https://doi.org/ 10.33472/AFJBS.6.Si3.2024.1-13

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

The Effect of Losartan Nanoparticles on Carbon Tetrachloride Induced Hepatic Fibrosis in Rats

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Article History

Volume 6,Issue Si3, 2024

Received:21 Mar 2024

Accepted : 08 May 2024

doi: 10.33472/AFJBS.6.Si3.2024.1-13

Abstract

Background: Following a persistent liver damage, a pathological process known as hepatic fibrosis arises due to an imbalance in the liver's repair system. Liver fibrosis was primarily caused by chemicals, metabolic intermediates, and biological agents such as viruses.

Aim: This study aims to investigate the biochemical and histopathological changes in the liver induced by Carbon Tetrachloride (CCl4) toxicity, and mitigating the potential effect losartan potassium (LP) and losartan potassium nanoparticles (LP-NPs) on experimentally induced CCl4 liver fibrosis.

Methods: A total of 32 adult male Sprague Dawley albino rats weighing (180-200) gm were included in this study. The rats were randomized into four groups (control group, model group, losartan potassium, and losartan potassium nanoparticles treated groups), in which all rats were given the intra-peritoneal injection of CCl_4 (2 ml/kg dissolved in a 1:1 ratio of olive oil, twice weekly for 6 weeks) except for rats of control group. Rats of losartan potassium, and losartan potassium nanoparticles treated groups were treated with LP and LP-NPs (orally in a dose of 10mg/kg/day along with ccl4 injection). After 10 weeks liver tissue and serum samples of all rats were examined. The structural and biochemical changes of the liver were measured.

Results: results showed that CCl4 toxicity induced abnormal liver function, severe liver architecture deformity with pseudoloboule formation and cellular inflammation with infiltration reported in most of the portal areas, fibrous septa and the liver lobules. In addition, collagen I and II were significantly expressed in model group compared to control rats. However, at both losartan potassium, and losartan potassium nanoparticles treated groups, these changes were reversed with strong advantage to LP-NPs. The administration of LP and LP-NPs significantly improved the liver function values, hepatic architecture and suppressing the overproduction of collagen fibrils

Conclusion: The present study highlights that LP or delivered LP-NPs nanoparticles could have a protective effect on liver functions and architecture in the rat liver of CCl4 induced liver injury.

Keywords: hepatic fibrosis, losartan-nanoparticles, Carbon tetrachloride, renin-angiotensin system inhibitors, liver enzymes.

1. Introduction

The treatments with any chemical drug or medicine-based agents showed to be associated with severe liver damage or hepatotoxicity (**Teschke et al.,2013**). In addition, it was reported that liver injury and hepatotoxicity proceed with the action of chemicals, metabolic intermediates, and viruses (**Shaaban et al.,2023**). Carbon tetrachloride (CCl4) is a well-known hepatotoxicant (**Ramaiah andRittlin,2007**). This substance is used to simulate pathophysiological lesions seen in humans in animal models. CCl4 toxicity is mediated by metabolites that react with antioxidant enzymes with significant rise in the extent of cellular inflammatory cytokines like tumor necrosis factor-alpha (TNF-α), interleukin-1 β (IL-1β), and nuclear factor (NF-κB) (**Rikans et al.,1994**). Moreover, in experimental studies, several organs, primarily the liver, kidneys and lungs were significantly intoxicated when exposed to CCl4 (**Guo et al., 2000**).

Previous data stated that CCl4 produces free radicals which initiates macrophages' activation, leading to the progression of cellular inflammatory and profibrogenic mediators (**Ebaid et al.,2013**). The production of cellular free radicals is considered the main and first step in the sequence of events leading to membrane lipid peroxidation, apoptosis, and necrosis (**Basu, 2003**; **Chen et al., 2015**).

Following a persistent liver damage, hepatic fibrosis is a pathological condition characterized by an imbalance in the liver's repair system. During this stage of recovery, the extracellular matrix (ECM) builds up instead of the hepatocytes (**Pinzani,2015; Seki and Brenner, 2015**), with significant excessive deposits of extracellular matrix proteins (**Arriazu et al., 2014**). This occurs when repetitive or long-lasting injury by chemical or biological agents which causes an excessive burden of scar tissue deposition distorting the normal liver architecture **(Altamirano-Barrera et al., 2017)**. Hepatic fibrosis can be treated in its early stages, such as steatosis and fibrosis, but left untreated over time can result in irreversible cirrhosis or even liver cancer (**Li et al.,2022; Sun et al.,2015; Atta et al.,2015**).

