Original Article

Assessing Platelet-derived Growth Factor and Liver Enzyme Levels in Rats Undergoing Carbon Tetrachloride Treatment and Bile Duct Ligation

Esraa Mohammed Kadhim Al- Huchimi[1], Hassan Falih Hassan Salman Al-Ardawi[2], Alshimaysawee Sadeq[3], Ameer Mohammed Ridha[1]

1. Department of Pharmacy, Faculty of Pharmacy, Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences, Najaf, Iraq.
2. Department of Medical Analysis, Faculty of Medical Science, Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences, Najaf, Iraq.
3. Department of Pharmacy, Faculty of Pharmacy, Islamic University, Najaf, Iraq

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ABSTRACT

Background: Platelet-derived growth factor (PDGF) is a major mitogen for connective tissue cells and certain other cell types.

Objectives: This study uses bile duct ligation (BDL) to assess PDGF levels in rats and the level of liver enzymes for two periods.

Methods: The experiment was divided into four groups (15 rats in each group). A total of 60 male rats were used and were divided into groups as follows: Group 1 included male rats administered with 0.5 mL/kg of drinking water as negative control; group 2 comprised male rats administered with 0.5 mL/kg of carbon tetrachloride (CCl_4) orally for one month; group 3 included male rats undergoing BDL for one week; and group 4 were male rats undergoing BDL for two weeks. At the end of the treatment period, which lasted for five weeks, male rats were sacrificed and blood samples were obtained to assess PDGF and levels of liver enzyme.

Results: The results showed a significant elevation ($P<0.05$) in the level of PDGF along with a significant elevation ($P<0.05$) in the level of liver enzymes (alkaline phosphatase, alanine aminotransferase, and aspartate transferase) in rats undergoing BDL and CCl_4 treatment compare to control groups.

Conclusion: In our current study, we concluded that a high level of PDGF is related to liver disease, and we can consider it as an indicator of cirrhosis.

Keywords: BDL, Platelet-derived growth factor (PDGF), Hepatic satellite cells, Kupffer cells

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*** Corresponding Author:**
Esraa Mohammed Kadhim Al- Huchimi, PhD.
Address: *Department of Pharmacy, Faculty of Pharmacy, Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences, Najaf, Iraq.*
Phone: *+ 964 (781) 623 9803*
E-mail: *esra.m.kadhim@jmu.edu.iq*

Introduction

The hepatic organ is the biggest organ inflexible in the body, weighing about 1.5 kg in the adult. Located in the upper part right quadrant of the abdomen, it is confined via the thoracic cage rib. It is completely enclosed by a peritoneal membrane, which is identified as Glisson's capsule (Abdel-Misih & Bloomston, 2010).

The liver is an extremely specific tissue consisting of mostly hepatic cells (hepatocytes) and controls a wide variety of biochemical reactions, as well as the production and break of tiny and complex molecules. These are essential for usual vital functions (Marieb & Hoehn, 2007). Liver fibrosis is an injury therapeutic response to a variety of chronic stimuli, including viral infection of the liver, disorders of metabolism, abuse of alcohol, and autoimmune sodden in the liver (Tortora & Derrickson, 2018).

Many achievable causes of liver fibrosis, and at times more than one cause is current in the same person. Worldwide, 57% of attributable cirrhosis is to hepatitis C (27%) or hepatitis B (30%) (Perz et al., 2006). Throughout the route of fibrogenesis, different disinterested parties, which are mainly created by Kupffer cells, tenant hepatic cells, and insightful inciting cells, stimulate myofibroblasts, which is the reason for overload extracellular matrix (ECM) accretion. Fibrosis is a consequential inequity among ECM resolution and production. The extreme ECM evidence (principally type 1 collagen evidence) disorders the normal planning of the liver, follow-on in fibrosis evolution, and following cirrhosis (Tahan, 2010).

ECM is a key part of the microenvironment in human hepatic. Forming a leathery, the ECM supplies outside for cell bonds, the gap for cell development and passage, and works as a tank for signaling molecules (Hynes, 2010). In addition, numerous ECM machinery, such as laminin, fibronectin, and collagen are dependable for promoting the idiom of hepatic exact task in addition to cell differentiation (Flaim et al., 2005). Also, the local severity of hepatic ECM is an essential mechanical effecter of cellular manners with tissue creation. Largely, responses of cells to usual signals of hepatic ECM comprise separation, proliferation, alterations, and relocation in cell bonds (cell in addition to matrix) (Wells, 2008). Injury fix is an active method where the hepatic ECM composition with inflexibility becomes significant. Continuous ECM remodels throughout chronic hepatic injuries guide to a change plus extreme buildup of 31 extracellular proteins proteoglycans as well as carbohydrates, hence fibrosis principal, which is dependable for the mortality and morbidity connected by hepatic collapse (Bataller & Brenner, 2005). Primary biliary cirrhosis is an autoimmune infection of the liver. Its consequences from slow, progressive damage to the small bile ducts of the liver, reason bile and other poison to grow in the liver, this circumstance is called cholestasis. A slower break to the liver tissue can lead to scarring, fibrosis, and eventually cirrhosis (Dan & Longo, 2012).

