared with control group. The activities of liver MDA and NO were significant decreased in (group3), decreased in (group4) and highly significant decreased in (group5) when compared with control group.

Histopathological results

The liver in group (2) showed congestion with severe dilatation

| Groups  | ALT (Units/ml) | AST (Units/ml) | Albumin (g/dL) | ALP (IU/L) | γ-GT (u/L) |
|---------|----------------|----------------|---------------|------------|------------|
| Group. 1 | Mean ± S. E. | Mean ± S. E. | Mean ± S. E. | Mean ± S. E. | Mean ± S. E. |
| 70.71 ± 1.90 | 77.86 ± 2.44 | 3.36 ± 0.173 | 64.35 ± 1.65 | 35.07 ± 1.42 |
| Group. 2 | 106.4 ± 3.50 | 119.9 ± 2.75 | 1.49 ± 0.171 | 104.1 ± 3.79 | 74.75 ± 2.01 |
| Group. 3 | 87.86 ± 1.57 | 91.43 ± 2.33 | 2.20 ± 0.141 | 87.66 ± 2.08 | 57.80 ± 2.61 |
| Group. 4 | 92.29 ± 1.63 | 98.43 ± 2.02 | 1.81 ± 0.169 | 91.73 ± 1.94 | 64.87 ± 2.24 |
| Group. 5 | 77.43 ± 2.20 | 81 ± 1.45 | 2.77 ± 0.169 | 73.67 ± 2.21 | 41.46 ± 1.75 |

Table 2: Effect of daily oral administration doses of Ursofalk drug (100 mg/kg body weight), Curcuma Longa (100 mg/kg body weight) and Curcuma Longa + Ursofalk drug after 30 days of treatment on serum ALT, AST, albumin, ALP and γ-GT levels of Albino rats, injected with CCl4 (1 ml/kg b. w.).

| Groups  | Creatinine (mg/dL) | Urea (g/dL) | Uric acid (mg/dL) | Glucose (IU/L) |
|---------|---------------------|-------------|-------------------|----------------|
| Group. 1 | Mean ± S. E. | Mean ± S. E. | Mean ± S. E. | Mean ± S. E. |
| 0.425 ± 0.05 | 29.7 ± 2.44 | 3.23 ± 0.24 | 88.26 ± 2.71 |
| Group. 2 | 0.912 ± 0.061 | 177.66 ± 2.91 | 1.43 ± 0.14 | 60.36 ± 2.14 |
| Group. 3 | 0.628 ± 0.054 | 63.80 ± 2.73 | 2.26 ± 0.17 | 73.23 ± 2.17 |
| Group. 4 | 0.757 ± 0.024 | 57.53 ± 1.92 | 2.06 ± 0.20 | 70.24 ± 2.19 |
| Group. 5 | 0.494 ± 0.027 | 36.77 ± 1.67 | 2.70 ± 0.186 | 82.71 ± 2.07 |

Table 3: Effect of daily oral administration doses of Ursofalk drug (100 mg/kg body weight), Curcuma Longa (100 mg/kg body weight) and Curcuma Longa + Ursofalk drug after 30 days of treatment on serum creatinine, urea, uric acid and glucose levels of Albino rats, injected with CCl4 (1 ml/kg b. w.).

| Groups  | Cholesterol (mg/dL) | Triglycerides (mg/dL) | HDL-Cholesterol (mg/dL) | LDL-Cholesterol (mg/dL) |
|---------|---------------------|-----------------------|-------------------------|------------------------|
| Group. 1 | Mean ± S. E. | Mean ± S. E. | Mean ± S. E. | Mean ± S. E. |
| 65.26 ± 2.69 | 62.75 ± 1.98 | 42.22 ± 2.10 | 28.87 ± 1.71 |
| Group. 2 | 89.19 ± 2.28 | 83.62 ± 1.87 | 29.26 ± 2.37 | 40.07 ± 2.91 |
| Group. 3 | 76.66 ± 1.60 | 74.32 ± 1.96 | 35.07 ± 2.07 | 33.36 ± 2.10 |
| Group. 4 | 81.90 ± 2.23 | 77.90 ± 1.46 | 33.25 ± 1.93 | 35.29 ± 1.87 |
| Group. 5 | 68.86 ± 2.52 | 66.74 ± 2.36 | 40.87 ± 2.23 | 29.24 ± 2.01 |

