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Curcumin Attenuated Oxidative Stress and Inflammation on Hepatitis Induced of by Fluvastatin in Female Albino Rats

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ABSTRACT

The current study was planned to investigate the hepatoprotective effect of curcumin (Cr) against oxidative stress insults characteristic for hepatitis induced by Fluvastatin **(**F) in female rats. Animals were divided into Three groups: group1 (negative control), group2 (F-control), group3 (F+Cr). Rats were received F for first 10 days while received Cr for all 20 days (duration of experimental period). Results obtained showed that F significantly increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AlP), gamma glutamyl transpeptidase (γ-GTP), total bilirubin, malondialdehyde (MDA), tumor necrosis factor α protein (TNFα), tumor growth factorβ1 (TGFβ1), nuclear factor Kappa B (NFKB), cyclooxygenase-1 and 2 (COX-1 and 2), while, decreased the levels of albumin and total protein, reduced glutathione (GSH), Glutathione peroxidase (GPx), catalase (CAT) and interleukin-10 protein (IL-10). Liver histological and immune-histochemical investigations of rats administered F showed sever congestion in portal vein associated with fibrosis and inflammatory cells infiltration. Also, collagen-I stained cells were found in the portal area and the central vein. On the other hand, animals treated with curcumin showed improvements for these parameters as well as in the histological and immune-histochemical feature of the liver. Therefore, the present results indicated that curcumin could reduce oxidative stress and inflammation characterizing hepatitis.

1. INTRODUCTION

The liver is a multifunctional vital organ with the primary role in maintenance of body homeostasis (Liu etal., 2012). The crucial role of liver in maintaining human health status means that liver diseases can severely affect health status and threaten human life (Åberg etal., 2009). Generally, hepatitis is the most common, prevalent and serious type of liver diseases. Hepatitis or liver inflammation is caused by one of the hepatitis viruses (A, B, C, D, and E) (Hu 2008; Sun and Karin 2008). Numerous medical studies have demonstrated the important role of oxidative stress (in the pathogenesis of liver diseases and have proved the ameliorative role of dietary antioxidants (Bishayee et al., 2010; Nabavi et al., 2012). Fluvastatin competitively inhibit 3-hydroxy-3- methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme necessary for cholesterol biosynthesis; they also act in several ways to decrease the level of low-density lipoprotein (LDL) and increase the stability of atherosclerotic plaques (Jacobson, 2006). Although it seems to be relatively safe and well tolerated, considerable attention has been paid to their adverse

effects including muscular toxicity and hepatotoxicity (Pasternak et al., 2002; MacDonald and Halleck, 2004). Fluvastatin is the first entirely synthetic HMG-CoA reductase inhibitor and is in part structurally distinct from the fungal derivatives of this therapeutic class and is sold under the brand name Lescol® in the United States. Fluvastatin appears in much higher concentrations in the liver than in non-target organs, and the liver is the drug's primary site of both action and side effects. LESCOL Prescribing Information stated the maximum fluvastatin tolerated dose in rats was determined to be 9 mg/kg/day (Steiner et al., 2001).

Curcumin is a bright yellow-colored phenolic compound that was initially isolated from *Curcuma longa* L. (turmeric) rhizomes in 1815 (Gupta et al., 2013). The genus Curcuma is a member of Zingiberaceae family, growing in India, Southeast Asia, and other tropical areas (Martin et al., 2012). Curcumin has a long history in traditional medicine and as a food ingredient (Benzie and Wachtel-Galor, 2011). It shows therapeutic actions in different liver diseases, namely, hepatitis B, hepatitis C, alcoholic liver disease, nonalcoholic fatty liver disease, drug-induced hepatotoxicity, liver cancer, biliary cirrhosis, and primary sclerosing cholangitis (Nabavi et al.,2014). It is well known that oxidative stress has a crucial role in the initiation and progression of several liver diseases and cancer (Nabavi et al.,2014 and Nabil et al., 2017). So, the phenolic hydroxyl groups of curcumin play an important role in the antioxidant activity of curcumin (Feng and Liu, 2009, Afrah, 2010). This study aimed to investigate the possible therapeutic effect of curcumin on oxidative stress and inflammation characteristic of hepatitis induced of by fluvastatin in female albino rats.

2. MATERIALS AND METHODS

2.1. Chemicals

Fluvastatin was purchased from Novartis Pharmaceuticals; Spain S.A. Curcumin was purchased from Sigma Co. USA.

2.2. Animals and management

Thirty adult female swiss albino rats weighting 200±20 g were obtained from National Research Center (NRC, Cairo, Egypt). Animals were housed (10 rats in each group) at the animal facility at Zoology Department, Faculty of Science, Tanta University, Egypt. In clean and dry plastic cages and kept under standard situation (temperature 22±2 °C, humidity of 54 ± 2% and 12h/12h light/dark cycle). The rats were fed with rodent pellets and tap water ad *libitum*. This study was performed in accordance to guidelines of the use of experimental animals in research at Zoology Department, Faculty of Science, Tanta University, Egypt.

2.3. Administration schedule of Fluvastatin and Curcumin

The animals were equally divided into three groups (n = 10). The first group served as negative control (G1) and received distilled water, Rats of the second group served as positive control (G2) were received Fluvastatin dissolved in drinking water at the dose of 75 mg/kg /day (Cokca *et al.* 2005) by gavage for 10 days and left another 10 days, Rats of the third group (G3) were treated with Cr at the dose of 200 mg/kg (Reyes-Gordillo et al., 2007) by gavage for 20 days (the duration of the experiment). At the end of the experimental period animals were left night fasted and at the next day they were euthanized following protocols and ethical procedures.