Carbon tetrachloride (CCl_4) is an organic solvent extensively used in chemical manufacturing. CCl_4 can reduce protein creation in the hepatic with early hepatotoxic break and its hepatotoxicity consists of hepatic necrosis and fatty liver (Masuda, 2006).

Materials and Methods

Study animals

Bile duct ligation (BDL) and animal treatment

The surgery was performed according to the standard procedure proposed by Maede et al. (2021), using 60 male rats sex adult (*Rattus norvegicus*) that weighed around 200-250 g. They were taken from the animal's domicile in a high center of fertility at Faculty of Medicin, University of Kufa, Kufa, Iraq. The animals were housed in the animal domicile of the Faculty of Science, University of Kufa, under standard environmental conditions (temperature around 25-28 °C and 12 h light/dark cycle) and had access to standard laboratory water and diet.

Experimental procedure

The rats were reserved in an animal domicile for acclimation to the laboratory state for two weeks before they were used for the test. Each group included 15 rats as follows: Group 1 included male rats that were administered with 0.5 mL/kg of drinking normal saline (as negative control); group 2 comprised male rats administered 0.5 mL/kg. CCl_4 orally as a positive control for one month; group were rats with BDL for one week; and group 4 included rats with BDL for two weeks. The period of one week and two weeks was adopted because the rats could not tolerate a longer period due to the living conditions in the experimental field and the death of a number of rats.

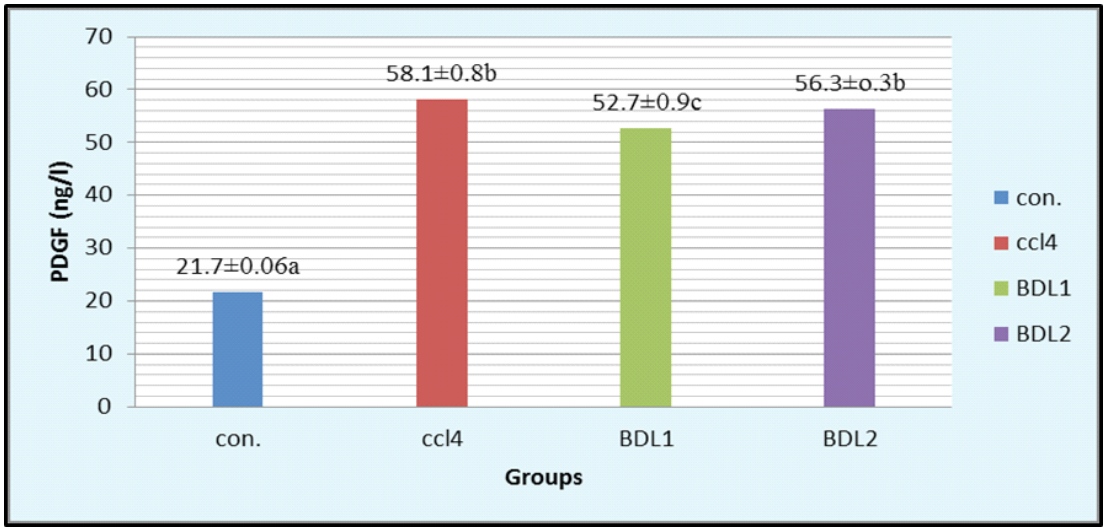

Figure 1. Changes in PDGF level in male rats treated with CCl_4 for one month and BDL for one week and BDL for two weeks

Notes: Values are Mean±SD. The variant letters mean significant differences (P<0.05).

Blood collection procedure

By the end of the experimentation, the animal was anesthetized by the fusion of ketamine 0.5 mL and xylazine 0.1 mL. Anesthesia was done by smell for experimental rats and they were scarified. Heart puncture was done with a 5 mL disposable syringe and 2-5 mL blood was drawn carefully and slowly. The blood was placed in a test tube that contained gel and gone for 30 min at room temperature and used to obtain serum through centrifugation at 3000 rounds per min for 15 min to isolate serum and placed in Eppendorf tubes which are saved at (-20 ˚C) in a cooler for promise biochemical examination.

