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# Evaluation of the Possible Antifibrotic effect of Aliskiren, Valsartan, Chloroquine, Zafirlukast and Colchicine on Liver Fibrosis Induced by Carbon Tetrachloride in Rats

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## ABSTRACT

The present work aims to study the possible anti-fibrotic effect of aliskiren, valsartan, chloroquine, zafirlukast in comparison to colchicine on prevention of liver fibrosis in experimental model of liver fibrosis induced by carbon tetrachloride (CCL4) in rats. Hepatic fibrosis was induced by CCL4 (33mg/kg, oral) for 6 weeks. The rats were treated with either aliskiren (10 mg/kg), valsartan (50 mg/kg), chloroquine (5 mg/kg) zafirlukast (5 mg/kg) or colchicine (50 µg/kg) simultaneously with CCL4 treatment. Department of Clinical Pharmacology, Faculty of Medicine, Mansoura University, Egypt, Between June 2013 and August 2014. We included 56 Spague Dawely rats. All are exposed to induction of hepatic fibrosis by CCL4 for 6 weeks. After the end of treatment, blood samples were obtained to assess liver function tests, plasma renin activity. Then, liver tissues were obtained to assess hepatic fibrosis markers (hydroxyproline and transforming growth factor-β1) and oxidative stress markers (reduced glutathione and malondialdehyde). Moreover, liver specimens from each group were processed for histo-pathological studies. Treatment with either colchicine, aliskiren, valsartan, chloroquine or zafirlukast simultaneously with CCL4 for 6 weeks resulted in marked decrease in the amount of collagen fibers around the central veins. There is also improvement liver function tests, fibrosis markers and oxidative stress markers. Sample size is 8 rats in each group. Data were analyzed using one way analysis of variance followed by post hoc test of Tukey's Honestly Significant Difference. *P-value* of less than 0.05 was considered to be significant. This study demonstrated the promising anti-fibrotic effect of aliskiren, valsartan, chloroquine, zafirlukast and colchicine. These drugs significantly prevent progression of liver fibrosis induced by CCL4 in rats; probably through, inhibition of renin angiotensin system, anti-inflammatory, anti-oxidant effects, and decrease TGF-β1 level so, inhibit hepatic stellate cells activation.

**Keywords:** liver fibrosis, carbon tetrachloride, Aliskiren, TGF-β1.

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## INTRODUCTION

Hepatic fibrosis results from excessive deposition of collagen fibers in liver tissue. After chronic liver injury; due to several causes, including persistent viral (as chronic hepatitis B and C virus) and helminthic (as bilhariziasis) infections, alcohol, autoimmune hepatitis, and metal overload[1]. The apoptotic bodies of injured hepatocytes, toxic free radicals metabolites and recruited inflammatory cells activate Kupffer cells to release cytokines as transforming growth factor β1 (TGF-ß1) and reactive free radicals which activate hepatic stellate cells (HSCs). The activated HSCs produce excess amounts of collagen fibers that cause fibrosis[2]. The activation of HSCs is the key event in hepatic fibrogenesis[3]. TGF-ß1 is the main pro-fibrogenic cytokine involved in liver fibrosis[4]. TGF-ß1 in turn, stimulates activated HSCs to produce type 1 collagen[5]. Angiotensin II induces HSCs contraction, proliferation and acquisition of myofibroblast like phenotype by activating angiotensin II type 1 receptors (AT1) receptors on HSCs[6]. Angiotensin II has a powerful capacity to initiate the signaling cascades that can produce liver fibrosis[7]. AT1 receptors have shown to be expressed on activated HSCs, and their ligand angiotensin II enhances fibrogenic action through stimulation of NADPH oxidase enzyme which increase oxidative stress, with consequent activation of HSCs[2]. Angiotensin II also increases TGF-β1 and the genetic expression of collagen 1 via AT1 receptors on HSCs[8]. In chronic liver injury, HSCs express 5-lipoxygenase enzyme. HSCs are also able to release leukotrienes (LTs) in a TGF-β1-regulated manner, potentially affecting pathogenesis of liver fibrosis. Moreover, the 5-lipoxygenase inhibitor (zileuton) and 5-lipoxygenase gene deletion were able to inhibit the TGF-β1-stimulated proliferation of HSCs, suggesting a role for LTs in HSC activation[9]. Chronic liver disease is a health problem worldwide, with significant morbidity and mortality. Treatment of liver fibrosis is not available until now and there is no drug approved to treat this condition[10]. This study aims to test the potential anti-fibrotic effect; of a direct renin inhibitor, aliskiren; AT1 Receptor blocker; valsartan, LT1 receptor antagonist; zafirlukast, anti-inflammatory drugs; chloroquine & colchicine on the progression of liver fibrosis induced by CCL4 in rats.

