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SULFASALAZINE AND MESALAZINE MODULATES LIGHT EXPRESSION IN EXPERIMENTAL HEPATIC FIBROSIS

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ABSTRACT
Background: LIGHT (TNFSF14), a tumor necrosis factor superfamily member expressed by activated T cells, binds to HVEM which is constitutively expressed by T cells and stimulates T cell activation in a CD28-independent manner. Given interest to proposed LIGHT fibrinogenic role in hepatocytes, we examined the role of LIGHT in experimental hepatic fibrosis and the impact of sulfasalazine and mesalazine on its expression in hepatocytes. **Objective:** Characterization of LIGHT expression pattern in in CCl_4 induced hepatic fibrosis model with investigation of possible modulatory effects of sulfasalazine and mesalazine. **Methods:** Thirty six adult male rats weighing 250–300 g were used. Animals were randomly divided into six groups 6 animals each: control group, 6 weeks group, DMSO group, sulfasalazine group, mesalazine group and 11 weeks group. All studied groups except controls were injected with CCl_4. Tissue and serum samples obtained from all groups of rats were taken followed by determination of enzyme levels (AST, ALT, and ALP), tissue oxidative stress markers (SOD and MAD) and histopathological analysis. LIGHT expression examined against control group and positive control groups by immunofluorescence analysis. **Results:** Quantitative analysis of immunofluorescence staining expressed as fluorescence intensity revealed that sulfasalazine decreased LIGHT expression by about 73% and mesalazine by about 57% with respect to that of CCl_4 treated group. Sulfasalazine concomitantly with CCl_4 significantly decreased ALT from CCl_4 alone or with DMSO groups but failed to retain it as the control, nevertheless AST and ALP did not significantly changed. Combination of sulfasalazine and CCl_4 increased MDA content to 23.4±1.17 compared to DMSO group (19.2±1.72) while mesalazine showed the minimal increase in MDA tissue content (7.53±0.71) compared which control group (4.36±0.31 nmol/g) and remarkably was significantly different when compared with 6 weeks CCl_4 treated group (19.7±1.52). SOD activity of hepatic tissue was 524±41.6 U/mg tissue. This content was significantly decreased in other groups to be 264±14.1 for 6 weeks group, 357±31.3 for DMSO, 264±14.1 for 11 weeks group, 283±27.8 for sulfasalazine group and 226±16.8 for mesalazine group. **Conclusion:** Both sulfasalazine and mesalazine modulates expression of hepatocytes LIGHT, however a dominance of sulfasalazine were detected. Hereafter both can protect against hepatic fibrosis.

KEYWORDS: *Sulfasalazine, Mesalazine, LIGHT, Hepatic Fibrosis.*

1. INTRODUCTION
Hepatic fibrosis develops due to an increase in fibrillary collagen synthesis and deposition along with insufficient remodeling.[1] It is associated with a number of pathological and biochemical changes leading to structural and metabolic abnormalities, as well as with increased hepatic scarring.[2] The progression of liver fibrosis leads to cirrhosis, a condition characterized by distortion of the normal architecture, septae and nodule formation, altered blood flow, portal hypertension, hepatocellular carcinoma, and ultimately liver failure.[3] Sulfasalazine is a synthetic drug obtained from the combination of sulfapyridine and 5-aminosalicylic acid (mesalazine), an antibiotic and an anti-inflammatory agent, respectively. This drug is commonly used in the inflammatory diseases of the large intestine and rheumatoid arthritis.[4] It has been demonstrated that sulfasalazine is a potent inhibitor of nuclear factor kappa-light-chain-enhancer of activated B-cell summarized as nuclear factor kappa B (NF-kB) activation, which explains, at least in part, its pharmacological immunomodulatory effects in chronic inflammation.[5] It was documented that a single administration of sulfasalazine to carbon tetra chloride (CCl_4)-injured rats promoted rapid clearance of alpha-smooth muscle actin (α-SMA)–positive myofibroblasts, promote apoptosis of activated hepatic stellate cells (HSC), reduced hepatic expression of procollagen I and tissue inhibitor of metalloproteinase 1 (TIMP1), increased hepatic matrix metalloproteinases (MMP) activity, and accelerated resolution of liver injury.[6] Mesalazine is active moiety of sulfasalazine which is responsible for its pharmacological action. It is released into the lumen by the action of gut flora in distal ileum