Measurement of platelet-derived growth factor (PDGF)

The test kit was used to assay the serum PDGF in the sample of rat serum supplied by Bioassay Technology Laboratory (Catalog No: E-EL-H0285).

Biochemical analysis

Activity measurement of serum transaminase: Colorimetric measurement of alanine transaminase and aspartate transferase activity agreement to the Reitman and Frankel method (Reitman & Frankel, 1957) via Biomerieux kit.

Measurement of alkaline phosphatase activity: Colorimetric determination of alkaline phosphatase was done according to Gerarde and Walter 1970 by using a linear chemicals kit.

Statistical analysis

The results were expressed as (Mean±SE) were performed by using the Megastat, and all comparisons were performed by the unpaired sample t-test, while the figures were constructed using the Excel software, version 2010. P<0.05 was considered statistically significant (Al-Rawi, 2000).

Results

Effects of CCl_4 and BDL on the level of PDGF

The results are shown in Figure 1 when contrasting male rats with control shows a significant increase (P<0.05) in serum levels of PDGF.

Effects of CCl_4 and BDL on rats' liver enzyme

The result in Figures 2, 3, and 4, when comparing male rats with controls shows a significant increase in serum level of aspartate aminotransferase, alanine transaminase, alkaline phosphatase in CCl_4 for one month, BDL1 for one week, and BDL2 for two weeks when compared with the control group.

Discussion

The findings suggest that the amount of PDGF releasable from each platelet is increased and concentrated in patients with chronic liver disease. The mean amount of PDGF released from platelets in patients with chronic liver disease was significantly greater than that in con-

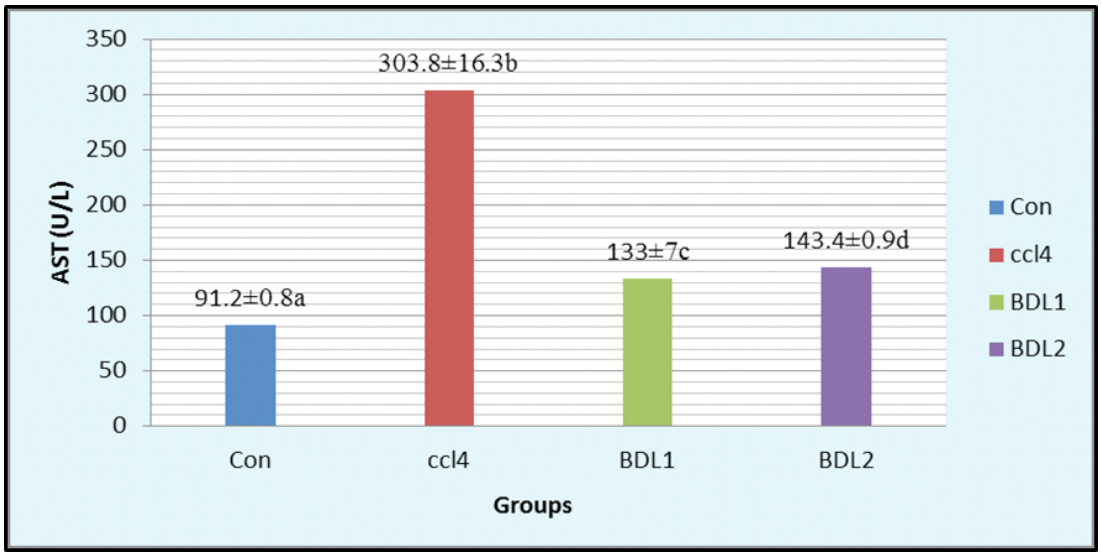

Figure 2. Changes in aspartate aminotransferase level in male rats treated with CCl_4 for four weeks and BDL for one and two weeks

Notes: Values are Mean±SD. The variant letters mean significant differences (P<0.05).

trol subjects. Accordingly, the large amount of PDGF released by platelet activation in patients with chronic liver disease may be involved in the pathophysiology of chronic liver diseases as a local factor (Stein & Harker, 1982).

The outcome acquired from statistical analysis demonstrates a significant rise in the level of PDGF in this group giving CCl_4 and BDL contrast with the control, for these elevated we have the same opinion as Erawan et al. (2008). Given that PDGF has a crucial role in hepatic fibrogenesis and works as a possible mutation on activate culture hepatic satellite cells. Stimulation of receptor of PDGF in hepatic satellite cells is healthy and recognized in only dosage of CCl_4 encourages severe liver damage. PDGFs were thought to participate in an essential function in stimulating hepatic satellite cells to encourage hepatic fibrosis and cirrhosis (Wong et al., 1994; Pinzani et al., 1996).

