DOI: https://doi.org/10.31351/vol34iss3pp227-236

The Possible Hepatoprotective Effects of Azilsartan against Carbon Tetrachloride CCl_4 - Induced Liver Fibrosis in Male Rats in Comparison with Silymarin: "*in vivo* Study"

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Received 1/6/2024, Accepted 5/11/2024, Published 20/9/2025

Abstract

Hepatic fibrosis is a pathophysiological result of continuous wound healing in response to chronic liver injury characterized by excessive accumulation of extracellular matrix proteins. Progressive hepatic fibrosis can be made by non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, chronic infection of hepatitis B or C, alcohol abuse, and other related conditions. Liver fibrosis and subsequent cirrhosis represent a serious medical challenge; yet, there is still an absence of approved strategies or medicines to reverse or prevent liver fibrosis. Therefore, effective antifibrotic agents are urgently needed. This study aimed to investigate the potential hepatoprotective effects of azilsartan in rats against carbon tetrachloride (CCl_4)-induced liver fibrosis. Methods: Forty white male albino rats were utilized in this study. During this study, liver fibrosis was induced by intraperitoneal (I.P) injection of CCl_4 50% in olive oil 1ml/kg twice weekly for 6 consecutive weeks. In the treatment groups, azilsartan and silymarin were daily orally administered together with I.P CCl_4. Following the end of the sixth week from the induction and treatment period, all animals were weighed individually, then euthanized and their livers were weighed to determine the relative liver weight percentage. Furthermore, a piece of each rat liver tissue was homogenized to determine tissue malondialdehyde and reduced glutathione. Moreover, liver tissue slices were prepared to study necroinflammation degree, and collagen deposition (fibrosis stage) histopathologically. Ultimately, transforming growth factor TGF-β_1, alpha-smooth muscle actin, and hydroxyproline were all assessed for immunohistochemistry expression levels in this study. The results revealed that intraperitoneal injection of CCl_4 in olive oil in rats resulted in inflammation and fibrosis induction and so, increased relative liver weight. Such intraperitoneal injection led to increased tissue level of MDA decreased GSH, and elevation in the immunohistochemical expression of TGFβ_1, alpha-smooth muscle actin, and hydroxyproline as compared to the normal control group. Finally, Oral administration of Azilsartan and silymarin reduced oxidative stress, inflammatory, and fibrosis markers representing hepatoprotection properties. Findings didn't show significant differences between azilsartan and silymarin treatment.

Keywords: Liver fibrosis, Azilsartan, CCl_4, Collagen, Silymarin.

Introduction

Liver fibrosis is a pathophysiological result of continuous wound-healing response to chronic injury made from repeated accumulation of extracellular matrix (ECM) proteins. Fibrosis is a dynamic process that involves intercomunication between hepatocytes, hepatic stellate cells (HSCs), sinusoidal endothelial cells, and both resident and infiltrating immune cells[(1)]. Progressive liver fibrosis can be caused by chronic infection of hepatitis B virus or hepatitis C virus, alcohol abuse, non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), and other relatively rare conditions such as autoimmune hepatitis, wilson's disease, hemochromatosis, and primary/secondary biliary cholangitis[(2)]. The hepatic fibrosis have high incidence rate and mortality throughout the world. Liver fibrosis and subsequent cirrhosis represent a serious medical challenge; yet, there is still an absence of approved strategies or medicines to reverse or prevent liver fibrosis except for the transplantation or removal of the cause of injury. Therefore, effective hepatic antifibrotic drugs are needed urgently[(3,4)]. After continuous liver injury, HSCs activate into myofibroblasts, start expressing proteins such