There is an urgent clinical need to develop an effective anti-fibrotic agent targeting attenuation of the fibrosis progression or even reversal of the fibrotic processes **(Friedman et al., 2013)**.

Losartan is anti-fibrotic agent that has been shown to reduce incidence of liver, cardiac and renal fibrosis. It has an inhibitory effect on progression and even led to regression of fibrosis stage **(Salama et al., 2016)**.

Globally, fibrosis and the ensuing failure of the organs are responsible for at least one-third of all deaths from diseases. Therefore, it is imperative that the molecular, histochemical, and cellular immunochemical mechanisms of liver fibrosis be understood in order to identify key therapy targets (**Melaibari et al.,2023**). In addition, new means of delivery of drugs to specific targeted sites such as nanomedicine using nanoparticles were extensively required. One method, to prevent the production of extracellular matrix (ECM) and to resolve hepatic fibrosis, was the use of nanoparticle-based antifibrotic therapy in recent years. It has the potential to deliver antifibrotic compounds with poor water solubility and bioavailability to the corresponding sites of fibrosis (**Devaraj et al.,2020**).

Previous studies on nanoparticles used in the management of liver fibrosis proved that some material as selenium when used as nanoparticles become more effective in liver fibrosis than ordinary selenium **(Ebaid et al., 2021)**. On the other hand, titanium dioxide nanoparticles induced more inflammation than the ordinary titanium dioxide when rats were exposed to an equal concentration of titanium dioxide **(Baranowska-Wójcik et al., 2020)**.

Therefore, this study aims to investigate the histopathological and biochemical changes in the liver fibrosis induced by CCl-4 toxicity, and mitigating the potential protective and enhancement effect of losartan potassium (LP) and losartan potassium nanoparticles (LP-NPs) on experimentally induced CCl-4 liver fibrosis.

2. Materials and methods

2.1. Animals

A total of 32 adult male Sprague Dawley albino rats weighing (180-200) gm were included in this study. All rats were maintained in normal conditions of temperature (23 ± 3 °C), relative humidity (60 ± 5%), and light-dark cycle of a 12 hours., the animals were kept under these conditions for two weeks prior to the experiment, in order to facilitate acclimation and maintain typical behavior and growth. The experiment was completed in Anatomy and Embryology Department and Medical Experimental Research Center (MERC), Mansoura University. This Experiment was performed in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals.

2.2. Chemicals and drugs

Carbon tetrachloride (CCl4; Cat. No. 289116) and Losartan potassium (Cat. No. 59489S) were purchased from Sigma-Aldrich Co (Saint Louis, MO 63103, USA). 2ml/Kg of sterile CCl4 was dissolved in olive oil, was procured from local markets in Egypt, with a ratio of 1:1 ratio, and the rats were intraperitoneally injected twice weekly for 6 weeks (**Zhao et al., 2016)**. LP and LP-NPs were dissolved in tap water, prepared as a 1% solution, and were given orally by gavage in a dose of 10 mg/kg/day, which is equivalent to the clinical dose for losartan **(Nassar et al., 2013; Namisaki et al., 2022)**.

2.3. Preparation and confirmatory the structure of losartan potassium nanoparticles:

2.3.1. Preparation

LP-NPs were prepared by shaking it in ball-mill apparatus of type Retsch MM2000 swing mill that made in China and delivered from USA. The mill has 10 cm^3 stainless steel tube, double-walled tube. Two stain steel balls of 12 mm diameter and 7 gm weight for each were used. Ball milling was performed at 20225 Hz for half an hour at room temperature (without circulating liquid and temperature did not rise above 30 °C) then particles were investigated under transmission electron microscope **(El-Shereafy et al., 2017)**.

2.3.2. Confirmatory of losartan potassium nanoparticles

2.3.2.1. Transmission electron microscope (TEM) images:

Transmission electron microscope is a special kind of electron microscope for imaging of different objects in contrast to other microscopes the electrons in TEM pass through and interact with atoms of the sample. Due to this interaction the electrons are being scattered. The final image is very complicated interference pattern of incident and diffracted beams. The images were measured by using JEOL HRTEM-JEM 2100 (JAPAN) at The Electron Microscope Unit at Faculty of Agriculture, Mansoura University.

