Journal of Liver
ISSN: 2167-0889

Salman et al., J Liver 2016, 5:3
DOI: 10.4172/2167-0889.1000200

Research Article Open Access

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 $CC1_4$ 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 CCl_4 (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 CCl_4 (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 CCl_4 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 ($CC1_4$) 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] CCl_4 induces liver necrosis, fibrosis, cirrhosis and acute tubular necrosis in the kidney [2] In addition, CCl_4 also alters the antioxidant profile of the liver including the antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GSH) [3]. Thus, CCl_4 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

- CCl_4 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

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% used as (normal group).

Group 2: The rats were injected i.p. with CCl_4 (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 CCl_4 (1 ml/kg per b.wt.), 3 times weekly for 2 weeks, followed by oral administrated doses of Ursofalk drug (100 mg/kg per body weight) daily for 30 days.

Group 4: The rats were injected i.p. with CCl_4 (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 CCl_4 (1 ml/kg per b.wt.), 3 times weekly for 2 weeks, followed by orally administrated with Ursofalk 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, CCl_4 [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, rspectivetly. 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 aminotranferease (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 metioned kis 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 4°C.

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 embbeding technique, sectioned at 5 μ thickeness 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 significant decreased (group 5) when compared with control group.

Biochemical results

The activities of serum ALT, AST, ALP and γ-GT were highly significant increased while 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

Groups	RBCs count (×10⁶/mm³)	WBCs count (×10³/mm³)	Hb Concn. (g/dl)	Platelets count (×10³/mm³)	P C V value (%)
	Mean ± S. E.	Mean ± S. E.	Mean ± S. E.	Mean ± S. E.	Mean ± S. E.
Group. 1	7.029 ± 0.21	7.043 ± 0.30	13.5 ± 0.28	505.0 ± 4.83	37.50 ± 1.51
Group. 2	5.35^{-a} ± 0.13	10.99^{++a} ± 0.25	11.49^{-a} ± 0.22	412^{-a} ± 3.395	29.5^{-a} ± 1.68
Group. 3	6.1^{+b} ± 0.23	8.48^{-b} ± 0.19	12.63^{+b} ± 0.23	451.6^{+b} ± 9.54	34.57^{+b} ± 1.31
Group. 4	5.80 ± 0.22	9.07 ± 0.27	12.29 ± 0.33	443.1 ± 3.47	33.59 ± 1.16
Group. 5	6.62^{++b} ± 0.21	7.33^{--b} ± 0.32	13.20^{++b} ± 0.41	485.7^{++b} ± 3.14	36.67^{++b} ± 0.83

-a=significantly decreased from normal at p<0.05
++a=highly significantly increased from normal at p<0.01
+b=significantly increased from control at p<0.05
--a=highly significant decreased from normal at p<0.01
-b=significantly decreased from control at p<0.05
++b=highly significant increased from control at p<0.01
--b=highly significantly decreased from control at p<0.01

Table 1: 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 complete blood count (RBCs, WBCs, Hb, platelets and PCV) of Albino rats, injected with CCl_4 (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 decreased in (group 5) 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 (group 3), increased (group 4) and a highly significant increase (group 5) when comared 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 hifhly 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)
	Mean ± S. E.	Mean ± S. E.	Mean ± S. E.	Mean ± S. E.	Mean ± S. E.
Group. 1	70.71 ± 1.90	77.86 ± 2.44	3.36 ± 0.173	64.35 ± 1.65	35.07 ± 1.42
Group. 2	106.4 $^{++a}$ ± 3.50	119.9^{++a} ± 2.75	1.49^{-a} ± 0.171	104.1^{++a} ± 3.79	74.75^{++a} ± 2.01
Group. 3	87.86 $^{-b}$ ± 1.57	91.43^{-b} ± 2.33	2.20^{+b} ± 0.141	87.66 $^{-b}$ ± 2.08	57.80 $^{-b}$ ± 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 $^{-b}$ ± 2.20	--b 81 ± 1.45	++b 2.77 ± 0.169	-b 73.67 ± 2.21	--b 41.46 ± 1.75

-a=significantly decreased from normal at p<0.05 ++a=highly significantly increased from normal at p<0.01
+b=significantly increased from control at p<0.05 ++b=highly significant increased from control at p<0.01
-b=significantly decreased from control at p<0.05 --b=highly significant decreased from control at p<0.01

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 CCl_4 (1 ml/kg b. w.).