Table 4: Effect of daily oral administration doses of Ursofalk drug (100 mg/kg body weight), Curcuma Longa (100 mg/kg body weight) and Curcuma Longa + Ursofalk drug after 30 days of treatment on serum cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol levels of Albino rats, injected with CCl4 (1 ml/kg b. w.).
in the blood sinusoid causing hepatic atrophy, besides vacuolation in some hepatic cells. Focal areas of diluted blood vessels replaced the necrotic hepatic cells (Figure 1). The kidneys in the same group showed thickening in the wall of the blood vessels with perivascular edema. Perivascularitis characterized by inflammatory edema surrounded the congested blood vessels, besides thrombosis in its components (Figure 2). Fibrous tissues proliferated among the renal tubules forming lobules in the kidneys (Figure 3).

The liver in the rats of group (3) noticed moderate degeneration and necrosis in some of hepatocytes. Congestion in the blood vessels with aggregation of rounded cells around it (Perivascularitis) was seen. Regeneration was detected in the hepatic cells (Figure 4). The hepatocytes displayed normal in the architecture of the hepatic cells adjacent to the central vein vascular degeneration in the hepatocytes with congestion in the central veins (Figure 5). The kidneys in group (3) showed perivascular edema with congestion and hyalinization in the wall of the blood vessels (Figure 6).

The Liver in group (4) showed few fibrous tissues proliferation surrounded the blood vessels and bile ducts in the portal tract (Figure 7). The kidneys in group (4) showed congestion in the glomerular capillaries. The renal tubules displayed mild necrosis and degenerative changes. Hyalinization in the wall of the blood vessels was detected. The liver in group (5) showed congestion in the blood vessels, besides necrosis in few of hepatic cells. The Kidneys in group (5) showed congestion in the glomerular capillaries and periglomerular blood vessels (Figure 8).

Discussion

In the present study carbon tetrachloride (1 ml/kg body weight) induced a highly significant decrease in RBCs count, platele count and PCV value and significant decrease in HB concentration. According to [31], depletion in RBCs count and HB content leads to iron deficiency anemia which is characterized by a microcytic hypochromic blood picture. Furthermore, the depression in RBCs count and HB content recorded in the present work could be attributed to disturbed hematopoiesis, destruction of erythrocytes, and reduction in the rate of their formation and/or their enhanced removal from circulation [32].

CCl4 induced a highly significant increase in WBCs count; this increase may be attributed to the defensive mechanism of immune system [33]. Treatment with CCl4 which reflecting decreased gluconeogenesis by the liver [46]. The improvements in glucose levels in (group3) could be due to the decreased hepatic glycogen content after treatment with CCl4 which caused structural integrity damage of the liver cell membrane and hence a leakage of the cellular enzymes in to the blood [37]. The reduction in serum albumin (control group) is due to the hepatic injury which caused by CCl4 [38]. The significant reduction of all the serum liver enzymes and the significant increase in serum albumin in case of ursofalk drug has been attributed to its mechanism on membrane stabilizing [39]. Curcuma longa may enhance the molecular mechanism of enzymes action; this could explain the reduction in serum liver enzymes [40]. Also this reduction in serum ALT and AST levels due to the antioxidative activity of curcuma longa [41].