and colon.[7] Peroxisome proliferator-activated receptors (PPAR) are members of the nuclear superfamily of receptors that are involved in the transduction of metabolic and nutritional signals into transcription events.[8] Mesalazine produce effects similar to activation of the c-form peroxisome proliferator-activated receptors (PPAR-c), e.g. modulation of inflammatory cytokine production, modulation of RELA/p65 dephosphorylation, leading to decreased transcriptional activity of NF-kB, and reduced prostaglandins and leukotrienes synthesis.[9] Tumor necrosis factors (TNFs) are well known as a critical factors in eliciting rapid inflammatory events acting through distinct receptors.[10] In general, ligation of these receptors results in activation of caspases, E3 ubiquitin ligases, or both. Death domain containing receptors, such as TNF receptor 1 (TNFR1), recruit caspase 8, whereas lymphotoxin-beta receptor (LTβR) forms an E3 ligase liberating the NF-kB-inducing serine kinase (NIK) from ubiquitination and degradation.[11] TNF superfamily member 14 (TNFSF14) is a cytokine in the TNF superfamily that is involved in innate and adaptive immune responses as well as in regulation of cell survival and proliferation known as LIGHT (lymphotoxin-like, exhibits inducible expression, and competes with herpes simplex virus (HSV) glycoprotein D (gD) for the herpes virus entry mediator (HVEM), a receptor expressed by T lymphocytes). LIGHT binds to three distinct members of the TNF receptor family, HVEM, LTβR, and the soluble decoy receptor 3 (Dcr3).[12] Studies in animal models indicate that LIGHT may be crucial for the development of various autoimmune disorders (e.g., inflammatory bowel disease and rheumatoid arthritis) through effects on T-cells and T-cell homing into inflamed tissues.[13] This cytokine has also been suggested to promote atherogenesis at least partly by inducing MMP activity in macrophages and inflammation in endothelial cells.[14] It was also shown that LIGHT was involved in regulation of lipid homeostasis.[15] In addition, potential involvement of LIGHT and LTβR in liver inflammation has been suggested by several observations.[16-18] Based on these properties, we hypothesized that LIGHT could also be involved in the progression of hepatic fibrosis. Herein we investigate this hypothesis using experimental approaches, including studies on the effect of sulfasalazine and mesalazine on the expression of LIGHT in hepatocytes.

2. MATERIAL AND METHODS

2.1 Animal model

Adult male Sprague-Dawley (SD) rats weighing 250–300 g were used in the current study. The animals were obtained from the breeding colony maintained at the animal house of the Nile Company for pharmaceuticals, Cairo, Egypt. Rats were housed in the animal house of Faculty of Pharmacy, Al-Azhar University at 23±1 °C, at 55 % relative humidity, with 12:12-h light: dark cycle, in wire-bottomed cubic cages, its side lengths were 40 cm, three animals per cage and maintained on a standard rodent chow composed of 54% carbohydrate, 6 % fats and 37.2 % proteins supplied by El-Nasr company, given *ad libitum* access to food and water. Animals were randomly divided into six groups/6 animals each: control group: 6 rats did not take any medications or solvents; dimethyl sulfoxide (DMSO) Group: were injected with DMSO, the vehicle of sulfasalazine (0.2 ml /200 g.bw/day, I.P) and CCl_4 (4 ml/kg of 1:1 corn oil twice weekly for 6 consecutive weeks S.C[19]; sulfasalazine group : 6 rats were injected with sulfasalazine (75 mg/kg/day, IP)[20] and CCl_4 as previously indicated dose; mesalazine group :rats were given mesalazine (100 mg/kg/day, orally by oral gavage)[21] and CCl_4 as previously indicated dose. Lastly 6 rats received the previously indicated dose of CCl_4 for 11 weeks and other 6 rats received the previously indicated dose of CCl_4 for 6 weeks. DMSO and CCl_4 were purchased from Sigma-Aldrich (MO, USA), whereas sulfasalazine and mesalazine were gifted from El-Kahera (Cairo, Egypt) and Pharopharm (Alexandria, Egypt) pharmaceutical companies respectively. On the day after the last dose, rats were anesthetized with diethyl ether and blood samples were collected for measurement of blood chemistry. The animals then were euthanized, and tissue samples from the livers were harvested and processed by standard histology and immunofluorescence techniques. All animal procedures were performed in accordance with the "international guide for the care and use of laboratory animals and general principles of laboratory animal care".[22]

