BIOCHEMICAL ROLE OF FOLIC ACID AND A-TOCOPHEROL IN EXPERIMENTALLY INDUCED LIVER CIRRHOSIS IN WHITE ALBINO RATS.

Afaf. A .El-Dosoky [1]; Mona Farouk [2]; and Abdelmaqsud M.A.[1]

[1]*Biochemistry Department, faculty of veterinary medicine, Benha University.* [2] *Pathology Department, faculty of veterinary medicine, Benha University.*

A B S T R A C T

Cirrhosis, the end stage of progressive fibrosis, is a major health problem worldwide which is caused by injury to the liver by a variety of etiological factors, It characterized by the accumulation of extracellular matrix proteins (including collagens I, III and IV), and distortion of the hepatic architecture. This study was designed to investigate the possible protective effect of folic acid and α-tocopherol on liver cirrhosis induced experimentally via administration of CCL_4 (100 mg/Kg b.w.) 50% in olive oil as a vehicle using orally three times per week for 20th weeks. In ddition, biochemical parameters such as MDA, hepatic antioxidants, cytokines and hepatic histopathology were performed. The obtained results revealed a significant elevations in γGT, AST, ALT, ALP, total and direct Bilirubin, lipid peroxidation (MDA), and cytokines. However a significant reduction in albumin, total protein, reduced Glutathione, Catalase and SOD in CCl_4 treated rats compared to the control group that indicated the hepatotoxic and pro-oxidant effect of CCl4. Administration of folic acid and α-tocopherol declared a protective effect against CCl4 induced oxidative hepatotoxicity as indicated by significant improvement in biochemical parametres, oxidative stress marker, cytokines and histopathological picture of liver.

Keywords: Cirrhosis, antioxidant, CCl4, Oxidative stress, folic acid and α-tocopherol.

(BVMJ-27(1):118-134, 2014)

1. INTRODUCTION:

Cirrhosis is one of the most cause of morbidity and mortality in developed countries, it had been seen to be not a single disease entity, but one that can be sub classified into distinct clinical prognostic stages, with 1-year mortality ranging from 1% to 57% depending on the stage. (Emmanuel, et al., 2014). Oxidative stress plays a basic role in initiation and development of liver damage. It induces necrosis and apoptosis of hepatocytes, inflammatory response and directly activates hepatic stellate cells (HSCs), resulting in the initiation of fibrosis and cirrhosis (Sanchez, et al., 2012) which is the end-stage of every chronic liver disease. Its natural history characterized by an asymptomatic phase, termed compensated cirrhosis followed by a rapidly progressive phase marked by the development of complications of portal hypertension and/or liver dysfunction, termed decompensated cirrhosis. In the compensated phase, portal pressure may be normal or below the threshold level identified for the development of varices or ascites ('clinically significant portal hypertension'). (Bataller and Brenner, 2005). Carbon tetrachloride (CCl4) is a well-known compound for the production of chemical hepatic injury (Brattin, et al., 1985) mediated by metabolites that react with antioxidant enzymes, such as reduced glutathione (GSH), catalase and superoxide dismutase, (Rikans, et al., 1994) and increase the level of inflammatory cytokines. Antioxidants exhibit a strong protection against CCl_4-induced hepatic

toxicity (Sheweita, et al., 2001). Folic acid, a water-soluble B vitamin, has recently gained considerable attention because of its great potential to prevent many disorders through supplementation for the general population. Folic acid was repeatedly reported to improve endothelial dysfunction in various clinical conditions, although the mechanisms of this beneficial effect are not fully understood. (Stanger, 2002). Vitamin E is synthesised only by plants and is therefore, found primarily in plant products, the richest source being plant oils. Animal tissues tend to have low concentrations of vitamin E, with the highest levels occurring in fatty tissues though this varies according to the intake of vitamin E. (Yusuf, et al., 2000). The protective effect of vitamin E is associated with its antioxidant properties as it possibly acts as a free radical scavenger, an inhibitor of lipid peroxidation and a plasma membrane stabilizer. It reacts with fatty acid peroxyl radicals, the primary products of lipid peroxidation and preventing them from doing any other reactions. (Claus, 2005). Despite liver cirrhosis irreversible disease, treatment could stop or delay further progression and reduce complications. A healthy diet is encouraged, as cirrhosis may be an energy-consuming process. Close follow-up is often necessary. Numerous experimental investigations have demonstrated that administration of vitamins with biologically active antioxidant property improve the liver damage. Thus, the present study established to investigate the biochemical effect of α-tocopherol and folic acid in experimentally induced liver cirrhosis in white albino rats.

2. MATERIALS AND METHODS:

2.1 *Experimental animals*:

One hundred and twenty male albino rats of 10-12 weeks old and weighing 170 – 200g used for the experimented investigation of this study. Rats were obtained from the laboratory animal's research center, faculty of veterinary medicine, Moshtohor, Benha University. Rats were housed under standard conditions of light and temperature and allowed free access of standard pellet diet and tape water was provided adlibitum. The animals were left 10 days for acclimatization before the beginning of the experiment.

2.2 Chemicals and drugs used:

All chemicals were of analytical grade and obtained from standard commercial suppliers. The chemicals used in the present study were: Folic acid was purchased from Amoun Co. For Trading Chemicals, Medicines and Medical Appliances, Egypt. α –tocopherol and CCl_4 were obtained from El-Gomhouria Co. For Trading Chemicals, Medicines and Medical Appliances, Egypt.