The concentrations of PDGF in the plasma of all normal subjects and patients with idiopathic thrombocytopenic purpura or chronic liver disease were below the detection limit. The concentrations of PDGF in serum

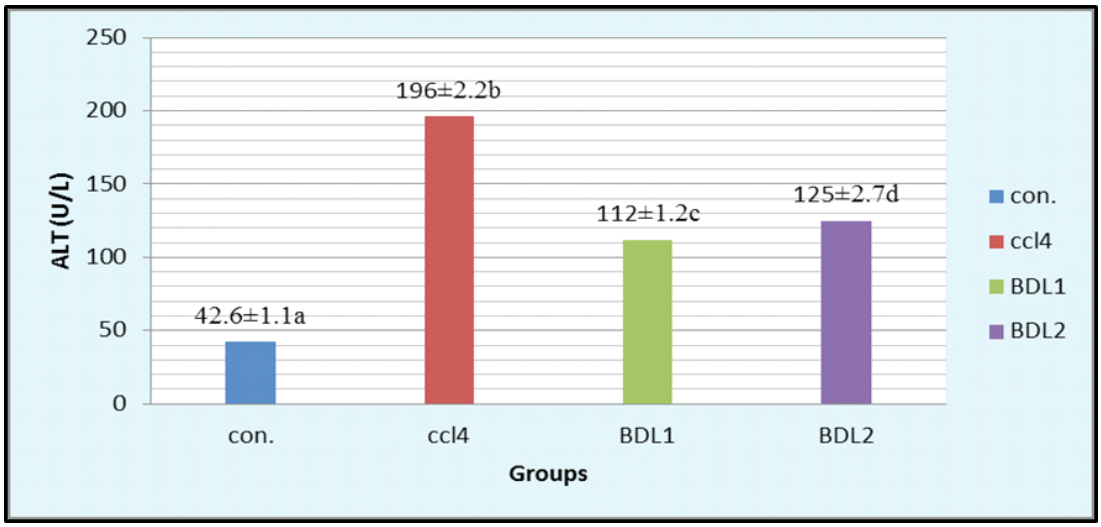

Figure 3. Changes in alanine transaminase level in male rats treated with CCl_4 for four weeks and BDL for one and two weeks

Notes: Values are Mean±SD. The variant letters mean significant differences (P<0.05).

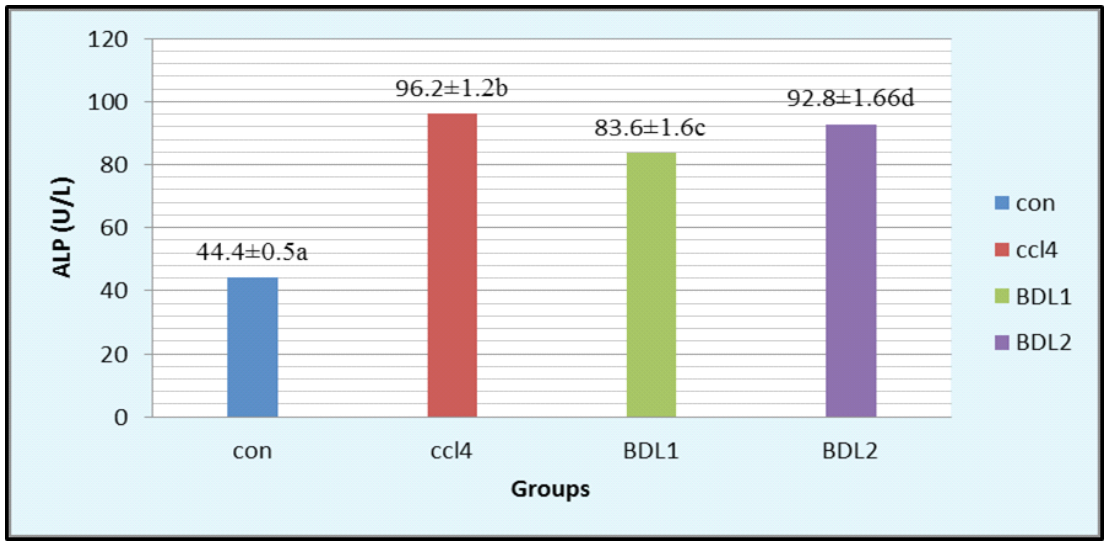

Figure 4. Changes in alkaline phosphatase level in male rats treated with CCl_4 for four weeks and BDL for one and two weeks

Notes: Values are Mean±SD. The variant letters mean significant differences (P<0.05).