2.2 Biochemical analysis

Blood samples were collected from retro-orbital plexus of each animal using heparinized capillary tubes and serum was separated by centrifugation for 20 min at 4000 rpm and used immediately for liver function parameters measurement. Serum enzymatic levels of transaminases (alanine transaminase (ALT) and aspartate transaminase (AST)) and alkaline phosphatase (ALP) were estimated by kinetic spectrophotometric method (JENWAY 6105 UV / VIS) using a kits purchased from Spectrum (Cairo, Egypt), Biosystems (Barcelona, Spain) and ELITech clinical systems (Paris, France) respectively according to the method of the international federation of clinical chemistry (IFCC).[23, 24] Isolated livers were homogenized in ice-cold 0.15 M KCl (w/v), pH 7 using ultrasonic homogenizer. The homogenate supernatant was then made into aliquots by centrifuging for 10 min in a refrigerated centrifuge at 4°C at 3000 rpm and used for the determination of liver contents of malondialdehyde (MDA)[25] and superoxide dismutase (SOD)[26] using standard spectrophotometric methods (JENWAY 6105 UV / VIS).

2.3 Immunofluorescence analysis

Liver obtained from animals were fixed in Davidson's fixative solution for 24 hours followed by 70% alcohol. Samples then dehydrated through gradient concentrations of alcohol (alcohol 70%, alcohol 95%, absolute alcohol), then dipped in xylene and immersed in paraffin at 56 °C subsequently, tissues were embedded in paraffin blocks

and cut using microtome into slices of 4 µm thickness and fixed on super frosted slides for immunofluorescence determinations. Rabbit polyclonal LIGHT antibodies were purchased from Santa Cruz Biotechnology (CA, USA). Cyanine dye 3 (Cy3)- conjugated goat anti-rabbit antibody was purchased from Jackson Immunoresearch (PA, USA). 4,6-Diamidino-2- phenylindole (DAPI) was purchased from Sigma-Aldrich (MO,USA). Slides containing liver tissues sections were deparafinized by heating at 56 °C for 20 min, then cleaned using xylene and rehydrated using gradient concentrations of alcohol followed by immersion in distilled water. After washing with phosphate- buffered saline (PBS) pH 7.4 containing 0.5 % Tween 20, the sections were boiled in Dako solution for antigen retrieval in microwave at 500 V. Fixation was done using cold methanol for 10 minutes. After washing, sections were blocked with PBS containing 10 % horse serum and 1 % bovine serum albumin to block the non-specific binding of antibodies. The primary antibody was diluted in blocking solution in the suitable dilution and left overnight in 4 °C. Secondary antibody diluted in the blocking solution was incubated for 30 min and the nuclei were counterstained using DAPI. Finally, all slides were mounted with the fluoromount solution, covered by covering slips, and allowed to stand for detection by immunofluorescence microscope (Leica DM 5500B).[27] Quantification of the fluorescence intensity was done using ImageJ software version 1.47. Fluorometric intensity of at least five microscopic fields for each section (minimally two rats in each group) was measured.[28]

2.4 Histopathological analysis

Autopsy liver samples were fixed in 10 % balanced formalin saline for 24 h. After washing with normal saline solution, liver tissue samples were dehydrated using serial dilutions of methyl, ethyl, and absolute ethyl alcohols and cleared in xylene then embedded in paraffin at 56 °C in a hot air oven for 24 h. Liver sections were embedded in paraffin blocks, sectioned at a thickness of 4 µm, stained with hematoxylin and eosin (H&E), and examined with light electric microscope.

2.5 Statistical analysis

Data were presented as the mean ± standard error of mean (M±SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer as a post hoc test, according to the number of groups. The 0.05 level of probability was used as the criterion of significance using GraphPad Prism software version 5 (GraphPad Software Inc, San Diego, CA, USA).

3. RESULTS

3.1 serum activity of ALT, AST and ALP: The serum ALT, AST and ALP activity of the control expressed as (M±SEM) were (48.2±3.53), (57.0±2.76) and (309±29.4) respectively. Treatment of animals with CCl_4 for 11 weeks or 6 weeks either alone or concomitantly with DMSO, sulfasalazine or mesalazine significantly elevated the serum activity of ALT, AST and ALP. Administration of sulfasalazine concomitantly with CCl_4 significantly decreased ALT from CCl_4 alone or with DMSO groups (198±17.7 versus 344±24.9 and 302±18.8 respectively) but failed to retain it as the control, nevertheless AST and ALP did not significantly changed. Administration of mesalazine concomitantly with CCl_4 did not produce any significant change from CCl_4 group as shown in table (1).

