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Original article

# Curcumin improves liver damage in male mice exposed to nicotine

Mohammadreza Salahshoor [a], Sabah Mohamadian [b], Seyran Kakabaraei [c], Shiva Roshankhah [a], Cyrus Jalili [a, *]

[a] Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran
[b] Student of medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran
[c] Anatomy Department, Kermanshah University of Medical Sciences, Kermanshah, Iran

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ABSTRACT

The color of turmeric (薑黃 jiāng huáng) is because of a substance called curcumin. It has different pharmacological effects, such as antioxidant and anti-inflammatory properties. Nicotine is a major pharmacologically active substance in cigarette smoke. It is mainly metabolized in the liver and causes devastating effects. This study was designed to evaluate the protective role of curcumin against nicotine on the liver in mice.

Forty-eight mice were equally divided into eight groups; control (normal saline), nicotine (2.5 mg/kg), curcumin (10, 30, and 60 mg/kg) and curcumin plus nicotine-treated groups. Curcumin, nicotine, and curcumin plus nicotine (once a day) were intraperitoneally injected for 4 weeks. The liver weight and histology, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and serum nitric oxide levels have been studied.

The results indicated that nicotine administration significantly decreased liver weight and increased the mean diameter of hepatocyte, central hepatic vein, liver enzymes level, and blood serum nitric oxide level compared with the saline group ($p < 0.05$). However, curcumin and curcumin plus nicotine administration substantially increased liver weight and decreased the mean diameter of hepatocyte, central hepatic vein, liver enzymes, and nitric oxide levels in all groups compared with the nicotine group ($p < 0.05$).

Curcumin demonstrated its protective effect against nicotine-induced liver toxicity.


## 1. Introduction

A large and increasing number of patients in the world use medicinal plants and herbs for health purposes.[1] *Curcuma longa* (turmeric; 薑黃 jiāng huáng), one of the oldest plants, belongs to the Zingiberaceae family, which has long been used in traditional medicine for blood purification, digestion, arthritis treatment, liver protection, and as an anti-inflammatory agent.[2] The color of turmeric is because of a chemical called curcumin, which comprises 3–4% of tumeric.[3] Numerous studies have reported the antioxidant properties, anti-mutation and antitumor effects, and carcinogenic characteristics of curcumin.[4] Curcumin affects the metabolism of arachidonic acid by inhibiting the phosphorylation of phospholipase A2 (PLA2), decreasing the expression of cyclooxygenase (COX)-2 gene and inhibiting the catabolic activity of COX-5. These effects induce the anti-inflammatory activity of curcumin.[5] In addition, curcumin decreases the expression of different inflammatory cytokines such as interleukin (IL)-1, tumor necrosis factor (TNF)- α, IL-6, and chemokines.[6] Curcumin shows efficacy in promoting wound healing as well as treating liver ailments, urinary tract diseases, and hepatitis.[7] Pharmacologically, curcumin exhibits a wide range of effects including anti-inflammatory, hypo-cholesterolemic, and anti-infection activities and as well as anticarcinogenic effects.[8,9] Although approximately 4000 components are present in the cigarette, nicotine is a highly toxic organic compound containing nitrogen and alkaloid, which are mostly found in tobacco, and it is responsible for some of the deleterious effects of smoking.[10] Nicotine affects a variety of cellular processes including altered gene expression.[11] Exposure to nicotine produces oxidative

* Corresponding author. Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.
*E-mail address:* cjalili@yahoo.com (C. Jalili).
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tissue injuries in the mouse, often resulting in a depletion of glutathione content and a decrease in the activity of some oxygen free radical scavengers, such as catalase and superoxide dismutase.[12] The liver is considered to be the major site of nicotine biotransformation, and nicotine exerts a number of adverse physiological effects on the liver.[13] Nicotine is absorbed through the lungs during smoking and is rapidly metabolized in the liver, which induces three major adverse effects on the liver: toxic (direct or indirect), immunological, and oncogenic.[14] Smoking causes liver cell injury and exerts genotoxic effects on rat liver.[15] Current studies on curcumin effects have not reported on the protective effect of curcumin against nicotine; therefore, the current study was conducted to analyze the protective effect of curcumin to offset the damage induced by nicotine in the liver of male mice.

## 2. Materials and methods

### 2.1. Chemicals

Curcumin (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; C21H20O6) powder (Merck, Germany) was purchased, The powder was dissolved in absolute ethanol (C2H5OH) and diluted with normal saline (0.9%) to prepare different doses. Also, the nicotine solution (1-metyl-2-3-pyridel-pirolidin; C10H14N2, Merck, Germany) was also purchased and was diluted by normal saline (0.9%) for administration (Fig. 1).