were higher than those in adenosine diphosphate-stimulated plasma (Bowen-Pope et al., 1984). In the present study, although the mean platelet count in peripheral blood was significantly lower in patients with chronic liver disease than in the control subjects, the mean serum PDGF level in patients with chronic liver disease was not lower compared to the control group. Jorgensen et al. reported that the mean volume of platelets in patients with chronic liver disease was smaller than that in normal subjects (Jorgensen et al., 1984). The results from this study showed that liver toxicity of CCl_4 was observed through a significant raised of alkaline phosphatase, alanine transaminase, and aspartate aminotransferase in CCl_4-treated rats in contrast with the control, as it has been previously reported that CCl_4 is considered as hepatotoxins in the experimental animals study to form damage of liver (Jaffat & Al-Huchimi, 2016).

The result of liver enzymes, including alkaline phosphatase, alanine transaminase, and aspartate aminotransferase showed a significant increase in septic arthritis calves when compared to healthy animals (Antora et al., 2023).

Serum tests of liver function also showed that alkaline phosphatase, alanine transaminase, and aspartate aminotransferase levels in mice exposed to zinc microparticles and alanine transaminase and alkaline phosphatase levels in mice exposed to zinc nanoparticles increased significantly compared to the control group (Negin et al., 2022).

The elevated level of alkaline phosphatase, alanine transaminase, and aspartate aminotransferase showed that these enzymes escape from the liver into the bloodstream which points to tissue damage, which is associated with liver necrosis (Negin et al., 2022).

Increases in the activity of hepatic enzymes are frequently regarded as expressions of cellular necrosis, principally in hepatocytes. The enlarge in the levels of transaminase reflect an indication of cellular leakage in addition to loss of functional integrity of the cell membrane. Estimation of the hepatic role can be made by estimating the action of serum alkaline phosphatase, alanine transaminase, and aspartate aminotransferase, which were originally present in superior concentrations in the cytoplasm. In hepatic injury, these enzymes escape into blood bloodstream in conformity to the extent of hepatic damage (Negin et al., 2022).

These enzymes mirror cellular harm due to CCl_4 administration which the liver toxicity of CCl_4 was observed through a significant rise of serum alanine transaminase and aspartate aminotransferase levels in CCl_4 group rats in contrast with the control (P<0.05). These results from the statistical analysis showed that vital rise in serum alkaline phosphatase, alanine transaminase, and aspartate aminotransferase concentration in BDL animals in contrast with the control group These heights show the severity of hepatic injury in addition to cholestasis that observed as a result of this enzyme current in bile duct epithelium and the canalicular covering of the hepatic cells. Numerous investigators recorded a noticeable rise in serum levels of alkaline phosphatase, alanine trans-

aminase, and aspartate aminotransferase concentration in rats following BDL (Marra et al., 2005).

The data in the present study demonstrate an important rise in the level of alkaline phosphatase activity after BDL in rats. This enhancement may be attributable to the preservation of bile salts that injured the membrane as well as accordingly guide to alkaline phosphatase enzyme that passes into circulation (Noble et al., 2011). Hepatic and bile duct disorders are followed by increased activity of alkaline phosphatase which is especially characteristic of the cholestasis syndrome (Han et al., 2013). Additionally, it has previously been proved in cholestasis a bile salts stimulate the production of original molecules of alkaline phosphatase (Jaeschke, 2011).

At-hand revision confirmed the finding of Olteanu et al., 2012, which explained the elevation in the serum enzymes aspartate aminotransferase and alanine transaminase to the increase in hepatic cell membrane fluidity that led to enzyme release into circulation, in line with the result of Esmat et al., 2013.

Further studies are required on the mechanism of increase of PDGF in platelets in patients with chronic liver disease, on whether PDGF is released at inflammatory sites in the liver in chronic liver disease, and on whether or not PDGF aggravates the progression of chronic liver diseases in vivo (Schiff, 1987).

Ethical Considerations

Compliance with ethical guidelines

The current study has been conducted according to the national research guidelines for the care and use of laboratory animals. All protocols have been approved by the High Committee for Review and Approval of Research Proposals of the Faculty of Medicine, University of Kufa, Kufa, Iraq (No.: #766, date:13/01/2020).

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Authors' contributions

Conceptualization, samples collection and experiments: Esraa Mohammed Kadhim Al- Huchimi; Methodology: Hassan Falih Hassan Salman Al-Ardawi; Statistic Analysis: Alshimaysawee Sadeq; Writing: Esraa Mohammed Kadhim Al- Huchimi, Hassan Falih Hassan Salman Al-Ardawi and Alshimaysawee Sadeq.

Conflict of interest

The authors declared no conflict of interest.

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