Table (1): Effect of CCl_4, 6 weeks and 11 weeks treatment, DMSO + 6 weeks CCl_4 treatment, sulfasalazine +6 weeks CCl_4 treatment and mesalazine +6 weeks CCl_4 treatment on liver function parameters (ALT, AST and ALP) and oxidative stress tissue parameters (MDA, and SOD).

	Serum ALT (IU/L)	Serum AST (IU/L)	Serum ALP (IU/L)	MDA (nmol/g tissue)	SOD (U/mg tissue)
Control	48.2±3.53	57±2.76	309±29.4	4.36±0.31	524±41.6
6 WKS CCL_4	344±24.9 [a]	429±24.7 [a]	567±34.8 [a]	19.7±1.52 [a]	264±14.1 [a]
6 WKS CCL_4 +DMSO	302±18.8 [a]	401±16.1 [a]	579±30 [a]	19.2±1.72 [a]	357±31.3 [a]
11 WKS CCL_4	299±9.31 [a]	363±14.1 [a]	792±33.5 [a]	29.5±1.57 [a, b]	232±14.3 [a]
6 WKS CCL_4 + Sulfasalazine	198±17.7[abc]	315±24.6 [a]	515±28.4 [a]	23.4±1.17 [a]	283±27.8 [a]
6 WKS CCL_4+ Mesalazine	323±19.1 [a]	485±45.9 [a]	683±60.2 [a]	7.53±0.71[b]	226±16.8 [a]

Data are expressed as Means ± SEM of six rats per group

[a] Signifcant difference from control group. (P = .05).

[b] Significant difference from 6 weeks CCl_4 (positive control) group. (P = .05).

[c] Significant difference from 6 weeks CCl_4 + DMSO group. (P = .05)

3.2 Hepatic tissue MDA and SOD activity: The hepatic content of MDA of control group was 4.36±0.31 nmol/g tissue. This content was significantly increased in CCl_4 non treated groups to be 19.7±1.52 for 6 weeks groups if given CCl_4 only and 19.2±1.72 if combined with DMSO. Treatment with CCl_4 for 11 weeks increased MDA content to 29.5±1.57. On the other hand combination of sulfasalazine and CCl_4 increased the content to 23.4±1.17 compared to DMSO group while mesalazine showed the minimal increase in MDA tissue content (7.53±0.71) compared which control group and remarkably was significantly different when compared with 6 weeks CCl_4 treated group. SOD activity of control group hepatic tissue was 524±41.6 U/mg tissue. This content was significantly decreased in other groups to be 264±14.1 for 6 weeks group, 357±31.3 for DMSO,

264±14.1 for 11 weeks group, 283±27.8 for sulfasalazine group and 226±16.8 for mesalazine group as shown in table (1).

3.3 LIGHT protein localization and expression in hepatic tissue: Figure (1) shows that the expression of LIGHT protein in liver tissues is located in the fibrotic area intracellularly around the nucleus of the hepatocyte. Expression of LIGHT was minimal and undetected in control group (A), highly obvious in 6 weeks CCl_4 treated group and CCl_4 + DMSO group (B and C) and associated with massive necrotic foci of hepatocytes in the 11 weeks CCl_4 group (F). Hepatocytes LIGHT expression were diminished in both sulfasalazine and mesalazine groups nevertheless the hepatocytes were free from necrotic cell death (D and E).

