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Research Article

Attenuation of CCl_4-Induced Hepatic Damage by Curcumin Extract and/or Folic Acid

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ABSTRACT

The objective of this study was to evaluate the hepatoprotective effects of curcumin, folic acid and their combination on CCl4 induced hepatic injury in rats. Oxidative stress, liver function, liver histopathology and serum lipid levels were evaluated. As well as the levels of protein kinase B (Akt1), interferon gamma (IFN-γ), programmed cell death-receptor (Fas) and Tumor necrosis factor-alpha (TNF-α) mRNA transcription was analyzed. After 2-weeks of experimental period, Carbon tetrachloride (CCl4) significantly elevated the levels of lipid peroxidation (malondialdehyde;,MDA), cholesterol, low-density lipoprotein (LDL), triglycerides, bilirubin and urea. CCl4 was found to significantly suppress the activity of both Catalase (CAT) and glutathione (GSH) and decrease the level of serum total protein. While, treatment with curcumin and folic acid for 2-weeks significantly reduced the impact of CCl4 toxicity on liver enzymes, AST, ALT and ALP. The overall potential of the antioxidant system was significantly enhanced by curcumin and folic acid supplements, the hepatic superoxide dismutase (SOD) and CAT activities and glutathione peroxidase (GSH-PX) protein level were elevated ($P < 0.05$). The mRNA transcriptional level of TNF-α and Fas was significantly upregulated in CCl4 group. Such upregulation was not recorded in treated groups. In addition, a downregulation of Akt1 gene expression was detected in CCl4-intoxicated rats. The mRNA transcriptional level Akt1 was markedly restored and clearly upregulated in rats that were treated with the combination of Curcumin and folic acid. The results indicated that curcumin and/or folic acid have a protective effect against acute hepatotoxicity induced by the administration of CCl4 partially through the restoration of AKT1 expression.

Keywords: Hepatic injury-markers, Curcumin, Folic acid, Oxidative stress, Anti-inflammatory effects.

INTRODUCTION

The last decade has observed increasing studies on extracts and biologically active substances obtained from plant species in proving of many health proplems, as it have little or no side effects.[1] Medicinal plants or herbal medicine are being used in the traditional systems of medicine from hundreds of years. In addition, the antioxidant activity of natural products has a beneficial effect in treatment of various diseases[2] .Among of these medicinal plants, Curcumin. Curcumin is the major component of turmeric and is obtained from the rhizome of Curcuma longa Linn.[3] mentioned that, Curcumin is mainly used from hundreds of years in many countries as a spice and a food-coloring agent,however, it has been shown to have several biological activities such as suppressing the proliferation of tumor cells in the skin, lung, colon, stomach and breast[4], as well as Curcumin has both anti-inflammatory and antioxidant activities[5]. Folic acid is a water-soluble vitamin (B9), has an antioxidant effect and alleviating role in hyper homocysteinemia reducing the risk of cardiovascular, hematological, neurological and neuropsychiatric disorders[6,7.] stated that, the anti-inflammatory role of folic acid is marked by decreasing the levels of interleukin as well as the C-reactive proteins**.**

Hepatotoxicity including, hepatocellular carcinoma, fibrosis, cirrhosis and hepatitis is presently the most widespread liver pathology, representing up to 83% of health problems[8]. Ethanol, acetaminophen carbon tetrachloride (CCl4) and many other chemicals, have various degrees of hepatocyte degenerative effect[9]**.** The

Table 1: Sequences of primers and probes of quantitative RT-PCR Primer (The primers used were chosen from the PubMed database, pubmed.com).

Primer	Sequence
Fas	F: CTGCCTCTGGTGCTTGCTGGC
	R: ACCCCACCCCCTTCTCCCAATTC
IFN-γ	F: TCTGGGCTTCTCCTCCTGCGG
	R: GGCGCTGGACCTGTGGGTTG
AKt1	F: ACGCCGCCTGATCAAGTTCTCC
	R: TGACGGACAGCGGGAGAGGG
TNF-α	F: GCGGAGTCCGGGCAGGTCTA
	R: GGGGGCTGGCTCTGTGAGGA

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Table 2: Concentrations of oxidative stress parameters in the different groups

Group	Total antioxidant	MDA	NO	Catalase	GST
Control	0.44± 0.01[c]	1.38±0.01[d]	17.8± 0.40[a]	389.6± 0.01[a]	2066.8±0.01[c]
CCL4	0.97± 0.24[a]	10.52±2.5[a]	11.95± 3.09[d]	117.33±10.1[d]	2940.12±35.3[a]
CCL4+CU	0.79±0.061[b]	7.96±6.91[b]	13.87±1.53[c]	283.8± 28.6[c]	2566.5±16.5[b]
CCL4+FA	0.76±0.063[b]	8.62±1.95[b]	12.17±2.15[c]	298.7±0.01[c]	2401.2±62.5[b]
CCL4+CU+FA	0.50±0.109[c]	3.87±0.79[c]	15.80±0.01[b]	316.8±0.01[b]	2101.2±54.50[c]
F value	7.71	6.39	8.26	2.31	6.77
P value	0.0014	0.0033	0.001	0.1057	0.0025
LSD	0.1893	5.1601	2.7554	208.67	1195.3

- Values are mean ±S.D, Values with the different letters in the same column are significantly different at (P< 0.05).