as alpha-smooth muscle actin (α-SMA), secrete cytokines, such as transforming growth factor-beta 1 (TGF-β_1), platelet-derived growth factor (PDGF), and connective tissue growth factor (CTGF) and migrate at the tissue repair site, and secrete a large amount of ECM. Fortunately, when the liver injury is removed, myofibroblasts may undergo inactivation and apoptosis, which lead to resorption of the fibrous scar and liver fibrosis regression[4,5]. Hydroxyproline (Hyp) is considered a non-proteinogenic amino acid which is generated during collagen synthesis through post-translational hydroxylation of proline[6]. Measurement of Hyp amino acid gives the researcher clear information for the liver fibrosis diagnosis[7]. It represents individual (signature) amino acids of collagens, being fibrillar collagen primary constituent of all given types of collagen[8]. Once exposed to harmful stimuli, the main effectors responsible for the generation of reactive oxygen species (ROS) are the Kupffer cells, which consequently affect HSCs and hepatocytes; where, ROS disrupts lipids, DNA, and proteins, induces necrosis and apoptosis of hepatocytes and promotes the inflammatory response[9]. Stellate cells secreted Angiotensin II, which has been noticed to stimulate fibrogenesis when binded to the angiotensin II (Ang II) receptor and activating different intracellular responses[10].

The renin-angiotensin-aldosterone system (RAAS) is expressed in fibroblasts, monocytes, macrophages, endothelial cells, and tumor cells, which is closely related to liver fibrosis, cell proliferation, metastasis, and angiogenesis in the development of HCC[11]. RAAS interacts with different pathways seems to be pro-fibrotic to establish fibrosis in different cell types, including TGF-β_1, PDGF, TNF-α, and Interleukins such as IL-6 and IL-13, to accelerate the proliferative phase of repair[12]. RAAS antagonism was investigated in many models and proved to have beneficial roles, in an experimentally induced colitis study; the inhibition of Ang II possesses anti-inflammatory actions involving antioxidant effect and inhibition of adhesion molecule synthesis in the colonic tissues[13]. Irbesartan, an angiotensin receptor blocker, reduced the behavioral sign and sequence of Parkinson's disease in mice[14]. Azilsartan is a newly approved potent, long-lasting angiotensin-receptor blocker. It is highly selective with a bioavailability of ~ 60%[15]. Compared to other angiotensin receptor blockers, azilsartan is superior, because it possesses a potent and persistent ability to inhibit angiotensin II binding to receptors. Preclinical studies have revealed that azilsartan may also have potentially beneficial effects on cellular mechanisms that could involve more than just blockade of angiotensin receptors and/or reduction in blood pressure[16]. Azilsartan protects the liver against injury in renal ischemic reperfusion[17]. Azilsartan exerts a favorable role against high-fat diet-induced NAFLD in rats[18]. Azilsartan exhibited anti-inflammatory and anti-proliferative actions which led it to possess an anti-psoriasis effect[19]. Furthermore, AZL has marked renoprotective[20], cardioprotective[21], and neuroprotective[22] effects in numerous experimental animal models. The effect of azilsartan on liver fibrosis is still elusive. Therefore, we hypothesized that RAAS blockade by azilsartan may exhibit beneficial effects against liver fibrosis induced by CCl_4.

This study aimed to investigate the possible antifibrotic effects of Azilsartan on CCl_4-induced liver fibrosis in male rats in comparison with silymarin.

Materials and methods

Materials and Reagents

Drugs (Chemicals)	Company (Origin)
CCl_4	THOMAS BAKER® (Chemicals) PVT. India
Silymarin	MADUAS®, Germany
Azilsartan medoxomil	BIDE PHARMATECH® Ltd. China
Carboxymethylcellulose	THOMAS BAKER®

Silymarin was freshly suspended 0.5% w/v carboxy methylcellulose[23], and Azilsartan was freshly prepared in a 0.5% w/v carboxy methylcellulose solution to be dispersed to obtain a uniform dosage[24].

Animals

Forty healthy white male albino rats were utilized in the study, weighed between (200-250 gm). The study was started in March 2023 (IRB NO. 2/3/29 in 8/1/2023). The animal house of the veterinary medicine college / Tikrit University supplied the animals. These animals were kept under standard conditions at a temperature between (23±2) °C and relative humidity of 50-60%, with a 12/12 hours light-dark cycle applied. The animals were acclimatized for 2 weeks before starting the work[25]. Furthermore, animals were randomly allocated into four (4) groups (each group contained ten animals; n=10).

Animals Grouping and Study Design

The experimental animal groups received the following:

Group I (Negative control): 10 healthy rats, not received any treatment.

Group II (positive control group; Induction Group): 10 rats were injected with 1ml/kg of 50% CCl_4 solution in olive oil intraperitoneally (I.P) twice a week for 6 weeks [26,27,28].

