Research Article

Effect of carbon tetra chloride (CCL4) on transforming growth factor -beta (TGF-β) expression in chronic liver disease.

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Abstract
Background: The liver is one of the most important organs in the human body because of its utmost importance, and it plays many essential roles for the body. Carbon tetrachloride affects the body in general and the liver, which sometimes leads to the occurrence of chronic hepatitis and may reach its climax and cause fibrosis. One of the effects of carbon tetrachloride is the stimulation of the beta-TGF pathway. **Results:** After CCL4 administration; There is highly statistically significant difference in expression level of TGF-β ($p < 0.005$). **Conclusions:** This study shows that carbon tetra chloride is promotor for several liver diseases that occur due to activation and upregulation of TGF-β.
Keywords: CCl4, TGF-β, chronic liver disease

Introduction
Hepatitis is a condition in which the liver tissue becomes inflamed. Hepatitis causes yellow discoloration of the skin and whites of the eyes (jaundice), as well as reduced appetite, fatigue, tiredness, stomach pain, and diarrhea Hepatitis is classified as acute if it clears up in six months or chronic if it lasts longer than that(Chow & Chow, 2006).

Aim of work.
The aim of our study is to evaluate changes in gene expression level of TGF-β in CCL$_4$ induced chronic hepatitis

Materials and Methods.
animals: Animals were divided into two separate groups, ten rats for each group. Group I received olive oil (*0.5 ml/kg, i.p., twice per week, for 6 weeks*) as normal control; group II received (*0.5 ml/kg as a 1:1 mixture with olive oil, i.p., twice per week, for 6 weeks*) as chronic hepatitis.

Total RNA Extraction:
In 1ml TRIzol™ reagent, 150 mg of hepatic tissue was homogenized using an ultrasonic homogenizer (Sonics-Vibracell, Sonics & Materials Inc., Newtown, USA) (Invitrogen, USA). The purity was measured using the ratio A260/A280 and the total RNA concentration was estimated at A260 nm.
Samples with a purity ≥ 1.7 used for qRT-PCR using GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) as a reference housekeeping gene for the determination of the relative expressions of TGF-β.

Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)
cDNA synthesis was performed for equal quantities of total RNA in all samples using the Revert-aid first strand cDNA Synthesis Kit (Thermo Scientific Fermentas, St. Leon-Ro, Germany) according to the manufacturer's instructions. Real-time PCR was carried out with single-stranded cDNAs. PCR reactions were performed by Maxima SYBR Green qPCR Master Mix (2X), with separate ROX vial (Thermo Scientific Fermentas St. Leon-Ro, Germany) using Step one Real-Time PCR Detection System (Applied Biosystems). Real-time polymerase chain reaction (qRT-PCR) was carried out using 12.5 μl of Maxima SYBR Green qPCR Master Mix [#K0251, Thermo Scientific Fermentas St. Leon-Ro, Germany] with cDNA, the volume of the cDNA added from the RT reaction with 10 Pmol of specific primers, for 40 cycles (95°C for 10 sec. and

60°C for 1 min). Comparative Ct (threshold cycle) method was used to determine the relative amounts of the products. The relative expression was calculated using the formula (2-ΔΔCt) (VanGuilder, Vrana, & Freeman, 2008). They were scaled relative to controls, where control samples were set at a value of 1.

Statically analysis.
The data was coded and entered using the statistical package Graph Pad prism version 7 software. Statistical differences between groups tested using the Chi-Square test for qualitative variables, independent sample T-test for quantitative normally distributed variables. P-values less than or equal to 0.05 are considered a statistically significant relationship. P-values more than 0.05 are considered a non-statistically significant relationship.

Results
Animals which treated with CCl4 show remarkable upregulation expression of TGF-β which responsible for inflammation (Table1).

