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Original Article

Hepatoprotective effects of naturally fermented noni juice against thioacetamide-induced liver fibrosis in rats

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Abstract

Background: Excessive reactive oxygen species (ROS) can result in inflammation and cytokine secretion in the liver, and then activate hepatic stellate cells that cause the accumulation of extracellular matrix proteins, especially collagen, in liver tissue. Naturally fermented noni juice (NJ; *Morinda citrifolia*) has been used for decades as a nutraceutical in humans. In this study, we intended to examine if NJ can ameliorate ROS-induced liver fibrosis via a thioacetamide (TAA)-induced rat model.

Methods: The 50 rats used in this study were separated into five groups of 10 rats each for 8 weeks as follows: (1) control group; (2) TAA; (3) TAA + low-dose NJ (2.51 mL NJ/kg); (4) TAA + medium-dose NJ (5.02 mL NJ/kg); and (5) TAA + high-dose NJ (7.52 mL NJ/kg).

Results: Treatment with TAA resulted in lower body weight and serum lipid levels ($p < 0.05$), while liver weight and collagen contents, and serum alanine aminotransferase and aspartate aminotransferase values were increased ($p < 0.05$). The protective effects of NJ on TAA treatment resulted from decreased endoplasmic reticulum stress-related gene expressions ($p < 0.05$), inflammatory cytokines, collagen accumulation, and matrix metalloproteinase (MMP-2 and MMP-9) activities, as well as upregulated ($p < 0.05$) tissue inhibitors of metalloproteinase (TIMP-1 and TIMP-3) in livers. NJ also increased hepatic antioxidant capacities ($p < 0.05$).

Conclusion: Naturally fermented NJ manifests a protective potential on liver fibrosis via the enhancement of antioxidant capacities, as well as decreasing endoplasmic-reticulum stress and MMP-2/MMP-9 activities.

Keywords: antioxidant capacity; ER stress; liver fibrosis; MMP-2/MMP-9 activity; noni juice

1. Introduction

Most chronic liver injuries including alcoholic disorder, viral hepatitis, biliary obstruction, or hemochromatosis consequently lead to hepatic fibrosis, a critical step which is instrumental in deciding the clinical outcome of liver disease.[1] The liver can function to facilitate the biochemical conversion of administered substances which significantly increase reactive oxygen species (ROS) generation.[2] A single dose of

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thioacetamide (TAA), a hepatotoxic agent, could produce centrilobular hepatic necrosis, while a chronic administration can lead to fibrosis or cirrhosis.[3] It is assumed that oxidative stress contributes to the development of TAA-induced liver fibrosis.[4] It has also been suggested that ROS is one of the important factors in cytokine-induced liver fibrogenesis by TAA induction.[5] A high ROS level effectively induces apoptosis, probably through an activation of the endoplasmic-reticulum (ER) stress-induced apoptotic pathway.[6] While transient and low-grade ER stress can be overcome by the unfolded protein response, persistent and severe ER stress results in cell apoptosis and also stimulates inflammatory responses.[7] Antioxidant supplements may emerge as potentially antifibrotic agents by either protecting hepatocytes from ROS or inhibiting the activation of hepatic stellate cells (HSCs).[8] Our previous reports indicated that enhanced liver antioxidant capacities in high-cholesterol/fat dietary hamsters[9] or alcohol-diet fed mice[10] supplemented with noni juice (*Morinda citrifolia*) (NJ) result from the polyphenolic contents in NJ. In addition, an excessive accumulation of extracellular matrix proteins (collagen) is often observed in liver fibrosis.[11] The injured liver cells stimulate HSCs to transform into myofibroblast-like cells which secrete large amounts of collagen, thereby producing liver fibrosis. Increasingly, ROS are viewed as a candidate driver of HSC activation and collagen I upregulation.[12] However, downstream mediators for the ROS on the activation of HSCs and the increased collagen synthesis could be a potential avenue to alleviate liver fibrosis and inflammation.