2.3 Induction of liver cirrhosis:

Liver cirrhosis induced by CCL_4 (100 mg/Kg b.w.) using oral gavage three times per week for 20^{th} weeks. The basal diet contained carbohydrates (56 g %), proteins (22 g %), fat (4 g %), fiber (4 g %) and mineral mixture (6 g %).

2.4 Experimental Design:

After 10 days adaptation period, Animals were randomly divided into six groups as: Group (1) Negative control: served as control group and received normal diet. Group (2) Positive control (CCL_4 group): received CCl_4 at a dose 100 mg/kg body weight of 50% in olive oil as a vehicle. Group (3) α- tocopherol-protected group: Received a daily dose of α- tocopherol (200 mg/kg/B.W) as a protection for one month then rats received the same dose combined by CCl4 as described above. Group (4) Folic Acid protected group: Received a daily dose of folic acid (250 mg/kg/B.W) as a protection for one month then rats received the same dose combined by CCl4 as described above. Group (5) α- tocopherol treated group: Received CCl4 as positive group for one month then accompanied with a daily dose of α- tocopherol (200 mg/kg/B.W) until the end of the experiment.

Group (6) Folic acid treated group: This group received CCl4 as positive group for one month then accompanied with a daily dose of folic acid (250 mg/kg/B.W) until the end of the experiment.

2.5 Sampling:

Blood samples and liver tissue specimens were collected from all animals groups, after overnight fasting, sacrificed under light anesthesia three times during the experiment at 8th, 12th and 20th weeks.

2.5.1 Blood samples:

Blood samples were collected by ocular vein puncture in dry, clean and screw capped tubes and serum were separated by centrifugation at 3500 r.p.m for 15 minutes. The clean, clear serum was separated by Pasteur pipette and kept in a deep freeze at -20°C until used for subsequent biochemical, MDA and cytokines analysis.

2.5.2 Liver tissue samples:

At the end of the experimental period, rats were sacrificed by cervical decapitation. The liver specimen was quickly removed and weighted, then perfused with cold saline to exclude the blood cells and then blotted on filter paper; and stored at -20°C. Briefly, liver tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were centrifuged at 5,000 r.p.m for 15 minutes at 4°C then the supernatant was used for the determination of superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH).

2.6 Biochemical Analysis:

γGT, ALT, AST, ALP, Albumin, Total protein and Bilirubin (Total and direct) were determined according to the methods of (Szasz, 1969), (Fischbach and Zawata, 1992) , (Z.Klin, 1970), (Gendler, 1984), (Koller, 1984) and (Kaplan, et al., 1984) respectively. Moreover, MDA was determined according to the method of (Yoshioka et al., 1979). The supernatant of hepatic tissue homogenate were used for the determination of Catalase (CAT), Superoxide dismutase (SOD) activities and reduced glutathione (GSH) concentration was also estimated according to the methods of (Sinha, 1972), (Kakkar, et al., 1984) and (Beutler, et al., 1963) respectively. Moreover, TNF-α, TGF-β, IL6 and IL10 were determined according to the methods of (So, et al., 2006), (Danielpour, et al., 1989), (Odeh, 1997), (Opdal, 2004).

2.7 Pathological study:

Specimens from liver were fixed in neutral buffer solution 10 % , after proper fixed it dehydrated in alcohol, cleared in xylene, imbeded in paraffin and sectioned of 5 μ thickness were stained either by H&E or with Sirius stain according to Bancroft & Gamble (2008). .

2.8 Statistical Analysis:

Statistical analysis were carried out by the aid of a digital computer, using Excel & SPSS version 15 programms according to the technique described by (Daniel, 1991).

3. RESULTS:

The obtained data table (1), (2) and (3) demonstrate that the administration of folic acid and α-tocopherol as treatment or protection has a significant effect in decreasing γGT, Alkaline phosphatase, AST, ALT, with significant increase in total protein and Albumin when compared to CCl4 group. Moreover, administration of folic acid and α-tocopherol show a significant improvement in the antioxidants status and lowering the MDA activity as presented in tables (4, 5) when compared to CCl4 group. A significant decrease in the cytokines were recorded after the administration of folic acid and α-tocopherol (TNF-α, TGF-β, IL6 and IL 10) when compared to CCl4 group as shown in tables (6, 7).

Table (1): Effect of Folic acid and α-tocopherol on γGT and alkaline phosphatase on cirrhotic & normal rats.

Animal Groups	γGT *(U/L)*			ALP*(U/L)*		
	8th	12th	20th	8th	12th	20th
NC	4.52±0. 28[b,d,e,f]	4.29±0. 28[b,c,d,e,f]	4.53±0.16[b,c,d,e,f]	220.9±0.38[b,c,d,e,f]	220.96±0.19[b,c,d,e,f]	222.01±1.19[b,c,d,e,f]
CCL4	5.76±0.27[a,c,d,e,f]	9.08±0. 13 [a,c,d,e,f]	16.55±0. 41 [a,c,d,e,f]	290.3±0.69[a,c,d,e,f]	343.54±0.97[a,c,d,e,f]	416.46±3.19[a,c,d,e,f]
CCl4+ Folic acid	4.74±0.16[b,d,e,f]	5.90±0.05[a,b,d,e.f]	8.00±0.08 [a,b,d,e.f]	230.5±0.43[a,b,d,e,f]	237.19±2.34[a,b,d,e,f]	250.97±1.39[a,b,d,e,f]
CCl4+ α-tocopherol	5.17±0.24[a,b,c]	7.07±0. 13[a,b,c,e,f]	10.26±0. 23 [a,b,c,e,f]	249.2±0.46[a,b,c,e,f]	262.55±0.49[a,b,c,e,f]	286.17±0.41[a,b,c,e,f]
Folic acid+ CCl4	5.10±0.13[a,b,c,f]	6.54±0.34[a,b,c,d,f]	12.36±0.43 [a,b,c,d,f]	239.03±0.2[a,b,c,d,f]	278.68±0.48[a,b,c,d,f]	318.7±0. 67[a,b,c,d,f]
α-tocophero +CCl4	5.39±0.07[a,b,c]	7.78±0.12[a,b,c,d,e]	13.03±0.14 [a,b,c,d,e]	256.6±0.77[a,b,c,d,e]	296.11±0.29[a,b,c,d,e]	345.22±0.78[a,b,c,d,e]

