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Protective effect of *Tetracera scandens* L. leaf extract against CCl_4-induced acute liver injury in rats

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PEER REVIEW

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Comments

This is a valuable research work in which authors have demonstrated the hepatoprotective activity of *T. scandens* L. extract in CCl_4-induced liver damage in rats. The activity was assessed based on biochemical parameters, antioxidant enzyme levels in liver homogenate. This traditional plant is found to be a promising hepatoprotective agent in CCl_4-indcued hepatitis in rat models.
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ABSTRACT

Objective: To investigate the protective potential of ethanolic extracts of *Tetracera scandens* L. (*T. scandens*) against CCl_4 induced oxidative stress in liver tissues.

Methods: Dried leaf powder of *T. scandens* was extracted with ethanol and concentrated to yield a dry residue. Rats were administered with 100 mg/kg of ethanolic extracts orally once daily for one week. Animals were subsequently administered with a single dose of CCl_4 (1 mL/kg body weight, intraperitoneal injection). Various assays, such as serum levels of alanine aminotransferase, aspartate aminotransferase, lipid peroxidation, protein oxidation (carbonyl protein group), tumor necrosis factor alpha, catalase, superoxide dismutase, and glutathione peroxidase, were used to assess damage caused by CCl_4 and the protective effects of the ethanol extract on liver tissues.

Results: Hepatotoxicity induced by CCl_4 was evidenced by a significant increase in serum aspartate aminotransferase and alanine aminotransferase level, lipid peroxidation, protein carbonyl group, and tumor necrosis factor alpha, as well as decreased activity of the hepatic antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase). Treatment with ethanolic *T. scandens* extracts prevented all of these typically observed changes in CCl_4-treated rats.

Conclusions: Our findings indicate that *T. scandens* has a significant protective effect against CCl_4 induced hepatotoxicity in rat, which may be due to its antioxidant properties.

KEYWORDS
Tetracera scandens L., Antioxidant, Carbon tetrachloride, Liver toxicity, Lipid peroxidation

1. Introduction

Liver is the principal organ which actively involves in metabolic functions. Liver performs an important function that detoxifies those hepatotoxicants, which can cause hepatic injury during metabolic reaction. Oxidative stress is considered as the imbalance between reactive oxygen species production and antioxidant protective mechanism. It is principal cause of the development of various hepatic disorders[1]. The reactive oxygen species plays an important role in both the initiation and progression of lipid peroxidation by inducing oxidative stress. Lipid peroxidation is the metabolism of lipids through pathways involving intermediate formation of lipid peroxides, hydroperoxides and endoperoxides. Lipid peroxidation, a type of oxidative degeneration of polyunsaturated lipids, has been implicated in a variety of pathogenic processes. It has been showed that lipid peroxidation is involved in the mechanisms of various disorders and diseases such as cardiovascular diseases, cancer, neurodegenerative diseases, and even aging[2]. CCl_4,

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a well-known hepatotoxin, has been widely used as a model to evaluate hepatotoxicity[3]. CCl_4 induces hepatotoxicity by increased oxidative stress, and a connection between oxidative stress and lipid peroxidation has been reported[4]. Firstly, CCl_4 is metabolized by action of cytochrome P450 oxygenase system to convert the trichloromethyl free radical, $CCl_3^{\cdot}$[4]. Secondly, $CCl_3^{\cdot}$ radical reacts with some biological molecular such as proteins, nucleic acids and lipids. Furthermore, the $CCl_3^{\cdot}$ radical is converted into the trichloromethyl peroxy radical ($CCl_3OO^{\cdot}$) when it reacts with oxygen. This radical is still more reactive and is capable to initiate the process of lipid peroxidation[4]. CCl_4 induces liver injury progressing from steatosis to centrilobular necrosis, and develops fibrosis and cirrhosis[5].