2.4. Sample collection and biochemical assays

After 20 days rats were euthanized, blood samples were collected after 12 hours fasting using the orbital sinus technique, under light anesthesia, according to the method of Van Herck *et al*. (2001). Each blood sample was left to clot in clean dry test tube and then centrifuged at 1800 x g for 10 min at 4 ºC to obtain serum. The clear serum samples were frozen at -20 ºC for the biochemical analyses. Also, liver tissues were directly obtained after separation from the attached tissues, washed in ice-cold saline and dried on filter paper. Pieces of each liver tissues were homogenized in 10 ml *(10% w/v)* cold buffer (50 mM potassium phosphate, pH 7.5) using tissue homogenizer. The homogenate was centrifuged at 4000 r.p.m for 15 minutes at

4 ºC and the supernatant removed for the different biochemical analyses. Parts of each liver were fixed in formalin buffer (10%) for the histological and immune histochemical investigations. The remaining liver was cut in pieces and rapidly frozen with liquid nitrogen for extraction of total RNA, hepatic proteins.

2.5. Biochemical parameters

Serum levels of transaminases (ALT, AST) were determined by the colorimetric method using available commercial kit (Diamond- Diagnostics, Egypt) according to the method of Reitman and Frankel, (1957). Serum alkaline phosphatase (ALP) activity was estimated colorimetrically according to the method of Wolf, (1986), obtained by Bio-diagnostic medical company. Serum gamma glutamyl transpeptidase (γ-GTP) activity was assayed by the Kinetic method using available commercial kit

(BioMed - Diagnostics, EGY- CHEM) according to the method of (Tiez, 1976).Serum albumin concentration was assayed by the colorimetric method using available commercial kit (Diamond- Diagnostics, Egypt) according to the method of (Walter and Gerade, 1970). Serum total bilirubin concentration was assayed by the colorimetric method using available commercial kit (Bio Diagnostic) according to the method described by (Young et al., 1975). Hepatic MDA level was assayed by the colorimetric method using available commercial kit (Bio Diagnostic, Egypt) according to the method described by Satoh, (1978) and (Ohkawa et al., 1979). Hepatic (GSH) concentration in cell lysates was assayed by the colorimetric method using available commercial kit (Bio Diagnostic, Egypt) according to the method described by (Beutler et al., 1963). Hepatic (GPx) activity was assayed by the UV method using available commercial kit (Bio Diagnostic, Egypt) which described by (Paglia and Valentine, 1967). Hepatic (CAT) activity was assayed by colorimetric method using available commercial kit (Bio Diagnostic, Egypt) according to the method of Aebi, (1984).

2.6. Western Blotting Analyses

Liver extracts were prepared from pieces of liver tissues excised from every rat in the rat model using ice-cold radioimmunoprecipitation assay lysis buffer containing 150 mM NaCl, 50 mM Tris, 0.1% SDS, 1% Triton x-100, and 0.5% sodium deoxycholate supplemented with protease and phosphatase inhibitors. Protein concentrations were determined using the Folin Lowry method. Forty micrograms of total proteins were subjected to 12% SDS-polyacrylamide gel electrophoresis. TNFα and IL-10 were detected by using primary antibodies against TNFα and IL-10 and secondary antibodies conjugated with horseradish peroxidase. β-Actin was probed as an internal control. Protein bands were visualized and the density of each band was normalized by β-actin.

2.7. RNA isolation and real-time PCR.

Total RNA was extracted from frozen liver tissues using total RNA Purification Kit following the manufacturer protocol (Thermo Scientific, Fermentas, #K0731) which includes Lysis buffer, Proteinase K solution, Wash buffer I and Wash buffer II. cDNA synthesis was performed using reverse transcription kits (Thermo Scientific, Fermentas, #EP0451). Total RNA was treated with DNase I to eliminate genomic DNA contamination, followed by synthesis of the first strand using reverse transcription system. Reverse transcription was carried out as follows: 42°C for 60 min, 70°C for 10 min, and 4°C for 5 min (one cycle). Real-time PCR with SYBR Green was used to measure expression of mRNAs of target genes in the liver tissues, with β- actin as an internal reference. The isolated cDNA was amplified using 2X Maxima SYBR Green/ROX qPCR Master Mix following the manufacturer protocol (Thermo scientific, USA, # K0221) and gene specific primers. The cycles for PCR were as follows: 95°C for 7 min, 40 cycles of 95°C for 20 s, 54°C for 30 s, and 72°C for 30 s. Melting curves were determined by heat-denaturing PCR products over a 35°C temperature gradient at 0.2°C/s from 60 to 95°C. The primers were as follows: ***TGFβ1,*** (forward) ⁄5-AAGAAGTCACCCGCGTGCTA-⁄3 and (reverse)⁄5-TGTGTGATGTCTTTGGTTTTGTCA-⁄3; ***NFKB***, (F) ⁄5-CCTAGCTTTCTCTGAACTGCAAA-⁄3 and (R) ⁄5-GGGTCAGAGGCCAATAGAGA -⁄3; ***Cox1***, (F) ⁄5-CCCAGAGTCATGAGTCGAAGGAG-⁄3and (R) ⁄5-CAGGCGCATGAGTACTTCTCGG-⁄3 ; ***Cox2***, (F) ⁄5-GATTGACAGCCCACCAACTT-⁄3 and (R) ⁄5-CGGGATGAACTCTCTCCTCA-⁄3 and **β-actin**, (F) ⁄5- ACCCACACTGTGCCCATCTA -⁄3 and (R) ⁄5-CGTCACACTTCATGATG -⁄3 . The quantities critical threshold (Ct) of target gene was normalized with quantities (Ct) of housekeeping gene (*ß-actin*) by using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.8. Histopathological examination

Autopsy samples were taken from the liver of rats in different groups and fixed in 10% formal buffer for twenty-four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty-four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slide microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained by Hematoxylin & eosin stain for routine examination through the light electric microscope (Bancroft and Gamble, 2002).