### 2.2. Experimental protocol

The mice were randomly divided into eight groups of six: control group (normal saline; 1 mL distilled water/daily); nicotine-treated group (2.5 mg/kg); 3) nicotine + curcumin 10 mg/kg treated group; nicotine + curcumin 30 mg/kg treated group; nicotine + curcumin 60 mg/kg treated group; curcumin 10 mg/kg treated group; curcumin 30 mg/kg treated group; and curcumin 60 mg/kg treated group. Nicotine was administered intraperitoneally once a day for 4 weeks. Curcumin and nicotine plus curcumin were administered intraperitoneally in animals.[16–18]

### 2.3. Animals

Forty-eight Balb/c male mice with weight range of 27–30 g were purchased from Tehran Razi Institute. Animals were kept at the temperature of 22 ± 2°C, under controlled environmental conditions, 12/12 hours light/dark cycle and free access to water and food. Maintenance and care of experimental animals complies with National Institutes of Health guidelines.[19] Experiments were designed to conform with the International Guiding Principles for Biomedical Research Involving Animals (1985).

### 2.4. Liver weight and collection of blood serum

At the end of the experimental period, all animals were deeply anesthetized with ether. Blood was collected from the right ventricle, and serum separated and stored at −80°C for measurement of nitric oxide. They were then killed. Livers were removed and weighed on a microbalance sensitive to 0.001 mg (Precisa 125A, Switzerland) and recorded.[20]

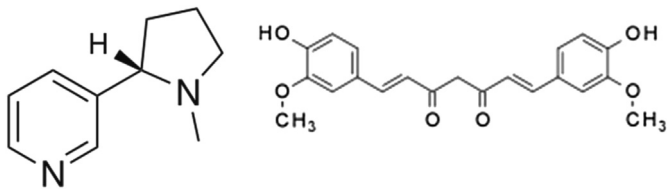


**Fig. 1.** Structure of nicotine and curcumin.

### 2.5. Histological analysis

For the histological evaluation of the hepatic structures, the lower 1-cm-long part of the right lobe of the liver in transverse pieces was removed, washed in saline, and fixed in 10% formalin at room temperature for 72 hours. After tissue fixation, it was thoroughly washed under running water and dehydrated in ascending concentration ethanol, cleared in xylene, and then embedded in soft paraffin. Thin sections (5 μm) were cut using a microtome (Leica RM 2125, Leica Microsystems Nussloch GmbH, Germany) and stained with hematoxylin and eosin. The preparation was examined with an Olympus BX-51T-32E01 research microscope connected to a DP12 Camera with 3.34-million pixel resolution and Olysia Bio software (Olympus Optical Co. LTD, Tokyo, Japan).[21]

### 2.6. Morphometric measurements

For each hepatocyte, the total cellular area was measured. The outline of each hepatocyte was measured after taking an image with a 40 × objective lens. The longest and shortest axis were measured in the drawing of each hepatocyte in order to estimate the mean diameter (mean axis). At least 50 hepatocytes from each zone (total, 100) were measured in each liver. A separate measurement for central hepatic vein was performed, using the same methodology.[22,23]

### 2.7. Griess assay

Nitric oxide was measured based on Griess colorimetric assay. Accordingly, N-1-naphthylenediamine (NEED), sulfonamide solutions, and nitrite standards were prepared. To measure nitrite concentration in serum, after defreezing the serum samples, 100 μL of the sample serum was deproteinized by zinc sulfate and transferred to the wells. One hundred microliters of chloride vanadium, 50 μL sulfonamide, and 50 μL NEED solutions were added afterward. The samples were incubated in the temperature of 30°C in darkness. The optical density of samples was measured using an enzyme-linked immunosorbent assay reader (Hyperion, Germany) at the wavelength of 540 nm.[24]

### 2.8. Biochemical analysis

The liver was minced and homogenized (10% w/v) in ice-cold 0.1M sodium phosphate buffer (pH 7.4). The homogenate was centrifuged twice at 10,000 rpm for 15 to 20 min at 4°C to obtain enzyme fraction. The resultant supernatant was used for various biochemical assays. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed using the method of Reitman and Frankel.[25] Activities of alkaline phosphatase (ALP) were determined according to the protocol described in the laboratory practical manual.[26]

### 2.9. Statistical analysis

All the quantitative data were presented as mean ± standard deviation. One-way analysis of variance followed by least significant difference *post hoc* test were performed to determine the statistical significance between different groups using SPSS software package 16.0 (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, Illinois, USA). A $p$ value $<0.05$ was considered significant.