Tetracera scandens L. (Dilleniaceae) (*T. scandens*) is an evergreen woody climbers and found widely in India, China, Indonesia, Myanmar, Philippines, Thailand, Malaysia and Vietnam. Different parts of *T. scandens* have been used in traditional medicine for lowering hypertension, lowering blood pressure, the treatment of rheumatism, inflammatory diseases, internal pains, urinary disorders, gout and hepatitis. In Vietnam, root and stem are used in treatment of hepatitis, gout and inflammation[6]. Some isoflavonoids have been isolated from the leaves of *T. scandens* and showed capacity to inhibit xanthine oxidase activity in a concentration-dependent manner *in vitro*[7]. Also genistein derivatives from *T. scandens* have been shown to exert significant glucose uptake effect in basal and insulin-stimulated L6 myotubes *in vitro*, suggesting its great potential in the management of diabetes[8]. The extract from leaves of *T. scandens* has also potential anti-diabetic efficacy in alloxan (2,4,5,6-pyrimidinetetrone) induced diabetic rats[9]. However, no scientific report of this plant *in vivo* has ever been recorded or mentioned in the literature showing the hepatoprotective efficacy. Therefore, the aim of the present study was to examine the effects of extract from *T. scandens* on CCl_4-induced acute hepatic injury in rats.

2. Materials and methods

2.1. Plant material

The leaves of *T. scandens* were collected in October 2013 from Nha Trang Province, Vietnam and authenticated by Prof. Nguyen Thanh Hai (School of Medicine and Pharmacy, Vietnam National University, Hanoi). A voucher specimen (No. SMP-2013-0012) was deposited at the Herbarium of School of Medicine and Pharmacy, Vietnam National University.

2.2. Ethanol extract of the leaves of T. scandens

The leaves of *T. scandens* (2.5 kg) were extracted with ethanol (10 L×3 times) at room temperature for a week. The combined ethanol extract was filtered then concentrated to yield a dry residue (251 g).

2.3. Animals

Adult male Wistar rats with body weights of 180-220 g were used in the study. The animals were maintained under standard environmental conditions (22-25 ℃, 12 h/12 h light/dark cycle) and had free access to standard rodent pellet diet and water *ad libitum*. The animals were acclimatized in the laboratory conditions for a week before begin of the study.

2.4. Hepatotoxicity and treated groups

Animals were divided into three groups (n=10): Group I was control group; Group II rats were injected intraperitoneally with a single dose of CCl_4 in corn oil (1 mL/kg body weight); Group III rats were preadministered with 100 mg/kg of ethanolic extracts orally by gastric tube, in the form of aqueous suspension once daily for one week. The animals were then simultaneously administered with a single intraperitoneal injection dose of CCl_4 (1 mL/kg body weight). The animals were sacrificed 24 h after the last treatment by decapitation. The collected serum samples were utilized for the estimation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) markers.

2.5. Tissue homogenization

Liver samples were dissected out and washed immediately with ice-cold saline to remove as much blood as possible. Liver homogenates (5% w/v) were prepared in cold 50 mmol/L potassium phosphate buffer (pH 7.4) using glass homogenizer in ice. The cell debris was removed by centrifugation at 5 000 r/min for 15 min at 4 ℃ using refrigerated centrifuge. The supernatant was used for the estimation of malondialdehyde (MDA), protein carbonyl groups, tumor necrosis factor alpha (TNF-α) levels and catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) activities. Protein concentration was determined by Bradford's method[10].

2.6. Hepatotoxicity study

Serum levels of ALT and AST as markers of hepatic function, were measured by using a ALT Activity Assay Kit and AST Activity Assay Kit (Sigma-Aldrich, Vietnam) according to the manufacturer's instructions.

2.7. Lipid peroxidation assay

Measurement of MDA has frequently been used to measure lipid peroxidation. Lipid peroxidation assay was performed by determining the reaction of malonaldehyde with two molecules of 1-methyl-2-phenylindole at 45 ℃[11]. The reaction mixture consisted of 0.64 mL of 10.3 mmol/L 1-methyl-2-phenylindole, 0.2 mL of sample and 10 μL of 2 μg/mL butylated hydroxytoluene. After mixing by vortex, 0.15 mL of 37% v/v HCl was added. Mixture was incubated at 45 ℃ for 45 min and centrifuged at 6 500 r/min for 10 min. Cleared supernatant absorbance was determined at 586 nm. A calibration curve prepared from 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, Singapore) was used for calculation. Peroxidized lipids are shown as nmol MDA equivalents/mg protein.

