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Original Article

Hepatoprotective effects of *Solanum nigrum* against ethanol-induced injury in primary hepatocytes and mice with analysis of glutathione S-transferase A1

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Abstract

Background: *Solanum nigrum* is a herbaceous perennial plant, which is widely used in traditional medicine systems for its antioxidant, anti-ulcerogenic, antitumorigenic, and anti-inflammatory characteristics. The purpose of this study was to investigate the protective effects of *S. nigrum* against alcoholic liver damage in primary hepatocytes and mice, using glutathione S-transferase alpha 1 (GSTA1) as an indicator.
Methods: Primary hepatocytes were obtained by the inverse perfusion method improved on Seglen two-step perfusion *in situ*.
Results: In the presence of *S. nigrum* aqueous extracts (100 μg/mL), no hepatocytic damage was observed in cells treated with ethanol, compared with the model group, and GSTA1 ($p < 0.01$) was more sensitive than alanine aminotransferase and aspartate aminotransferase ($p < 0.05$). Mice that received *S. nigrum* aqueous extracts (150 mg/kg) with ethanol showed marked attenuation of ethanol-induced hepatotoxicity, as evidenced by significant reductions of serum transaminases ($p < 0.01$), and variation of hepatic oxidative indices ($p < 0.05$) and GSTA1 ($p < 0.05$), compared with the model group and mice that received *S. nigrum* aqueous extracts (200 mg/kg). All the detection indexes were significantly different ($p < 0.01$) from those of the model group, and the protective effects were almost the same as that of the positive drug group.
Conclusion: These results suggested that *S. nigrum* has hepatoprotective effects against ethanol-induced injury both *in vitro* and *in vivo*, and can protect the integrity of hepatocytes and thus reduce the release of liver GSTA1, which contributes to improved liver detoxification.

Keywords: ethanol; glutathione S-transferase alpha 1; hepatic injury; primary hepatocytes; *Solanum nigrum*

1. Introduction

Solanum nigrum (SN) is a species in the family Solanaceae, native to Eurasia and introduced in America and Australia.[1] It is an herbaceous perennial plant, which is widely used in traditional medicine systems for its antioxidant, anti-ulcerogenic, antitumorigenic, and anti-inflammatory characteristics.[2] The plant has two significant alkaloids, solamargine and solasonine, that produce carbohydrate glycone solasodine, which is bioactive and in great demand in the pharmaceutical industry.

Alcoholic liver damage is one of the most common hepatic injuries worldwide. However, ethanol abuse and dependence are becoming increasingly more serious and are presently a topic of significant discussion associated with liver injury.[3] Excessive consumption of ethanol can cause a series of hepatic injuries, contributing to the development of alcoholic

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liver diseases, which are characterized by fat accumulation and inflammation in the liver, leading to cirrhosis and hepatocellular carcinoma.[4] Progression of alcoholic liver diseases is a multifactorial process, which involves a number of genetic, nutritional, and environmental factors.[5] Presently, there is substantial evidence that oxidative stress is involved in the pathogenesis and progression of liver injuries.[6] Ethanol intake increases the production of free radicals or reactive oxygen species and causes oxidative stress by compromising the antioxidant defense system.[7]

Glutathione S-transferases (GSTs) are widely used Phase II drug-metabolizing enzymes that have the function of detoxification in humans and animals. They are the most important part of the antioxidant defense system in an organism, contributing to the resistance against attacks of electrophilic substances and catalyzing the reaction of exogenous chemicals to protect organisms.[8] GSTs are usually divided into eight superfamilies, alpha (A), kappa (K), mu (M), pi (P), sigma (S), theta (T), zeta (Z), and omega (O), where the dominant component in the human liver is alpha class [alpha-GST (GSTA)].[9] GSTA is encoded by a P12 gene cluster located in chromosome 6, which contains five subunits (GSTA1−A5). GST alpha 1 (GSTA1) is a predominant member of the GSTA family[10] and accounts for 65−75% of the total GSTs in the human liver. It plays an important role in the antioxidative defense system, which can catalyze many xenobiotics such as carcinogens, environmental toxins, and certain drugs; it can also combine with glutathione (GSH), promoting its degradation in the cells to remove and protect the body.[11] With some efficacy, therefore, GSTA1 can be used as a marker of liver injury. The changes of GSTA1 can be detected at a low level during the early stage of acute hepatic injury, and GSTA1 is a more sensitive and accurate indicator than alanine aminotransferase (ALT).[12]