CCl4 (1 ml/kg body weight) in the present study induced alternations in creatinine urea and uric acid (control group) the increase in serum creatinine and urea levels may indicate a reduction in the glomerular filtration rate (GFR) as a result of acute renal dysfunction as mentioned by Gavin [42]. The reduction in serum uric acid level in the present study may be attributed to the increased utilization of uric acid against increased production of the free radicals since it has a capable especially of reacting with free radicals [43]. Treatment with ursofalk drug (group3) and curcuma longa (group4) ameliorates the elevation of kidney functions parameters, as mentioned by Wong [44]. Ursodeoxycholic acid is an antioxidant, and its vascular effects could be mediated via a reduction in oxidative stress. The improvements in renal function markers in (group4) may be due to the protective effect of curcumin against renal injury by suppressing oxidative stress, increasing kidney GSH content and glutathion peroxidase activity [45]. The present study declared that, administration of CCl4 induced a significant reduction in serum glucose compared with normal group this may be due to the decreased hepatic glycogen content after treatment with CCl4 which reflecting decreased gluconeogenesis by the liver [46]. The improvements in glucose levels in (group3) could be explained by Zavodnik [47] who mentioned that ursodeoxycholic acid completely normalized the blood glucose level in alloxan-treated rats where it may prevents induction and development of diabetes and its complications by protecting the β-cell membrane. In (group4) curcuma longa improvement mechanism explained by Kanitkar [48] who stated that curcuminoid, which is component of turmeric, inhibited the formation of oxygen species associated with damage and dysfunction of langerhans islets. The present results showed that CCl4 induced a highly significant increase in serum Cholesterol, Triglycerides and LDL-Cholesterol levels while serum HDL-Cholesterol level was significantly decreased, it has been shown that micro-viscosity of a membrane increase markedly with increases in cholesterol and phospholipids ratio thus leading to cellular rigidity. Intoxication of rats with CCl4 levels while it induced a reduction in serum albumin, compared with normal animals, the hepatotoxic effects of CCl4 are largely due to its active metabolite, trichloromethyl radical [36]. These elevations in the serum liver marker enzymes could be attributed to the free radicals which caused structural integrity damage of the liver cell membrane and hence a leakage of the cellular enzymes in to the blood [37]. The reduction in serum albumin (control group) is due to the hepatic injury which caused by CCl4 [38]. The significant reduction of all the serum liver enzymes and the significant increase in serum albumin in case of ursofalk drug has been attributed to its mechanism on membrane stabilizing [39]. Curcuma longa may enhance the molecular mechanism of enzymes action; this could explain the reduction in serum liver enzymes [40]. Also this reduction in serum ALT and AST levels due to the antioxidative activity of curcuma longa [41].

In the present study carbon tetrachloride (1 ml/kg body weight) induced a highly significant decrease in RBCs count, platele count and PCV value and significant decrease in HB concentration. According to [31], depletion in RBCs count and HB content leads to iron deficiency anemia which is characterized by a microcytic hypochromic blood picture. Furthermore, the depression in RBCs count and HB content recorded in the present work could be attributed to disturbed hematopoiesis, destruction of erythrocytes, and reduction in the rate of their formation and/or their enhanced removal from circulation [32].

CCl4 induced a highly significant increase in WBCs count; this increase may be attributed to the defensive mechanism of immune system [33]. Treatment with CCl4 which reflecting decreased gluconeogenesis by the liver [46]. The improvements in glucose levels in (group3) could be explained by Zavodnik [47] who mentioned that ursodeoxycholic acid completely normalized the blood glucose level in alloxan-treated rats where it may prevents induction and development of diabetes and its complications by protecting the β-cell membrane. In (group4) curcuma longa improvement mechanism explained by Kanitkar [48] who stated that curcuminoid, which is component of turmeric, inhibited the formation of oxygen species associated with damage and dysfunction of langerhans islets. The present results showed that CCl4 induced a highly significant increase in serum Cholesterol, Triglycerides and LDL-Cholesterol levels while serum HDL-Cholesterol level was significantly decreased, it has been shown that micro-viscosity of a membrane increase markedly with increases in cholesterol and phospholipids ratio thus leading to cellular rigidity. Intoxication of rats with CCl4
may have altered membrane structure and function as suggested by the increase in cholesterol level [49]. Ursodeoxycholic acid may exert significant changes on cholesterol metabolism in patients with primary biliary cirrhosis and, ultimately, significantly reduce the risk associated with hypercholesterolemia [50,51]. The hyperlipidemic effect of CCl₄ ameliorated in rats treated with *curcuma longa* (group 4) [52], reported that, the effect of curcumin on cholesterol could be due to an effect on cholesterol absorption, degradation or elimination.