## MATERIAL AND METHOD

### Drugs and chemicals

Carbon tetrachloride purchased from Sigma-Aldrich Chemical Co. (St Louis, Missouri, USA; Catalog number, 48604). Aliskiren (Tekturna, 150 mg, tablet, Novartis, USA). Valsartan (Diovan, 160 mg, tablet, Novartis, USA). Chloroquine (Alexoquine, 250 mg, tablet, Alexandria Co., Egypt). Zafirlukast (Accolate, 20 mg, tablet, AstraZeneca, UK). Colchicine (Colchicine, 500 micrograms, tablet, El-Nasr Co., Cairo, Egypt).

**Animals**

This study was carried out on 56 Sprague–Dawley rats of either sex, weighing 150-200 g. Animals were allowed free access to food and water. They were exposed to the same environmental conditions. All experimental procedures were performed in accordance with the guidelines of the National Institutes of Health and the Research Ethics Committee Criteria for Care of Laboratory Animals at Mansoura University.

**Method of hepatic fibrosis induction**

CCL4 dissolved in corn oil. Then, rats were given carbon tetrachloride (CCL4) once daily at a dose of 33 mg/kg/day in 0.1 ml corn oil (5 times weekly) orally for successive 6 weeks[11].

**The design of the experiment**

Rats were randomly equally divided into 7 groups (8 rats each):

Control group: rats received only 0.1 ml corn oil orally.

CCL4 treated group: rats received CCL4 at a dose of 33 mg/kg/day in 0.1 ml corn oil, orally, 5 times weekly, for successive 6 weeks[11].

Aliskiren treated group: rats received CCL4 and aliskiren (10 mg/kg/day), orally, for successive 6 weeks.

Valsartan treated group: rats received CCL4 and valsartan (50 mg/kg/day), orally, for successive 6 weeks.

Chloroquine treated group: rats received CCL4 and chloroquine (5 mg/kg/day), orally, for successive 6 weeks.

Zafirlukast treated group: rats received CCL4 and zafirlukast (5 mg/kg/day), orally, for successive 6 weeks.

Colchicine treated group: rats received CCL4 and colchicine (50 µg/kg/day), orally, for successive 6 weeks.

After the end of the experimental period, rats were sacrificed by overdose of anesthesia.

**BIOCHEMICAL MEASUREMENTS**

**Measurement of liver function test**

Blood withdrawn from the heart of rat and then divided into two parts: the first part of blood sample was collected in a dry test tube and then centrifugation of the tubes at 3000 xg, for 15 minutes to separate the serum and stored at -20°C. Serum aspartate amino-transferase (AST) and alanine amino-transferase (ALT) enzymes levels were analyzed using colorimetric kits (Biomerieux, France; Catalog number, 61 691, 61 692, respectively). AST or ALT enzymes in serum sample transfer amino group from aspartate or alanine to α-ketoglutarate giving glutamate and either oxaloacetate or pyruvate, respectively which is measured in their derivatives coloured forms by spectrophotometer at wave length 505 nm and the results are

expressed in international units per liter. Serum total bilirubin was measured using colorimetric kits (Diamond diagnostics, Cairo, Egypt; Catalog number, 265 ml). Bilirubin in serum sample reacts with diazotized sufanilic acid giving coloured azo-bilirubin and absorbance was measured by spectrophotometer at wave length 578 nm and the results expressed in mg/dl. Serum albumin level was measured using colorimetric kits (Biomed diagnostics, Cairo, Egypt; Catalog number, ALB 100250). Albumin in serum sample binds to bromocresol green and absorbance was measured by spectrophotometer at wave length 623 nm and values are expressed as g/dl.

**Plasma renin activity analysis**

The other part of blood sample was collected on EDTA centrifuged at 3000 xg, for 15 minutes, the plasma was separated and stored at -20°C. Plasma renin activity was measured using ELISA kits (Diagnostic Biochem Canada, Canada; Catalog number, CAN-RA-4600) and the results are expressed as a mass of angiotensin-1 generated per volume of plasma in unit time i.e., ng/ml.h.