2.4. Experimental Design

The rats were randomly divided into four groups (8 rats each);

Control group: For six weeks, rats were intraperitoneally injected with 2 mg/kg of olive oil twice a week. After four weeks from the previous injection, these rats were sacrificed.

Carbon tetrachloride group (CCl4 group): Throughout a 6-week period, rats were intraperitoneally injected twice a week with a dose of CCl4 (2 milliliters per kilogram diluted in a 1:1 ratio of olive oil); the rats were sacrificed four weeks following the last injection.
Losartan group (LP group): CCl4 (2 mg/kg diluted in a 1:1 ratio of olive oil) was administered intraperitoneally twice a week for six weeks, and LP was taken orally at a dose of 10 mg/kg each day along with CCl4 injection and four weeks after last CCl4 injection and then were sacrificed.
Losartan nanoparticles group (LP-NPs group): For six weeks, rats were given intraperitoneal injections of CCl4 (2 milliliters per kilogram dissolved in a 1:1 ratio of olive oil) twice a week and oral administration of LP-NPs (10 mg/kg/day) along with CCl4 injection and four weeks after last CCl4 injection and then were sacrificed.

2.5. Blood and liver samples Collection

Before being dissected, the rats received an intraperitoneal injection of 300 mg/kg chloral hydrate to induce sleep. For the purpose of evaluating liver function tests, blood samples were drawn directly from the ventricle into polyethylene tubes (AST and ALT). Following abdominal incision, the liver was meticulously extracted, conserved in 10% buffered formalin, and subjected to standard histopathological techniques. It was then embedded in paraffin, sectioned at 6 μm intervals. Hematoxylin and eosin staining was used for histological evaluation, while Sirius red staining was used to detect collagen fibers.

2.6. Biochemical analysis

All groups' serum samples were tested for liver function indicators such as aspartate transaminase (AST) and alanine transaminase (ALT). In this investigation, the sera were collected by centrifuging blood for 10 minutes at 5000 g at 4°C. Clinical test kits (Elitech, UK) were then used to estimate the liver function indicators colorimetrically in accordance with the standard biochemical analysis technique.

2.7. Light microscopic study

Paraffin sections (4-6μm) will be prepared and stained with hematoxylin and eosin (H & E) for routine histopathological examination (**Bancroft and Stonard, 2013**). In addition, Picro - Sirius red stain **(Chun and Inoue, 2014)** was applied to histological liver tissues for visualization of collagen I and III in all studied animals. Finally, they were examined under a light microscope.

2.8. Morphometric analysis

Image analysis of the liver's collagen fiber-occupied region was identified as mentioned before (**Varghese et al., 2014**). Image analysis was applied to all of the groups' sections that had been stained with 0.1% Picro sirius red, which specifically stains collagen. A total of 100 magnification was achieved by measuring at least six random fields on each slide using an objective lens of magnification 10, which was obtained from multiple readings taken from the various slides containing the eight animals of each group. The Olympus® SC100 digital camera installed on the Olympus® CX41 light microscope was used to photograph the liver of each rat in all groups. The morphometric study was conducted using the NIH Image J program (National Institutes of Health, Bethesda, MD, USA), as directed by the program.

2.9. Statistical analysis:

An IBM-SPSS program was used for statistical analysis. Results will be expressed as the mean ± standard deviation (SD). The normality of the data was assessed by Shapiro-Wilk’s test and boxplots were analyzed to check for any notable outliers. Given that the data lacked any notable outliers and was regularly distributed across all variables and groups. Comparison of the studied variables between the studied groups will be performed using one way ANOVA for parametric

values to compare between more than two groups of numerical (parametric) data followed by post hoc tukey test for multiple comparisons Least. The p values less than 0.05 will be considered statistically significant. Correlations among liver enzymes and Sirius red were plotted, and the Pearson's correlation coefficients were labelled.