2.8. Detection of protein carbonyl groups by slot blotting

Protein carbonylation was performed as indicated by Robinon[12], based on a combination of 2,4-dinitrophenylhydrazine (DNPH)

derivatization. Blanks were prepared by treatment with 20 mmol/L $NaBH_4$ and incubation at 37 ˚C for 90 min. Then samples and corresponding blanks were prepared at final concentration at 0.5 mg/mL by diluting in 70% trifluoroacetic acid. About 1 μL protein samples were slot-blotted onto a polyvinylidene difluoride membrane. Polyvinylidene difluoride membrane was incubated with 50 mL of 0.1 mg/mL DNPH in acetic acid for 15 min, then washed extensively in acetic acid (3×5 min) and immersed in a solution of 7% acetic acid and 10% methanol for 15 min at room temperature. Membrane was washed with deionized water four times for 5 min each. Then the membrane was incubated in SYPRO Ruby blot stain reagent for 15 min to determine protein loading. After washing with deionized water (3×1 min) fluorescence was monitored for quantification of the total protein loading. After that, membrane was blocked with 5% skim milk dissolved in 0.5 mmol/L Tris–HCl (pH 7.5), 150 mmol/L NaCl, and 0.1% Tween-20 for 1 h at room temperature. Further, it was incubated with the primary antibody anti-DNPH (Sigma-Aldrich, Singapore) at a 1:5 000 dilution overnight at 4 ˚C. After three washes with Tris-buffered saline with 0.1% Tween-20, it was incubated with secondary horseradish peroxidase conjugated goat anti-rabbit antibody (Sigma-Aldrich, Singapore) in Tris buffered saline with Tween with 5% skim milk at a 1:10 000 dilution for 1 h at room temperature. Slot blot detection was developed using an enhanced chemiluminescence detection substrate Immobilon TMWestern Chemiluminescent HRP Substrate (Millipore). Carbonylated proteins were visualized by the ChemiDoc™ XRS+ System and compiled with Image Lab™ 4.0.1 Software (Bio-Rad Laboratories) for quantification.

2.9. Measurement of TNF-α

Liver's TNF-α was determined with commercially available ELISA (Thermo Fisher Scientific, Pierce, USA) kits according to the manufacturers' instructions. Analysis of TNF-α were performed using a sandwich ELISA method. Briefly, 96-well plates were coated overnight at 4 ˚C with 100 μL of monoclonal antibody against TNF-α (1 μg/mL) in phosphate buffer solution (PBS) 1× (pH 7.2). The plate was then washed four times with wash buffer (PBS 1× +0.05% Tween-20), blotted dry, and then incubated with blocking solution (PBS 1× +1% bovine serum albumin) for 1 h. The plate was then washed and 100 μL of each homogenate sample or standard was added. Then the plate was incubated at room temperature for 2 h, followed by washing, and addition of 100 μL of detection antibody TNF-α (0.25 μg/mL). The antibody was incubated at room temperature for 2 h. Following additional washing, 100 μL of avidin conjugated with horseradish peroxidase (1:2 000) was added to each well, followed by a 30 min incubation. After thorough washing, plate development was performed using ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) liquid substrate solution. Then the plate was incubated at room temperature for color development and the color was monitored using a microplate reader at 405 nm with wavelength correction set at 650 nm. The standard curve for the ELISA was established by using murine standard TNF-α diluted in PBS 1× buffer. All standard curves obtained an r^2 value between 0.98 and 1. Results were normalized to total protein content in the liver samples, determined by Bradford's method[10]. Data were reported as pg TNF-α per mg protein. TNF-α standard curves were prepared in ELISA buffer, and samples from the tissue homogenates were calculated from these standard curves.