2.9. Immunohistochemistry

Rat liver was embedded in optimal cutting temperature compound and stored at−80 °C until use. Cryosections (5mm) were fixed in acetone/methanol (1:1) and incubated with normal goat serum to reduce non-specific binding. Tissue sections were incubated with anti-collagen-I monoclonal antibody overnight in

a humidified chamber at 4 °C. Horseradish peroxidase (HRP) labeled secondary antibody included in the MaxVision™ HRP-Polymer anti-mouse/rabbit IHC kit (Fuzhou Maixin, China), was applied for 30 min at room temperature, and followed by incubating at room temperature with diaminobenzidine (DAB) Chromogen for color development. Tissue sections were counterstained with Mayer's Hematoxylin (Sigma-Aldrich) (Bai et al., 2014)**.**

2.10. Statistical analysis

In the present study, all results were expressed as Mean ± S.E of the mean. One-way analysis of variance (ANOVA) was used to assess significant differences among treated groups and controls using Graph Pad Prism 7 (La Jolla, CA, USA). The Tukey Test was used to compare all groups with each other and to show the significant effect of treatment. Values were considered statistically significant when $p<0.05$.

3. RESULTS

3.1. Curcumin restored biochemical profile

The result in Table (1) revealed that serum (ALT), (AST), (ALP), (γ-GTP) activities and total bilirubin concentration were significantly increased ($P < 0.001$) in F induced group when compared to normal control group (G1). ALT, AST, ALP and γ-GTP enzyme activities and total bilirubin concentration in serum were significantly decreased in (F+Cr) ($P < 0.01$) in comparison with the (F) induced group (G2). In contrast, Serum albumin concentration was significantly decreased ($P < 0.001$) in (F) when compared to normal control group. Albumin concentration in serum was significantly increased in (F+Cr) ($P < 0.001$) in comparison with the (F) group (G2).

The result in Table (2) showed that hepatic total protein, (GSH) concentrations and (GPx), (CAT) activities were significantly decreased ($P < 0.001$) in (F) induced group when compared to normal control group. Hepatic total protein, GSH concentrations, (GPx), (CAT) activities were significantly increased in (F+Cr) ($P < 0.001$) in comparison with the (F) group. In contrast, Hepatic lipid peroxidation products (MDA) was significantly increased ($P < 0.001$) in (F) when compared to normal control group. Hepatic MDA was significantly decreased in (F+Cr) ($P < 0.001$) in comparison with the (F) group.

3.2. Tumor necrosis factor alpha (TNF-α) and interleukin-10 (IL-10) levels by western blotting

The result in Table (3)) showed that the expression of the apoptotic protein TNFα was significantly increased ($P < 0.001$) in F-induced hepatitis group (G2), as compared to normal group control group. This elevated expression was significantly decreased ($P < 0.001$) in Cr-treated group as compared to F-induced hepatitis group. On the other hand, the expression of anti-inflammatory IL-10 protein in liver tissue was significantly decreased ($P < 0.001$) in F-induced hepatitis (positive control) group as compared to normal and group. The expression of IL-10 protein level was significantly increased in Cr-treated group in comparison with the F-induced hepatitis group.

3.3. Molecular analysis by using RT-PCR for TGF-β1, NFKB, COX-1 and COX-2 genes

The result in Table (4) showed that a significant ($P < 0.001$) increase in the expression of the pro-inflammatory genes *TGFβ1, NFKB, COX1, COX2* in the liver of F-induced hepatitis rat as compared to normal control group. Cr-treated group showed significant decrease ($P < 0.001$) in the expression level of *TGFβ1, NFKB, COX1, and COX2* as compared to F-induced hepatitis group.

3.4. Histopathological findings

Microscopic examination of liver tissue sections of rats in the negative control group (G1) showed no histopathological alteration, with normal histological structure of the central vein and surrounding hepatocytes in the parenchyma (Fig.1.a). Photomicrograph of liver section of induced rats with fluvastatin showed sever congestion in the portal vein associated with fibrosis and inflammatory cells infiltration in the portal area as well as nuclear pyknosis in some of the hepatocytes (Fig.1.b). Micrograph of liver section of fluvastatin induced rats treated with curcumin showed few inflammatory cells infiltration with edema in the portal area (Fig.1.c).

3.5. Immunohistochemical investigations

After fluvastatin treatment, collagen-I stained cells presented in the portal area and the central vein, and those intensities significantly increased (Fig.1.e). Curcumin restored the liver to normal structure in group 3 (F+ Cr) showed less collagen-I deposition (Fig.1.f).

Table (1): Effect of treatment with Curcumin on Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP) enzyme activities and concentration of albumin, total bilirubin in hepatitis model.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	γ-GTP (U/L)	Albumin (g/dl)	Total bilirubin (mg/dl)
Normal control (G1)	42.1 ± 4.1	140.6 ± 11.3	100.35 ± 3.9	7.32 ± 0.79	4.29 ± 0.157	0.76 ± 0.135
F-treated (G2)	182.3 ± 4.88 [a]	388.9±5.76[a]	302.5 ± 3.7 [a]	28.97 ± 3.2[a]	1.73 ± 0.13 [a]	3.76 ± 0.14 [a]
F+ Cur (G3)	78.94 ± 6.3[b]	238.9±12.6[b]	177.2±5.5[b]	14.8±1.45 [b]	3.46 ± 0.18[b]	1.9 ±0.14[b]

Data are expressed as mean ± SEM (n= 10 in each group).
Means within superscript letter (a) were significantly different (P≤ 0.001) as compared to normal control group.
Means within superscript letter (b) were significantly different (P≤ 0.001) as compared to positive control (F-induced hepatitis).
The significance of difference was analyzed by one – way ANOVA and Tukey test using computer program (Graph pad prism 7).