Table 3: Concentrations of liver functions biomarkers (ALT (U/L), AST (U/L), ALP (U/L) and LDH (U/L)) in the different groups.

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	LDH (U/L)
Control	28.08±8.31[d]	48.63±7.62[d]	234.8 ±50.5[c]	253.55±91.5[c]
CCL4	350.14±92.8[a]	348.44±128.9[a]	408.07± 33.5[a]	525.31±26.4[a]
CCL4+curcumin	213.29±32.7[b]	255.84±62.8[b]	305.14± 52.6[b]	379.57±42.5[b]
CCL4+folic acid	210.14±33.53[b]	243.28±117.9[b]	296.9±47.19[b]	366.01±13.6[b]
CCL4+Curcumin+folic	165.63±23.26[c]	135.84±97.73[c]	229.11±48.8[c]	249.40±19.8[c]
F value	18.3	4.72	8.09	1.02
P value	0.0001	0.0212	0.0035	0.4405
LSD	86.973	170.87	85.567	308.66

- Values are mean ±S.D, Values with the different letters in the same column are significantly different at (P< 0.05).

hepatic injury induced by CCl4 have been mediate by metabolites that react with glutathione (GSH), Catalase, superoxide dismutase and other antioxidant enzymes[10]. Free radicals of CCl4 reduce both glutathione (GSH) of phase II detoxificatiting enzymes and GSH dependent antioxidant enzymes, leading to induction of oxidative stress, which considered an important factor in acute and chronic hepatic injuries [11]. Lipid peroxidation, caused by CCl4, has been extensively used in the experimental models; to know the cellular mechanisms behind the oxidative damage and further to evaluate the therapeutic effect of drugs and dietary antioxidants[12].

MATERIALS AND METHODS

Drugs

Curcumin: A known amount of fresh Curcumin powder was purchased from a local market and used for ethanolic extraction in a Soxhlet apparatus until exhaustion.

*Folic acid***:** Was purchased from Biomedical company for pharmaceutical industries, Egypt. (PubChem CID: 6037).

CCl4: Was purchased from Biomedical company for pharmaceutical industries, Egypt (PubChem CID: 5943).

Kits for biochemical analysis

Serm concenterations of malondialdehyde (MDA), total antioxidant capacity (TAC) concentration, erythrocytic reduced glutathione (G-SH) content, erythrocytic SOD activity and Catalase activity in whole blood (CAT) and Nitric oxide (NO) in the liver homogenate were determined using commercially available kits (Biodiagnostic Co, Egypt).

Curcumin extraction

Dried powder (50 g) of curcumin was extracted in soxhlet apparatus with 500 ml of 95% ethanol. The soxhelation process was carried out until the solvent was found to be colorless. The dark brown ethanolic extract was then filtered, concentrated using a rotary evaporator under reduced pressure at 50 °C. The dried ethanolic extract was subjected to extensive column chromatography using various compositions of n- hexane/dichloromethane and then dichloromethane/ethyl acetate to get the purified 1 (1.98 g). Its purity was checked on pre-coated thin layer chromatography (TLC) plate giving single spot [12]

Experimental animals

The animals were procured from the Central Animal House, Faculty of Medicine, Mansoura University. They were housed in well ventilated hygienic experimental animal house under constant environmental and nutritional conditions.

Experimental protocol

The present study was conducted in accordance of approve by the Ethical Committee for Animal Experiments at Mansoura University, Egypt. The animals were randomly divided into five groups each of five rats : groups (1) received physiological saline (3 ml/kg body weight/week) by subcutaneous injection, whereas groups (2), (3), (4) and (5) received a single dose of CCl4 (1 ml/kg) through an intraperitoneal (IP) injection[14], group (3) treated with curcumin extract a daily dose of 150 mg / kg for 15 days[15] and rats of group (4) were injected subcutaneously with 2.5 mg/kg folic acid (FA) a daily dose for 2 weeks[16]. Group (5) was received curcumin extract and FA with the previous doses.

Sampling

Blood samples, from all experimental groups, were collected via retro-orbital bleeding 12 h after fasting at the end of the experiment. Each blood sample was left in plain test tube at room temperature for 1 hour and then centrifuged for 10 min at 3000 rpm to obtain the serum. Serum samples were stored at −80°C for further biochemical analysis. Rats were sacrificed by

Table 4: Concentrations of lipid profile biomarkers (Triglycerides (mg/100 ml), Cholesterol (mg/100 ml), LDL (mg/100 ml) , HDL (mg/100 ml))in the different groups.

Group	Triglycerides (mg/100 ml)	Cholesterol (mg/100 ml)	LDL (mg/100 ml)	HDL (mg/100 ml)
Control	65.53±4.26^{c}	56.03±12.75^{b}	35.06±6.45^{c}	32.39±4.51^{a}
CCL4	126.14±9.43^{a}	83.90±18.71^{a}	50.43±13.93^{a}	12.18±4.23^{c}
CCL4+curcum	81.63±6.39^{b}	57.65± 12.19^{b}	36.95±2.63^{c}	17.32±5.64^{c}
CCL4+folic	70.08±5.25^{c}	71.19±14.90^{c}	44.06±4.20^{b}	25.90±5.77^{b}
CCL4+Curcum+folic	65.97±3.93^{c}	58.77± 9.70^{b}	37.10±5.37^{c}	29.84±5.25^{a}
F value	51.99	2.92	2.13	8.77
P value	0.0001	0.0772	0.1509	0.0026
LSD	11.252	25.444	13.841	9.3188

- Values are mean ±S.D, Values with the different letters in the same column are significantly different at (P< 0.05).