The present study showed that administration of CCl₄ produced marked oxidative impact as evidenced by the highly significant decreased in the hepatic SOD, catalase, and GSH activities. Peroxidative damage by CCl₄ is the result of reductive dehalogenation, which is catalysed by P-450 and forms the highly reactive trichloromethyl-free radical CCl₃. This then readily interacts with molecular oxygen to form the trichloromethyl peroxy radical CCl₃OO. Both radicals are capable of binding to proteins or lipids or of abstracting a hydrogen atom from an unsaturated lipid, which initiates liver damage and plays a significant role in the pathogenesis of diseases [53]. In the present study, administration of CCl₄ induced a highly significant increase in MDA and nitric oxide levels in liver of treated rats, this increase explained by Fraga et al. [54], who stated that the high significant elevation of MDA level in liver homogenate of rats treated with CCl₄ indicated excessive formation of free radicals and activation of lipid peroxidation of cell damage [55], proposed that a high level of nitric oxide is associated...
with CCl₄-induced acute liver injury. In rats treated with ursofalk drug, the elevation in GSH, SOD, catalase, MDA and NO activities explained by Lukivskaya et al. [56] who stated that ursodeoxycholic acid administration reduced the production of reactive oxygen forms in the liver, the content of lipid peroxidation carbonyl-containing products (alkenals, alkanals, ketones, oxyalkenals and MDA), and the activities of antioxidant defense enzymes (SOD). Moreover, ursodeoxycholic acid normalized liver microsomal cytochrome P-450 level. The ability of *Curcuma longa* to increase GSH, catalase and SOD activities and decreased MDA and NO levels in liver tissue of treated rats explained by Sreejayan et al. [57], who stated that the presence of phenolic groups in the structure of curcumin is fundamental to explain these pathological changes, even more (group 5) showed a marked improvements in liver and kidney tissues. It could be concluded that [62] CCl₄ induced severe destruction in most organs, which showed a clear improvement by using combination of ursofalk and *Curcuma longa*.

### Acknowledgment

We are most grateful to Prof. Dr. Abd-Elrahim A. Elshater - Faculty of Science and Prof. Dr. Mouchira M. Mohi El-din Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, at the University of South Valley University in Egypt for their invaluable insights and suggestions.

### References

1. Abd El Dayem SM, Moawad KM (2001) Toxicity of CCl₄ in rat liver and the effects of antioxidant treatments. J Egypt Soc Zool 36: 415-442.
2. Manibusan MK, Odmir D, Eastmond DA (2007) Postulated carbon tetrachloride mode of action: a review. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 25: 185-209.
3. Shewelita SA, Abd El-Gabbar M, Bastawy M (2001) Carbon tetrachloride-induced changes in the activity of phase II drug-metabolizing enzyme in the liver of male rats: role of antioxidants. Toxicology 165: 217-224.
4. Hagey LR, Crombie DL, Espinosa E, Carey MC, Igini H, et al. (1993) Ursodeoxycholic acid in the Ursidae: biliary bile acids of bears, pandas, and related carnivores. J Lipid Res 34: 1911-1917.
5. Tabouy L, Zamora AJ, Oliwa L, Montel AM, Beauge F, et al. (1998) Ursodeoxycholate protects against ethanol-induced liver mitochondrial injury. Life Sci 63: 2259-2270.
6. Oliwa L, Beaugé F, Choquet D, Montel AM, Guitaoui M, et al. (1998) Ursodeoxycholate alleviates alcoholic fatty liver damage in rats. Alcohol Clin Exp Res 22: 1538-1543.
7. Kandasamy CS, Shima TP, Mohammed BE, Arul RP, Gopal V, et al. (2010) Anti-hepatotoxic activity of polyherbal formulation in carbon tetrachloride induced toxicity in rats. RJPBCS 1: 342-346.
8. Miquel J, Bernd A, Sempere JM, Diaz-Alperi J, Ramirez A (2002) The curcuma antioxidants: phaemacological effects and prospects for future clinical use. A review. Arch Gerontol Geriatr 34: 37-46.
9. Asai A, Miyazawa T (2001) Dietary curcuminoinds prevent high-fat diet-induced lipid accumulation in rat liver and epididymal adipose tissue. J Nutr 131: 2932-2935.
10. Venkatesan N (2000) Pulmonary protective effects of curcumin against paraquat toxicity. Life Sci 66: PL21-28.
11. Somchit MN, Sulaiman MR, Norutulunirina R, Ahmed Z (2002) Hepatoprotective effects of *Curcuma Longa* rhizomes in paracetamol-induced liver damage in rats. Proceedings of the Regional Sym Environ. Natural Reso 1: 698-702.
12. Basu L (2003) Carbon tetrachloride-induced lipid peroxidation: eicosanoid formation and their regulation by antioxidant nutrients. Toxicol 189: 113-127.
13. Zhao Y, Zhai D, He H, Liu J, Li T, et al. (2009) Matrine improves 17alpha-ethinyl estradiol-induced acute cholestasis in rats. Hepatol Res 39: 1144-1149.
14. Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28: 56-63.
15. Bellfield A, Goldberg DM (1971) Revised assay for serum phenyl phosphatase activity using 4-amino-antipyrine. Enzyme 12: 561-573.
16. Doumas BT, Watson WA, Biggs HG (1971) Albumin standards and the measurement of serum albumin with bromocresol green. Clin Chim Acta 31: 87-96.
17. Bartels H, Bohmer M, Heierli WA (1972) Serum creatinine determination without protein precipitation. Clin Chim Acta 37: 193-197.
18. Fawoett JK, Scott JE (1960) A rapid and precise method for the determination of urea. J Clin Pathol 13: 156-159.
19. Barham D, Trinder P (1972) An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 97: 142-145.
20. Trinder P (1969) Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J Clin Pathol 22: 246.