a significant compared to negative control
b significant compared to CCl4 group.
c significant compared to α tocopherol protected
d significant compared to Folic acid protected
e significant compared to α-tocopherol treated
f significant compared to Folic acid treated

Table (2): Effect of Folic acid and α-tocopherol on total protein and Albumin on cirrhotic & normal rats.

Animal Groups	Total protein *(mg/dl)*			Albumin *(mg/dl)*		
	8th	12th	20th	8th	12th	20th
NC	6.69±0.06b[c,d,e,f]	6.67±0.05[c,d,e,f]	6.68±0.06 ,[c,d,e,f]	3.77±0.09 [b,c,d,e,f]	3.77±0.06 [b,c,d,e,f]	3.75±.09 [b,c,d,e,f]
CCL4	5.63±0.08a[c,d,e,f]	4.34±0. 12[c,d,e,f]	3.07±0.07,[c,d,e,f]	3.07±0. 10 [a,c,d,e]	2.64±0.06 [a,c,d,e]	1.53±.09a[c,d,e,f]
CCl4+ Folic acid	6.22±006[a,b,d, f]	5.91±0.08 [a,b,d,e,f]	5.78±0.04 [a,b,d,e,f]	3.57±0. 04 [a,b,f]	3.33±0.06 [a,b,e,f]	3.30±.39 [a,b,e,d,e,f]
CCl4+ α-tocopherol	5.94±0.07[a,b,c,e,f]	5.53±0. 10 [a,b,c, f]	5.17±0. 07 [a,b,c,e,f]	3.41±0. 09 [a,b]	3.30±0.19 [a,b,e,f]	2.78±0.10 [a,b,c,e,f]
Folic acid+ CCl4	6.09±0.06[a,b,d,f]	5.55±0. 28[a,b,c,f]	4.77±0. 06 [a,b,c,d,f]	3.39±0. 17 [a,b]	2.93±0. 14 [a,b,c,d]	2.43±0.13 [a,b,c,d,f]
α-tocophero +CCl4	5.77±0.13 [a,b,c,d,e]	5.19±0. 15 [a,b,c,d,e]	4.03±0. 12 [a,b,c,d,e]	3.24±0. 12 [a,c]	2.77±0. 17 [a,c,d]	2.01±0.22 [a,b,c,d,e]

a significant compared to negative control
b significant compared to CCl4 group.
c significant compared to α tocopherol protected
d significant compared to Folic acid protected
e significant compared to α-tocopherol treated
f significant compared to Folic acid treated

Table (3): Effect of Folic acid and α-tocopherol on ALT and AST on cirrhotic & normal rats.

Animal Groups	AST (U/L)			ALT (U/L)		
	8th	12th	20th	8th	12th	20th
NC	90.99±0.59 b,c,d,e,f	90.63±0.49 b,c,d,e,f	91.41±0.51 b,c,d,e,f	29.94±1.26b,c,d,e,f	30.62±0.61 b,c,d,e,f	30.58±1.2 b,c,d,e,f
CCL4	115.69±0.74 a,c,d,e,f	187.19±0.67 a,c,d,e,f	253.89±0.76 a,c,d,e,f	54.78±0. .99a,c,d,f	97.68±0.71 a,c,d,e,f	135.51±.6 a,c,d,e,f
CCl4+ Folic acid	93.50±0.57 a,b,d,e,f	110.10±0.86 a,b,d,e,f	125.68±0.84 a,b,d,e,f	38.26±0.34a,b,d,e,f	45.67±0.58 a,b,d,e,f	60.13±1.0 a,b,d,e,f
CCl4+ α-tocopherol	98.35±0.86a,b,c,f	124.80±0.83 a,b,c,e,f	152.37±0.81 a,b,c,e,f	41.44±0.61a,b,c,e,f	53.88±0.36 a,b,c,e,f	72.72±1.4 a,b,c,e,f
Folic acid+ CCl4	97.38±0.72a,b,c,f	132.60±1.39 a,b,c,d,f	183.37±1.34 a,b,c,d,f	53.76±1.30a,c,d,f	71.03±.76 a,b,c,d,f	95.16±1.5 a,b,c,d,f
α-tocophero +CCl4	102.31±0.49a,b,c,d,e	148.79±1.12 a,b,c,d,e	203.27±0.62 a,b,c,d,e	50.79±0.71a,b,c,d,e	83.16±1.69 a,b,c,d,e	103.5±1.1 a,b,c,d,e

a significant compared to negative control
b significant compared to CCl4 group.
c significant compared to α tocopherol protected
d significant compared to Folic acid protected
e significant compared to α-tocopherol treated
f significant compared to Folic acid treated

Table (4): Effect of Folic acid and α-tocopherol on heptic SOD and CAT on cirrhotic & normal rats.