Table 5: Concentrations of Plasma Protein (g/100 ml), Urea (Mg/100 ml) and Bilirubin (Mg/100 ml) in the different groups

Group	Total Protein(g/100 ml)	Bilirubin (Mg/100 ml)	Urea (Mg/100 ml)
Control	7.723±0.74^{a}	0.285±0.011^{c}	40.50±5.13^{c}
CCL4	5.513± 0.70^{c}	0.511±0.014^{a}	53.73±12.45^{a}
CCL4+curcum	5.816±0.87^{c}	0.379±0.044^{b}	46.53±11.3^{b}
CCL4+folic	5.613±0.60^{c}	0. 362 ± 0.018^{b}	45.34±6.8^{b}
CCL4+Curcum+folic	6.886±0.85^{b}	0. 215 ±0.024^{c}	42.41 ±3.15^{c}
F value	4.31	181.32	1.19
P value	0.0279	0.0001	0.3724
LSD	1.3904	0.0312	15.57

- Values are mean ±S.D, Values with the different letters in the same column are significantly different at (P< 0.05).

decapitation. Liver was quickly removed from all rats then washed with ice-cold normal saline. Slices from liver were used to prepare tissue homogenates (10% weight/volume) in phosphate buffered saline (pH 7.4). A part of the homogenate was used for estimation of reduced glutathione (GSH) and lipid peroxidation (MDA). The remaining homogenate was centrifuged at 5000 rpm for 10 min at 4°C; after removal of the cell debris, the supernatant was stored at −80°C until further analysis of nitric oxide (NO) and RNA extraction. The remaining specimens from liver were fixed in 10% buffered formaldehyde for haematoxylin and eosin staining of paraffin-embedded sections.

Biochemical analysis

Total antioxidant capacity concentrations were measured according to[17]. Erythrocytic GSH content was determined using the method described by[18] and[19]. Erythrocytic SOD activity was determined using the method described by[20]. Serum Malondialdehyde (MDA) were measured by a colorimetric spectrophotometric method of[21]. Nitric oxide **(**NO) in the liver homogenate was estimated according to[22]. Catalase activity in whole blood was determined according to[23]. Serum protein fractions were determined using cellulose acetate electrophoresis according the methods described by[24]. Serum total protein was determined calorimetrically according to the method described by[25]. Serum bilirubin concentration was determined calorimetrically according to the method described by[26]. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to method of[27]. Serum LDL-cholesterol was determined according to[28]. Serum cholesterol, HDL-cholesterol levels were determined according to[29]. Serum triglyceride was done according to[30]. Urea was determined according to the method of [31]. Measurement of serum MDA, TAC concentration, erythrocytic reduced GSH content, erythrocytic SOD activity and CAT activity in whole blood and Nitric oxide (NO) in the liver homogenate was performed using kits obtained from Biodiagnostic, Egypt, and manufacturer's instructions were followed by using Jenway 7305, UV-VIS spectrophotometer (Jenway Scientific Equipments, UK).

RNA extraction and cDNA synthesis

Total RNA from liver tissue homogenates was isolated using TRIzol reagent (Invitrogen). The isolation was performed according to the manufacturer's instructions and quantified by measuring the absorbance at 260 nm. The cDNA synthesis was performed using the high-Capacity cDNA reverse transcription kit (Applied Biosystems) and manufacturer's instructions were followed. A total of 1.5 µg of total RNA from each sample was added to a mixture of 2.0 µl of 10x reverse transcriptase buffer, 0.8 µl of 25x dNTP mix (100 mM), 2.0 µl of 10x reverse transcriptase random primers, 1.0 µl of MultiScribe reverse transcriptase, and 3.2 µl of nuclease-free water. The final reaction mixture was kept at 25°C for 10 min, heated to 37°C for 120 min, heated to 85°C for 5 s, and then cooled to 4°C.

Quantification of mRNA expression by real-time polymerase chain reaction (RT-PCR)

Quantitative analysis of the transcriptional level of mRNA of target genes was achieved by RT-PCR. The cDNA from the above preparation was subjected to PCR amplification using 96-well optical reaction plates in the ABI Prism 7500 System (Applied Biosystems). 25-µl