2.10. CAT activity determination

CAT activity was measured in triplicate according to the method of Aebi by monitoring the disappearance of H_2O_2 at 240 nm. A total of 30 μL homogenate was suspended in 2.5 mL of 50 mmol/L phosphate buffer (pH 7.0)[13]. Assay started by adding 0.5 mL of 0.1 mol/L hydrogen peroxide solution and absorbance at 240 nm was recorded every 10 seconds during 2 min and used to calculate CAT activity. Hydrogen peroxide solution was substituted by phosphate buffer in the negative control. CAT activity was determined by using the molar extinction coefficient 39.4 M^{-1} cm^{-1} for H_2O_2 and was expressed as nmol of hydrogen peroxide converted per min per mg total protein where 1 IU activity=1 μmoL H_2O_2 converted to H_2O per min.

2.11. SOD activity determination

Total SOD activity in tissue homogenates was determined following the procedure of Marklund and Marklund with some modifications[14]. The method is based on the ability of SOD to inhibit the autoxidation of pyrogallol. In 970 μL of buffer (100 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.2), 10 μL of homogenates and 20 μL pyrogallol 13 mmol/L were mixed. Assay was performed in thermostated cuvettes at 25 ˚C and changes of absorption were recorded by a spectrophotometer (EVO 210, Thermo-Fisher) in triplicate at 420 nm. One unit of SOD activity was defined as the amount of enzyme can inhibit the auto-oxidation of 50% the total pyrogallol in the reaction.

2.12. GPx activity determination

GPx activity was measured with a coupled enzyme assay[15]. The 1 mL assay mixture contained 770 μL of 50 mmol/L sodium phosphate (pH 7.0), 100 μL of 10 mmol/L GSH, 100 μL of 2 mmol/L nicotinamide adenine dinucleotide phosphate (NADPH), 10 μL of 1.125 mol/L sodium azide, 10 μL 100 IU/mL glutathione reductase and 10 μL homogenate. The mixture was allowed to equilibrate for 10 min. The reaction was started by adding 50 μL of 5 mmol/L H_2O_2 to the mixture and NADPH oxidation was measured during 5 min at 340 nm. One unit of glutathione peroxidase was defined as the amount of enzyme able to produce 1 μmol $NADP^+$ from NADPH per min. GPx activity was determined using the molar extinction coefficient 6 220 M^{-1} cm^{-1} for NADPH at 340 nm and reported as IU per mg total protein.

2.13. Statistical analysis

All results are expressed as mean±SEM. Serial measurements were analyzed by using Two-way ANOVA with Tukey's *post hoc* test using SigmaStat 3.5 program and figures were performed by

using SigmaPlot 10.0 program (Systat Software Inc). The critical significance level α was 0.050 and, then, statistical significance was defined as $P<0.05$.

3. Results

3.1. Damages in liver by CCl_4 administration

3.1.1. Hepatotoxicity

Serum ALT and AST activities were increased significantly in CCl_4-treated group (Group II) as compared with control group (Table 1). In Group III, ALT and AST activities were significantly decreased as compared to the CCl_4-treated group.

Table 1

Serum ALT and AST activities were changed significantly in mice receiving CCl_4.

Parameters	Group I	Group II	Group III
ALT (IU/L)	25.8±3.8	305.6±21.7*	45.4±24.6#
AST (IU/L)	19.4±4.2	289.3±23.2*	39.8±27.5#

*: Significantly different from control mice ($P<0.05$); #: Significantly different from CCl_4-treated mice ($P<0.05$).

3.1.2. Lipid peroxidation

Lipid peroxidation of biomembranes is one of the principal degenerative effects of free radicals. Figure 1 shows the amount of lipid peroxidation in the three groups of animals.