Table (2): Effect of treatment with Curcumin on hepatic parameters [total protein concentration (g/g tissue), MDA (nmole/g tissue), GSH (mmol /g), GPx(mmol/g), catalase (U/g)]in hepatitis model.

Groups	Total protein (mg/g tissue)	MDA (nmol\g)	GSH (mmol /g)	GPx (U/g)	Catalase (U/g)
Normal control (G1)	6.74 ± 0.14	4.54 ± 0.25	114.47 ± 4.23	24.13 ± 0.42	48.0 ± 0.6
F-treated (G2)	3.48 ±0.15 [a]	17.12 ± 0.49[a]	45.77± 1.09[a]	5.24 ± 0.16[a]	13.68 ±0.42 [a]
F+ Cur (G3)	5.04 ±0.11[b]	9.46 ± 0.38[b]	71.38 ± 1.0[b]	9.11±0.26[b]	24.93 ± 0.36[b]

Data are expressed as mean ± SEM (n= 10 in each group).
Means within superscript letter (a) were significantly different (P≤ 0.001) as compared to normal and control group.
Means within superscript letter (b) were significantly different (P≤ 0.001) as compared to positive control (F-induced hepatitis).
The significance of difference was analyzed by one – way ANOVA and Tukey test using computer program (Graph pad prism 7).

Table (3): Band quantification of TNFα and IL-10 proteins expression by image J software.

Group	TNFα protein		IL-10 protein	
	Relative density	SEM	Relative density	SEM
(G1) Normal control	**1.09**	**0.1**	**1.02**	**0.09**
(G2) F-treated	**5.85**	**0.51 [a]**	**0.12**	**0.01 [a]**
(G3) F+ Cur	**3.70**	**0.23 [b]**	**0.47**	**0.06 [c]**

Data are expressed as relative expression (density) versus control. Each column represents the mean ± SE of three independent experiments.
Means within superscript letter (a) were significantly different (P≤ 0.001) as compared to normal control group.
Means within superscript letter (b) were significantly different (P≤ 0.001), (c) were significantly different (P≤ 0.01) as compared to positive control (F-induced hepatitis).
The significance of difference was analyzed by one – way ANOVA and Tukey test using computer program (Graph pad prism 7).

Table 4: Effect of Curcumin on TGFβ1, NFKB, COX1, COX2 gene expressions.

Group	*TGFβ1*	*NFKB*	*COX1*	*COX2*
(G1) Normal control	1.00 ± 0.07	1.00 ± 0.07	1.00 ± 0.07	1.00 ± 0.07
(G2) F-treated	8.51 ± 0.38 [a]	14.32 ± 0.38 [a]	7.73 ± 0.38 [a]	11.39 ± 0.38 [a]
(G3) F+ Cur	3.86 ± 0.19 [b]	4.72 ± 0.29 [b]	5.90 ± 0.19 [b]	6.5 ± 0.19 [b]

Data are expressed as Fold change mean ± SEM (n= 10 in each group).
Means within superscript letter (a) were significantly different (P≤ 0.001) as compared to normal control group.
Means within superscript letter (b) were significantly different (P≤ 0.001) as compared to positive control (F-induced hepatitis).
The significance of difference was analyzed by one – way ANOVA and Tukey test using computer program (Graph pad prism 7).

4. DISCUSSION

The current study revealed that Fluvastatin administration in a dose of 75 mg/Kg B.W. for 10 days induced significant elevation in Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP) enzyme activities, concentration of total bilirubin and reduction of albumin level in adult female albino rats. These results are in conformity with (Cokca et al., 2005 and Kim et al., 2017) who stated that high-dose therapy with statins leaded to toxicological situation that causes viral hepatitis. Thus, statins were considered in establishing the new experimental model of hepatitis. Measurement of aminotransferases levels is considered as the most important laboratory test for hepatitis. Viral hepatitis and toxic drugs may cause hepatic necrosis, which indicted by 20-50-fold elevation in the serum aminotransferases. Alcoholic hepatitis causes aminotransferases to increase up to 8-10 folds, with AST greater than ALT due to release of AST from injured hepatic cell mitochondria (Nalpas et al., 1984; Salama, et. Al. 2006). Statins are metabolized mainly by the liver and increase aminotransferases levels with hepatic potential toxicity that might be attributed to alteration of the hepatocyte cellular membrane rather than direct liver injury (Clarke and Mills, 2006). Other study of (Swislocki et al., 1997; Lee and Kim, 2019) supported our results and indicated increased level of serum aminotransferases (ALT) and (AST) that occurred in patients who received fluvastatin for an extended period of time.

Also, Heba et al. (2016) stated that mild-to-moderate elevations in liver transaminases are the most commonly seen side effect of statin treatment as fluvastatin significantly elevated serum ALT and AST contents in liver homogenate.

This increase in aminotransferase release with Fluvastatin could be caused by necrotic degeneration of hepatocyte membrane, or by changes in membrane composition induced by reduction of lipid components, especially cholesterol (Tolman, 2002). Moreover, elevated aminotransferases levels alone do not usually predict or indicate serious liver injury, it just indicates more hepatocytes cytoplasmic contents leakage or cell necrosis (Segarra-Newnham *et al*., 2007).

