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Phytochemical study and protective effect of *Trigonella foenum graecum* (Fenugreek seeds) against carbon tetrachloride-induced toxicity in liver and kidney of male rat

Sakhria Mbarki[a,*], Hichem Alimi[b], Hafsia Bouzenna[a], Abdelfettah Elfeki[a], Najla Hfaiedh[a]

[a] *Laboratory of Environmental pathophysiology, Development of bioactive molecules and mathematical modeling, Faculty of Science, 3018 Sfax, Tunisia*
[b] *Research unit of Macromolecular Biochemistry and Genetics, Faculty of Sciencces of Gafsa, 2112 Gafsa, Tunisia*

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ABSTRACT

Liver and kidney diseases are a global concern, therefore considerable efforts to obtain fine herbs useful as drugs from medicinal plants are currently in progress. The aim of this work was to study the antioxidant effects of previous supplementation with fenugreek seeds (FS) against carbon tetrachloride (CCl_4) toxicity in the liver and kidney. CCl_4 toxicity was induced by one dose (i.e. 5 ml CCl_4/kg of body weight, 50% CCl_4 in olive oil) after 7 weeks of normal diet or diet rich in 10% of grinded fenugreek seeds (20 g of pellet rat food/rat/day). 24 h after the treatment with CCl_4, all animals were scarified and biological analyses were performed. A phytochemical study of fenugreek seed extract (FSE) was also carried out. The phytochemical analysis of FS and FSE revealed the presence of polyphenols (5.92 ± 0.02 mg EGA/g DM), flavonoids (0.44 ± 0.19 mg ER/g DM), polysaccharides and trace elements. DPPH radical-scavenging activity of FSE showed an EC50 of 285.59 ± 2.01 μg/ml. *In vivo*, CCl_4 administration significantly ($p < 0.05$) induced an increase liver and kidney biomarkers. A significant ($p < 0.05$) alteration of the antioxidant enzyme activities was also observed. In animals pretreated with FS, the studied parameters were much less shifted. These results indicate that the supplementation with fenugreek seeds is significantly effective in protecting the liver and kidneys from CCl_4 toxicity.

1. Introduction

Carbon tetrachloride (CCl_4) was largely used as solvent in many industries, but now it is frequently used to induce oxidative stress in experimental animals [1]. In fact, the administration of carbon tetrachloride induces free radical generation in many tissues such as liver, testis, kidney, lung and blood [2–5]. The mechanism of liver injury induced by CCl_4 has received more attention than that induced by any other chemical [6]. Indeed, Al-Sayed and Abdel-Daim [7] and Al-Sayed et al. [8] found that the carbon tetrachloride induced a hepatotoxicity manifested by a significant change in the levels of hepatic biomarkers and an alteration of the status of antioxidant enzymes. CCl_4 is activated by CYP2E1, CYP2B or CYP3A to form various radicals, causing fatty degeneration, fibrosis, hepatocellular death, and carcinogenesis [9]. The metabolism of CCl_4 into trichloromethyl ($—CCl_3$) and peroxytrichloromethyl ($—OOCCl_3$) free radicals has been reported to cause damage to cell membranes, to change enzymes activity and to finally induce hepatic necrosis [9]. Kidney tissue has also a greater affinity for CCl_4 due to the predominant presence of the cytochrome P450 in the renal cortex, which intensified CCl_4 induced alteration in this organ [10]. Similarly, Ogeturk et al. [11] suggested that CCl_4 administration is in the origin of acute and chronic renal injuries.

Several studies were concerned with finding protective effects of many antioxidant molecules against CCl_4 induced damages. The seeds of fenugreek are commonly used in India and in oriental countries as a spice in food preparations due to their strong flavor and aroma and are reported to have restorative and nutritive properties and to stimulate the digestive process [12]. Fenugreek seeds are used as a traditional remedy for the treatment of diabetes [13]. Clinical and experimental studies have also documented antiatherosclerotic effects [14] of fenugreek seeds. Others Studies show that fenugreek seeds have antioxidant properties. Many findings show that supplementation of fenugreek seed powder in the diet leads to a reduction in biomarkers of oxidative damage in alloxan-diabetic rats [15]. Furthermore, polyphenols from

* Corresponding author at: Department of Biology, Faculty of Science, Sidi Ahmed Zarroug 2112 Gafsa, Tunisia.
E-mail address: mbarki_sakhria@yahoo.com (S. Mbarki).

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fenugreek seeds prevented oxidative hemolysis and lipid peroxidation induced by H_2O_2 in vitro in human erythrocytes [16]. Dixit et al. [17] reported that an extract of the germinated fenugreek seeds has the highest antioxidant activities in vitro. The aqueous extract of fenugreek seeds also reduces body weight gain, which is induced by high fat-diet and ameliorates dyslipidemia in obese rats [18]. Fenugreek oil has a protective effect against the toxicity of acrylamide through its free radical scavenging and potent antioxidant activities [19]. Fenugreek has also a beneficial action and antioxidant effects against the toxicity of CCl_4 [20] and other toxins such as deltamethrin [21], aluminum [22] and cadmium [23].

In this study, using the carbon tetrachloride-induced injury model, we investigated the protective actions of fenugreek. The serum levels of transaminases (AST, ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatinine, urea and proteins were measured. Blood parameters, lipid peroxidation and the activity of the antioxidant enzymes (superoxide dismutase (SOD), glutathione peroxidase (GPx) and Catalase (CAT)) in the extracts of liver and kidney were also investigated. In addition, a phytochemical study was conducted in order to identify some bioactive compounds from fenugreek and to evaluate their antioxidant effects *in vitro* and *in vivo*.