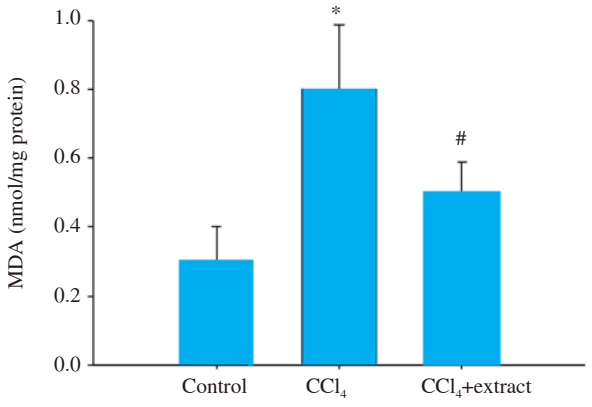

Figure 1. Effects of *T. scandens* extract on CCl_4-induced hepatic lipid peroxidation.
The bars represent the mean±SEM ($n=10$). *: Significantly different from control mice ($P<0.05$); #: Significantly different from CCl_4-treated mice ($P<0.05$).

There was a significant increase in the levels of MDA in CCl_4-treated rats. Treatment with extract significantly decreased the elevated levels of MDA in CCl_4-treated rats.

3.1.3. Protein oxidation: carbonyl group

Formation of carbonyl groups produces conformational and functional alterations in proteins, which can lead to a loss of enzymatic activity and to an enhanced susceptibility to proteolytic digestion[16]. Similar to the case of lipid peroxidation, the content of carbonyl groups was increased significantly by treatment of CCl_4 as showed in Figure 2. However, interestingly, in rats fed with *T. scandens* extract, the level of protein carbonyl group was reduced significantly.

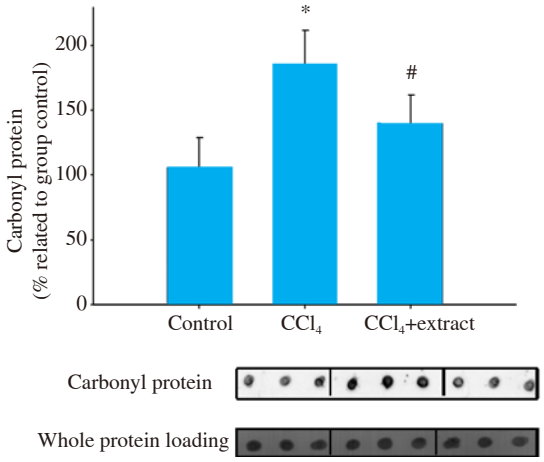

Figure 2. Effects of *T. scandens* extract on CCl_4-induced hepatic protein oxidation.
The bars represent the mean±SEM ($n=10$). *: Significantly different from control mice ($P<0.05$); #: Significantly different from CCl_4-treated mice ($P<0.05$).

3.1.4. TNF-α–marker of inflammation

TNF–α is considered as a special biomarker that reflects inflammatory status. The level of TNF-α was showed in Figure 3. CCl_4 significantly increased the level of this biomarker in rats liver. The treatment with *T. scandens* extract in Group III significantly reduced the levels of TNF-α.

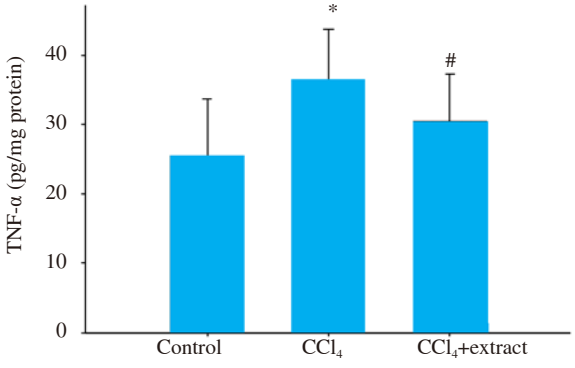

Figure 3. Effects of *T. scandens* extract on CCl_4-induced hepatic TNF-α.
The bars represent the mean±SEM ($n=10$). *: Significantly different from control mice ($P<0.05$); #: Significantly different from CCl_4-treated mice ($P<0.05$).

3.2. Antioxidant enzymes

Antioxidant enzymes are thought to be the first line of cellular defense that protects cellular components from oxidative damage. Among them SOD, CAT and GPx are important enzymes in the elimination of reactive oxygen species. Then, we measured SOD, CAT and GPx activities as an index of antioxidant status of liver tissues.