Animal Groups	SOD *(U/g)*			Catalase *(U/g)*		
	8th	12th	20th	8th	12th	20th
NC	44.35±0.82 b,c,d,e,f	44.75±0.49 b,c,d,e,f	44.58±0.92 b,c,d,e,f	3.10±0.019 b	3.25±0.32 b,c,d,e,f	3.1±.05 b,c,d,e,f
CCL4	38.64±0.74 a,c,d,e,f	29.81±0.97 a,c,d,e,f	20.74±0.76 a,c,d,e,f	2.575±0.29 a,c,d,e,f	2.15±0.2 a,c,d,e,f	1.64±.2 a,c,d,e,f
CCl4+ Folic acid	42.20±0.88 a,b	40.88±1.07 a,b,d,e,f	39.57±1.06 a,b,d,e,f	3.07±0.04 b	2.77±0.27 a,b	2.62±0.23 a,b,f
CCl4+ α-tocopherol	41.40±0.94 a,b	39.60±0.80 a,b,c,e	37.29±1.20 a,b,c	3.028±0.008 b	2.768±0.19 a,b	2.48±0.06 a,b,f
Folic acid+ CCl4	41.32±0.50 a,b	38.24±1.23 a,b,c,d	38.15±1.64 a,b,c,f	2.99±0.016 b	2.633±0.19 a,b	2.539±0.24 a,b,f
α-tocophero +CCl4	40.55±0.53 a,b,c	38.85±0.60 a,b,c	36.27±0.56 a,b,c,e	2.978±0.04 b	2.552±0.38 a,b	2.13±.08 a,b,c,e

a significant compared to negative control
b significant compared to CCl4 group.
c significant compared to α tocopherol protected
d significant compared to Folic acid protected
e significant compared to α-tocopherol treated
f significant compared to Folic acid treated

Table (5): Effect of Folic acid and α-tocopherol on MDA and Reduced Glutathione on cirrhotic & normal rats.

Animal Groups	MDA *(nmol/L)*			Reduced Glutathione *(mg /g)*		
	8th	12th	20th	8th	12th	20th
NC	35.922±0.14 b,c,d,e,f	35.72±0.20 b,c,d,e,f	35.53±0.44 b,c,d,e,f	15.04±0. 09 b,d,e,f	14.93±0. 15 b,c,d,e,f	14.95±0. 14 b,c,d,e,f
CCL4	49.65±0.42 a,c,d,e,f	65.39±0.56 a,c,d,e,f	77.41±2.85 a,c,d,e,f	12.07±. 13 a,c,d,e,f	8.89±0. 14 a,c,d,e,f	6.02±0.109 a,c,d,e,f
CCl4+ Folic acid	38.66±1.47 a,b,d,e,f	41.91±1.01 a,b,d,e,f	48.57±1.05 a,b,d,e,f	14.86±0.162 b,d,e,f	13.85±0. 22 a,b,d,e.f	12.68±0. 16 a,b,d,e.f
CCl4+ α-tocopherol	41.35±0.46 a,b,c,f	46.43±2.34 a,b,c,d,f	54.79±1.93 a,b,c,d,f	14.04±0.08 a,b,c,e,f	12.54±0.12 a,b,c,f	10.87±0.15 a,b,c, f
Folic acid+ CCl4	40.61±0. 37 a,b,c,f	50.93±0.69 a.b,c,d,f	62.29±0.46 a.b,c,d,f	14.55±0.34 a,b,c,d,f	12.75±0.32 a,b,c,f	11.12±0.35 a,b,c,f
α-tocophero +CCl4	43.99±0.75 a.b,c,d,e	52.56±0.63 a.b,c,d,e	66.97±1.63 a.b,c,d,e	13.72±0.44 a,b,c,d,e	11.30±0.33 a,b,c,d,e	10.02±0.15 a,b,c,d,e

a significant compared to negative control
b significant compared to CCl4 group.
c significant compared to α tocopherol protected
d significant compared to Folic acid protected
e significant compared to α-tocopherol treated
f significant compared to Folic acid treated

Table (6): Effect of Folic acid and α-tocopherol on IL6 and IL10 on cirrhotic & normal rats.

Animal Groups	IL6 *(ng/L)*			IL10 *(ng/L)*		
	8th	12th	20th	8th	12th	20th
NC	81.88±1.09 b,c,d,e,f	87.26±0.63 b,c,d,e,f	103.90±0.81 b,c,d,e,f	22.88±0.3 b,c,d,e,f	26.16±0.55 b,c,d,e,f	25.6±.6 b,c,d,e,f
CCL4	159.59±2.26 a,c,d,e,f	289.7±9.24 a,c,d,e,f	352.63±1.6 a,c,d,e,f	50.01±1.08 a,c,d,e,f	71.33±1.05 a,c,d,e,f	90.12±.92 a,c,d,e,f
CCl4+ Folic acid	96.75±2.33 a,b,d,e,f	139.80±.73 a,b,d,e f	188.83±2.8 a,b,d,e f	25.64±0.51 a,b,d,e,f	42.50±1.17 a,b,d,e,f	51.54±1.04 a,b,d,e,f
CCl4+ α-tocopherol	105.90±2.22 a,b,c, f	169.09±2.1 a,b,c,e, f	226.73±1.3 a,b,c,e ,f	30.48±0.72 a,b,c,e,f	47.79±0.60 a,b,c,e,f	59.79±0.87 a,b,c,e,f
Folic acid+ CCl4	107.30±1.79 a,b,c, f	152.6±20.4 a,b,c,d. f	240.27±0.8 a,b,c,d. f	33.30±1.20 a,b,c,d,f	50.600±0.9 a,b,c,d,f	62.25±1.84 a,b,c,d,f
α-tocophero +CCl4	123.25±1.22 a,b,c,d.e	187.09±1.0 a,b,c,d. e	258.49±1.2 a,b,c,d. e	38.61±1.22 a,b,c,d,e	58.55±1.2 a,b,c,d,e	71.47±1.15 a,b,c,d,e