**Analysis of hepatic hydroxyproline content**

The rat abdomen was incised and the liver was irrigated several times by saline via a cannula introduced through thoracic aorta to wash blood out of the liver. Then, two pieces of liver tissue were excised. One piece of liver tissue was placed in a dry tube and stored at -20°C for determination of hepatic hydroxyproline level. Hydroxyproline content in wet liver tissue was measured by the use of chloramine T method via colorimetric technique by spectrophotometer at wave length 560 nm and the results are expressed as ng/g wet liver tissue [2].

**Analysis of hepatic TGF- β1**

The other piece of excised liver was placed in a Falcon tube containing 5 mL Phosphate buffered solution (PBS) and stored at -20°C. Liver tissue homogenate preparation was done using cold buffer (i.e., 5-10 mL, 500 milli-Mole of potassium phosphate at a PH of 7.5 & 1 milli-Mole EDTA/1 gm of liver tissue) by the use of pestle and morter; after that centrifugation of the homogenized liver tissue for 15 minutes at 4°C at 4000 rpm then, supernatant fluid collected into 2 Eppendorf tubes and stored at -80°C for further determination of TGF-β1 and oxidative stress markers in liver tissue homogenate. TGF-β1 level in liver tissue homogenate was measured using ELISA kits (eBioscience, Vienna, Austria; catalog number, BMS62313) and the results are expressed as pg/mg of liver tissue.

**Analysis of hepatic oxidative stress markers**

*Measurement of reduced glutathione* content in liver tissue homogenate was measured using colorimetric kits (Biodiagnostic: diagnostic and research reagents, Giza, Egypt; catalog

number, GR 25 11). Reduced glutathione causes reduction of nitrobenzoic acid; then the absorbance was measured by spectrophotometer at wave length 405 nm and the results are expressed as mg/g liver tissue.

*Measurment of malondialdehyde* (MDA) *lipid peroxide* in liver tissue homogenate was measured using colorimetric kits (Biodiagnostic: diagnostic and research reagents, Giza, Egypt; catalog number, MD 25 29). MDA binds to thiobarbituric acid; then the absorbance was measured by spectrophotometer at wave length 534, and the results are expressed as nmol/g liver tissue.

**HISTO-PATHOLOGICAL EXAMINATION OF HEPATIC TISSUE**

**Histo-pathological examination of liver sections**

Then, the rest of liver tissue was perfused in situ by 10% neutral buffered formalin through a cannula and then removed and the portion of the liver tissue was fixed in 10% phosphate buffered formalin, (dehydrated, cleared in xylene) then the liver specimens processed into paraffin blocks and sections of 6 μm thickness made. Histo-pathological examination of liver sections stained with Sirius red stain for collagenous fibrous content evaluation.

**Fibrosis score**

Fibrosis score was divided into eight grades according to the extent of fibrous tissue: Grade 1, Peri-central fibrosis in some central veins. Grade 2, Peri-central fibrosis in most central veins. Grade 3, Short septa. Grade 4, Central-central fibrosis. Grade 5, Nodules corresponding to portal lobule. Grad 6, Focal sub-segmentation of portal lobules. Grade 7, Diffuse sub-segmentation of portal lobules. Grade 8, Small separate cirrhotic islets separated by wide fibrous septa.

**Image analysis of the area occupied by collagen fibers**

Image analysis of the area occupied by collagen fibers: Quantitative assessment of liver fibrosis was performed on sections stained with Sirius red stain. The data were obtained using Imagej software computer program. In each chosen picture the Sirius red-stained area was enclosed inside the standard measuring frame & then the red coloured area was masked by a blue binary colour to be measured. The percentage of the area of fibrosis over the whole observed field was assessed to represent the degree of hepatic fibrosis[12].

**Statistical analysis**

It was carried out via Statistical Package for Social Science (SPSS) version 16 (USA). Sample size is 8 rats in each group. Data were presented as mean ± standard error of mean (M±SEM). Comparisons between groups were analyzed using one way analysis of variance (ANOVA) followed by post hoc test of Tukey's Honestly Significant Difference. P-value of less than 0.05 was considered to be significant.