2. Materials and methods

2.1. Chemicals

All the chemicals are of analytical grade and were purchased from E-Merck (Germany), Sigma Aldrich, Sharlau, NORMAPUR, BIOTECH, Panreac, CHIMICA PLUS, etc.

2.2. Phytochemical study of fenugreek seed extract (FSE)

2.2.1. Plant extraction

The dried seeds of *Trigonella foenum-graecum* L. were finely grinded in an electric grinder and extracted by soxhlet apparatus with ethanol (95°) until completely exhausted. Ethanol was evaporated by a rotary evaporator. The yield was determined as 4.95% (w/w).

2.2.2. UV-Vis spectrophotometer

A fraction of FSE was diluted in 1 ml methanol then it was scanned using UV–vis Spectrophotometer (Analytic Jenna, Nov AA 400), at a range of 200–800 nm, in order to detect the maximum of flavonoid and phenolic compounds.

2.2.3. HPLC analysis conditions

The High-performance liquid chromatography (HPLC) analysis of the FSE was carried out using a Varian ProStar HPLC system equipped with a ProStar 230 ternary pump, a manual injector and a ProStar 330 diode array detector were used as well. The chromatographic analyses were performed on a 5 μm particle C-18 reversed-phase column (Varian, 250 × 4.6 mm). Final chromatographic conditions were a gradient elution made up from solvent A: 2% acetic acid aqueous solution and solvent B: H_2O/Acetonitrile/acetic acid (58:40:2). The flow rate was 1 ml/min and the injection volume was 20 μl at 25°C. The identification of phenols and flavonoids was based on the comparison with the retention times of the peaks in the injected sample extracts to those of HPLC standard compounds.

2.2.4. Determination of total phenolic content

The determination of total polyphenols was performed according to the method of Folin and Ciocalteu [29]. The absorbance was measured at 765 nm. The total polyphenol content was determined by using the calibration equation of gallic acid (25–100 μg/ml) which was used as a phenol standard: $Y = 0.0096\ X + 0.0244$ ($R^2 = 0.9966$); Where X is the concentration of gallic acid expressed in mg/ml and Y is the absorbance at 765 nm. The flavonoid rate was expressed as mg of equivalent of gallic acid per gram of dry matter (mg EGA /g DM). The phenol assay test was repeated three times.

2.2.5. Determination of total flavonoid content

Total flavonoid content was determined according to the method described by Zhishen et al. [30]. The absorbance was measured at 510 nm. Rutin (25–400 μg/ml) was used as a standard flavonoid to produce the calibration curve, giving the following equation: $Y = 0.0014\ X + 0.0325$ ($R^2 = 0.9973$); Where X is the concentration of rutin expressed in mg/ml and y is the absorbance at 510 nm. The flavonoid rate was expressed as mg of equivalent of rutin per gram of dry matter (mg ER /g DM). The flavonoid assay test was repeated three times.

2.2.6. DPPH radical-scavenging activity

The free radical scavenging activity of FSE was evaluated with the DPPH radical assay according to the method reported by Grzegorczyk et al. [31]. The absorbance was measured at 517 nm. All analyses were run in triplicate and the values were averaged. Radical scavenging activity (RSA) was expressed as the inhibition percentage calculated using the formula: $RSA\% = [1-(Abs_{sample} - Abs_{control}/Abs_{DPPH})] \times 100$; where A_{DPPH} is the absorbance of DPPH solution without sample extract, A_{sample} is the absorbance of sample extract mixed with DPPH solution and $A_{control}$ is the absorbance of the sample extract tested without DPPH. Ascorbic acid was used as standard antioxidant and the results were presented as the percent change of the RSA in function of the concentration. The EC_{50} value was defined as the concentration (in μg/ml) of the FSE required for scavenging 50% of DPPH radical formation.

2.3. Phytochemical study of fenugreek seeds (FS)

2.3.1. Extraction of polysaccharides

The extraction method of polysaccharides was performed as follows: the powdered fenugreek seeds were extracted with boiled distilled water (1:15, w/v) under magnetic stirring for one hour. The residue was removed by centrifugation (12000*g*/10 min). The polysaccharides dissolved in the supernatant are then precipitated by the addition of absolute ethanol (1:4, v/v) overnight at 4°C. The solution was then centrifuged at 4500*g* for 10 min. The resulting precipitate was again dissolved in 20 ml of distilled water and the proteins were removed by the Sevag reagent (chloroform/butanol 4:1, v/v) by the method described by Navarini et al. [32]. The resulting fraction was centrifuged (4000 g/5 min) and the polysaccharides dissolved in the supernatant were again precipitated with absolute ethanol, recovered by centrifugation (10000*g*/5 min) and dried at 60°C overnight.

2.3.2. Fourier-transform infrared spectra analysis

Fourier transform infrared (FT-IR) spectra of the fenugreek polysaccharides (FP) was obtained using a Shimadzu 8400S FT-IR Spectrophotometer, equipped with IR solution 1.10 Shimadzu software in the range of 4000–500 cm^{-1}. FT-IR scans were collected on completely dried thin films of the FP cast on KBr discs. The spectra covered the infrared region 4000–500 cm^{-1}, the number of scans per experiment was 10 and resolution was 6 cm^{-1}.

2.3.3. Mineral element analysis

The determination of mineral element content (Zn, Cu, K and Na) in FS was performed using a Flame atomic absorption