Group III (Silymarin Treatment Group): 10 rats received 1 ml/kg of 50% CCl_4 solution in olive oil I.P twice a week + Silymarin (hepatoprotective agent) (100mg/kg) once daily orally for six weeks concurrently with CCl_4[27,28,29].
Group IV (Azilsartan Treatment Group): 10 rats received 1 ml/kg of 50% CCl_4 solution in olive oil I.P twice a week + azilsartan (1mg/kg) once daily orally for six weeks concurrently with CCl_4 [21].
After 6 weeks and 24hrs of the treatment period, all animals were kept withholding from food overnight and were anesthetized with Ketamine and Xylazine at a dose of 50 mg/kg, and 5 mg/kg body weight respectively injected intramuscularly in the limb muscle[30]. Diethyl ether was used as a backup[31]

Following the end of the sixth week, all animals were weighed individually, then euthanized and their livers were weighed to determine the relative liver weight percentage. Furthermore, a piece of each rat liver tissue was homogenized to determine oxidative stress markers, tissue malondialdehyde (MDA), and reduced glutathione (GSH). Liver tissue slices were prepared to study necrosis and inflammation degree after being stained with hematoxylin and eosin stain, and collagen deposition (fibrosis stage) after being stained with Masson's trichrome stain. Ultimately, this study assessed TGF-β1, α-SMA, and hyp for their immunohistochemistry (IHC) expression levels [32].

Measurement of hepatic oxidative stress

A piece of the liver tissue is homogenized with a homogenization buffer solution which contained a 1% protease inhibitor cocktail. The lysates mix was homogenized on ice using a homogenizer, then centrifuged for 5 minutes at 12,000 rpm and 4◦C. The supernatant was aliquoted and levels of MDA and GSH in hepatic tissue homogenates were assessed using commercially available kits (Sunlong Biotech Co. LTD), following the manufacturer's instructions[33,34,35].

Histological assessment

Specimens of liver tissue were fixed in 10% neutral buffered formalin and then embedded in paraffin. Five-µm-thick slices were prepared, then depar-affinized, and undergoed the processing for hematoxylin& eosin (H&E) and Masson Trichrome (MT) stains[36,37,38]. H&E slides were examined under the light microscope for assessment of the general tissue architecture and inflammation grade. Masson Trichrome slides were also examined for assessment of collagen deposition and fibrosis stage. The necroinflammation grade and fibrosis stage were scored according to the Batts-Ludwig scoring system, which is used for staging fibrosis and grading histological specimens obtained from the liver. Values of both stage and grade range from 0 to 4. Grading is based on the necroinflammation and portal/periportal activity or lobular activity, while Staging is based on the presence of portal/periportal fibrosis and septa formation with/without cirrhosis, which corresponds to stage 4[39].

Immunohistochemical assessment

The samples were fixed in 10% neutral buffered formalin for 48 hours and stained with hematoxylin and eosin. Immunohistochemistry was performed on tissue slices after dewaxing in xylene, immersing in ethanol, and washing in phosphate-buffered saline. Endogenous peroxidase was suppressed for 30 minutes using a 3% hydrogen peroxide solution in methanol. After freezing at 25°C for an hour, tissue slices were treated with primary antibodies at 4°C overnight[40]. Anti-rat immunohistochemistry was performed on paraffin-embedded tissue using α-SMA polyclonal antibody E-AB-34268 at a 1:200 dilution. Hydroxyproline is visible in paraffin-embedded tissues using the Hyp Antibody. This experiment uses Hyp Antibody #73812 at 1:200. TGF β Receptor I Immunohistochemistry of paraffin-embedded tissue with a polyclonal antibody at 1:70 dilution. After triple washing, the sections were treated with poly-HRP goat anti-mouse IgG (1:200, Wuhan Biotech, China) for 60 minutes at 37°C. Biotin and avidin were used for detection. The slices were dried and coated after 60 seconds of hematoxylin staining[41]. IHC evaluation was done according to the following semiquantitative scores that represented the percentage of positively stained cells as follows: Score 1: equal or Less than 25% positive cells. Score 2: 26–50% positive cells. Score 3: 51–75% positive cells. Score 4: 76-100% positive cells[42].