3. Results

3.1. Assessments of LP and LP-NPs particles by TEM images analysis

TEM showed losartan potassium particles of different shapes. The dimensions of the particles ranging from 0.24 µm to 0.35µm with a magnification of x15000 while the dimensions of the prepared LP-NPs particles ranging from 24 nm to 31.02 nm with a magnification of x20000 and the morphology was either in the form of irregular or distorted spheres **(fig. 1.A & fig. 1.B) respectively.**

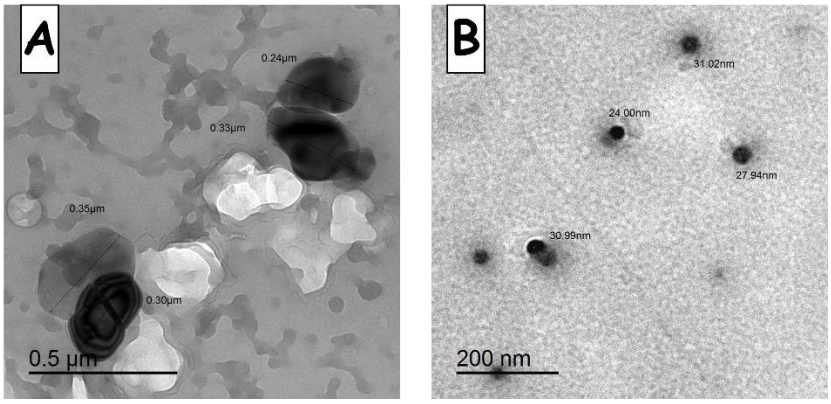

Fig. (1): TEM of losartan potassium particles of different shapes. Some particles were oval and others were irregular and the dimensions of the losartan particles ranging from 0.24 µm to 0.35µm with magnifications of **x15000 (A),** the dimensions of the losartan nano particles ranging from 24 nm to 31.02 nm with magnifications of **x20000(B).**

3.2. Assessment of liver enzymes

The liver functions of all studied rats were identified as shown in Table (1). The results showed that there was a highly significant difference between all studied groups ($P<0.001$) as shown in table (1). By using post hoc tukey, pairwise comparisons showed high significant increase in the serum levels of ALT and AST of CCl4 treated groups, Losartan and LP-NPs treated group as compared to that of control group ($P<0.001$). whereas, there were a significant marked reduction in the serum levels of ALT and AST of Losartan and LP-NPs treated group in comparison to CCl4 groups ($P<0.001$). By comparing LP-NPs group to losartan group in samples of ALT and AST showed high significant decrease ($P<0.001$), significant decrease ($P=0.001$) respectively.

Table 1: liver enzymes in different studied groups (ANOVA test)

Liver enzymes	groups				
	Control group (n=8) Mean ± SD	CCl4 group (n=8) Mean ± SD	CCl4-LP group (n=8) Mean ± SD	CCl4-LP-NPs group (n=8) Mean ± SD	P value
ALT (U/L)	24.17 ± 6.22	80.25±10.06	60.5±7	37.67 ± 6.62	P <0.001**
AST (U/L)	127.47± 12.15	273.56± 37.3	188.14± 13.92	145.18± 17.17	P <0.001**

SD= standard deviation; P: significance in differences of variables between the 4 studied groups
P > 0.05 = non-significant, P < 0.05 = significant*, P < 0.001= highly significant**

3.3. Liver histopathology

The control group's liver's histological appearance revealed that it was composed of central hepatic venules and classical hepatic lobules. Hepatocyte cords that formed flat, anastomosing plates spreading from the central vein and divided by broad hepatic sinusoids gave rise to the lobules. The hepatocytes' cytoplasm featured one or two central spherical vesicular nuclei and was often acidophilic. There were connective tissue-based portal tracts at the corners of the lobules that contained a small bile duct, a branch from the portal vein, and a small branch from the hepatic artery. There were unclear interlobular septa (fig. 2.A).

The liver sections of rats treated with CCl4 showed a considerable quantity of connective tissue in the portal tracts and surrounding the central veins; fibrous tissue septa (bridging fibrosis) were evidently connected the portal tracts together, the central vein and the portal tracts (fig. 2. B). Furthermore, rats given Losartan potassium (LP) demonstrated a notable improvement in liver architecture. In addition to fibrous tissue around the central vein and thin bridging fibrous septa encircling the hepatic lobules, the portal tracts were thick and infiltrated with inflammatory cells (fig. 2.C). Additionally, liver slices from rats receiving LP-NPs revealed a largely normal hepatic architecture. Figure 2.D showed that a small number of short, thin, and fibrous septa were visible extending from the portal tract into the adjacent parenchyma.