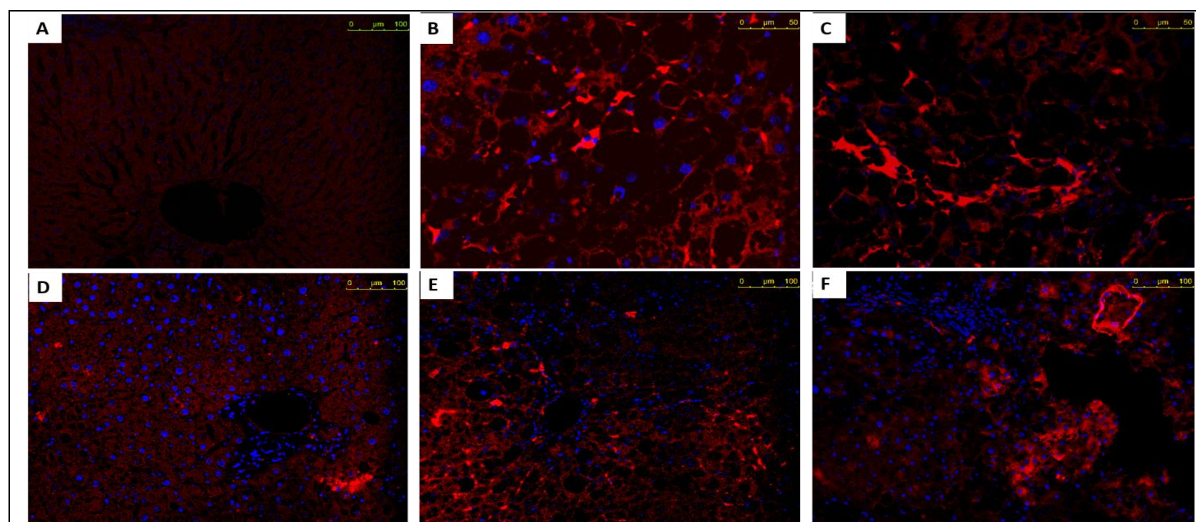

Figure (1): Expression and localization of LIGHT in hepatic tissue of different studied groups., (A) Control group, (B) 6 weeks CCl_4 group (positive control), (C) 6 weeks CCl_4 + DMSO group, (D) 6 weeks CCl_4 +sulfasalazine group, (E) 6 weeks CCl_4 + mesalazine group and (F) 11 weeks CCl_4 group., Blue (DAPI) = Nucleus, Red (Cy3) = LIGHT. 100X magnification power

Quantitative image analysis for immunofluorescence staining expressed as fluorescence intensity showed that there is minimal expression of LIGHT in negative control animals. Administration of CCl_4 for 6 weeks and 11 weeks significantly increases the expression of LIGHT by 459% and 289% respectively versus 100% for control as shown in figure (2A). Injection of CCl_4 in combination with DMSO negligibly decrease the expression of LIGHT compared to the 6 weeks CCl_4 group (97.3 % versus 100%). Figure shows that LIGHT expression in sulfasalazine and mesalazine treated groups was decreased significantly from positive control. Sulfasalazine showed maximal decrease by about 71.2% and mesalazine by about 56.7% with respect to that of CCl_4 treated group (28.8% and 43.3% respectively versus 100% as shown in figure (2B)).

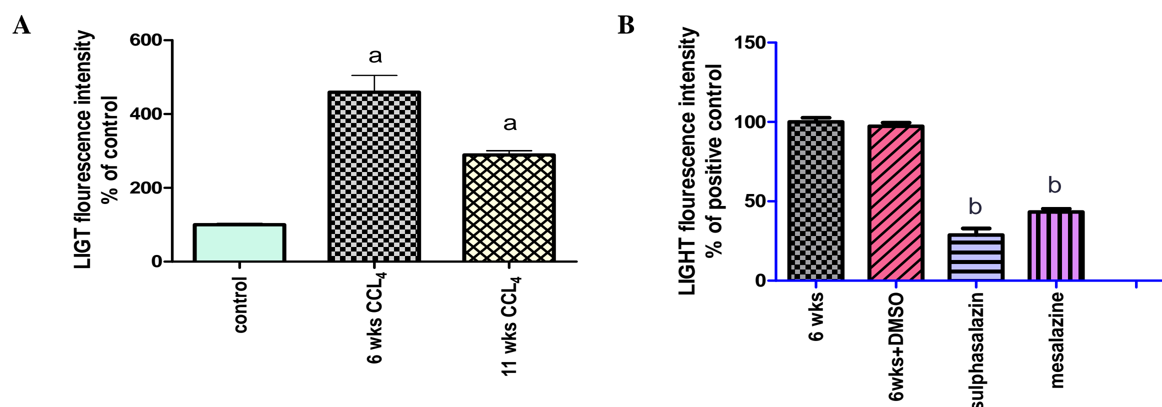

Figure (2): LIGHT fluorescence intensity % change. (A) Quantitative image analysis for immunofluorescence staining expressed as fluorescence intensity in control, 6 weeks CCl_4 and 11 weeks CCl_4. (B) Quantitative image analysis for immunofluorescence staining expressed as fluorescence intensity in positive control (6 weeks CCl_4), 6 weeks CCl_4 + DMSO, 6 weeks CCl_4 + sulfasalazine and 6 weeks CCl_4 + mesalazine.

Data are presented as Mean ± SEM.
a significant difference from control group, (P = .05).
b significant difference from positive control group (6 weeks CCl_4 group), (P = .05).