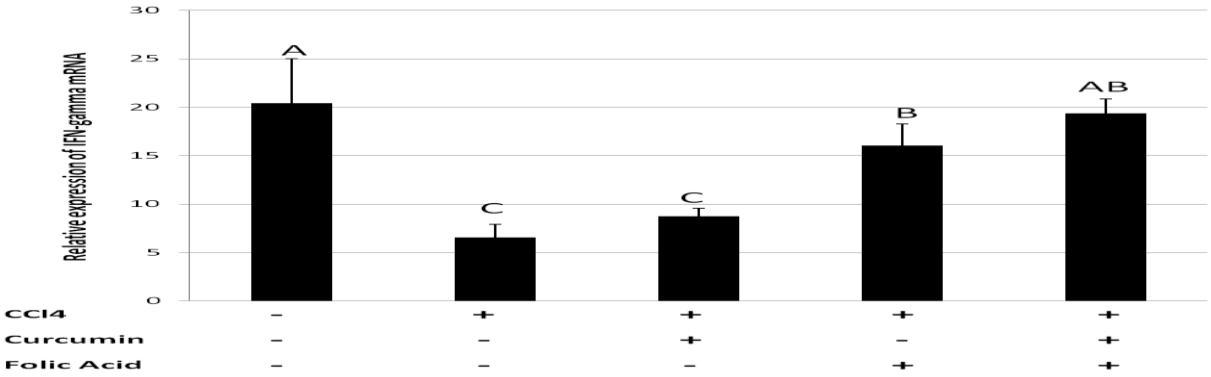

Figure 1: Relative mRNA expression of IFN-alpha in the different groups. Values with the different letters in the same column are significantly different at (P< 0.05)

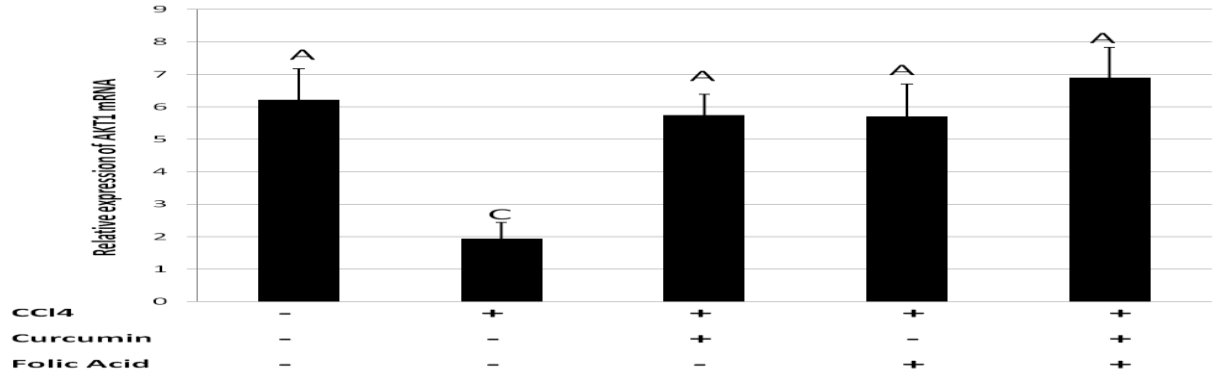

Figure 2: Relative mRNA expression of AKT in the different groups. Values with the different letters in the same column are significantly different at (P<.05)

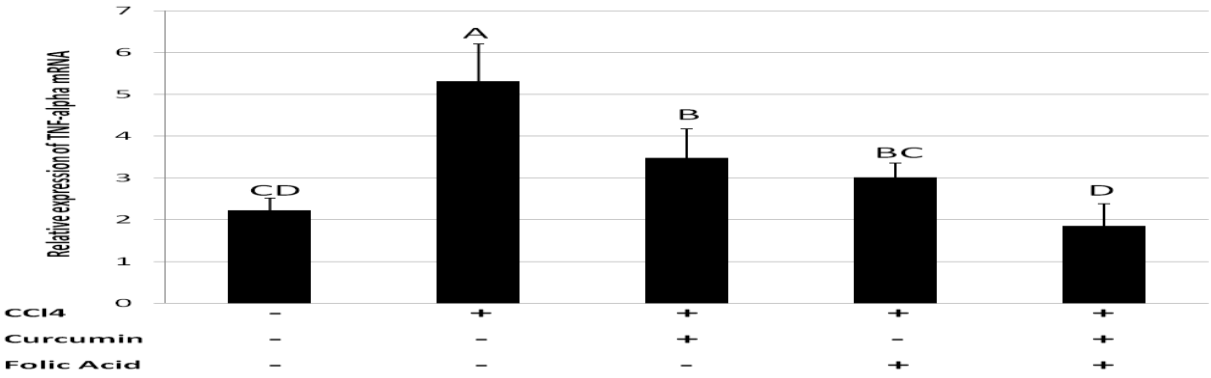

Figure 3: Relative mRNA expression of TNF-alpha in the different groups. Values with the different letters in the same column are significantly different at (P< 0.05).