Statistical Analysis

To compare the study groups; one-way ANOVA (with Tukey post hoc test) for normally distributed variables, or Kruskal-Wallis test (The Two-stage linear step-up procedure of Benjamini, Krieger, and Yekutieli for post hoc test) for non-normally distributed variables. All analyses used GraphPad Prism version 10.0.0. The significance level was defined by p-value ≤ 0.05.

Results And Discussion

Effect of CCl_4, silymarin, and azilsartan on the study parameters

The study results as shown in Table 1 show a significant dropping in (relative liver weight percentage; tissue level of MDA; IHC score of TGF-β_1, Hyp, α-SMA; histopathological score of necroinflammation grade, fibrosis stage), significant elevation in tissue level of GSH in control group in comparison with induction group (P value<0.001). Moreover, the study results as shown in Table 1 show a significant dropping in (relative liver weight percentage; tissue level of MDA; IHC score of TGF-β_1, Hyp, α-SMA; histopathological score of necroinflammation grade, fibrosis stage), significant elevation in tissue level of GSH in treated

(Silymarin and Azilsartan) groups in comparison with induction group (P value<0.001). The relations among studied groups (Silymarin, and Azilsartan groups) didn't show significant differences.

Table 1. Assessment of the parameters in the study groups

Parameter	Control	Induction	Silymarin	Azilsartan
Relative liver weight percentage	3.01±0.09	5.59±0.17$^{\#}$	4.19±0.08$^{\$\&}$	4.30±0.05$^{*\&}$
MDA (ng/ml)	3.34±0.12	6.97±0.50$^{\#}$	4.40±0.14$^{\$\&}$	5.26±0.31$^{*\&}$
GSH (ng/l)	67.70±3.43	48.65±3.12$^{\#}$	60.35±8.46$^{\$\&}$	61.81±8.99$^{*\&}$
TGF-β_1 score	0.19±0.04	0.91±0.04$^{\#}$	0.42±0.05$^{\$\&}$	0.46±0.04$^{*\&}$
Hyp score	0.08±0.03	0.93±0.03$^{\#}$	0.26±0.04$^{\$\&}$	0.32±0.03$^{*\&}$
α-SMA score	0.07±0.03	0.78±0.06$^{\#}$	0.28±0.05$^{\$\&}$	0.32±0.06$^{*\&}$
Necroinflamation grade score	0.40±0.52	4.00±0.00$^{\#}$	2.50±0.53$^{\$\&}$	2.60±0.52$^{*\&}$
Fibrosis stage score	0.20±0.42	3.40±0.16$^{\#}$	1.50±0.17$^{\$\&}$	2.00±0.15$^{*\&}$

Data presented as mean ± SD (standard deviation), n=10

$^{\#}$ If p-value ≤0.05 between induction and control.

$^{\$}$ If p-value ≤0.05 between induction and silymarin.

* If p-value ≤0.05 between induction and azilsartan.

$^{\&}$ If p-value >0.05 between silymarin and azilsartan.

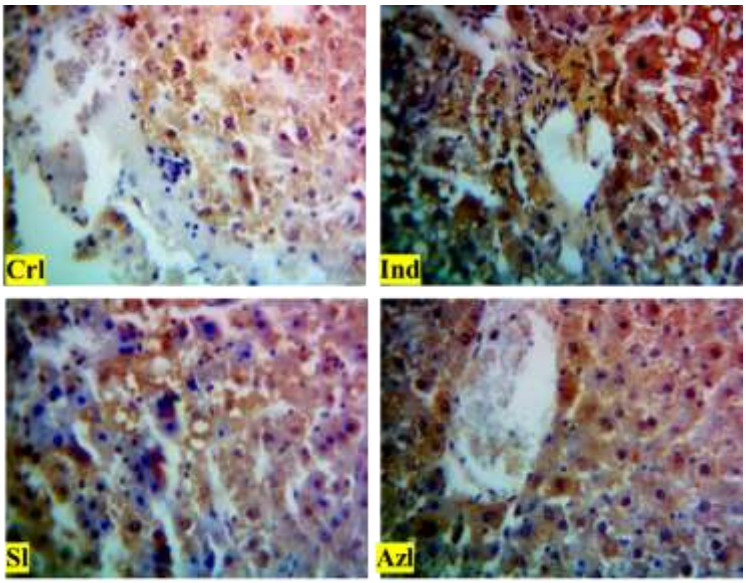

Figure 1. Immunohistochemical staining for TGF-β_1 in the liver. Crl (control), Ind (induction), Sl (silymarin), Azl (Azilsartan).