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b significant compared to CCl4 group.
c significant compared to α tocopherol protected
d significant compared to Folic acid protected
e significant compared to α-tocopherol treated
f significant compared to Folic acid treated

Table (7): Effect of Folic acid and α-tocopherol on TNF-α and TGF-β on cirrhotic & normal rats.

Animal Groups	TNF-α *(ng/L)*			TGF-β *(pg/ml)*		
	8th	12th	20th	8th	12th	20th
NC	43.67±0.55 b,c,d,e,f	37.28±0.35 b,c,d,e,f	45.11±0.41 b,c,d,e,f	42.01±0.94 b,c,d,e,f	48.82±1.22 b,c,d,e,f	33.89±0.79 b,c,d,e,f
CCL4	78.90±0.61 a,c,d,e,f	126.00±0.68 a,c,d,e,f	144.03±0.98 a,c,d,e,f	60.43±0.83 a,c,d,e,f	164.54±3.93 a,c,d,e,f	149.65±1.26 a,c,d,e,f
CCl4+ Folic acid	46.14±0.68 a,b,d,e,f	52.16±0.31 a.b,d,e,f	56.13±0.23 a,b, d,e,f	45.50±0.51 a,b, d,e, f	73.54±0.57 a,b, d,e, f	61.15±0.46 a,b, d,e, f
CCl4+ α-tocopherol	50.30±0.42 a,b,c ,e,f	69.40±0.53 a,b,c ,e,f	79.17±0.80 a,b,c ,e,f	49.95±0.67 a,b,c, f	85.95±0.49 a,b,c,e,f	77.77±0.36 a,b,c,e,f
Folic acid+ CCl4	51.30±0.35 a,b,c ,d,f	65.20±0.29 a,b,c ,d,f	75.62±0.61 a,b,c ,d,f	50.61±0.75 a,b,c, f	107.08±1.02 a,b,c,d,f	96.79±0.48 a,b,c,d,f
α-tocophero +CCl4	58.33±0.55 a,b,c,d,e	72.98±0.58 a,b,c,d,e	90.60±0.59 a,b,c,d,e	56.10±0.41 a,b,c,d,e	135.86±.45 a,b,c,d,e	119.65±0.64 a,b,c,d,e