Discussion.
Our results revealed that, gene expression of TGF-β1 were significantly elevated in CCl4 group compared with control group. (Drago et al., 2006) and (Gressner, Weiskirchen, Breitkopf, & Dooley, 2002) reported increased levels of both TGF-β1 in chronic liver disease are positively correlated with the stage of liver damage. Similar results have been reported by (Drago et al., 2006). This finding is suggesting that locally released TGF-β1 may be responsible for upregulation of fibrogenic cytokines observed in patients with HCV related chronic liver disease. It is well documented that, the activated hepatic stellate cells (HSCs) and myofibroblast produce a large amount of TGF-β1 (Boudreau, Sympson, Werb, & Bissell, 1995). Several studies demonstrated that

TGF-β could inhibit the growth of quiescent and early activated HSCs but fully activated cells and myofibroblast are released from this inhibitory effect (Broekema et al., 2005). On the other side, TGF-β have been found to mediate extracellular matrix (ECM) gene expression in HSCs and to a great extent in myofibroblast through auto-crine stimulation (Lohse et al., 1999)and this pro-matrix effects of TGF-β1 are recognized to play a key role in the fibrotic process characterizing chronic liver diseases (Simian et al., 2001). Therefore, the production of the ECM by these cells could be determine the extent of fibrotic process after binding of TGF-β1. Prolife-ration and an inducer of apoptosis. The major portion of TGF-β1 is secreted as part of an inactive complex and the details of the activation process in liver have not yet been elucidated. The initially striking simplicity of the core TGF-β /Smad signaling pathways is rapidly giving way to a much more complex view of intracellular signal transduction mechanisms (Gressner et al., 2002). In other study by (Seki, Umezawa, Urano, & Shinozaki, 2007) defines HSC-mediated Kupffer-cell chemotaxis and sensitization to TGF-β –induced signals as two independents, yet complementary, mechanisms by which Toll-like receptor 4 (TLR4) enhances HSC activation and hepatic fibrosis. TGF-β is considered the most powerful mediator of HSC activation in vitro and in vivo. Kupffer cells are a main source of TGF-β in the liver and promote HSC activation and fibrogenesis. Therefore, more promising strategies may be to prevent latent TGF- β1 activation in a cell-type-specific manner rather than blocking already active TGF- β1 (Hinz & Gabbiani, 2010). In conclusion, our study shows that expression of TGF-β1 is a promising marker of hepatic injury.

Table (1): TGF-β gene expression:

	Control	CCL4	N	p value
GAPDH	1.68±0.18	2.910±0.37	10	0.0429*
TGF-β	1.32±0.12	3.560 ±0.58	10	

- Data expressed as range/ mean ± SD.
- Independent Samples T test for quantitative data between the two groups
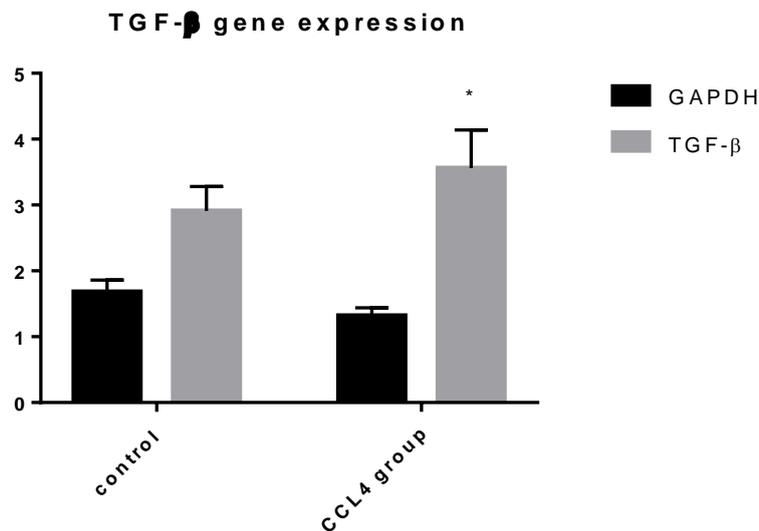- *: Significant level at P value ≤ 0.05

Figure (1): Relative gene expression of TGF-β Abbreviations: P > 0.05: non-significant (ns), P < 0.05: significant (*).

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