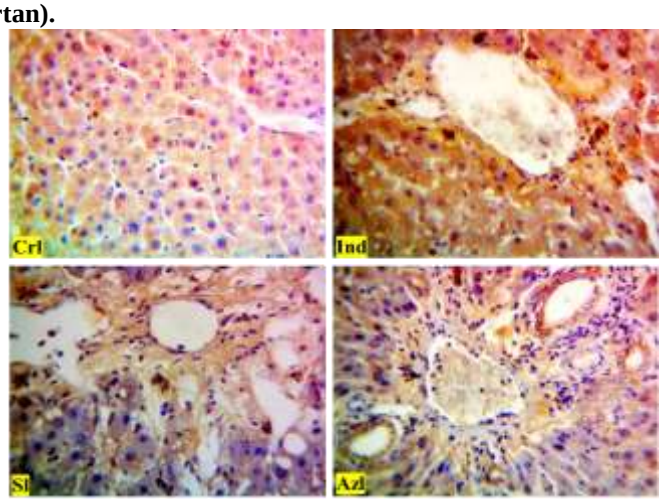

Figure 2. Immunohistochemical staining for Hydroxyproline in the liver. Crl (control), Ind (induction), Sl (silymarin), Azl (Azilsartan).

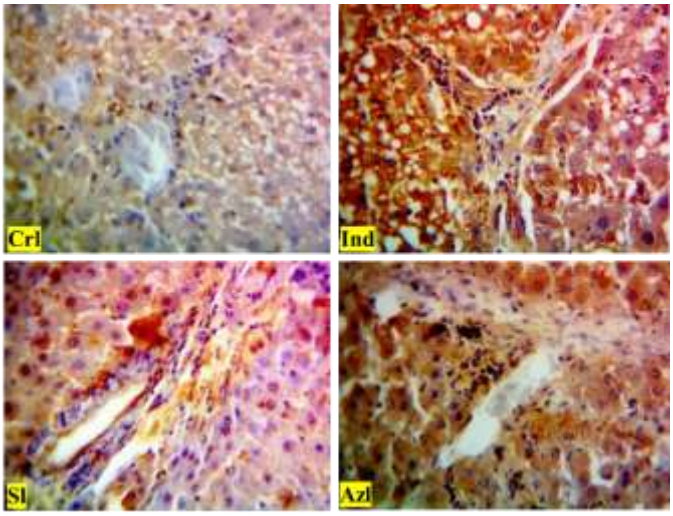

Figure 3. Immunohistochemical staining for α-SMA in the liver. Crl (control), Ind (induction), Sl (silymarin), Azl (Azilsartan).