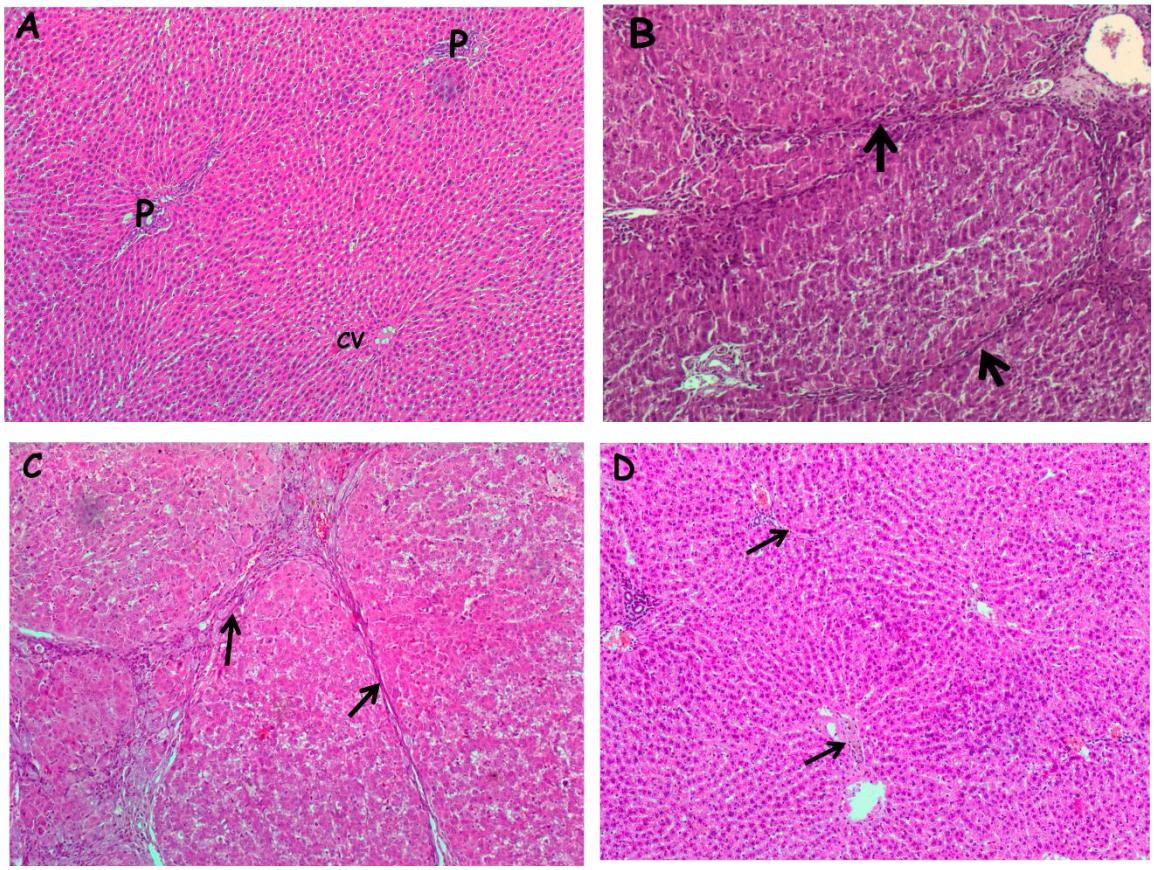

Fig. (2): A microscopic photo of liver section of control, LP, LP-NPs treated and non-treated ccl4 intoxicated rats. A) A microscopic photo of liver section of control group shows normal architecture. Hepatic lobules formed of cords of hepatocytes with flat, anastomosing plates radiating from central vein (CV) and at the corners of the lobules, there were portal tracts (P). B) A microscopic photo of liver section of ccl4 treated rats shows distortion of liver architecture with pseudolobules formation and multiple thick fibrous septa (black arrows) C) Rats of losartan with ccl4 group exhibit distorted liver architecture on a photomicrograph of their liver segment. There were signs of fibrous septa (black arrows). D) A microscopic photo of the liver segment of LP-NPs with the ccl4 group reveals that the hepatic architecture has mostly been retained, and a few thin, short septa are visible extending from the portal tracts into the parenchyma around them (black arrows) (HX. & E. stain; x100).