# RESULTS AND DISCUSSION

**Liver function tests**

CCL4 intoxicated rats showed significant deterioration of liver function tests in the form of significant increase in serum level of AST, ALT enzymes, total bilirubin and decrease in albumin level in comparison to control group. Treatment with either aliskiren 10 mg, valsartan 50 mg, chloroquine 5 mg, zafirlukast 5 mg or colchicine 50 µg/kg/day orally for 6 weeks concurrently with CCL4 caused significant decrease in mean serum level of AST, ALT enzymes & total bilirubin levels and increase in serum albumin level as compared to the CCL4-treated group. Notably, zafirlukast caused significantly less increase in the serum AST and ALT levels as compared to other groups (Table 1).

**Plasma renin activity (PRA)**

CCL4 intoxicated rats showed significant increase in plasma renin activity as compared to control group. Treatment with either chloroquine, zafirlukast or colchicine for 6 weeks caused non-significant changes in PRA versus CCL4-treated group. Aliskiren caused significant decrease in PRA level, while valsartan caused significant increase in PRA level as compared to CCL4 treated rats (Figure 1).

**Hepatic hydroxyproline content in liver tissue**

**CCL4 intoxicated rats showed** significant increase liver fibrosis marker (hepatic hydroxyproline content in liver tissue) in comparison to control group (Figure 2). Treatment with either colchicine, aliskiren, valsartan, chloroquine or zafirlukast concomitantly with CCL4 for 6 weeks caused significant decrease in hepatic hydroxyproline content as compared to CCL4 treated rats (Figure 2).

**Transforming growth factor-β1 (TGF-β1)**

CCL4 intoxicated rats showed significant increase in hepatic TGF-β1 in comparison to control group. Treatment with either aliskiren, valsartan, chloroquine, zafirlukast or colchicine; concomitantly with CCL4 for 6 weeks caused significant decrease in TGF-β1 level as compared to CCL4 treated rats, but still significantly higher than control group (Figure 3).

**Oxidative stress markers**

CCL4 intoxicated rats showed significant deterioration of oxidative stress markers in the form of significant decrease in reduced glutathione and significant increase in MDA levels in comparison to control group. Treatment with either aliskiren, valsartan, chloroquine, zafirlukast, or colchicine concomitantly with CCL4 for 6 weeks caused significant improvement in oxidative stress markers (significant increase in reduced glutathione and

significant decrease in MDA levels) as compared to CCL4-treated group, but still significantly different from control group (Figure 4 and 5).

**Liver histo-pathological studies**

In CCL4 treated rats; the histo-pathological examination of liver sections stained with Sirius red showed the picture of hepatic fibrosis, in the form of peri-central fibrosis with central-central septa incompletely encircling the portal vein as compared with normal histo-pathological picture. Rats that received either aliskiren, valsartan, chloroquine, zafirlukast or colchicine simultaneously with CCL4 for 6 weeks showed marked decrease in the amount of collagen fibers around central veins (peri-central). Marked decrease or no fibrous septa can be seen radiating into surrounding parenchyma as compared to the CCL4-treated group (Figure 6). Image analysis and fibrosis score showed that rats treated with CCL4 for 6 weeks showed significant increase in liver fibrosis score and percent of area occupied by collagen fibers in liver tissue. Treatment with either aliskiren, valsartan, chloroquine, zafirlukast or colchicine concurrently with CCL4 for 6 weeks caused significant decrease in liver fibrosis score and percent of area of collagen fibers by image analysis as compared to CCL4-treated group (Table 2). The current study showed that oral administration of CCL4 at a dose of 33 mg/kg/day in 0.1 ml corn oil, 5 times weekly, for successive 6 weeks caused significant fibrosis associated with significant deterioration of liver function tests, significant increase in hydroxyproline, TGF- β1 levels, significant deterioration of oxidative stress markers and significant increase in plasma renin activity. CCL4 induced liver fibrosis in rat is frequently used as an experimental model for the study of liver fibrosis[13, 14], because experimentally induced liver fibrosis in rats by CCL4 has been shown to resemble liver fibrosis in human[15]. The deterioration of liver functions as a result of CCL4 may be attributed to CCL4 metabolites which may induce lipid peroxidation and liver injury due to tissue injuries and cell death[16] or loss of the structural integrity of the hepatocytes which released the soluble enzyme AST when injury involves organelles such as mitochondria[17]. CCL4 intoxicated rats showed significant increase in hepatic hydroxyproline content and also TGF-β1 level. Hydroxyproline, is a characteristic component of collagen, so, hydroxyproline content in liver tissue can reflect the amount of collagen in liver tissue[18, 19]. This may be due to chronic liver inflammation which involved excess deposition to extra-cellular collagen fibers[20]. Also, TGF-β1 which is the most powerful and widely distributed pro-fibrogenic cytokine. It induces the deposition of the extra-cellular matrix as a consequence of liver injury, which produce liver fibrosis[21]. Elevations of TGF-β1 is associated with hepatic fibrosis[22]. Also, CCL4 administration causes oxidative stress in rats which play an essential role in development of liver fibrosis[23], in which liver NADPH oxidase enzyme produce excess free