a significant compared to negative control
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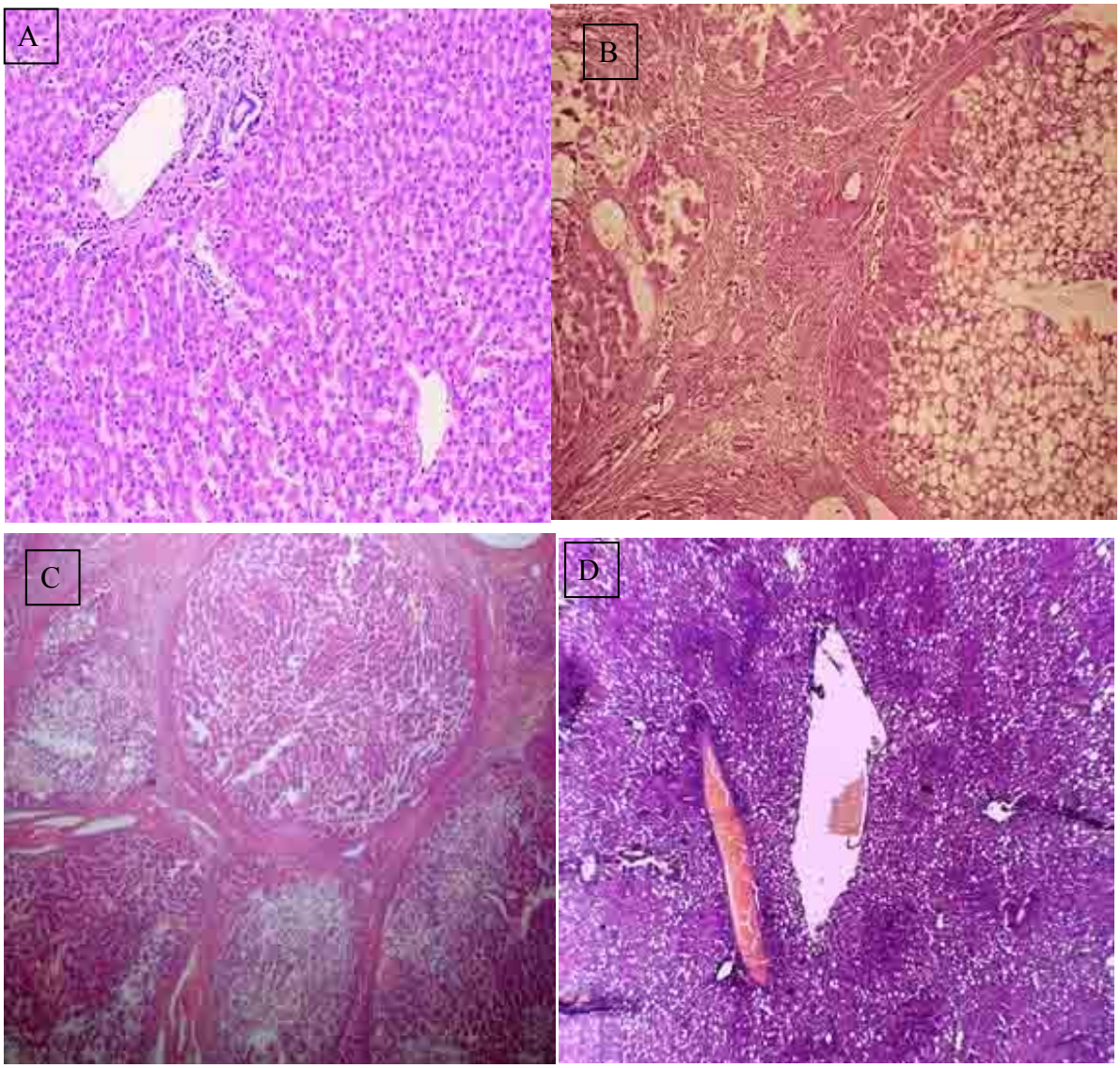
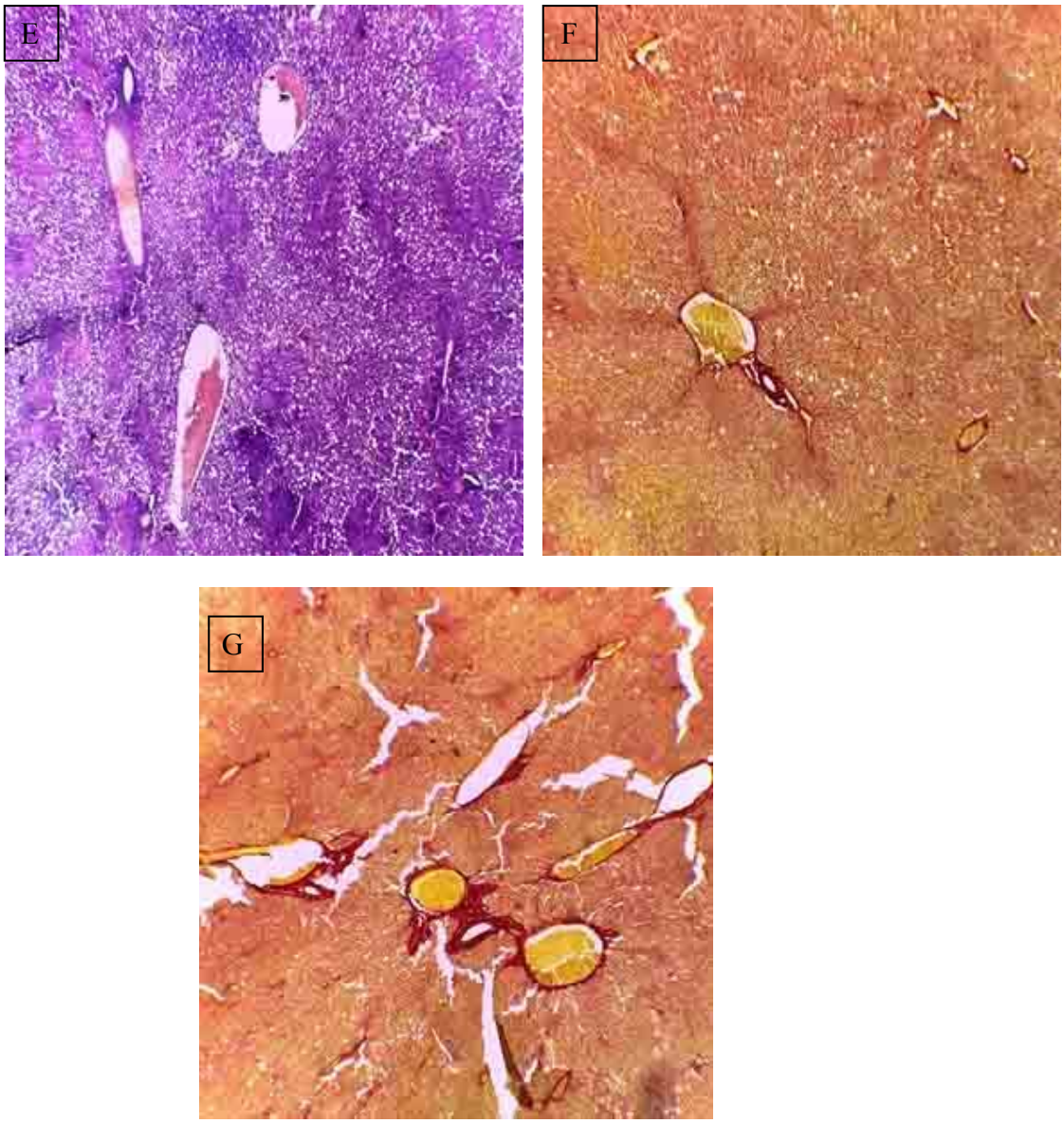
f significant compared to Folic acid treated



Photomicrographs for liver sections of the different groups: (A) Photomicrograph of a control liver section showing the normal histological architecture of hepatic tissues (H&E stain, 40x). (B, C) The histological features of a representative liver section from CCl4-treated rats (Sirius stain highlighted fibrosis around hepatocytic nodules, 40x) which shows complete cirrhosis, steatosis score (3), fibrous septa contains inflammatory cells mainly lymphocytes.(D) A representative liver section from α-tocopherol protected group (H&E stain, 40x) shows congestion of central vein and steatosis score (2).