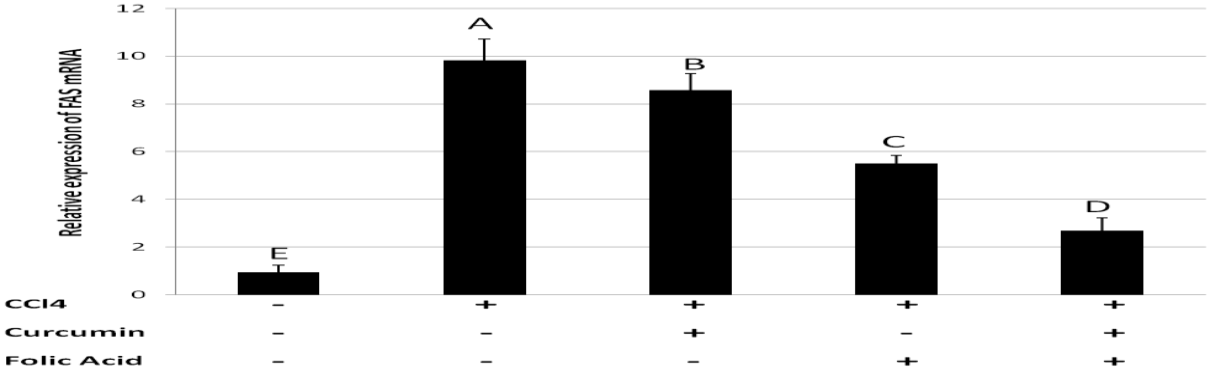

Figure 4: Relative mRNA expression of death receptor (FAS) in the different groups. Values with the different letters in the same column are significantly different at (P< 0.05).

reaction mixture in each well contained 0.1 µl of 10 µM forward primer and 0.1 µl of 10 µM reverse primer for a final concentration of 40 µM of each primer, 12.5 µl of SYBR Green Universal Mastermix (Applied Biosystems), 11.05 µl of nuclease-free water, and 1.25 µl of the cDNA sample. RT-PCR data were analyzed using relative gene expression method, as described by the Applied Biosystems User Bulletin No. 2. The data are therefore presented as the fold change in gene expression normalized to the endogenous reference gene and relative to a calibration gene.

Histopathological study

Liver collected from all experimental groups was processed for haematoxylin and eosin staining of paraffin-embedded tissue sections at the Histology Department, Faculty of Medicine, Mansoura University, Egypt. Tissue sections were examined and photos were taken using XSZ-07 Series of light biological microscope. Histopathological scoring for the recorded hepatic alterations was created as shown in (Figure 1).

Statistical analysis

Gene expression and serological data were statistically analysed using one-way analysis of variance (ANOVA), using the general liner model procedure of SAS [32]. Followed by T-test (student T-test) test for pairwise comparison Data are presented as the mean ± SD. p-value of less than 0.05 was considered statistically significant.

Histopathological scoring data were analysed using Statpages website (http://statpages.org/). All experimental groups were compared with the negative control group using one-way ANOVA, followed by Tukey's test for pairwise comparison.

RESULTS

Effects of Curcumin and or Folic acid on CCl4-induced oxidative stress (The antioxidant activity).

The results revealed that, the antioxidant activity and hepatic lipid peroxidation MDA, GST were increased in the liver of rats treated with CCl4 when compared with those of control non treated rats. Curcumin, folic acid and their combination were found to significantly reduce this high concentration of total antioxidant, although it was still significantly higher than that of the control group. Interestingly, the amount of total antioxidant was restored to the normal baseline concentration observed in the control group by these two treatments. While the levels of NO and Catalase activities were significantly ($p < 0.05$) decreased in CCl4 treated group which then significantly increased in curcumin and\or folic acid treated groups. (Table, 2).

*Effects of Curcumin and or Folic acid on CCl4-induced liver enzyme concentrations***.**

The results presented in Table (3) showed that CCl4 significantly ($p \leq 0.05$) increased the levels of ALP, ALT, LHD when compared to those of control values. While rats treated with Curcumin (G3) and Folic acid (G4) attenuated the increased levels of the serum enzymes (AST, ALT and ALP) induced by CCl4 (G2). Although the individual treatments with curcumin or folic acid did not normalize the concentrations of ALT and AST to the values observed in the control group, they did significantly decrease the concentrations of these enzymes compared to that observed in the CCl4-group. In addition, the combination of curcumin and folic acid (G5) significantly restored the concentrations of both ALP and LDH to their baseline concentrations.

The *previous results were further confirmed by the histopathological examinations.*

Effects of Curcumin and or Folic acid on CCl4-induced lipid profile.

Our results showed significantly ($p \leq 0.05$) elevated levels of cholesterol, LDL and triglycerides (TG) in the liver of CCl4-treated rats as compared with those of the control rats. In addition, the levels of cholesterol phospholipids (PL), and TG were elevated significantly in the circulation. Administration of folic acid and Curcumin effectively reduced these levels when compared with those of CCl4-treated rats. Furthermore, the HDL level was significantly decreased in the liver of CCl4-treated rats and was positively modulated by folic acid and Curcumin treatment (Table, 4).

Effects of Curcumin and or Folic acid on CCl4-induced concentration of total protein, bilirubin and urea.

The results of the present study revealed that CCl4 significantly decreased the concentration of total protein in the serum. Treatment with either curcumin or folic acid individually failed to restore the normal concentration of total protein. However, the synergistic combination of folic acid and curcumin was uniquely able to restore the concentration of total protein to a value that is close to that measured in the control group. Unlike its effect on the total protein concentration, the concetrtion of bilirubin and urea in the serum of CCl4-treated rats were higher than the concentration in the serum of control rats. We generally observed that the treatments with curcumin alone or in combination with folic acid were better able to restore the concentrations of these two parameters. (Table, 5).