In view of the present data, Fluvastatin administration induced significant elevation in serum ALP and GGT levels, these results are in agreement with (Hwang et al., 2000) who indicated that ALP and GGT levels signify the cells membrane integrity; and also GGT related to glutathione metabolism, and glutamic acid uptake, thus ALP and GGT are an extremely sensitive enzymes for identifying cholestatic diseases, and they have been taken into account in the evaluation of patients with chronic HCV infection. These results are also supported by (Sugatani et al., 2010) who observed that Fluvastatin caused hepatocellular damage to Chang liver cells such as significant rise of GGT activity.

Also, it has been demonstrated that Fluvastatin induced significant elevation in serum total bilirubin and decreasing Albumin levels. Jaundice (an accumulation of bilirubin in the blood caused by inefficient bilirubin conjugation by the hepatocytes indicating liver injury. Statin-related drug-induced liver injury by (atorvastatin, simvastatin, fluvastatin and rosuvastatin (Russo et al., 2009; Bjornsson et al., 2012).

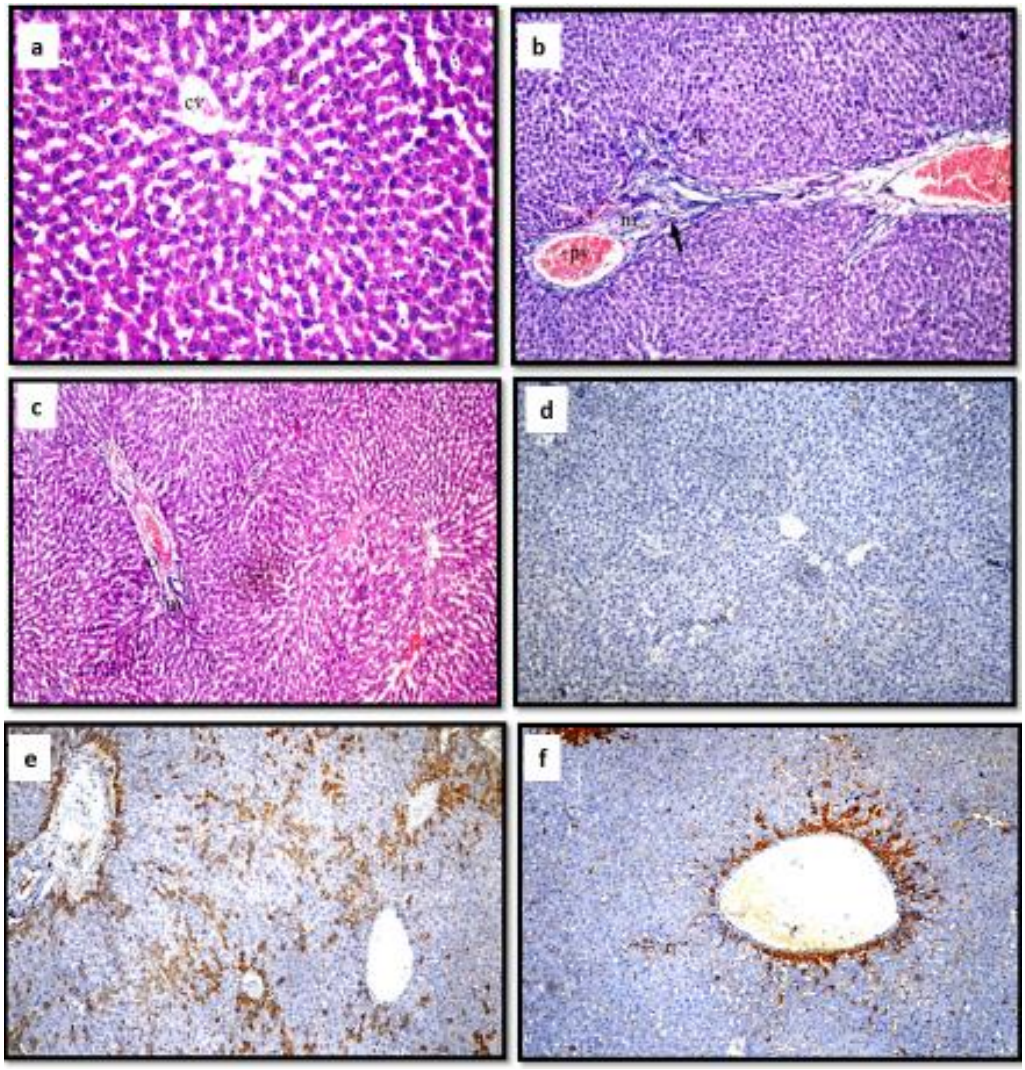

Fig. (1.a): Micrograph of liver section of normal control group showing normal histological structure of the central vein (**CV**) and surrounding hepatocyte (**h**) in the parenchyma "H&E 40".
Fig. (1.b): Micrograph of liver section of fluvastatin induced rat showing sever congestion in portal vein (**PV**) associated with fibrosis and inflammatory cells infiltration (**m**) in the portal area with nuclear pyknosis (**K**) in some hepatocytes "H&E 16". ARROW = fibroblastic cells proliferation
Fig. (1.c): Micrograph of liver section of fluvastatin induced rat treated with curcumin showing few inflammatory cells infiltration (**m**) with edema (**O**) in the portal area "H&E 16".
Fig. (1.d): Immunohistochemical staining showed no deposition of collagen-I antibody in liver of normal control group "X16 magnification ".
Fig. (1.e): Immunohistochemical staining showed sever deposition of collagen-I antibody in liver of positive control group 2 "X16 magnification".
Fig. (1.f): Immunohistochemical staining showed mild deposition of collagen-I antibody in liver of (F+Cr) group "X16 magnification".