Groups	Creatinine (mg/dL)	Urea (g/dL)	Uric acid (mg/dL)	Glucose (IU/L)
	Mean ± S. E.	Mean ± S. E.	Mean ± S. E.	Mean ± S. E.
Group. 1	0.425 ± 0.05	29.70 ± 2.44	3.23 ± 0.24	88.26 ± 2.71
Group. 2	0.912^{++a} ± 0.061	75.96^{++a} ± 1.91	1.43^{-a} ± 0.14	60.36^{-a} ± 2.14
Group. 3	0.628^{-b} ± 0.054	48.80-b ± 2.73	2.26^{+b} ± 0.17	73.23^{+b} ± 2.17
Group. 4	0.757 ± 0.024	57.53 ± 1.92	2.08 ± 0.20	70.24 ± 2.19
Group. 5	0.494^{-b} ± 0.027	36.77^{-b} ± 1.67	2.70^{++b} ± 0.166	82.71^{++b} ± 2.07

-a=significantly decreased from normal at p<0.05 ++a=highly significant increased from normal at p<0.01
+b=significantly increased from control at p<0.05 ++b=highly significant increased from control at p<0.01
-b=significantly decreased from control at p<0.05 --b=highly significant decreased from control at p<0.01

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 CCl_4 (1 ml/kg b. w.).

Groups	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-Cholesterol (mg/dL)	LDL-Cholesterol (mg/dL)
	Mean ± S. E.	Mean ± S. E.	Mean ± S. E.	Mean ± S. E.
Group. 1	65.26 ± 2.69	62.75 ± 1.98	42.22 ± 2.10	28.87 ± 1.71
Group. 2	89.19 $^{++a}$ ± 2.28	83.62^{++a} ± 1.87	29.26^{-a} ± 2.37	40.07^{++a} ± 2.91
Group. 3	76.66 $^{-b}$ ± 1.60	74.32^{-b} ± 1.96	35.07^{+b} ± 2.07	33.36 $^{-b}$ ± 2.10
Group. 4	81.90 ± 2.23	77.90 ± 1.46	33.25 ± 1.93	35.29 ± 1.67
Group. 5	68.86^{-b} ± 2.52	66.74^{-b} ± 2.36	40.87^{++b} ± 2.23	29.24^{-b} ± 2.01

-a=significantly decreased from normal at p<0.05 ++a=highly significantly increased from normal at p<0.01
+b=significantly increased from control at p<0.05 --a=highly significant decreased from normal at p<0.01
-b=significantly decreased from control at p<0.05 ++b=highly significant increased from control at p<0.01
--b=highly significantly decreased from control at p<0.01

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 CCl_4 (1 ml/kg b. w.).

Groups	GHS (u/L)	Catalase (u/g)	SOD (u/g)	MDA (n mol /g)	NO µmol / L
	Mean ± S. E.	Mean ± S. E.	Mean ± S. E.	Mean ± S. E.	Mean ± S. E.
Group. 1	302.9 ± 30.92	1.86 ± 0.172	598 ± 39.25	5.594 ± 0.372	65.12 ± 3.723
Group. 2	194.4^{-a} ± 18.87	0.847^{-a} ± 0.231	434^{-a} ± 37.98	13.56^{++a} ± 1.142	90.62^{++a} ± 5.329
Group. 3	243.9^{+b} ± 18.47	1.291^{+b} ± 0.225	545^{+b} ± 39.48	8.917^{-b} ± 1.174	75.05^{-b} ± 4.561
Group. 4	234.6 ± 16.86	1.130 ± 0.223	508.6 ± 43.82	9.930 ± 1.098	80 ± 4.490
Group. 5	279.9^{++b} ± 24.18	1.653^{++b} ± 0.154	580.6^{++b} ± 40.92	6.481^{--b} ± 0.912	68.69^{--b} ± 3.033