Effects of Curcumin and or Folic acid on CCl4-induced TNF-α, Akt1, Fas and IFN-γ mRNA expression

The expected CCl4-induced hepatocytotoxic effects were confirmed by the significantly elevated apoptosis receptor Fas and TNF-α mRNA expression levels, but either Curcumin, Folic acid or their combination down regulated these expression levels to those observed in the control group (Fig, 3&4). while, it was found a significantly upregulation in the expression of the Akt1 and IFN-γ mRNA in (G. 3) , (G. 4) and (G. 5) when compared to the CCl4 group. This restoration of the Akt1 and IFN-γ gene expressions in animals treated with the combination of Curcumin and Folic acid was superior to that observed in the animals treated with only one of these compounds (Fig. 1&2).

*Effects of Curcumin and Folic acid on CCl4-induced on the histology of liver***.**

Our histopathological observations were also in correlation with the biochemical parameters.All Ccl_4-treated groups showed marked hepatic pathological alterations, compared to the negative control group (Fig. 5). However, the highest score was recorded in the

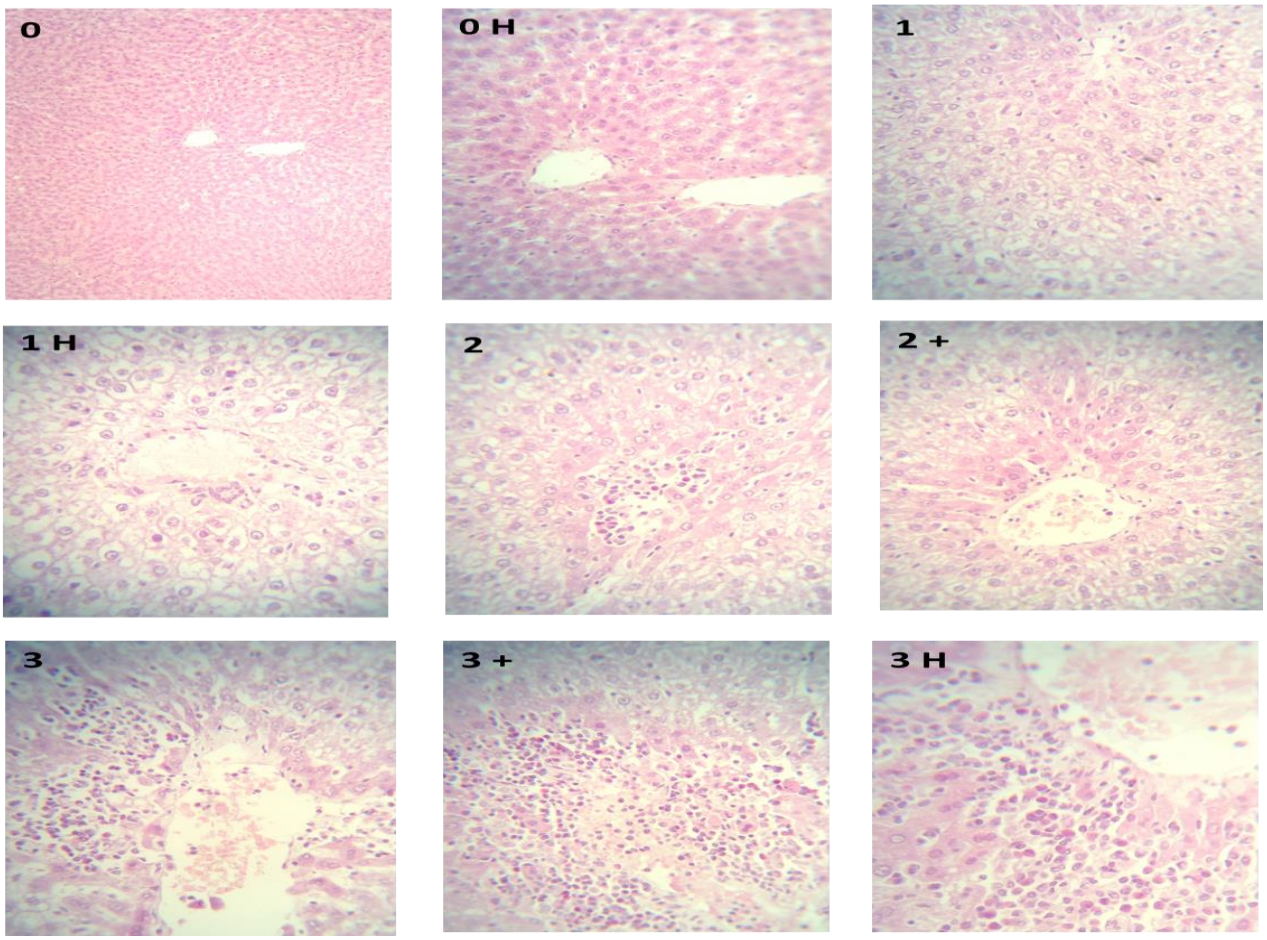

Figure 5: Liver histopathology scoring. Scoring varied from 0 to 3, where 0 represents no histopathology:
1 represents vacuolar and hydropic degeneration affecting <25% of hepatic parenchyma and minimal hepatocyte atrophy and necrosis.
2 represents vacuolar and hydropic degeneration affecting 50-75% of hepatic parenchyma, and moderate hepatocyte atrophy and necrosis, and minimal leukocytic infiltration (whether in the hepatic lobule (2) or adjacent to the central vein (2+).
3 represents vacuolar and hydropic degeneration affecting >75% of the hepatic parenchyma, and moderate to severe hepatocyte atrophy and necrosis, and moderate leukocytic infiltration of the hepatic parenchyma (whether adjacent to the central vein (3) or in the hepatic lobule (3+), which consisted mainly of mononuclear and polymorphnuclear leukocytes.
0 H: a higher power of 0 showing normal liver histology.
1 H: a higher power of 1 showing hydropic degeneration of hepatic parenchyma. Images were taken using XSZ-07 Series of biological microscope, where 0 is 10x; 0 H to 3 + are 20x; 3 H is 40x.