3.4. Histological analysis of collagen I and III in liver sections

Collagen fibrous were identified in the tissue sections of control CCl4, LP and LP-NPs groups by using Sirus red stain as shown in figure (3.A, B, C&D). Within the control group, collagen fiber-based connective tissue encircled the portal tracts located at the corners of each lobule. In the portal tract and surrounding the central vein, fine collagen fibers were seen (figs. 3.A). Collagen fibers in the portal tracts and surrounding central veins were much more abundant in the group treated with carbon tetrachloride. In every portion, there were thick, well-developed septa joining the central veins and portal tracts. The architecture of the liver was noticeably distorted (figs. 3.B).

Thin fibrous tissue was observed in the portal tract and surrounding the central vein in LP with ccl4 group. The liver's pseudoloboule was fully encircled by thin, fibrous septa (figs. 3.C). Additionally, there was a little increase in the quantity of collagen fibers in the portal tracts and

surrounding central veins in the LP-NPs with ccl4 group. A few thin fibrous septa were visible, partially joining the portal tract and central veins (figs. 3.D).

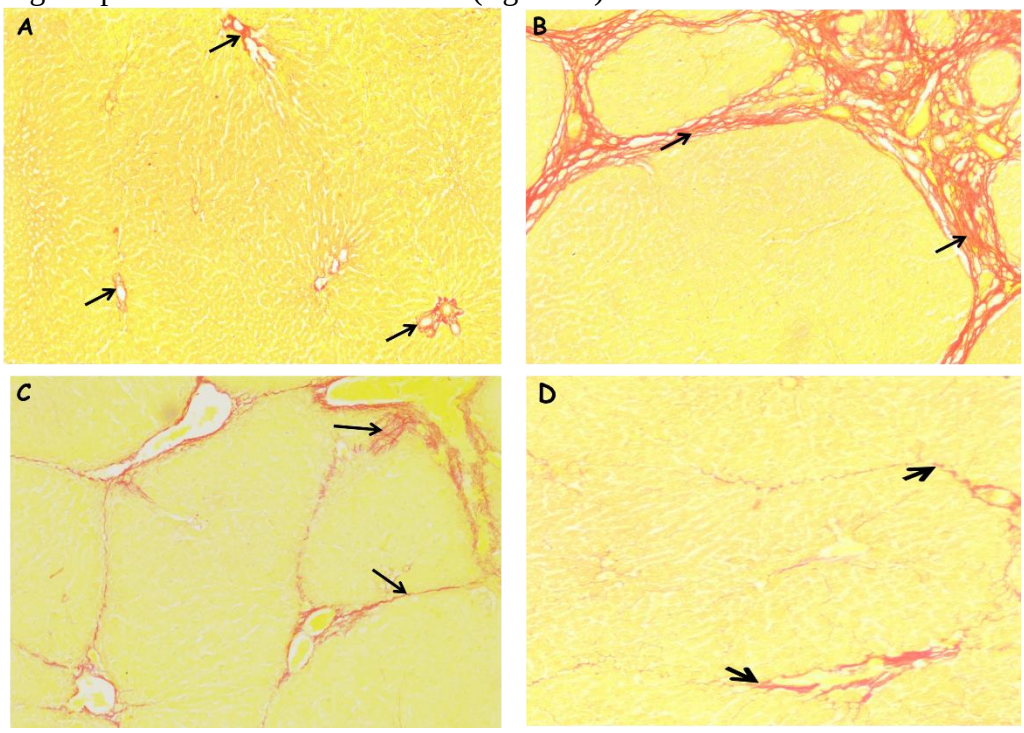

Fig. (3): microscopic photos of liver section of control, LP, LP-NPs treated and non-treated ccl4 intoxicated rats. **A**) liver sections of control group show central vein and portal tract surrounded by few collagen fibers (black arrow). **B**) A microscopic photo of liver sections of ccl4 treated group shows increased periportal and collagen fibers with thick fibrous septa radiating from the central veins and portal tracts (black arrows). **C**) A thin fibrous tissue was observed in the portal system and surrounding the central vein in a microscopic photo of the liver segment of an LP with the ccl4 group. The liver's pseudolobule was entirely surrounded by thin, fibrous septa (black arrows). **D**) A little increase in the quantity of collagen fibers in the portal tracts and surrounding central veins is visible in the liver section of a microscopic photo of LP-NPs with the ccl4 group. A small number of thin fibrous septa were visible, partially joining the portal tract and central veins (arrows) (**Sirius red stain; ×100).**