-a=significantly decreased from normal at p<0.05
+b=significantly increased from control at p<0.05
-b=significantly decreased from control at p<0.05
--b=highly significantly decreased from control at p<0.01
++a=highly significantly increased from normal at p<0.01
--a=highly significant decreased from normal at p<0.01
++b=highly significant increased from control at p<0.01

Table 5: 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 GSH, catalase, SOD, MDA and NO of Albino rats, injected with CCl_4 (1 ml/kg b. w.).

in the blood sinusoid causing hepatic atrophy, besides vacuolation in some hepatic cells. Focal areas of dilated 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. Perivasculitis 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 (Perivasculitis) 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 vacuolar 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, plateles 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]. CCl_4 induced a highly significant increase in WBCs count; this increase may be attributed to the defensive mechanism of immune system [33]. Oral treatment with ursofalk drug (group 3) and *curcuma longa* (group 4) ameliorates the blood picture parameters which may be attributed to their antioxidant effects; since it has been shown that Ursodeoxycholic acid has antioxidative properties [34]. It is evident that *curcuma longa* may stabilize the cell membrane and restore various blood variables [35]. The present study declared that, administration of CCl_4 induced a highly significant increase in serum ALT, AST, ALP and γ-GT levels while it induced a reduction in serum albumin, compared with normal animals, the hepatotoxic effects of CCl_4 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 CCl_4 [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]. *Curcum alonga* 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 antioxidant activity of *curcuma longa* [41].

CCl_4 (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 (group 4) may be due to the protective effect of curcumin against renal injury by suppressing oxidative stress, increasing kidney GSH content and gluthation peroxidase activity [45]. The present study declared that, administration of CCl_4 induced a significant reduction in serum glucose compared with normal group this may be due to the decreased hepatic glycogen content after treatment with CCl_4 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 (group 4) *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 CCl_4 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 CCl_4

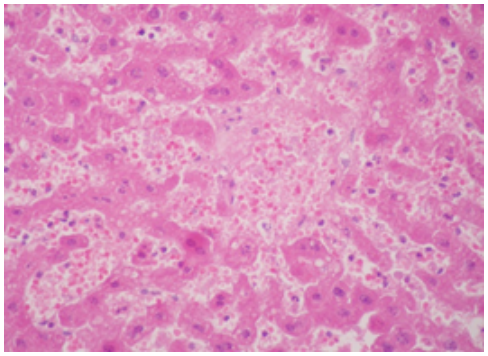

Figure 1: Liver (group 2) showing focal areas of dilated blood vessels replaced the necrotic hepatic cells (H&E 300X).

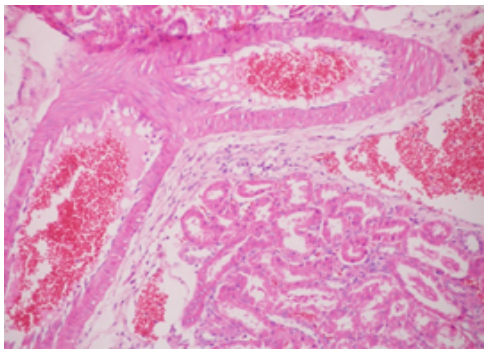

Figure 2: Kidneys (group 2) showing perivasculitis characterized by inflammatory edema surrounded the congested blood vessels (H&E 300X).

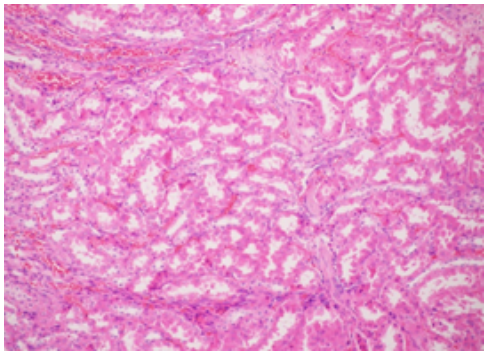

Figure 3: Kidneys (group 2) showing fibrous tissues proliferation forming lobules in the kidney (H&E 300X).