positive control (Ccl4-treated) group. Both folic acid and curcumin were able to significantly reduce the histopathological score in the liver, when compared to the positive control (Ccl4-treated) group. The combined use of folic acid and curcumin decreased the histopathological score in the liver to its lowest detectable level, though, still comparable to the negative control group (Fig. 6).

DISCUSSION

Oxidative stress is one of the priciple causes responsible for liver damage and disease progression. On the other hand, antioxidants try to combat the oxidative stress and minimize its deteriorated effects[33]. A Severe hepatic injuries caused by toxic chemicals is difficult to manage; however, plant extracts have a helpful role in treating hepatic failure owing to severe oxidative stress [34]. An attempt was made in this study to explore the hepato protective effect of curcumin alone as well as in combination with folic acid in CCl4-induced liver injury in rat.

The present study showed that, Ccl4 signifecntly increased raising lipid peroxidation levels, which reflect its hepatotoxic effect. This hepatotoxic effect might be due to production of free radicals as well as, activation of both Kupffer cells and macrophages which create inflammatory and profibrogenic mediators. The increase production of free radicals is the first step in a chain of events that eventually leads to lipid peroxidation and finally cellular necrosis[13]. The probable mechanism of chemical-induced liver necrosis includes raising in cytokine concentrations and/or oxidative stress[35]. In

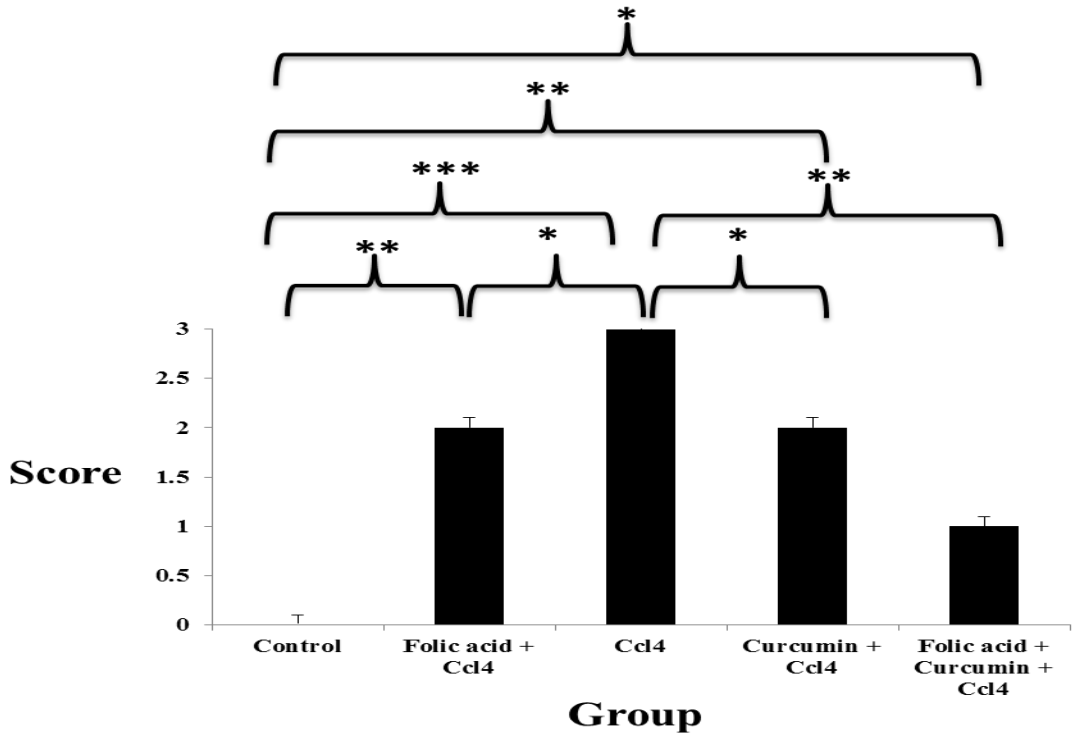

Figure 6: Group liver histopathology scoring. Using the liver histopathology scoring in figure (5), experimental groups were scored for alteration in liver histology caused by toxicity and/or treatment. Data were analysed using ANOVA, followed by Tukey's test for pairwise comparison. *= $p< 0.05$; **= $p<0.01$; ***= $p<0.0001$. Error bars represent standard error of the mean.