The present study aimed to investigate whether SN actually has hepatoprotective effects against ethanol-induced hepatic injury *in vivo* and *in vitro,* and further explore the role of GSTA1 in liver detoxification as adjusted by SN. Regulation of GSTA1 might aid in the prevention of hepatopathy and the development of new drugs.

2. Methods

2.1. Reagents

The ethanol used in our study was purchased from the Shanghai Chemical Reagent Factory (Shanghai, China). Type IV collagenase, dimethyl sulfoxide, insulin, transferrin, heparin, dexamethasone, and trypan blue were purchased from the Sigma Chemical Co. (St Louis, MO, USA). GSTA1 detection kit was purchased from American Rapidbio Company (RB; 23830 Arminta Street, West Hills, CA 91304, USA). SN and silymarin were purchased from Harbin Jiacheng Dispensary (Harbin, China). The detection kits of ALT, aspartate aminotransferase (AST), malondialdehyde (MDA), superoxide dismutase (SOD), GSH, and glutathione peroxidase (GSH-Px) were obtained from the Nanjing Jiancheng Institute of Biotechnology (Nanjing, China).

2.2. Primary hepatocyte extraction and culture

The liver of mice was rinsed and digested by the use of the inverse perfusion method improved on Seglen two-step perfusion *in situ*. After washing three times with Hank's balanced salt solution, the liver was put into emundans culture medium to end the digestion and extract cells gently with tweezers. The hepatocytes were mixed fully with adherent culture medium after filtration and centrifugation (500 rpm, 3 minutes). The number and survival rate of hepatocytes were calculated after a trypan blue dyeing experiment. The hepatocytes were seeded to a culture plate containing 24 wells (5×10^4 cells/well) with rat tail collagen under the conditions of 5% CO_2 and 37°C for 6 hours, and then the nonadherent hepatocytes were discarded along with the culture fluid. The adherent culture medium was replaced with a growing culture medium without serum, after which the growing culture medium was substituted with the same culture medium after 24 hours.

2.3. Animals and treatment

Adult male Kunming mice (18−22 g body weight) were obtained from the Central Laboratory of Harbin Pharmaceutical Group Co., Ltd (Harbin, Heilongjiang Province, China). The animals were housed in a controlled environment under standard conditions at a temperature of 20 ± 2°C and a relative humidity of 40−60%; they were allowed free access to food (standard mice pellets) and water, and were acclimatized for at least 1 week prior to use. All procedures involving animals complied with the China National Institutes of Healthy Guidelines for the Care and Use of Laboratory Animals.

2.4. Preparation of SN aqueous extracts

The powder of SN (800 g) was dipped into 5000 mL water for 30 minutes, and then extracted continuously for 40 minutes at 100°C to remove most of the colored materials, oligosaccharides, and small-molecule compounds. The supernatant was thereafter concentrated using a rotatory evaporator at 90°C and dried by a vacuum drying oven at 70°C to afford SN aqueous extracts 185 g. The SN aqueous extracts accounted for 23% of the original herb of *Solanum nigrum.*

2.5. Hepatoprotective activities against ethanol-induced cytotoxicity

The hepatocytes were randomly divided into five groups ($n = 6$): control group, ethanol model group, high-concentration group of SN aqueous extracts (SN-H, 100 μg/mL), middle-concentration group of SN aqueous extracts (SN-M, 75 μg/mL), and low-concentration group of SN aqueous extracts (SN-L, 50 μg/mL), respectively. The control and ethanol model groups were treated with an equal volume of culture medium. After 12 hours, we replaced the culture medium, and each group was administered the culture medium with 100 mmol/L ethanol except for the control group. After