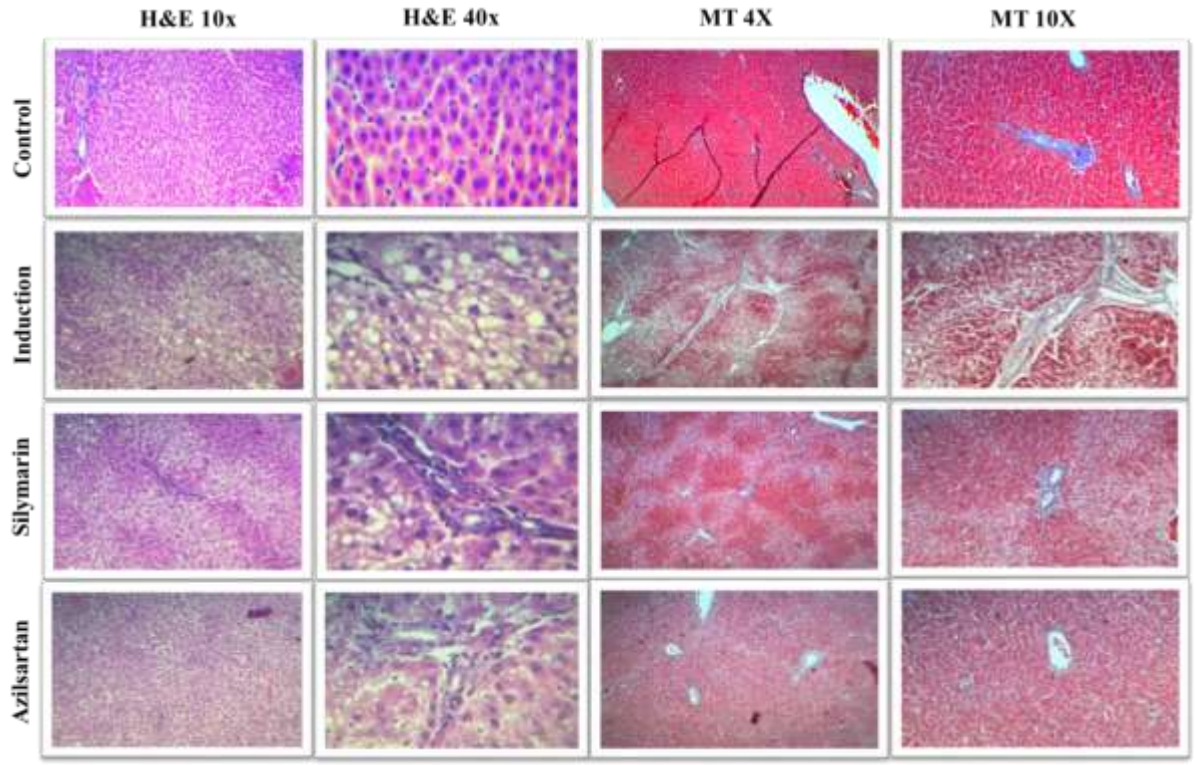

Figure 4. Representative Photomicrographs Showing: in the first and second columns, slides stained with hematoxylin and eosin (H&E), 10x and 40x respectively viewed necroinflammation grade comparison among groups. In the third and fourth columns, slides stained with Masson Trichrome (MT), 4x and 10x respectively viewed fibrosis stage (collagen deposition) comparison among groups. In H&E stained slides, the induction group showed severe diffuse hepatocellular damage (grade 4), and silymarin and Azilsartan groups showed mild hepatocellular damage and mild portal inflammation (grade 2). In MT-stained slides, the induction group showed fibrous bands and nodules,(stage 3-4), and silymarin and Azilsartan groups showed mild portal fibrosis (grade 2).

Discussion

Drug repurposing (drug re-profiling, or repositioning) which is an attractive proposition now increasingly considered as a strategy for identifying new uses for already approved and de-risked agents that are in use currently for other purposes or indications, such as azilsartan in the current study[43]. RAAS is overproduced at different stages of liver fibrosis. Several investigations concluded that RAAS inhibition is a promising approach for liver fibrosis treatment. However,

conduction of further clinical trials is needed[44]. In a study of cisplatin-induced Hepatotoxicity in rats, control and azilsartan groups showed normal hepatic architecture, this finding proves the hepatoprotection of azilsartan and agrees with our findings regarding histopathological findings[24]. Azilsartan alone or in combination with aliskiren exerted a protective role against high-fat diet-induced NAFLD in rats, by attenuating early signs of liver fibrosis, fatty changes, and necrosis seen in non-treated animals[18]. Losartan improved fibrosis in 50% of hepatitis C patients after 18 months of treatment, coupled with a significant decrease in the expression of several profibrogenic genes[45].