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 CCl_4 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 CCl_4 produced marked oxidative impact as evidenced by the highly significant decreased in the hepatic SOD, catalase, and GSH activities. Peroxidative damage by CCl_4 is the result of reductive dehalogenation, which is catalysed by P-450 and forms the highly reactive trichloromethyl-free radical CCl_3. This then readily interacts with molecular oxygen to form the trichloromethyl peroxy radical CCl_3OO. 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 CCl_4 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 $CC1_4$ 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

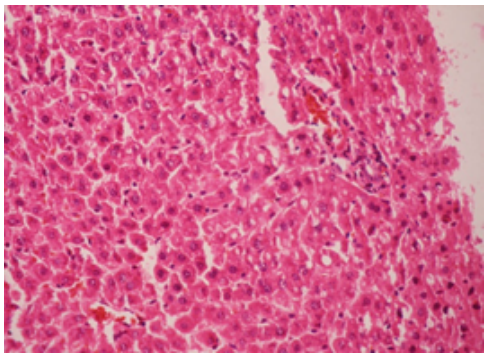

Figure 4: Liver (group3) showing regeneration was detected in the hepatic cells (H&E 300X).

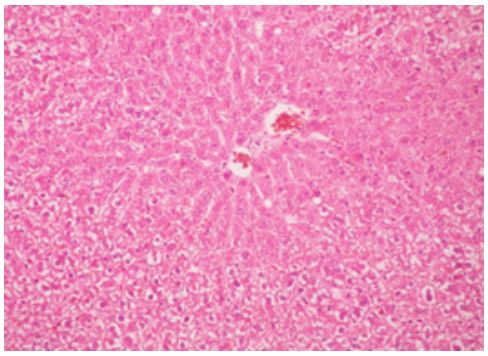

Figure 5: Liver (group3) showing normal in the architecture in the hepatic cells adjacent to the central vein vacuolar degeneration in the hepatocytes with congestion in the central veins (H&E 150X).

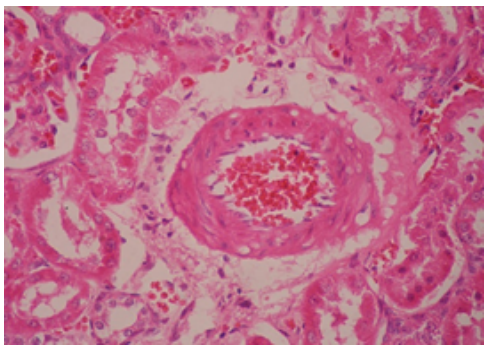

Figure 6: Kidneys (group3) showing perivascular edema with congestion and hyalinization in the wall of the blood vessels (H&E 600X).

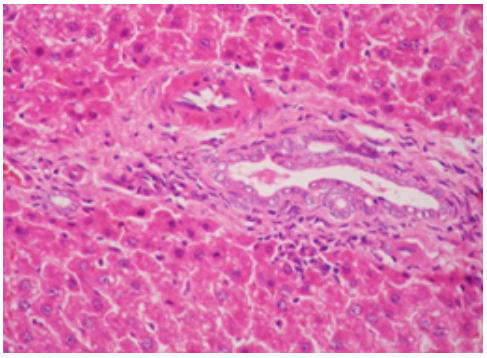

Figure 7: Liver (group4) showing fibrous tissues proliferation surrounded the blood vessels and bile ducts in the portal tract formed periporal fibrosis (H&E 300X).

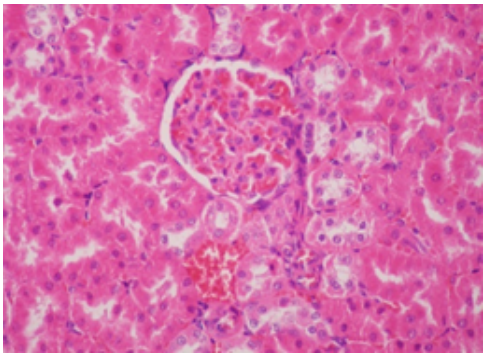

Figure 8: Kidneys (group5) showing congestion in the glomerular capillaries and periglomerular blood vessels (H&E 300X).

with CCl_4-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 its ability to eliminate oxygen free radicals from the medium and that methoxy group increases this activity. Besides, the phenolic moiety of the curcumin structure can donate hydrogen atoms to deleterious oxy radicals and form the less reactive phenoxy radicals in the process [58,59], stated that *Curcuma longa* extracts produced significant reduction in NO level which may be attributable to the bioactive substance curcumin, which scavenges free radicals and inhibits nitric oxide synthesis activity. Rats treated with ursofalk drug plus *curcuma longa* (group5) showed marked improvements in all biochemical parameters this may be attributed to the strong antioxidant effects of the both treatments [60,61].