Serum albumin concentration, which is usually used to evaluate synthetic function of hepatocytes, is influenced by mitochondrial integrity and function. In addition, this may indicate that when hepatocytes proceed in apoptotic pathway, they also stop synthesizing essential body elements, such as albumin. So, the influence of liver diseases on albumin homeostasis, it varies according to the severity and type of the disease. In acute and in chronic persistent hepatitis serum albumin is usually normal or minimally decreased. Also, Rats administered fluvastatin showed signs and symptoms of liver toxicity, especially a decrease in albumin level Wolf, (1999).

In light of the present findings, treatment of hepatitis group with curcumin experienced significant decrease in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP) enzyme activities, concentration of total bilirubin and increasing Albumin level in adult female albino rats. (Fu et al., 2008 and Hadisoewignyo et al., 2019) stated that administration of curcumin significantly reduced

the levels of serum alkaline phosphatase, ALT, and AST in CCl4-induced liver injury and these findings demonstrated that curcumin not only protected the liver against injury but also inhibited HSC activation, and it reduced hepatic fibrogenesis caused by chronic CCl4 intoxication. Also, In agreement with Mohan et al. (2019) who estimated that a novel formulation of curcumin as curcumin-galactomannosides (CGM) reduced the elevation in (SGPT, SGOT, and ALP) activities of liver alcoholic toxicity exposure. Moreover, curcumin treatment prevented the indicators of necrosis (alanine aminotransferase) and cholestasis (γ-glutamyl transpeptidase and bilirubins) increases after CCl4 intoxication (Reyes-Gordillo et al., 2007). Curcumin alone provide to be beneficial in decreasing the levels of free radicals and lipid peroxidation and improving the activities of the antioxidant enzymes. Also, curcumin could be used as a spice in food and as a food additive to be beneficial in alleviating either cadmium or any environmental toxins that human may be exposed, Salama, 2010).

Galaly et al. (2014) demonstrated that the treatment of gentamicin-administered rats with curcumin significantly reduced the elevated serum AST, ALT and bilirubin level. Soetikno et al., (2012) revealed that curcumin significantly protects the liver from injury as indicated by reducing the serum AST, ALT, and ALP activities, and by amending the histological architecture of the liver in carbon tetrachloride and streptozotocin treated rats. Khedr and Khedr, (2014) reported that plasma albumin level was also increased by curcumin administration in CCl4 treated rats. Galaly et al. (2014) demonstrated that oral supplementation of gentamicin-administered rats with curcumin significantly increased the declined total protein and albumin levels.

The current study revealed that fluvastatin administration induces significant elevation in hepatic MDA and significant reduction in hepatic GSH, GPx, catalase and total protein. Oxidative stress plays an important role in various liver diseases. It is a common pathogenic mechanism contributing to initiation and progression of hepatic damage. Increased oxidative stress in statin-treated groups is attributed to increased lipid peroxidation-associated with tissue damage. Increased lipid peroxidation is generally believed to be an important underlying cause of the initiation of oxidative stress-related various tissue injuries, cell death, and the progression of many acute and chronic diseases (Heba et al., 2016). Also, oxidative stress can lead to cancer (Abdel-Hamid, et al., 2017).

Heba et al. (2016) also, indicated that administration of Fluvastatin significantly elevated NO and MDA contents. Moreover, they reduced GSH, TAC and SOD activity in liver homogenate. Srichan et al. (1999) demonstrated that Fluvastatin also exhibited the inhibitory effect on the elevation of peroxisomal enzymes catalase level. Moreover, it could reduce the total liver protein and microsomal protein content induced by grmfibrozil-treated group.

In view of our results, treatment of hepatitis-induced rats with curcumin resulted in significant decrease in hepatic MDA. On the other hand, treatment of hepatitis group with curcumin produced significant increase in hepatic GSH, GPx, catalase and total protein. Nissar et al. (2013) indicated that hepatic MDA level and oxidative stress which increased in CCl4 treated rats leading to formation of reactive metabolites due to biotransformation by Cytochrome P450 2E1. Once formed, free radicals trigger a cascade of reactions that culminate in lipoperoxidation. So, treatment with curcumin which induced the significant decline in the concentration of these constituents in the liver of rats indicated anti-lipid peroxidative effects. Also, studies suggested that curcumin inhibits CYP2E1 activity (Guangwei et al., 2010). (Fu et al., 2008) also indicated that administration of curcumin significantly diminished the CCl4-elevated level of lipid hydroperoxide in the rat model and this suggested that curcumin might protect the liver against CCl4-caused injury by attenuating oxidative stress. Curcumin reduces the oxidative stress in animals, by its high ROS scavenging capacity and by protecting the antioxidant enzymes from being denatured (Madkour, 2012). It has also been suggested that hydrogen-bonding interaction between the phenol rings (hydroxyy and o-methoxy groups) in demethoxycurcumin, significantly affects the energy of O–H bond and hydrogen-donating potential, leading to better antioxidant action. Transition metal chelation property is known as another antioxidant mechanism of curcumin, which is attributed to the diketone and the o-methoxy phenol moieties (Anand et al., 2008). Curcumin, as a strong inhibitor of cytochrome P450, can normalize antioxidant enzymes and nonenzymatic antioxidant compounds such as GSH (Oetari et al., 1996; Afrah, 2010).

