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Potential antioxidant properties and hepatoprotective effects of *Juniperus phoenicea* berries against CCl_4 induced hepatic damage in rats

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ABSTRACT

Objective: To investigate the antioxidant and hepatoprotective properties of *Juniperus phoenicea* (*J. phoenicea*) berries against CCl_4-induced oxidative damage in rats.
Methods: Hepatotoxicity was induced in albino Wistar rats by single dose of CCl_4 dissolved in olive oil (1 mL/kg BW, 1/1 in olive oil, *i.p.*). Aqueous extract of *J. phoenicea* berries (AEJP) was administered at the dose of 250 mg/kg/day by gavage for 12 days.
Results: Obtained results revealed that administration of CCl_4 caused a significant increase in plasma ASAT, ALAT, ALP and LDH activities and total bilirubin concentration, compared to the control group. While, albumin and total protein concentration were significantly lower. Additionally, a significant decrease in the level of hepatic GSH, GPx and GST activities associated with a significant increase of MDA content in CCl_4 group than those of the control. However, the treatment of experimental rats with AEJP prevented these alterations and maintained the antioxidant status. The histopathological observations supported the biochemical evidences of hepatoprotection.
Conclusions: The results of the present investigation indicate that *J. Phoenicea* possesses hepatoprotective activity and this effect was may be due to its antioxidant properties.

1. Introduction

The liver as a vital organ has a wide range of functions in the body, including detoxification, plasma protein synthesis, and glycogen storage. Oxidative stress is considered as the imbalance between reactive oxygen species (ROS) production and antioxidant protective mechanism. It is principal cause of the development of hepatic disorders [1]. Various types of liver disorders are characterized by cirrhosis, jaundice, tumors, metabolic and degenerative lesions and liver cell necrosis *etc* [2]. The management of liver disorders is still a challenge to the modern medicine [3]. Conventional or synthetic drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects [4]. Carbon tetrachloride (CCl_4) is a highly toxic chemical and a well known hepatotoxin used extensively to investigate the hepatotoxicity in animal models [5]. CCl_4 by itself does not have cytotoxic effects on the liver but its metabolic products such as generated trichloromethyl free radicals are responsible for the toxicity and the production of lipid peroxidation [6]. It has

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been reported that antioxidants appear to act against disease processes by increasing the levels of endogenous antioxidant enzymes and decreasing lipid peroxidation [7]. Lots of studies indicate that natural substances from edible and medicinal plants exhibited strong antioxidant activity that could act against CCl_4-induced liver damage, because they contain lots of free radical scavenger such as phenolic acids and flavonoid compounds [8].

The genus *Juniperus* belongs to the Cupressaceae family, comprising about 67 species all over the world [9]. Plants from the *Juniperus* genus have found application in different European cuisines as a spice, flavouring for alcoholic drinks, as well as in cosmetics [10]. Furthermore, these plants have an extensively history of use in global folk medicine for various disorders, such as common colds, urinary and kidney infections and dermatological disorders [11]. Among their species, *Juniperus phoenicea* (*J. phoenicea*) is an evergreen tree indigenous to the North Africa. In Algeria, *J. phoenicea* grows on the steppes, commonly known as 'Arar'. This plant is considered as an important medicinal plant largely used in the Algerian folk medicine as a diuretic, stimulative, stomach tonic, pulmonary and depurative disinfectant. A decoction of the leaves and berries has been used to treat diarrhea, rheumatism, and diabetes. Previous phytochemical studies reveal that *J. phoenicea* contains a large variety of compounds, mainly diterpenoids, biflavonoids, lignans, phenyl propane glucosides, two furanone glucosides, norterpene and sesquiterpene glucosides [9]. There are many papers report on the chemical composition of leaves and berries essential oils of *J. phoenicea* grown in north Mediterranean basin [12]. However, little attention has been given to the phenolic contents, antioxidant and biological activities of *J. phoenicea* berries. Therefore; the aim of the present study was to evaluate the antioxidant and hepatoprotective properties of *J. phoenicea* berries against CCl_4-induced oxidative damage in rats.