3.4 Effect of CCl_4, CCl_4 + DMSO, CCl_4 + sulfasalazine and CCl_4 + mesalazine treatment on liver histopathological features

The effect CCl_4, CCl_4 + DMSO, CCl_4 + sulfasalazine and CCl_4 + mesalazine treatment on liver histopathological features are presented in table (2) and illustrated in figure (3). The histopathological examination of liver tissues of the control group showed normal histological structure. Administration of CCl_4 for 6 weeks resulted in irregular surface by fibrous septa extending to underlying portal tract (PT), marked steatosis (micro- and macro-vesicular steatosis) in peri-venular areas, dilated central veins with disrupted wall and with underlying fibrosis. Nevertheless Administration of CCl_4 for 11 weeks resulted in markedly expanded PT, complete nodular formation, marked micro- and macro-vesicular steatosis and necrotic hepatocyte. The histological features of tissues obtained from CCl_4 + DMSO treated group matching the results obtained from CCl_4 treated group described before. Interestingly, sulfasalazine and mesalazine treated groups showed average PT, average hepatocytes, irregular surface, underlying short fibrous septa, average central veins and moderate steatosis.

Table (2): Effect CCl_4, CCl_4 + DMSO, CCl_4 + sulfasalazine and CCl_4 + mesalazine treatment on liver histopathological features.

	Control	6 weeks CCl_4	6 weeks CCl_4 +DMSO	11 weeks CCl_4	6 weeks CCl_4 +Sulfasalazine	6 weeks CCl_4 +Mesalazine
Central veins	0	++	0	++	0	0
Steatosis	0	+++	++	+++	++	+
Hepatocytes	0	+	0	+	0	0
Spotty necrosis	0	0	+	+	0	0
Interface activity	0	+	0	++	0	0
Portal tract	0	+	++	++	0	++
Fibrosis	0	++	++	++++	++	+++

Central vein (CV): 0: within normal +: dilated ++: markedly dilated
Steatosis: 0: no steatosis +: mild ++: moderate +++: marked
Hepatocytes: 0: within normal +: single cell necrosis ++: confluent or diffuse necrosis
Spotty necrosis: 0: no steatosis +: mild ++: moderate to marked
Interface activity: 0: no +: mild ++: moderate to marked
Portal tract (PT): 0: within normal +: expanded ++: expanded with inflammatory infiltrate
Fibrosis: 0: no fibrosis
+: fibrosis confined to enlarged portal zones
++: fibrosis of peri-portal or portal-portal septa with intact architecture
+++: architectural distortion (septal or bridging fibrosis) without obvious cirrhosis
++++: probable or definite cirrhosis.

4. DISCUSSION

A line of animal models of liver toxicity including fibrosis and cirrhosis has been generated using chemicals (e.g., CCl_4 or dimethylnitrosamine). CCl_4-induced liver toxicity is a well-established model in terms of its detrimental effects and mechanistic basis in animals.[29] There is now considerable interest in the discovery of compounds that selectively promote the apoptosis of activated HSC because proof-of-principle studies have shown that in vivo stimulation of HSC apoptosis will promote recovery from liver fibrosis.[30]

In our model, administration of chronic S.C. doses for 6 or 11 weeks of CCl_4 were done. CCl_4 significantly elevated serum transaminases and enzymes level which indicates hepatocellular damage. These results are in agreement with previous reports that CCl_4 significantly increased serum transaminases.[31, 32] Interestingly, concomitant administration of sulfasalazine with CCl_4 significantly decreased serum activities of ALT compared to 6 weeks group a result which supposes the hepatoprotective effect of sulfasalazine. This resembles what has been reported by Oakley *et al.* who reported that ALT decreased significantly when sulfasalazine administered with CCl_4 although our results concerning AST not in accordance with his result as he reported significant difference between the previously mentioned group, the alteration which was not significant in our work.[6]

ALP activity shows non-significant increase from control group, a result that oppose what was reported by Kalu *et al.*[32] Interestingly Posen and Doherty have reported that ALP elevation is more predominant in obstructive conditions than in hepatocellular disorders and also they reported that ALP elevates 3-10 times than control in obstruction conditions. On the other hand; In contrast, hepatocellular disorders, such as hepatitis and cirrhosis,

show only slight increases, usually less than 3 times.[33] In our study ALP elevation in chronic models including 6 weeks, 11 weeks and DMSO group shows significant increase than control which is less than 3 times as control; a result that matches with that previously reported by Posen and Doherty.[33] Contrary to our prospects, Deltenre *et al.*, reported that mesalazine can cause most of the sulphasalazine induced adverse effects, and hepatic side effects as development of chronic hepatitis and liver fibrosis.[34] In the mediocre between our finding and Deltenre *et al.* conflicting conclusion Szabò *et al.* reported that mesalazine has no significant effects, profibrotic or antifibrotic.[35]