Sharma et al. (2001) showed that curcumin increases glutathione S-transferase activity which involved in the synthesis of GSH. Moreover, Curcumin elevates the level of cellular GSH and induces de novo synthesis of GSH in HSC by stimulating the activity

and gene expression of glutamate cysteine ligase, a key rate-limiting enzyme in GSH synthesis. These effects may recommend curcumin as a hepatoprotective agent (Zheng *et al.,* 2007). Also, oral administration of curcumin not only increased the level of total hepatic GSH but also significantly improve the ratio of GSH/GSSG in the liver (Fu *et al*., 2008).

(Mahmoud et al., 2015 and Mohan et al., 2019) also evidenced that curcumin treatment significantly restoring the antioxindant enzymes SOD, CAT and GPx activities as well as non-enzymatic antioxidant GSH concentration in the liver. Nabavi et al. *(*2014) stated that Curcumin significantly modulated the activities of SOD and GPx after CCl4 withdrawal. Also, Galaly et al. (2014) showed that the treatment of gentamicin-administered rats with curcumin significantly decreased the elevated lipid peroxidation whereas they detectably increased the glutathione content, glutathione peroxidase and superoxide dismutase activities. Khedr and Khedr, (2014) reported that plasma total protein and albumin level was also increased by curcumin administration after CCl4 treatment.

The current study revealed that fluvastatin administration induces significant reduction in hepatic anti-inflammatory marker IL-10 protein but it induces significant elevation in hepatic apoptotic marker TNFα protein and pro-inflammatory cytokine genes *(TGFβ1, NFKB, COX1* and *COX2*) levels. As mentioned before, Statin-drugs such as (atorvastatin, simvastatin, fluvastatin and rosuvastatin) induced liver injury due to increasing oxidative stress. Also, Johar et al. (2004) showed that rosuvastatin could interact with proteins and enzymes of the hepatic interstitial tissue by interfering with the antioxidant defense mechanism and leading to reactive oxygen species (ROS) generation which in turn may imitate an inflammatory response. investigations focusing on the relationship and interaction of oxidative stress and inflammation have attracted great attention as accumulated evidences indicated that they are tightly correlated and orchestrated to drive the pathophysiological procedure of liver diseases (Biswas, 2016). A number of reactive oxygen species (ROS) or reactive nitrogen species (RNS) can augment proinflammatory gene expression by provoking intracellular signaling cascade. On the other hand, inflammatory cells could produce more ROS/RNS, resulting in exaggerated oxidative stress at inflammatory lesion that promotes the pathogenesis of liver diseases (Ambade and Mandrekar, 2012). Oxidative stress can lead to chronic inflammation because many transcription factors and receptors such as NFKB, activator protein-1 (AP-1), p53, hypoxia-inducible factor 1-alpha (HIF-1α), PPAR-γ, β-catenin/Wnt, and erythroid 2-related factor 2 (Nrf2) are activated by oxidative stress, which could regulate the expression of many genes, including those inflammatory cytokines and anti-inflammatory molecules (Mittal et al., 2014) . ROS and lipid peroxidation induce inflammation through promoting the release of proinflammatory cytokines, resulting in neutrophil chemotaxis (Videla et al., 2004). ROS activate NF-κB signaling pathway, leading to the synthesis of TNF-α and the upregulated TGF-β, IL-8, IL-6, and Fas ligand.Also, the generation of proinflammatory cytokines like cyclooxygenase-1 (COX-1), COX-2, TNF-α, IL-1, IL-26, IL-8, IL-18, and macrophage inflammation protein-1 (MIP-1α) via activation of NFKB alters the hepatic microenvironment and leads to fibrosis and carcinogenesis (Barash et al., 2010). ROS can stimulate the production of profibrogenic mediators from infiltrated inflammatory cells by interacting directly with HSCs, which are the main executors of fibrogenesis to generate ECM (Yamamoto et al., 2016). Treatment of hepatitis group with curcumin produced significant increase in hepatic anti-inflammatory marker IL-10 protein but it induces significant reduction in hepatic apoptotic marker TNFα protein and pro-inflammatory cytokine genes *(TGFβ1, NFKB, COX1* and *COX2*) levels.

Curcumin is in fact a potent free radical scavenger and inhibit COX2 and 5-lipooxygenase (5-LOX) expression and catalytic activity. Curcumin is a potent immunomodulatory agent, as well. Curcumin can modify immune cell activity including T and B cells, macrophages, neutrophils, dendritic cells (DC) and natural killer cells (NKC). Curcumin down-regulates pro-inflammatory cytokines such as IL-1, IL-2, IL-6, IL-8, IFN-γ, as well as monocyte chemotactic protein 1-1 (MCP-1), iNOS and NO production in many pathological conditions (Derosa et al., 2016; Karimian et al., 2017and Stoica et al., 2018). Curcumin inhibits the proliferation of lymphocytes and their ability to secrete IL-4, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF). On the other hand, curcumin can induce the expression and production of IL-10 to counteract inflammatory conditions. Some of the anti-inflammatory effects of curcumin include a reduction in TNF-α, IL-12 activity and macrophage infiltration. Curcumin inhibits TGF-β and NF-κB signaling *(*Sunagawa et al., *2011)*.