radicals that produce oxidative stress and induce liver fibrosis[24, 25]. CCL4 induced liver fibrosis is mainly due to oxidative stress induced by CCL4. As CCL4 is metabolized in the liver by cytochrome P-450 2E1 to highly reactive tri-chloro-methyl free radicals. These free radicals deplete liver content of reduced glutathione and cause lipid peroxidation by binding to malondialdehyde which cause damage to the cell membranes[26, 27]. CCL4 free radicals activate Kupffer cells to secrete pro-inflammatory cytokines (e.g., TNF-α) and chemokines which attract neutrophils, monocytes, and lymphocytes to the site of injury causing damage to liver tissue[28]. Repeated rounds of injury, inflammation and repair produce fibrosis[29]. Moreover, CCL4 intoxicated rats showed significant increase in plasma renin activity (PRA). The renin angiotensin system is involved in development of liver fibrosis[30] and its complications as portal hypertension and hepato-cellular carcinoma[31, 32]. Angiotensin II produced mainly through endothelial cleavage of angiotensin I (which is synthesized from angiotensinogen by hepatocytes) by renin enzyme[30]. Renin enzyme is secreted from the juxtaglomerular cells in the kidneys. Renin cleaves angiotensinogen to form angiotensin I which is then converted by angiotensin converting enzyme to the active angiotensin II[33]. Plasma renin activity and angiotensins were elevated in advanced liver disease[34, 35, 36]. So that, inhibition of angiotensin II synthesis or blockage of angiotensin II type I receptors may attenuate hepatic fibrosis[2]. In the current study, treatment with either aliskiren or valsartan for 6 weeks simultaneously with CCL4 resulted in significant decrease in the amount of collagen fibers around the central veins and shorter fibrous septae extending through the parenchyma as compared to CCL4 alone. This improvement in the histological picture is associated with significant improvement in liver function tests, hepatic hydroxyproline content, TGF-β1 level and oxidative stress markers. Moreover, both aliskiren and valsartan produce improvement of renin angiotensin system through inhibition of angiotensin II formation and angiotensin II action, respectively. The beneficial effects of aliskiren in preventing progression of liver fibrosis in rats is mostly due to its direct renin inhibition to reduce PRA through interaction with the active site of the renin enzyme and reduction of the formation of angiotensin II; aliskiren may additionally inhibits renin angiotensin system. As angiotensin II is a powerful pro-oxidant agent in the liver [37]. Angiotensin II increases oxidative stress by stimulation of NADPH oxidase and thus, HSCs activation[6]. Also, aliskiren inhibits Kupffer cells activation, reduces TGF-β1 level and has anti-oxidant effect[38, 39]. Valsartan, angiotensin II type 1 receptor (AT1) blocker; prevents progression of liver fibrosis in rats by blocking the binding of angiotensin II to angiotensin II type 1 receptors expressed on the surfaces of activated HSCs[2]. Also, blockage of AT1 receptors by valsartan leads to increase in local angiotensin II concentration that stimulates the unblocked AT2 receptors[40]. The anti-fibrogenic effect of