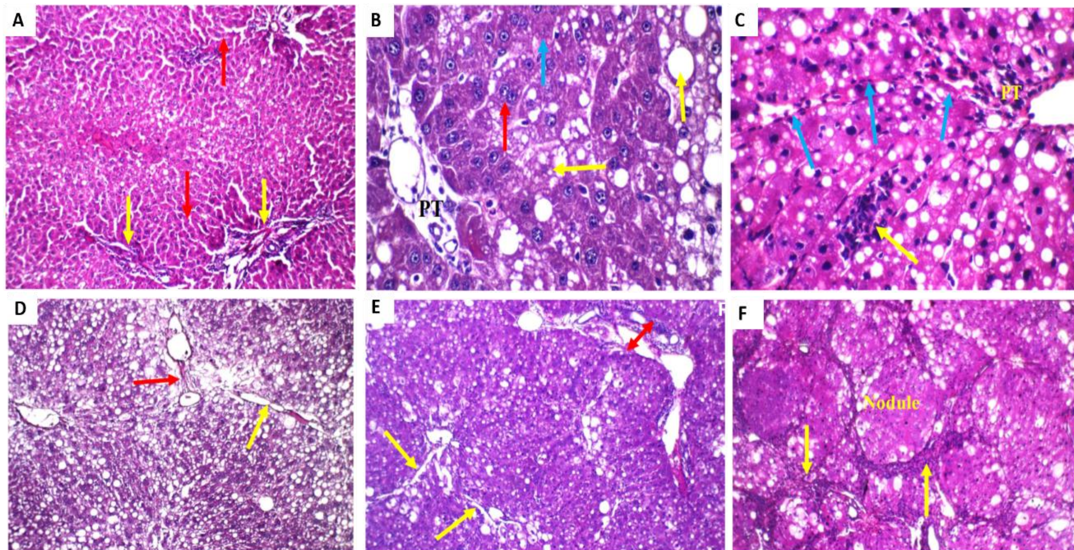

Figure (3) Histopathology of liver sections of control group and after treatment with CCl_4, CCl_4 + DMSO, CCl_4 + sulfasalazine and CCl_4 + mesalazine groups.

Figures show different degrees of liver injury such as steatosis, fibrosis and cirrhosis. (A): liver tissue from all control rats revealed normal cellular architecture showing average PT (yellow arrows), and average hepatocytes, arranged in cords (red arrows) (H&E x 235) (B): liver tissue after treatment with CCl_4 showing showing irregular surface with underlying fibrosis, marked micro- and macro-vesicular steatosis (yellow arrows), bi-nucleated cells (red arrow), single cell necrosis (blue arrow), mildly expanded PT with average hepatocytes in peri-portal area (H&E x 360). (C): in liver tissue from CCl_4 + DMSO group showing fibrous septa extending from PT (blue arrows), spotty necrosis (yellow arrow), average hepatocytes and moderate steatosis (H&E x 360) (D): liver tissue of CCl_4 + sulfasalazine for 6 weeks group showing average PT (yellow arrow), short fibrous septa (red arrow), average hepatocytes and moderate steatosis (H&E x 235) (E): CCl_4 + meslazine for 6 weeks group liver tissue showing expanded PT (red arrow), fibrous septa extending from PT (yellow arrows), average hepatocytes and mild steatosis (H&E x 235) (F): CCl_4 for 11 weeks liver tissue showing markedly expanded PT (yellow arrows), complete nodular formation, marked micro- and macro-vesicular steatosis (H&E x 235).