addition, lipid peroxidation, has been concerned in the pathogenesis of liver and consequent liver fibrogenesis that was observed in experimental rats. These results were matched with those of[36] who mentioned that CCl4 elevate the level of lipid peroxidation. As well as [37] investigated that oxidative stress manifest by increased levels of MDA (end product of LPO) and decrease levels of GSH (primary redox regulator). In the other hand, curcumin (G. 3) and folic acid (G. 4) alone or in compination (G. 5) effectively reduced oxidative stress, restored the normal concentrations of anti-oxidant enzymes, and showed evidence of hypolipidemic effect. The data presented in this study were also in agreement with many other investigators who reported that curcumin has protective effects against chemicals induced liver injury.[38] elucidated that curcumin supplementation resulted in improving liver steatosis (fatty changes) and inflammatory cells' infiltration and finally, lowering the elevation of hepatic lipid peroxidation in ethanol treated rats. The previous observations were confirmed by those of[39] who claimed that the presence of phenolic groups in the structure of curcumin are essential in elucidation its ability to eradicate oxygen-derived free radicals from the medium responsible for the lipid peroxidation.

Our results demonsterated that, the levels of AST, ALT and ALP were significantly increased in CCl4 treated group indicating its hepatocellular injury. it is well known that, exposure to CCl4 results in hepatic structural damage that leads to the realse of these enzymes to the circulation when hepatocellular damage has been occurred. These results were agree with those of[40] who concluded that hepatic injury elevate serum AST and ALT and increase severity of histopathological hepatic lesions. Moreover,[36] mentioned that, the low activity of antioxidant enzymes in liver tissue caused by CCl4 toxicity appears to be a result of not only oxidative tissue damage but also to altered enzyme structure, function, and expression. While in curcumin treated group, there was a marked decrease in the previous levels. These results were consistent with those of[41] who revealed that, curcumin has a protective role during liver injury which manfestied by lowering serum AST, ALT, and ALP levels and improving the histological architecture of the liver. In fact, Folic acid and Curcumin significantly attenuated these increased concentrations of the liver enzymes that were induced by CCl4 and thus led to their restoration to the normal concentrations. This effect was further confirmed by histopathological examinations.

HDL plays an essential role in the transport of cholesterol to the liver for excretion into bile acids, thus plaing a cytoprotective role in hepatocytes[39.] In the present study, the combination of Curcumin and Folic acid was able to restore the elevated concentrations of cholesterol, triglycerides than the individual treatments. These results were in agreement with those of[42] who investigated that, oral Curcumin prevented oxidation of LDL and lowering cholesterol level in rabbits received diet high in cholesterol.

Oxidative stress and TNF-α are known as activators of NF-kB which has a crucial role in proinflammatory gene

induction during the onset of inflammation[43.] Agents acting as inhibitors to TNF-α and NF-kB exert a therapeutic effect on liver injury in rats with bile duct ligation (BDL) through anti-inflammatory and antioxidant actions[44.] In the present study, TNF-α and Fas mRNA expression were significantly up regulated in CCl4 treated rats. Similarly,[45] found Fas expression is upregulated in CCl4 induced liver fibrosis.

Our findings gave evidence of the antioxidant and anti-inflammatory effects of Curcumin and folic acid, as their combination restored normal oxidative stress concentrations, which in turn might inhibit NF-κB and therefore down regulate both TNF-α and Fas mRNA expressions. Furthermore, this effect resulted in significant improvements to the liver function and the histological architecture. Thus, the hepatic injury markers were significantly declined in animals received these treatments. These findings supported those of[46] who showed that, curcumin able to control the inflammatory response via either inhibiting the expression of inflammatory cytokines, TNF- α, or by lowering the release of inflammatory indicating enzymes**.** Also,[47] concluded that, Curcumin is a potent modulator of the transcription factors , NF-κB that exhibit critical role in signal transduction pathways in inflammatory illnesses. So curcumin has a favorable effect on inflammatory disease.[48] The Akt1 signal has a vital role in cell survival that is triggered by growth factors and the extracellular matrix , thus, there is impaired Akt in cirrhotic livers .Previous studies have shown that Mr-AKT gene therapy can restore AKT activation in cirrhotic livers, which suggests that this therap ma be helpful in treating portal hypertention[48.] We found a down regulation of Akt1 gene expression in CCl4- challenged rats. The concentration of Akt1 mRNA expression was markedly restored and clearly upregulated in CCl4-treated rats that were treated with the combination of Curcumin and folic acid.

CONCLUSIONS

From the results obtained, we could conclude that curcumin and folic acid compination have a protective effect against CCl4 associated liver injury that was superior than either treatment alone.

ABBREVIATIONS

Akt1: protein kinase B; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; CAT: catalase; CCl4: carbon tetrachloride; FA: folic acid; Fas: programmed cell death-receptor; GSH: glutathione; GSH-PX: glutathione peroxidase; HDL: high density lipoprotein; IFN-γ: interferon gamma; IL: interleukin; LDH: lactate dehydrogenase; LDL: low-density lipoprotein; MDA: malondialdehyde; NO: nitric oxide; ROS: reactive oxygen species; RT-PCR: reverse transcriptase-polymerase chain reaction; SD: standard deviation; SOD: superoxide dismutase; TAC: total antioxidant capacity; TNF-α: tumor necrosis factor-alpha.

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