AT2 receptors is due to reduction of oxidative stress in the liver[41]. Moreover, valsartan down-regulates α-SMA, TNF-α, MCP-1 expression[42], TGF-β1, TGF-β1 type II receptor mRNA and smad-3 and up-regulates Smad-7[43]. In the present research, treatment with chloroquine for 6 weeks simultaneously with CCL4 for 6 weeks resulted in significant decrease in liver fibrosis as well as significant improvement in liver function tests, fibrosis markers and oxidative stress markers. These results are in agreement with several studies. It was found that chloroquine attenuated CCL4-induced liver fibrosis[44]. As chloroquine inhibits autophagy and consequently inhibits HSCs activation[45]. Autophagy, is an energy-dependent process involved in the cell death process, degrades and recycles sub-cellular organelles[46]. The process of liver fibrosis induced by HSCs activation is associated with intra-cellular depletion of lipid droplets. When HSCs undergo autophagy, intra-cellular lipids are degraded in lysosomes[45, 47]. Suppression of autophagy process inhibits the process of activation of HSCs. Also, chloroquine restored the oxidative stress markers to near normal levels[48]. As chloroquine decreases malondialdehyde (lipid peroxide) and increases reduced glutathione levels [49]. Additionally, chloroquine downregulates α-smooth muscle actin (α-SMA) and TGF-β1 gene expression, which are profibrogenic genes *in vivo*[50, 51]. In the current study, treatment with zafirlukast concomitantly with CCL4 caused significant attenuation of histopathological picture of liver fibrosis that accompanied by significant improvement in laboratory measurements. Human studies show that leukotrienes (LTs) are involved in the pathogenesis of hepato-renal syndrome and liver cirrhosis[52]; furthermore, experimental studies indicate that LTs production is increased in CCL4-induced hepatopathy[53]. The effect of zafirlukast in attenuating liver fibrosis in rats induced by CCL4 may be through its anti-inflammatory effect as a LTs receptor blocker[54]. Also, leukotriene receptor blockers suppress the release of inflammatory and oxidative stress markers via its antioxidant activities[55]. Also, montelukast decrease hepatic expression of TGF-β1, NF-κB And MMP-9/TIMP-1 ratio [56]. Zafirlukast through inhibition of leukotriene synthesis or action may have a protective role in inflammatory bowel disease[57]. Notably, zafirlukast caused significantly less improvement in the serum AST and ALT levels as compared to other drugs. There were 2 reports of hepatitis related to zafirlukast, which may be a rare drug-induced idiosyncrasy or drug interaction between zafirlukast and concomitant drugs as amoxicillin-clavulinic acid. So, zafirlukast related hepatitis still needs further study to know the exact factor that increase the risk of zafirlukast related hepatitis[58]. The present study showed that, treatment with colchicine simultaneously with CCL4 for 6 weeks resulted in significant improvement of the hepatic fibrosis picture. Image analysis and fibrosis score confirm these pathological changes. This improvement in histological picture is accompanied by significant improvement in liver

function tests, fibrosis markers and oxidative stress markers. These findings are in agreement with several studies. It was reported that colchicine resolves collagen fibers in liver fibrosis and inhibits TGF-β1 expression[59]. Moreover, colchicine prevents the progression of hepatic fibrosis in human[1]. The anti-fibrotic effect of colchicine in preventing progression of liver fibrosis may be attributed to the anti-inflammatory effect of colchicine as colchicine can decrease the phagocytic activity of Kupffer cells[60]. Moreover, colchicine is an alkaloid, prefers to accumulate in neutoropils. Colchicine reduces destruction, chemotaxis and phagocytic activities of neutrophils; thus reducing initiation and perpetuation of inflammation[61].

## CONCLUSION

In conclusion, aliskiren and valsartan prevent the development of liver fibrosis mainly through; improvement of renin angiotensin system by inhibition of angiotensin II production, angiotensin II action, decrease TGF-β1 level and anti-oxidant effects, thus, inhibiting HSC activation. Chloroquine has antifibrotic effect via its antioxidant, anti-inflammatory effects, decrease TGF-β1 level, and inhibition of autophagy with consequent inhibition of HSCs activation. Zafirlukast exerted an anti-fibrotic effect mainly through its anti-inflammatory, antioxidant effect and decreased TGF-β1 level thus inhibiting HSCs activation. However, it produced less improvement in liver function tests, so monitoring of liver function parameters is required during treatment with zafirlukast. Moreover, colchicine has exerted a hepato-protective, antifibrotic effect, mainly through its anti-inflammatory effect by inhibiting the phagocytic activity of Kupffer cells, inhibition of TGF-β1 expression and its anti-oxidant effects. Either aliskiren, valsartan, chloroquine, zafirlukast or colchicine may be tried in strategy for treatment of chronic liver injury especially if associated with concomitant diseases suitable for treatment by these drugs e.g., hypertension, rheumatoid arthritis, bronchial asthma, familial Mediterranean fever, gout.

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