Curcumin treatment suppressed inflammation caused by CCl4 by decreasing the levels of TNFα, IFNγ, and IL-6 which might lead to the protection of the liver from injury (Fu et al., 2008). Mohan et al. (2019) stated that Curcumin and CGM significantly downregulated the respective mRNA expression of TNFα and IL6 indicating the anti-inflammatory role of curcumin and enhanced effect of CGM in ethanol-induced hepatic damage. Jobin et al. (1999) also demonstrated that the hepatoprotective effects of curcumin that mediated through the inhibition of the NFKB system in acutely liver-damaged rats. As curcumin blocks gene expression by inhibiting the signal leading to IκB kinase (IKK) activation without directly interfering with NFKB-inducing kinase or IKK kinase complexes. Blockade of IKK activation causes inhibition of IκBα phosphorylation/degradation and NFKB activation.

Bulku et al. (2012) also demonstrated that curcumin ameliorates acetaminophen-induced liver damage through normalization of proapoptotic (Bax, caspase-3) and antiapoptotic signaling pathways the curcumin chemopreventive potential is further shown by it capacity to inhibit COX-2 and iNOS expression through NF-kB pathways that are considered another possible ameliorative mechanism against drug-induced liver damage (Puri and Sanyal, 2012).

Fu et al. (2008) indicated that curcumin significantly reduced the levels of PDGF and *TGFβ1* in the rat model, which might result in the inhibition of HSC activation stimulated by CCl4.

Micrograph of liver section of fluvastatin induced rat showed severs congestion in portal vein associated with fibrosis and inflammatory cells infiltration in the portal area with nuclear pyknosis in some hepatocytes. Also, collagen-I stained cells were found in the portal area and the central vein. Micrograph of liver section of fluvastatin induced rat showed severs congestion in portal vein associated with fibrosis and inflammatory cells infiltration in the portal area with nuclear pyknosis in some hepatocytes. Also, collagen-I stained cells were found in the portal area and the central vein. Heba et al. (2016) histopathological studies showed that the harmful effect increased in a fluvastatin dose-dependent manner and showed mild dilatation and congestion of some blood sinusoids, aslight cellular infiltration near the central vein, in addition to deformation of the normal architecture and vacuolar degeneration of various degrees in many hepatocytes.

Al-Doaiss et al. (2013) stated that the liver of rosuvastatin -treated rats had lost some hepatic architecture characteristics and demonstrated several histological and histochemical alterations such as marked parenchymal necrosis in hepatocytes with eosinophilic cytoplasm. Also, Dilatation and congestion of hepatic sinusoids became evident.

Oxidative stress-related molecules and pathways due to fluvastatin induction can modulate tissue and cellular events involved in the pathogenesis of liver fibrosis (Sánchez-Valle et al., 2012). The presence of oxidative stress and decreased antioxidant defenses caused by stimulus has been detected in almost all settings of fibrosis and cirrhosis in clinical and animal model. The disruption of lipids, proteins, and DNA caused by oxidative stress will induce necrosis and hepatocytes death and intensify the inflammatory response, resulting in the initiation of fibrosis (Heeba and Mahmoud, 2014). Liver fibrosis, a reversible multicellular wound healing process that results from chronic liver injuries with excessive collagen and extracellular matrix (ECM), is characterized by perpetuation of parenchymal necrosis, infiltration of inflammatory cells, and activation of HSCs, macrophages, and KCs (Devi and Anuradha, 2010).

Micrograph of liver section of hepatitis induced rats treated with curcumin revealed few inflammatory cells infiltration with edema in the portal area and Congestion in central and portal veins associated with nuclear pyknosis in hepatocytes and less collagen-I deposition that indicated curcumin could modulate collagen-I deposition, and then alleviate the progress of hepatic fibrosis.

Fu et al. (2008) reported that curcumin significantly reduced the levels of TNFα and IL-6 in the liver and in serum in the CCl4 rat model. TNFα stimulates the development of hepatic fibrosis (Yin et al., 1999). IL-6 induces hepatic inflammation and collagen synthesis in vivo (Choi et al., 1994). IL-6 produced by activated HSC facilitates the production of ECM, including type I collagen, leading to hepatic fibrosis (Natsume et al., 1999).

Fu et al. (2008) also indicated that curcumin significantly reduced the levels of platelet-derived growth factor (PDGF) and TGFβ in the rat model, which might result in the inhibition of HSC activation stimulated by CCl4. Activation of hepatic stellate cells (HSC) is triggered by various cytokines and chemokines, including the fibrogenic factor TGFβ (Gressner et al., 2002), released from kupffer cells and

activated HSC (Friedman, 1999). The process of HSC activation is associated with sequential up-regulation of corresponding receptors, including PDGFβR (Pinzani et al., 1996), epidermal growth factor receptor (EGFR) (Kömüves et al., 2000), and type I and II TGFβ receptors (Friedman et al., 1994). Curcumin significantly suppressed gene expression of PDGFβR, EGFR, and type I and II TGFβ receptors in HSC (Zhou et al., 2007).

Fu et al. (2008) stated that curcumin treatment remarkably reduced the size stained with Sirius red in the liver suggesting a decreasing high level of collagen deposition resulted from hepatic fibrogenesis. (Reyes-Gordillo et al., 2007 and Hadisoewignyo et al., 2019) indicated that treatment of CCl4- group with curcumin resulted in the absence of necrotic areas and this histopathological analysis is in agreement with the observed result in the ALT activity, a biochemical indicator of necrosis that was significantly prevented by curcumin. Also, Mohan et al. (2019) stated that the histopathology of the rat liver treated with CGM showed no hemorrhage, necrosis, or inflammatory cells.

5. CONCLUSION

Curcumin provided considerable hepatoprotective and phototherapeutic effects against Fluvastatin-induced hepatitis and improved the oxidative stress and inflammation on liver tissues by enhancing antioxidant activities through ROS scavenger.

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7. DECLARATION OF INTEREST

All authors declare that they have no conflict of interests.

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