Valsartan showed a hepatoprotective effect in a study of CCl4–induced liver fibrosis in rats, according to histopathological findings observed by HE and MT staining; valsartan significantly reduced the degree of liver fibrosis [46]. Administration of ramipril and candesartan in patients with chronic hepatitis C with liver fibrosis was effective in improving liver fibrosis[47]. Based on the above, any agent block or reverse angiotensin action may have a favorable effect on attenuating or even reducing fibrosis progression, and therefore, this idea complies with our findings regarding relative liver weight, and histopathological score of fibrosis. Azilsartan significantly inhibited the inflammatory state in the liver in renal ischemic reperfusion, as viewed by decreased levels of MDA and TNF-α, and there was also an elevation in GSH content in the livers of rats. These findings demonstrated the anti-oxidant, anti-inflammatory properties of azilsartan and provided the first proof of azilsartan's effect on endoplasmic reticulum stress and mitochondrial biogenesis[17]. In a study of cisplatin-induced hepatotoxicity, azilsartan was able to counteract ROS and reduce lipid peroxidation, representing a significant improvement in antioxidant defenses. these important results strengthen our findings [24]. Experiment evidence pointed out that Ang II is capable of inducing ROS synthesis by activated HSCs. Ang II caused a marked elevation in ROS production. When HSCs are treated with the angiotensin receptor blocker, (losartan) Ang II-induced ROS production by HSCs is blocked[48]. Such findings are consistent with the findings of the current study. Because of the histomorphological similarities shared by fibrosis in all organs, there is an attractive concept of common tissue fibrosis pathways that might consider potential therapeutic targets in all organs[49]. Azilsartan reduces oxidative stress in kidney tissue in a study of cisplatin-induced nephrotoxicity[50], and it attenuates Lipopolysaccharide-induced acute lung injury and exerted anti-inflammatory action through amelioration of production of inflammatory factors, and anti-oxidant action[51]. Another study proved that azilsartan might suppress Lipopolysaccharide-induced inflammation in U937 macrophages by suppressing oxidative stress[52]. Prajapati P, et al. 2023 stated that azilsartan increases GSH and decreases MDA muscle tissue level and collagen deposition in muscle[53]. All the above-mentioned studies and findings regarding azilsartan and oxidative stress revealed that azilsartan possesses antioxidant properties which may benefit in protection against fibrosis and agree with our study results. Komaki H, et al. in 2018 viewed that azilsartan decreased cardiac expressions of TGF-ß$_1$, cardiac interstitial and perivascular fibrosis in rats with high salt intake[21], and significantly reduced expression of TGF-ß$_1$ and cardiac fibrosis in a diabetic cardiomyopathy mouse model[54], so these findings strengthen our results.

A study done in 2020 by Hashim et al. highlights the hepatoprotective effect of aliskirin (direct renin inhibitor) and fenofibrate and implies that their anti-fibrotic mechanism involves blockade of TGF-β_1/Smad signaling pathway, induction of Hepatic Growth Factor expression, besides modulation of inflammation as well as oxidative stress[55]. In a randomized open-label controlled study in patients with alcoholic liver disease, candesartan reduced the area of fibrosis, hyp, α-SMA, and TGF-$\beta_1$levels[56]. Candesartan treatment for 6 months showed ameliorated liver fibrosis and reduced fibrotic area, scores, and markers of α-SMA and hydroxyproline levels in patients with alcoholic liver fibrosis[40]. α-SMA expression was attenuated by Azilsartan in the kidneys of rats treated with cisplatin to induce EMT[46]. Based on our study findings and discussed studies agreements, it is preferable to choose azilsartan in indicated cases like hypertension which is accompanied by liver fibrosis or any condition that worsens the general state of the liver.

Study limitations

The study's limitations were that one dose of azilsartan was used rather than different doses. The absence of genetic parameters which may have different beneficial effect in the evaluation of the potential protective use of azilsartan against fibrosis.

Conclusion

This study revealed that orally administered azilsartan significantly increased or potentiated hepatoprotective activity against CCl_4-induced hepatotoxicity in white albino rats. Findings didn‘t show significant differences between azilsartan and silymarin treatment.

Acknowledgment

The author is thankful to Dr. Youssef Shakuri, Sammarra Drugs Company for his efforts in the preparation of animals.

Conflicts of Interest

The authors declare no conflict of interest.

Funding

No funding source

Ethics Statements

All experiments were carried out following the study protocol that was reviewed by the Institutional Review Board of the College of Medicine/Al-Nahrain University after permission from the scientific committee of the Department of Pharmacology at the College of Medicine/Al-Nahrain University. The best effort was made to reduce the animals' distress and pain throughout all experiment procedures.