The protective effect of sulfasalazine and mesalazine may be due to it is a selective and potent inhibitor of NF-κB activation and preceding work suggests that this property is due to the ability of sulfasalazine to inhibit the autophosphorylation of inhibitor of kappa B kinase (IKK)α and IKKβ and the subsequent activation of the IKK complex.[36] Sulfasalazine treatment of activated HSC caused a powerful dose-dependent diminution of the persistently increased basal NF-κB transcriptional activity that is characteristic of these cells.[30,37] It has previously been speculated that NF-κB may function as a survival factor for HSC by preventing apoptosis.[30,37-39]

The in vivo studies with sulfasalazine clearly show that the drug promotes recovery from fibrosis not only by removal of collagen-producing HSC, but also by reducing hepatic TIMP1 expression and promoting the collagenolytic activity of the liver. It was hypothesized that the administration of sulfasalazine or mesalazine under conditions of ongoing injury would be protective against the development or progression of fibrotic disease.[6] Although sulfasalazine and mesalazine has strong anti-inflammatory properties, which would be expected to affect the injury process in the CCl_4 disease model and complicate the interpretation of its potential antifibrogenic characteristics. However, it is now recognized that models of fibrosis reversion are acceptable alternatives to progressive liver injury models for predicting a true antifibrotic effect.[40] The characterization of LIGHT expression enhances the postulation about their protective role.

Understanding the molecules that directly promote fibrosis or might drive activity of other fibrotic factors may prove useful for future therapeutic approaches. Because of the importance of inflammation in fibrotic

cascade in different organs. The inflammatory mediators and cytokines are extensively studied in fibrosis of many organs. LIGHT involved in innate and adaptive immune responses as well as in regulation of cell survival and proliferation.[12] Doherty *et al.* have shown that blockade or absence of LIGHT reduced subepithelial fibrosis, smooth muscle hypertrophy and hyperplasia in mouse model of chronic asthma. They also showed that the production of the profibrotic cytokines transforming growth factor-β (TGF-β) by macrophages is important in LIGHT induced remodeling of asthma from acute to chronic.[41] Yamamoto has shown that direct injection of recombinant LIGHT into naïve mice promoted dermal and epidermal thickening. Additionally, fibrotic features in the skin were abrogated in LIGHT-deficient mice treated with bleomycin in a mouse model that produces symptoms reminiscent of those exhibited in scleroderma.[42]

Based on what is formerly mentioned; we could conclude that there is a relationship between LIGHT and collagen related diseases mainly fibrosis. We therefore asked whether there is a similar relationship between LIGHT and liver fibrosis in rats and also if there is effect of the fore mentioned drugs on it.
In the present study, the immunofluorescence assay of LIGHT protein in the liver tissues showed minimal expression in control animals. It was found that 6 weeks S.C. administration of CCl_4 induced the expression of LIGHT by about 459 % in case of CCl_4 only and by about 446% in case of CCl_4 with DMSO. The expression declines again on continuous administration of CCl_4. After 11 weeks treatment; the expression declines considering 6 weeks treatment and was about 289 % relative to control group. LIGHT expression in sulfasalazine and mesalazine treated groups was decreased significantly from positive control. Sulfasalazine showed maximal decrease by about 71.2% and mesalazine by about 56.7% with respect to that of CCl_4 treated group.

From the fore mentioned data we can conclude that the expression of LIGHT reaches its peaks after about 6 weeks S.C. administration of CCl_4. In other words maximal expression of LIGHT occurs at the stage of collagen synthesis so it can be said that there is a significant relationship between LIGHT and collagen accumulation in liver. This conclusion quietly agrees with Doherty *et al.*[41] and Herro *et al.*[43] Doherty *et al.* has reported that LIGHT mediated collagen deposition in the lungs in response to allergen. It was also reported by Herro *et al.* that LIGHT promotes collagen deposition in skin and LIGHT as a soluble molecule can very rapidly induced a fibrotic phenotype in the skin even in the absence of any other stimulus.

Sulfasalazine and its metabolite mesalazine are reasonably well tolerated by humans. Given the remarkable protection achieved with the administration of the drugs on the rat liver, the potential therapeutic benefit of use of the drugs in combination with therapies that treat the underlying cause of liver disease should be explored. Moreover, our demonstration that LIGHT expression increased in hepatocytes and by joining the postulations about its role in fibrosis indicates that sulfasalazine and mesalazine to lesser extent may be a good antifibrogenic agents.

4. CONCLUSION

LIGHT expression is increased in experimentally CCl_4 induced hepatic fibrosis. However a dominance of sulfasalazine were detected, both sulfasalazine and mesalazine protect against experimentally CCl_4 induced hepatic fibrosis via modulation of expression of hepatocytes LIGHT protein evidenced by alteration of serum hepatic enzymes, tissue biochemical changes, Immunofluorescence analysis and histopathological analysis.

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COMPETING INTERESTS

We declare that we have no conflict of interest.

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