3.2.1. CAT activity

The CAT activity was showed in Figure 4. It was significantly decreased in CCl_4-treated rats compared to that in normal controls. However, activity of this enzyme was a near normal in rats treated with CCl_4 and extract.

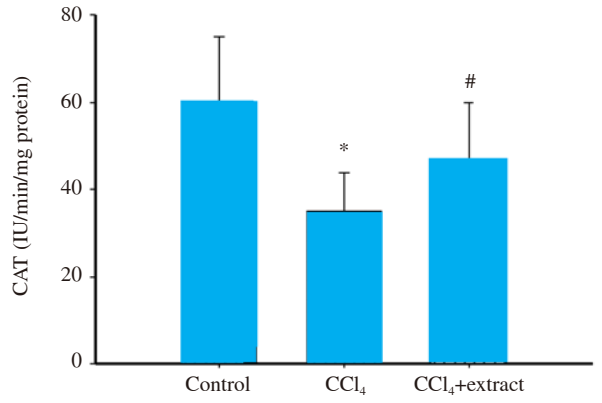

Figure 4. Effects of *T. scandens* extract on CCl_4-induced hepatic CAT activity.

The bars represent the mean±SEM (n=10). *: Significantly different from control mice (P<0.05); #: Significantly different from CCl_4-treated mice (P<0.05).

3.2.2. SOD activity

Total SOD activity was also decreased by CCl_4 as shown in Figure 5. Significantly lower activities of liver SOD were observed in CCl_4-treated group as compared to the normal control group. There were significant increases in SOD activity in the extract-treated groups compared to the CCl_4-treated group (P<0.05).

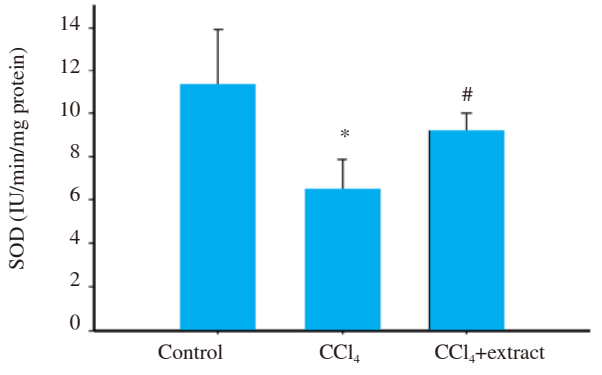

Figure 5. Effects of *T. scandens* extract on CCl_4-induced hepatic SOD activity.

The bars represent the mean±SEM (n=10). *: Significantly different from control mice (P<0.05); #: Significantly different from CCl_4-treated mice (P<0.05).

3.2.3. GPx activity

GPx is a group of important antioxidant enzymes that converts hydrogen peroxide and lipid peroxides to their corresponding alcohols whereas glutathione is oxidized to glutathione disulfide. Enzymatic activity of GPx showed a significant drop by CCl_4 as showed in Figure 6. This activity was also increased significantly by treatment with *T. scandens* extract.

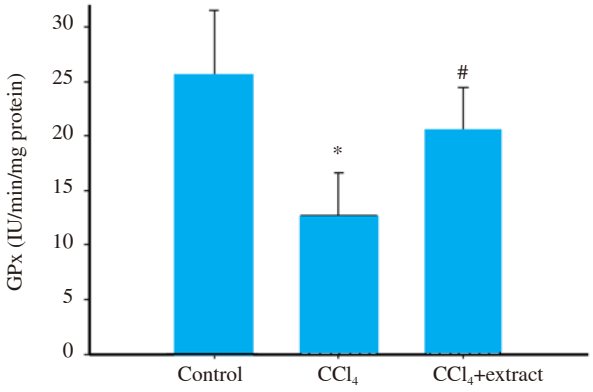

Figure 6. Effects of *T. scandens* extract on CCl_4-induced hepatic GPx activity.