The results of biochemical alterations were insured by histopathological examination of the liver and kidney in the intoxicated rats (control group). Treatment with Ursofalk drug (group3) and *curcuma longa* (group 4), as antioxidants treatment, could improve these pathological changes, even more (group 5) showed a marked improvements in liver and kidney tissues. It could be concluded that [62] CCl_4 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- Elraheim 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, Odin M, Eastmond DA (2007) Postulated carbon tetrachloride mode of action: a review. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 25: 185-209.
3. Sheweita SA, Abd El-Gabar 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, Igimi 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, Oliva L, Montet AM, Beauge F, et al. (1998) Ursodeoxycholate protects against ethanol-induced liver mitochondrial injury. Life Sci 63: 2259-2270.
6. Oliva L, Beaugé F, Choquart D, Montet AM, Guitaoui M, et al. (1998) Ursodeoxycholate alleviates alcoholic fatty liver damage in rats. Alcohol Clin Exp Res 22: 1538-1543.
7. Kandasamy CS, Shimna 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 Geronto Geriatr 34: 37-46.
9. Asai A, Miyazawa T (2001) Dietary curcuminoids 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, Noratunlina R, Ahmed Z (2002) Hepatoprotective effects of Curcuma Longa rhizomes in paractamol-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. Belfield 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 C (1972) Serum creatinine determination without protein precipitation. Clin Chim Acta 37: 193-197.
18. Fawcett 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.

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, Prencipe 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 polyanions. 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 (1984) 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: 351-8.

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

30. Drury RAB, 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 haematotoxicity in Swiss albino mice. J Med Plants Res 4: 1977-1986.

33. Patrick-Iwuanyanwu KC, Wegwu MO, Ayalogu EO (2007) Prevention of CCl4-induced liver damage by ginger, garlic and vitamin E. Pak J Biol Sci 10: 617-621.

34. Joo SS, Kang HC, Won TJ, Lee DI (2003) Ursodeoxycholic acid inhibits pro-inflammatory repertoires, IL-1 beta and nitric oxide in rat microglia. Arch Pharm Res 26: 1067-1073.

35. Sharma V, Sharma C, Sharma S (2011) Influence of curcuma longa and curcumin on blood profile in mice subjected to aflatoxin B1 JPSR 2: 1740-1745.

36. Johnston DE, Kroening C (1998) Mechanism of early carbon tetrachloride toxicity in cultured rat hepatocytes. Pharmacol Toxicol 83: 231-239.

37. Patel BA, Patel JD, Raval BP, Gandhi TR (2010) The Protective Activity of Saccharum officianarum Against CCl4 Induced Hepatotoxicity in Rats. Int J Pharm Res 2: 5-8.

38. Etuk EU, Agaie BM, Ladan MJ, Garba I (2009) The modulatory effect of Cochlospermum tinctorium a rich aqueous root extract on liver damage induced by carbon tetrachloride in rats. A J Pharmacy Pharm 3: 151-157.

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

40. 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 Biomedic pp: 193-199.

41. 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.

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

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

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

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

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

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

48. Kanitkar 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.

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

50. Poupon RE, Ouguerram K, Chrétien Y, Verneau C, Eschwège E, et al. (1993) Cholesterol-lowering effect of ursodeoxycholic acid in patients with primary biliary cirrhosis. Hepatology 17: 577-582.

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

52. Arafa HM (2005) Curcumin attenuates diet-induced hypercholesterolemia in rats. Med Sci Monit 11: BR228-234.

53. Brent JA, Rumack 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.

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

55. Tipoe GL, Leung TM, Liong E, So H, 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.

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

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

58. 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.

59. El-Sayed EM, Abd El-azeem AS, Afify AA, Shabana MH, Ahmed HH (2011) Cardioprotective effects of Curcuma longa L. extracts against doxorubicin-induced cardiotoxicity in rats. J Med Plants Rese 5: 4049-4058.

60. 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.

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

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