The expression of fibrous collagen and its condensation in the liver tissues of several rat groups. Table (2) presents the results, which indicated a highly significant difference ($P<0.001$) between all tested groups. Pairwise comparisons with post hoc Tukey analysis revealed a highly significant increase ($P<0.001$) in the area occupied by collagen fibers from the CCl4 treated groups, Losartan treated group, and LP-NPs treated group compared to the control group. A highly significant decrease in the area filled by collagen fibers was seen when comparing the groups treated with Losartan and LP-NPs to the ccl4 groups ($P<0.001$). Samples from the LP-NPs group to the losartan group revealed a highly significant decrease ($P<0.001$).

Table 2: Area percentage (%) occupied by collagen fibers in different studied groups (ANOVA test)

	Groups				
	Control group (n=8) Mean±SD	CCl4 group (n=8) Mean±SD	CCl4-LP group (n=8) Mean±SD	CCl4-LP-NPs group (n=8) Mean±SD	P value
Area occupied by collagen fibers (%)	1.82 ±0.2	18.69±6.47	8.54± 1.72	2.5±0.67	P <0.001**

SD= standard deviation; **P**: significance in differences of variables between the 4 studied groups. P > 0.05 = non-significant, P < 0.05 = significant*, P < 0.001= highly significant**

3.5. Correlation (table3)

Pearson's correlation test was done between area percentage occupied by collagen fibers and liver enzymes. There was a significant positive correlation between area percentage occupied by collagen fibers and each of AST (r= 0.878, p<0.001) and ALT (r= 0.864, p<0.001)

Table3: correlation between area percentage occupied by collagen fibers and liver enzymes

parameters	r value	P value
AST	0.878**	<0.001
ALT	0.864**	<0.001

r= Correlation coefficient value; ** Correlation is significant the 0.01 level (2-tailed)

4. Discussion

Hepatic fibrosis is the major health problem proceeds as the end result of chronic liver illness among most chronic liver diseases. Histological analysis reported that liver scaring is the common histological feature appeared following the exposure of the liver to any such chemical and biological agents. A marked increase in collagen fiber synthesis and deposition of glycoproteins and proteoglycans, which all form an extracellular matrix were reported following exposure of the liver to infection with chronic viral infections (e.g., hepatitis B and C), or chemical toxicants (**Albillos et al., 2014**). Moreover, excessive alcohol consumption, non-alcoholic fatty liver disease, and metabolic syndrome were also an additional causes of liver fibrosis (**Rajapaksha et al., 2021**). The progression of liver fibrosis leads to irreversible cirrhosis, which eventually causes further complications such as hepatocellular carcinoma, and even death (**Ebrahimi et al., 2018**).

In this study, the potential antifibrotic activity of losartan potassium (LP) alone or in nanoparticle format (LP-NPs) were studied in carbon tetrachloride (CCL4) induced hepatic fibrosis in albino rats. The results showed the administration of LP and LP-NPs significantly improved liver biomedical function, histological structure.

In this study, liver function markers such as ALT and AST were significantly increased following the treatment with CCl4 toxicant compared to that healthy control rats. However, a significant reduction in the analyzed liver biomarkers ALT and AST compared to either LP or LP-NPs treated groups.

Like thioacetamide and dimethyl nitrosamide, which are frequently used in animal models to induce liver fibrosis, CCl4 is one of the most fibrotic agents. The fibrosis produced in this way bears some resemblance to the mechanisms of hepatic fibrosis in humans **(Crespo Yanguas et al., 2016)**. The first sign of liver injury and fibrosis, is the cellular release of liver enzymes which significantly increased within a short time **(Li et al., 2021)**. According to our findings, inflammatory markers such as TNF-α significantly increase levels of liver enzymes like ALT and AST, which in turn caused an increase in the synthesis of collagen fibers within injured liver cells **(Abdelghffar et al., 2022)**.