The bars represent the mean±SEM (n=10). *: Significantly different from control mice (P<0.05). #: Significantly different from CCl_4-treated mice (P<0.05).

4. Discussion

The hepatotoxicity of $CC1_4$ is extensively investigated and it results in generation of damaging free radicals during the oxidation of this compound by hepatic enzyme. $CC1_4$ induced lipid peroxidation leading to changes of structures of the endoplasmic reticulum and other membranes, loss of metabolic enzyme activation and reduction of protein synthesis results in liver damage[17]. CCl_4 induced hepatic damage by generation of lipid peroxidation, decreasing activities of antioxidant enzymes and increasing the levels of free radicals[18]. Cytochrome P450 is the enzyme responsible for the conversion of CCl_4 to $CC1_3$ radical. Then, the toxic metabolite $CC1_3$ radical reacts with oxygen to give the chloromethyl peroxy radical. Those radicals bind covalently to macromolecules and cause peroxidative degradation of lipid membrane of hepatocytes. In the present study, we assessed the liver damage by measurement of serum ALT and AST level as markers of liver injury, level of MDA as an indicator of lipid peroxidation, carbonyl protein group as an indicator of protein oxidation and TNF-α levels as an indicator of inflammation.

First, in our study, CCl_4 developed significant hepatic damage in rats as presented by a significant increase in activities of AST and ALT. AST and ALT are markers of hepatocyte damage and reflect the severity of liver injury. Extract protects the rats from CCl_4-induced acute liver injury *in vivo*. After CCl_4 administration, serum ALT and AST levels in rats were dramatically higher than those in control group, and extract can reduce those levels. These results indicate that extract protects hepatocytes from damage induced by CCl_4 administration *in vivo*.

Second, lipid peroxidation products are formed when reactive oxygen species attack polyunsaturated fatty acids, leading to membrane structural and/or functional damage[2]. Lipid peroxidation conducts to the formation of highly reactive aldehydes which are

extremely diffusible and attack or form covalent links with farther cellular components. Markers of lipid peroxidation have been found to be elevated in liver fibrosis induced by CCl_4[19]. Among the many secondary products during lipid peroxidation, MDA is a commonly used biomarker for the assessment of lipid peroxidation[11]. MDA is a very highly reactive and toxic aldehyde formed as a consequence of peroxidation of polyunsaturated fatty acids. MDA can alter the membrane permeability as well as impair fluidity of the membrane lipid bilayer[11]. MDA is also the most mutagenic product of lipid peroxidation[20]. In this study, we have showed that the level of MDA, a marker of lipid peroxidation, was increased significantly in rats by administration of CCl_4, and in rats treated with the extract it can be decreased to nearly normal level.

Third, the level of carbonyl protein group is useful for measuring oxidative damage to proteins. The oxidative inactivation of enzymes by free radicals and the intracellular accumulation of oxidized proteins may play a critical role in the alteration of cellular function and cell death[21]. However, the damage effects of CCl_4 on cell proteins have not been studied well. Our data have showed that the administration of CCl_4 in rats increased the level of carbonyl protein group, and the level in animals treated with the extract can be nearly decreased to that in control group.

Fourth, CCl_4 induced liver injury is also associated with increased cytokine levels including TNF-α[22]. We evaluated the effects of extract treatment on the liver TNF-α level. TNF-α is one of the pro-inflammatory cytokines, which are early mediators of tissue damage and repair. The release of TNF-α is linked to cytotoxicity induced by CCl_4. Kupffer cells in liver produce TNF-α in rapid response to tissue injury[23]. We have demonstrated that the administration of CCl_4 in rats increased the levels of TNF-α and rats fed with extract can inverse significantly this level to that in control group.

CCl_4 increased damages in liver by raising the level of MDA, TNF-α and carbonyl group. Our data are in line with many previous reports[3,17,19,23]. Our finding showing that the *T. scandens* extract can protect against the oxidative stress led us to assess the possible antioxidant defense mechanism against oxidative hepatic damage.