Photomicrographs for liver sections of the different groups: (E) A representative liver section from Folic acid+CCl_4 group (H&E stain, 40x) shows congestion of central vein and steatosis score (3).(F) A representative liver section from CCl_4 + α-tocopherol group (Sirius stain, 40x) shows congestion in central vein Thin, short fibril septa extended from central vein "Fibrosis score 2". (G) A representative liver section from CCl_4 + folic acid group (Sirius stain, 40x) shows congestion, thick scattered fibrous septa present "Fibrosis score 3".

4.DISCUSSION:

Cirrhosis represents the end stage of any chronic liver disease and it is one of the most common causes of mortality worldwide because hepatic dysfunction constitutes a potentially lethal condition. Its etiology is variable being the most important alcoholic liver disease, viral chronic hepatitis and there is no treatment for prevention or regression of this pathology (Victoria, et al., 2011). The metabolic role of the liver makes it a preferred target for oxidative stress and antioxidant action that had been shown to confer hepatoprotective effects. (Gutierrez, et al., 2010). The administration of CCL_4 induced significant deterioration in liver function tests and oxidative stress tested. CCl4 group show high levels of ALT, AST and γGT indicate liver damage as well as cellular leakage and loss of functional integrity of cell membrane in liver while the increase in serum alkaline phosphatase level is due to increased synthesis in the presence of increasing biliary pressure *(*Ranawat, et al., 2010**).** The elevated serum total and direct bilirubin indicated a defect in hepatic biotransformation. (Chen, et al., 2009) and the Significant decline in serum albumin and total protein indicated the toxicant induced changes in protein biosynthesis via a substantial deficit in ribosomal RNA methylation and a decrease in polyamine synthesis (Weber and Stampf, 2003). In this study, the data shown in tables (1,2,3) revealed a significant decrease in biochemical markers: ALT, AST, γGT, ALP activities, Bilirubin total and direct level with significant increase in albumin and total protein levels in α-tocopherol and folic acid groups as compared to the CCL_4 group. These obtained results agree with (Ali, et al., 2012) Study which indicate that the administration of α-tocopherol had hepatoprotective effect against liver toxicity and with the study of (Conni, et al., 2006) which indicate that folic acid supplementation offers a hepatoprotective effect. The data shown in tables (4,5) show a significant increase in the antioxidants concentration SOD, Catalase and reduced glutathione with a significant decrease in the MDA concentration in α-tocopherol and folic acid groups as compared to the CCL_4 group. These results are nearly similar to the studies of Hossam, et al., (2013) and Ali, et al., (2012) which indicate that α tocopherol and folic acid ameliorate the antioxidant status, which protect liver from the oxidative stress, exerts by CCL_4.

The hepatic content of GSH as well as SOD activity were found to be decreased significantly in CCL4-intoxicated rats as compared with control rats. In addition, the decrease in CAT in CCL_4 treated rats might indirectly lead to an increase in oxidative DNA damage. (Abdel-Aziz, et al., 2005). Vitamin E is recognized as the most important antioxidant, and a large number of experimental studies (Chow, et al., 2001) supports its antioxidant function in vivo. The protective effect of vitamin E is associated with its antioxidant properties as it possibly acts as a free radical scavenger, an inhibitor of lipid peroxidation and a plasma membrane stabilizer. It reacts with fatty acid peroxyl radicals, the primary products of lipid peroxidation and preventing them from doing any other reactions (Claus, 2005). Administration of Vitamin E show a significant improvement in all of the tested parameters affected by CCl_4 although most of them were significantly different from normal control values with a significant increase in antioxidant enzymes as GSH, SOD and Catalase when compared to the CCl_4 group and significant decrease in MDA as oxidative stress parameter. Which indicate the protective effect of α-tocopherol and its ability to protect cell membranes from lipid peroxidation-mediated damage. (Fariss, 1990). Several studies have shown that folic acid supplementation can reduce the risk of cardiovascular and hematological diseases, neurological and neuropsychiatric disorders, neural tube defects and several types of cancer, including cervical, lung, brain, pancreatic, colorectal and breast cancer (Duthie, et al., 2002). The