**Figure 7**: Liver (group 4) showing fibrous tissues proliferation surrounded the blood vessels and bile ducts in the portal tract formed perportal fibrosis (H&E 300X).

**Figure 8**: Kidneys (group 5) showing congestion in the glomerular capillaries and periglomerular blood vessels (H&E 300X).
21. Richmond W (1973) Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem 19: 1350-1356.

22. Fassati P, Principe L (1982) Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Hem 28: 2077-2208.

23. Burstein M, Scholnick HR, Morfin R (1970) Rapid method for the isolation of lipoproteins from human serum by precipitation with polyamions. J Lipid Res 11: 583-595.

24. Wieland H, Seidel D (1983) A simple specific method for precipitation of low density lipoproteins. J Lipid Res 24: 904-909.

25. Beutler E, Duron O, Kelly BM (1963) Improved method for the determination of blood glutathione. J Lab Clin Med 61: 882-888.

26. Nishikimi M, Appaji N, Yagi K (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 46: 849-854.

27. Aebi H (1948) Catalase in vitro. Enzymol 105: 121-126.

28. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides. In: Animal tissue by thiobarbituric acid reaction. Anal Biochem 95: 351-358.

29. Montgomery HAC, Dymock JF (1961) Colorimetric method for dedetermination of nitrite. Analyst 86: 414.

30. Drury BM, Wallington EA (1980) Carleton's histological technique, 5th Ed. Oxford University Press, New York.

31. Ballinger A (2007) Gastroenterology and anemia. Medicine 35: 142-146.

32. Essawy AE, Hamed SS, Abdel-Moneim AM, Abou-Gabal AA, Alzergy AA (2010) Role of black seeds (Nigella sativa) in ameliorating carbon tetrachloride induced haematoxotoxicity in Swiss albino mice. J Med Plants Res 4: 1977-1986.

33. Miettinen TA, Vuoristo M, Karvonen AL, Leino R, et al. (1995) Cholesterol-lowering effect of curcuminoid mixture in patients with primary biliary cirrhosis. Hepatology 17: 577-582.

34. Patel BP, Gandhi TR (2010) The Protective Activity of Saccharum officinarum Against CCI4 Induced Hepatotoxicity in Rats. Int J Pharm Res 2: 5-8.

35. Eltuk EU, Agaie BM, Ladun MJ, Garba I (2009) The modulatory effect of Cochlospermum viticaria a rich aqueous root extract on liver damage induced by carbon tetrachloride in rats. J Pharmacia Pharm 3: 151-157.

36. Beuers U, Boyer JL, Paumgartner G (1998) Ursodeoxycholic acid in cholestasis: potential mechanisms of action and therapeutic applications. Hepatology 28: 1449-1453.

37. Sengupta M, Sharma GD, Chakraborty B (2011) Hepatoprotective and immunomodulatory properties of aqueous extract of Curcuma longa in carbon tetra chloride intoxicated Swiss albino mice. Asian Pac. J of Trop Biomed pp: 193-199.

38. Shi M, Cai Q, Yao L, Mao Y, Ming Y, et al. (2006) Antiproliferation and apoptosis induced by curcumin in human ovarian cancer cells. Cell Biol Int 30: 221-226.