2. Materials and methods

2.1. Chemicals

Carbon tetrachloride and all other chemicals and reagents used in the study were obtained from Sigma-Aldrich Corporation (St. Louis, Missouri, USA).

2.2. Plant material

The plant material consisted of mature 'berries' of *J. phoenicea*, collected in month of March 2013, from Aouinet province of Tebessa (Algeria). The identity of plant was confirmed by Professor A. Chefrour in the botany laboratory, Department of Pharmacy, Faculty of Medicine, Badji Mokhtar University, Annaba, Algeria. The berries were cleaned, dried in shade and powdered then stored in air tight container.

2.3. Preparation of plant extracts

2.3.1. Preparation of aqueous extract

Five grams of the dried berries powder of *J. phoenicea* were boiled in 50 mL of distilled water and heated for 15 min. The extract was then filtered through Whatman filter paper and directly administered orally by gavage to the animals at a volume of 250 mg/kg body weight (BW).

2.3.2. Preparation of methanol extract

Twenty grams of the dried berries powder of *J. phoenicea* were extracted at room temperature for 72 h, in 100 mL methanol (85%) three times. The hydro-alcoholic extract was filtered through Whatman filter paper, and stored at 4 °C. The filtrate was concentrated under reduced pressure at 60 °C. The extract was weighed and stored at 4 °C in storage vials.

2.4. Chemical characterization

2.4.1. Total phenolic contents

The total phenolic content was determined by the Folin-Ciocalteu method of Waterman and Mole [13]. A volume of 10 μL of the AEJP and MEJP was mixed with 50 μL of Folin-Ciocalteu reagent. After 5 min, 150 μL of 20% Na_2CO_3 was added and the mixture was shaken once again for 1 min. Finally, the solution was brought up to 790 μL by adding distilled water. After 90 min, the absorbance was measured at 760 nm and the total phenolic content was calculated from the calibration curve using gallic acid as a standard. The results were expressed as mg of gallic acid equivalent per g (mg GAE/g) of dry weight DW of extract.

2.4.2. Total flavonoid contents

Total flavonoid contents were measured by a colorimetric assay according to the method of Zhishen *et al* [14], and quercetin was used as a standard to construct the calibration curve. Briefly 250 μL of extracts were mixed with 1.25 mL of distilled water and 75 μL of 5% $NaNa_2$ solution. After 6 min, 150 μL of 10% $AlCl_3$ solution were added. 6 min later, 0.5 mL of 1M NaOH solution were added and then the final volume was adjusted to 2.5 mL with distilled water and mixed thoroughly. The absorbance was measured at 510 nm versus a blank prepared without extract and data were expressed in mg quercetin equivalent per g (mg QE/g) of DW of extract.

2.4.3. Total condensed tannin contents

Condensed tannins were determined according to the method of Julkunen-Tiitto [15]. A volume of 50 μL of extracts was added to 1.5 mL of 4% vanillin solution in methanol, and then 750 μL of HCl were added. The mixture was allowed to stand in the dark for 20 min, and absorbance was measured at 500 nm against methanol as a blank. Catechin was used to make the standard curve and the amount of total condensed tannins is expressed as mg catechin equivalent per g (mg CE/g) of DW of extract.

2.5. In vivo study

2.5.1. Animals and treatments

Twenty-four male albino Wistar rats (aged between 8 and 9 weeks) with an average weighing (180–200) g were provided from Pasteur Institute (Algiers, Algeria). They were housed in cages at room temperature of (22 ± 2) °C and kept under standard conditions of a 12 h light/dark cycle and minimum relative humidity of 40%. The rats were fed with a standard food supplied by (ONAB, El harrouch, Algeria) and water was offered *ad*