The cells have an effective mechanism (the antioxidant system, such as SOD, CAT and GPx) to prevent and neutralize the free radical-induced damage. The lost of balance between reactive oxygen species production and antioxidant defense results in oxidative stress, leading to deregulation of the cellular functions. SOD, CAT and GPx are the main endogenous enzymatic defense systems against reactive oxygen species. SOD is the main antioxidant enzyme that catalyzes the conversion of superoxide anion ($O_2^{\bullet-}$ to H_2O_2) and protects cells and tissues from the reactive oxygen species generated from endogenous and exogenous sources. CAT is heme-containing enzyme that converts H_2O_2 to water and O_2, and it is largely localized in subcellular organelles such as peroxisomes, thus protecting the cell from oxidative damage by H_2O_2 and $OH^{\cdot}$. GPx belongs to a class of enzymes that catalyze the reduction of H_2O_2, phospholipid-hydroperoxide and other organic hydroperoxides. GPx removes H_2O_2 by coupling its reduction with the oxidation of reduced glutathione. GPx can also reduce other peroxides, such as fatty acid hydroperoxides. Our data have showed the decline in the activities of these enzymes in CCl_4-treated animals and their reversal to near normalcy in rats treated with CCl_4 and extract.

The nuclear factor erythroid 2–related factor 2 (Nrf2) is an important regulator of cellular resistance to oxidants. Nrf2 controls the activation of antioxidants enzymes by regulating their transcription[24]. Under basal conditions, Nrf2 is sequestered in the cytoplasm in association with the actin cytoskeleton, by Kelch-like ECH-associated protein-1. Upon oxidation, Nrf2 dissociates from Kelch-like ECH-associated protein-1, translocates to the nucleus and binds to antioxidant response elements, promotes the expression of Nrf2 target genes, and increases the effect of antioxidative enzymes, such as CAT, SOD and GPx[25]. Recent study demonstrated that glycyrrhetinic acid has hepatoprotective action upon CCl_4-induced chronic liver fibrosis due to its ability to promote Nrf2 nuclear transcription and enhance the Nrf2 target genes' expression, leading to decrease in the MDA content and increase in antioxidant SOD, CAT, GPx activities[26]. So, we suggest that *T. scandens* extract may have the similar mechanism; it is able to increase the activity of Nrf2 in tissues where it is dysregulated. Mechanisms involved in this effect need to be study in deep.

In summary, this study demonstrates that *T. scandens* extract had a protective effect against CCl_4-induced acute hepatic damage in rats. The hepatoprotective effect of *T. scandens* extract is likely due to its ability to scavenge free radicals and in combination with the ability to reduce inflammatory responses.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Liver is the key organ which metabolises most of the drugs and chemicals, and it plays important role in the detoxification of chemicals and drugs. *T. scandens* have been used in traditional medicine for the treatment of hepatitis. It is important to investigate whether this natural plant can protect liver in toxic-regent-induced acute hepatitis.

Research frontiers

The present research work depicts hepatoprotective activity of *T. scandens* extract against CCl_4-induced hepatic injury and assesses by estimating different biochemical paradigms and *in vivo* antioxidant parameters.

Related reports

CCl_4 is reported to cause hepatic necrosis due to formation of free radicals. This model is a classic animal model of acute hepatitis.

The traditional medicine has evidence of effectiveness of herbs in treating various liver disorders.

Innovations and breakthroughs

T. scandens extract is a medicinal plant used in various diseases. In the present study, authors have demonstrated the hepatoprotective activity of *T. scandens* extract in CCl_4-induced acute hepatitis in rat models.

Applications

From the literature survey, it has been found that *T. scandens* extract is safe to humans and good for oral administration. This scientific study supports and suggests the use of this plant as an drug along with commonly used hepatoprotective agent.

Peer review

This is a valuable research work in which authors have demonstrated the hepatoprotective activity of *T. scandens* extract in CCl_4-induced liver damage in rats. The activity was assessed based on biochemical parameters, antioxidant enzyme levels in liver homogenate. This traditional plant is found to be a promising hepatoprotective agent in CCl_4-indcued hepatitis in rat models.

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