39. Gavin JB (1995) Assessment of renal function. The Md GR J 23: 102-105.

40. Hasegawa T, Kuroda M (1989) A new role of uric acid as an antioxidant in human plasma. Rinsho Byori 37: 1020-1027.

41. Wong F, Borzon A, Allard J, Liu P, Blendsis L (1999) Effects of ursodeoxycholic acid on systemic, renal and forearm haemodynamics and sodium homeostasis in cirrhotic patients with refractory ascites. Clin Sci (Lond) 96: 467-474.

42. Venkatesan N, Punithavathi D, Arumugam V (2000) Curcumin prevents adriamycin nephrotoxicity in rats. Br J Pharmacol 129: 231-234.

43. Muriel P, Alba N, Perez-Alvarez VM, Shibayama M, Tsutsui VM (2001) Kupffer cells inhibition prevents hepatic lipid peroxidation and damage induced by carbon tetrachloride. Comp Biochem Physiol C Toxicol Pharmacol 130: 219-26.

44. Zavodnik I, Lukivskaya O, Lapshina E, Boku V (2010) Antioxidant correction of diabetic complications. Molec Mech and Pharm of Diab Comp pp: 263-283.

45. Kanikar M, Bhonde RR (2008) Curcumin treatment enhances islet recovery by induction of heat shock response protein, Hsp 70 and heme oxygenase-1, during cryopreservation. Life Sci 82: 182-189.

46. Ojo OO, Radno MS,Tella I0 (2006) Protection of rats by extract of some common Nigeria trees against acetaminophen induced hepatotoxicity. Afric J Biotechnol 5: 755-760.

47. Poupon RE, Ogugmam K, Chretien Y, Verneau C, Eschewege E, et al. (1993) Cholesterol-lowering effect of ursodeoxycholic acid in patients with primary biliary cirrhosis. Gastroenterology 95: 577-582.

48. Miettinen TA, Täkäriä M, Karvonen AL, Leino R, et al. (1995) Serum cholesterol, cholesterol precursors, and plant sterols during placebo-controlled treatment of primary biliary cirrhosis with ursodeoxycholic acid or colchicine. Hepatology 21: 1261-1268.

49. Afra HM (2005) Curcumin attenuates diet-induced hypercholesterolemia in rats. Med Sci Monit 11: BR226-234.

50. Brent JA, Rummack BH (1993) Role of free radicals in toxic hepatic injury. II. Are free radicals the cause of toxin-induced liver injury? J Toxicol Clin Toxicol 31: 173-196.

51. Fraga CG, Leibovitz BE, Tappel AL (1987) Halogenated compounds as inducers of lipid peroxidation in tissue slices. Free Radic Biol Med 3: 119-123.

52. Tipoe GL, Leung KM, et al. (2006) Inhibitors Of Inducible Nitric Oxide (No) Synthase Are More Effective Than An No Donor In Reducing Carbon Tetrachloride-induced Acute Liver Injury. Histol Histopathol 21: 1157-1165.

53. Luikivskaya O, Zavodnik L, Knas M, Boku V (2006) Antioxidant mechanism of hepatoprotection by ursodeoxycholic acid in experimental alcoholic steatohepatitis. Adv Med Sci 51: 54-59.

54. Sreejayan N, Rao MNA, Priyadarshini KI, Devasagayam TPA (1997) Inhibition of radiation induced lipid peroxidation by curcumin. Int J Pharma 151: 127-130.

55. Arora A, Nair MG, Strasburg GM (1998) Antioxidant activities of isoflavones and their biological metabolites in a liposomal system. Arch Biochem Biophys 356: 133-141.

56. El-Sayed EM, Abd El-azeem AS, El-Sayed AH, El-Sayed SM (2001) Cardioprotective effects of Curcuma longa L. extracts against doxorubicin-induced cardiotoxicity in rats. J Med Plants Res 5: 4049-4058.

57. Shih CC, Wu YW, Lin WC (2005) Aqueous extract of Anoectochilus formosanus attenuate hepatic fibrosis induced by carbon tetrachloride in rats. Phytomedicine 12: 453-460.

58. Hubbell WL, McConnell HM (1971) Molecular motion in spin-labeled phospholipids and membranes. J Am Chem Soc 93: 314-326.

59. Szasz G (1969) A kinetic photometric method for serum gamma-glutamyl transpeptidase. Clin Chem 15: 124-136.