In addition, the improved cellular levels of liver enzymes in LP or LP-NPs CCl4 treated rats significantly supported the antifibrotic activity of LP to ameliorate the progression of hepatic fibrosis induced by CCL4 via controlling of the cellular expression of fibrotic markers like; Ang II type 1 receptor, and TGF-β, reducing in such away the leak of cellular liver enzymes **(Ogata et al., 2016)**. Previous research demonstrated that the administration of losartan potassium greatly prevented the overproduction of angiotensin II in the damaged liver, which was implicated in the activation of hepatic stellate cells and the subsequent fibrogenesis in individuals with chronic HCV infection. These findings suggested that the fibrosis stage should recede, but they had no bearing on the degree of inflammation (**Salama et al.,2016**).

In the present study, H&E and Sirus red -stained liver sections of the CCL4 treated group revealed distortion of hepatic architecture and intense inflammatory cellular infiltration which progressed to both septal and non-septal fibrosis. In addition, a statistically significant increase in the percentage area of fibrosis was significantly increased in CCL4 treated group as compared to control group. Like others, CCL4 toxicity was found to significantly increase the amount of fibrous tissue in the liver **(Zhang et al., 2016)**. The mechanism of inducing of fibrosis, goes through metabolizing of CCL4 in the liver which in turn leads to the release of cellular free radicals, triggers oxidative stress which contribute in initiation and progression of liver injury through the production of inflammatory cytokines. These inflammatory cytokines can cause necrosis of hepatocytes, induce inflammation, activate hepatic stellate cells and promote the progression of hepatic fibrosis.

In LP-treated rats, the liver section showed that many hepatocytes restored their normal histological appearance. The results showed that the administration of LP was followed significantly by a high reduction in the area percentage occupied by collagen fibers as compared to CCL4 treated group. Moreover, in LP-NPs treated rats, showed that the liver restored its normal histological appearance with few and thin fibrous septa could be seen. There was reduction of the area occupied by collagen fibres in LP-NPs group as compared to both losartan and CCL4 groups. Matched to our results, past studies showed that both liver fibrosis and necroinflammation were significantly reduced in patients with chronic hepatitis C following the treatment of ACE inhibitors (captopril, enalapril, lisinopril, quinapril, and trandolapril), and ARBs (losartan, valsartan and irbesartan) as a treatment for hypertension. The results showed a significantly decrease in level of fibrosis**(Corey et al., 2009;Stokkeland et al., 2018; Rajapaksha et al., 2021).)**

In the current approach, nanotechnology involves various nanoparticles systems as inorganic, liposomal, polymeric albumin and nano micelles **(Sharma et al., 2019)**. These nanparticles are small enough in the nanoscale range with nontoxic, non-injurious, and harmless properties to

prevent any probable immunological response in the biological system. These compounds serve as means of delivery of drugs to specific targeted sites **(Patra et al., 2018)**. Thus, the improvement of liver fibrosis with drugs based upon nanotechnology was currently approached **(Giannitrapani et al., 2014)**.

Like other nanoparticles used in management of liver fibrosis, LP-NPS particles become more effective in the enhancement of liver fibrosis than LP-alone. This similar to selenium nanoparticles which showed more efficient activity against liver fibrosis compared to their ordinary selenium **(Ebaid et al., 2021)**. On the other hand, titanium dioxide nanoparticles induced more inflammation than the ordinary titanium dioxide **(Baranowska-Wójcik et al., 2020)**. Therefore, the enhancement effects of losartan potassium nanoparticles against liver fibrosis were evaluated and compared with effects of the ordinary losartan.

In conclusion: The present study highlights that ordinary LP or delivered LP-NPs nanoparticles could improve chemical and histochemical parameters in the rat liver of CCl4-induced liver injury. These effects may be largely attributed to its antifibrotic improvement properties. However, LP-NPs nanoparticles provide better protective effect than the ordinary LP.

5. Recommendation

Future studies will confirm the exact cellular mechanism of nanoparticle approaches for the improvement of liver fibrosis.

6. Acknowledgment

"We acknowledge 'Medical Experimental Research Center - MERC' of Mansoura faculty of medicine for its support"

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