antioxidant activity of folic acid is thought to be involved in these effects of folic acid on health (Nakano, et al., 2001). In fact, folic acid has been reported to have an antioxidant effect against ROS and an alleviating role in hyperhomocysteinemia and its associated endothelial dysfunction (Moens, et al., 2008).Moreover, the anti-inflammatory effect of folic acid is manifested by a decrease in the levels of interleukin and C-reactive proteins (Solini, et al., 2006). With this model, circulating cytokines changes also revealed including TNF-α, TGF-β, IL-6 and IL-10 during cirrhosis development. The roles of Cytokines facing hepatic damage are complicated: they may be responsible for the establishment and progression of hepatic injury, fibrosis and cirrhosis or participate in liver regeneration. (Simpson, et al., 1997). The present study, show significant decrease in IL6, IL10, TNF-α and TGF-β which indicate that α - tocopherol administration significantly reduced nuclear factor kappa B activity that had been shown to enhance the expression of cytotoxic cytokines. In addition, it effected the inflammatory response through reducing serum level of TNF-α. (Liu, et al., 1995). Folic acid markedly up regulated the cell survival signal, and down regulate TNF- α concentrations. Furthermore, the restoration of the survival signaling genes that was induced by folic acid also resulted in significant improvements to the liver function and the histological architecture and restore normal oxidative stress concentrations. (Hossam, et al., 2013). The oxidative stability that is induced by α-tocopherol and folic acid may mediate a down regulation of NF-κB activation, which results in the suppression of the inflammatory cascade and the low concentrations of TNF-α that were observed. Thus, the hepatic injury markers were significantly retarded in the animals that received any of these treatments. In fact, α-tocopherol and folic acid significantly attenuated the increased concentrations of the serum liver enzymes that were induced by CCl_4 and therefore led to the subsequent restoration near to normal concentrations during the experimental period. Histopathological examination of the liver sections of rats treated for long period with CCl_4 showed classical cirrhotic appearance consistent with that already described by Ehrinpreis, et al., (1980). These sections revealed extensive collagen deposition and show complete cirrhosis in which the fiubrous septa contain inflammatory cells mainly lymphocyte with steatosis score (3). Liver sections obtained from treated groups with either α-tocopherol or folic acid showed consistent reduction of liver necrosis and inflammation with short and scattered collagen bands. While in the protected groups with α-tocopherol or folic acid collagen bands connecting central regions with portal areas and pseudolobules were not present whereas fatty changes were present. It was found that pre-treatment with the α-tocopherol and folic acid had broad anti-inflammatory effects and attenuated the allergic inflammation in the CCl4 challenged rats. This amelioration of the hepatic tissues by α-tocopherol and folic acid seemed to be mediated by the inhibition of oxidative stress and therefore the suppression of NF-κB, the key regulator of inflammatory production, which results in the decreased production of pro-inflammatory cytokines.

Conclusion &recommendation: The present study demonstrates that the administration of folic acid and α-tocopherol as a protection or therapeutic doses is effective in improving the liver conditions and improving the liver damage by the inhibition of oxidative stress and therefore the suppression of NF-κB, the key regulator of inflammatory production, which results in the decreased production of pro-inflammatory cytokines.

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التأثير الكيميائي الحيوي لحمض الفوليك والألفا توكوفيرول على التليف الكبدي المحدث تجريبيا في الفئران

عفاف عبدالمجيد الدسوقي[1]،منى فاروق[2]، مروة عبدالمقصود أحمد[1]

[1]قسم الكيمياء الحيوية-كليه الطب البيطري – جامعة بنها

[2]قسم الباثولوجي-كليه الطب البيطري – جامعة بنها

الملخص العربي

يعتبر تليف الكبد أحد أكبر المشاكل الصحية الموجودة في العالم والذي يحدث عادة نتيجة العديد من الأسباب التي تحدث إصابة في الكبد ويصحب تليف الكبد بتجمع البروتينات الخلوية خارج الخلايا (تشمل الكولاجين I، III،IV) وتشوه شكل الخلية الكبدية. كان الهدف من هذه الدراسة هو معرفة تأثير حمض الفوليك والألفا توكوفيرول على تليف الكبد المحدث تجريبيا في الفئران. وقد تم إحداث التليف الكبدي بواسطة تجريع الفئران رباعي كلوريد الكربون بجرعة (100مجم/كجم/الوزن) بنسبة 50% محمل على زيت الزيتون ثلاث مرات أسبوعيا لمدة عشرين أسبوع وقد تم استخدام حمض الفوليك والألفاتوكوفيرول كحماية وعلاج بجرعات (250مجم/كجم/الوزن) و(200مجم/كجم/الوزن) بالترتيب. تم قياس معدل إنزيمات الكبد والبروتينات وقياس نشاط MDA في مصل الدم وقياس نشاط إنزيمات السوبر أوكسيد ديسميوتيز والكتاليز والجلوتاثيون ريداكتيزو السيتوكينز باستخدام الطرق القياسية المحددة لذلك بالإضافة إلى فحص أنسجة الكبد. وقد نتج عن رباعي كلوريد الكربون ارتفاع في نشاط γGT, AST ,ALT و الألكالين فوسفاتيز و في مستوى بليروبين الدم الكلي و المباشر و MDA والسيتوكينز مع انخفاض ملحوظ في البروتين الكلي و الألبومين و السوبر أوكسيد ديسميوتيز والكتاليز والجلوتاثيون ريداكتيز مقارنة بالفئران الموجودة في المجموعة السالبة مما يظهر التأثير السام لرباعي كلوريد الكربون على الكبد. وقد أوضحت النتائج أيضا أن تناول حمض الفوليك والألفاتوكوفيرول يساعد على حماية الكبد من تأثير رباعي كلوريد الكربون والذي ظهر في تحسن الدلالات البيو كيميائية ومضادات الأكسدة والسيتوكينز وأيضا في فحص أنسجة الكبد.

(مجلة بنها للعلوم الطبية البيطرية: عدد 27(1):118- 134, سبتمبر 2014)