Author Contribution

The authors confirm their contribution to the paper as follows: study conception and design: Mohammed Jasim Mohammed and Haitham Mahmood Kadhim; data collection: Haitham Mahmood Kadhim; analysis and interpretation of results: Mohammed Jasim Mohammed; draft manuscript preparation: Mohammed Jasim Mohammed and Haitham Mahmood Kadhim. The authors reviewed the results and approved the final version of the manuscript.

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التأثيرات الوقائية المحتملة للأزيلسارتان على الكبد ضد تليف الكبد الناجم عن رابع كلوريد الكربون في الجرذان الذكور بالمقارنة مع السليمارين: دراسة في الجسم الحي

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الخلاصة

التليف الكبدي هو نتيجة فيزيولوجية مرضية تحصل استجابةً لإصابة الكبد المزمنة والتي تتميز بالتراكم المفرط للبروتينات المصفوفة خارج الخلايا. يمكن أن يحدث التليف الكبدي المتقدم بسبب العدوى المزمنة لإلتهاب الكبد الڤايروسي C & B, التعاطي المفرط الكحول، مرض الكبد الدهني غير الكحولي، التهاب الكبد الدهني غير الكحولي مع بعض الحالات الأخرى. تليف الكبد وما قد يتبعه من تشمع الكبد يمثل عبئاً طبياً خطيراً؛ ومع ذلك، لا توجد اساليب أو أدوية معتمدة لمنع أو معالجة تليف الكبد. ولذلك، هناك حاجة ماسة للأدوية الفعّالة المضادة للتليف الكبدي. كان الغرض من هذه الدراسة هو دراسة التأثيرات الوقائية المحتملة للأزيلسارتان ضد تليف الكبد الناجم عن رابع كلوريد الكربون CCl_4 في الجرذان. الطرائق: تم استخدام أربعين جرذاً نوع البينو من الذكور البيضاء في هذه الدراسة. خلال هذه الدراسة تم إستحداث تليف الكبد عن طريق حقن مادة ال CCl_4 بنسبة خمسين بالمئة والممزوجة مع زيت الزيتون داخل الصفاق وبجرعة واحد مل/كجم مرتين أسبوعياً لمدة ستة أسابيع متتالية. تم إعطاء أزيلسارتان وسيليمارين عن طريق الفم يوميًا بالتزامن مع الحقن داخل الصفاق لمادة CCl_4. بعد نهاية الأسبوع السادس من فترة الاستحداث والعلاج، تم وزن جميع الحيوانات بشكل فردي، ومن ثم تطبيق القتل الرحيم وتم وزن جميع أكباد الحيوانات لتحديد النسبة المئوية لوزن الكبد النسبي. كذلك، تم اجراء تجانس لجزء من جميع اكباد الجرذان لتحديد المالوندايالدهايد (MDA) والجلوتاثيون المهدرج (GSH) في الانسجة. علاوة على ذلك، تم تحضير شرائح من أنسجة الاكباد لغرض التشريح المرضي لدراسة درجة الالتهاب النخري وترسيب الكولاجين (درجة التليف). في النهاية،تم تقييم عامل النمو المحول بيتا-١ ($TGF\text{-}\beta_1$) ، وأكتين العضلات الملساء ألفا (α-SMA) ، والهيدروكسيبرولين لمستويات التعبير مناعيا في هذه الدراسة. أظهرت النتائج أن حقن مادة CCl_4 ممزوجة مع زيت الزيتون داخل الصفاق للجرذان أدى إلى حدوث التهاب وتليف في الكبد وبالتالي زيادة في وزن الكبد النسبي.كما أدى هذا الحقن إلى زيادة مستوى الأنسجة من MDA وانخفاض في مستوى GSH وزيادة مستويات التعبير مناعيا ل ($TGF\text{-}\beta_1$) , (α-SMA) والهايدروكسيبرولين مقارنة مع مجموعة السيطرة. وأخيرًا، تناول عقار أزيلسارتان وسيليمارين عن طريق الفم قد قلل وبشكل ملحوظ مؤشرات الاجهاد التاكسدي،الالتهاب والتليف وهذا بدوره يمثل خاصية حماية للكبد. النتائج لم تظهر اختلافات ملحوظة بين علاج الأزيلسارتان والسيليمارين.

الكلمات المفتاحية: تليف الكبد، ازيلسارتان، سي سي ال فور، كولاجين، سليمارين.