Polysaccharides, fatty acid esters, glycosides, iridoids, anthraquinones, flavonoids, phytosterols, carotenoids, vitamin A, antraquinones, potassium, and others have been identified as putative active ingredients in NJ.[13] Our previous report indicated that gentisic, *p*-hydroxybenoic, and chlorogenic acids have been characterized as the major phenolic acids in our fermented NJ, while the hepatic antioxidant and antiinflammation effects of NJ in a high-fat diet were partially attributed to its phenolic acid.[9] Furthermore, the major mineral in NJ is potassium (K), followed by magnesium (Mg), and sodium (Na). Interestingly, some trace minerals, i.e., zinc (Zn), manganese (Mn), and selenium (Se) were also found in this fermented NJ.[10] In addition, this naturally fermented NJ contains polysaccharides (2141.52 mg/100 mL), and its anti-inflammatory effects against alcoholic liver disease also significantly result from its polysaccharide contents. It has been reported that polysaccharides can downregulate the phosphorylation of ERK and JNK, and then suppress NFκB activation, which influences tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) secretions.[14] Therefore, we speculated that the bioactive compounds (polyphenols, polysaccharides, and minerals) in the naturally fermented NJ may also contribute to increased hepatic antioxidant capacities and antiinflammatory responses in TAA-induced liver fibrosis.

Although NJ showed hypolipidemic, antioxidative, and antiinflammatory effects in a high-fat/cholesterol diet[9] and liquid alcohol diet[10] fed to hamsters and mice, respectively, the protective mechanism of NJ against TAA-induced rat liver fibrosis is still lacking. Therefore, by employing a TAA-induced liver fibrosis rat model, the present study addressed the protective effects of NJ via: (1) increased antioxidative capacities; (2) downregulation of inflammatory and ER stress; and (3) inhibited collagen accumulation.

2. Methods

2.1. NJ preparation

NJ was sourced from the same batch as the one used in our previous studies.[9,10] On the basis of our previous studies,[10] the major identified phenolic acids in NJ are gallic acid, gentisic acid, chlorogenic acid, *p*-hydroxybenzoic acid, caffeic acid, ferulic acid, and *p*-anisic acid. Identified flavonoids in NJ include epicatechin, hesperidin, and naringin. Gentisic, *p*-hydroxybenzoic, and chlorogenic acid were the dominant phenolic acids in NJ. To ensure accurate measurements of phenolic acid, flavonoids, condensed tannin, ascorbic acid, and polysaccharides were obtained, the NJ stored at −20°C for 1 year was measured based on the previous methods.[10]

2.2. Animals and experimental design

Fifty male Wistar rats (6 weeks old, 200–220 g) were purchased from BioLASCO Taiwan Co. Ltd. (Taipei, Taiwan), and acclimated under an environmentally controlled room at 22 ± 2°C and 12/12-h light/dark cycle. After 1 week of acclimation, the 50 rats were randomly divided into five groups: (1) the control group: intraperitoneal (i.p.) saline + normal distilled water (NDW) (ddH_2O); (2) TAA (i.p.); (3) TAA (i.p.) + low-dose NJ [NJ-L; rats were given 2.51 mL NJ/kg body weight (BW) orally]; (4) TAA (i.p.) + medium-dose NJ (NJ-M; rats were given 5.02 mL NJ/kg BW orally); and (5) TAA (i.p.) + high-dose NJ (NJ-H; rats were given 7.52 mL NJ/kg BW orally). The doses and schedules of NJ were calculated, compared, and associated with the dose from our previous report[10] involving mice and rats.[15] During the experimental period, liver fibrosis was induced in rats by i.p. administration of TAA (100 mg/kg) three times weekly on Monday, Wednesday, and Friday; and the ddH_2O or NJ oral gavages on Tuesday, Thursday, and Saturday. TAA was purchased from Sigma-Aldrich (St Louis, MO, USA) and dissolved in sterile saline. Ultimately, body weight, liver weight, serum biochemical values, and histopathological results were analyzed at the end of the 8-week experiment.

Rats were fasted overnight (approximately 10 hours) and then sacrificed by CO_2 asphyxiation on the last experimental day. Blood was collected for biochemical analyses and other measurements, and livers were removed and individually weighed. The liver tissues were fixed or stored later in Bouin's solution or RNA (Ambion, Austin, TX, USA) in a deep freezer (−70°C) for further analyses. For this study, the animal use and protocol were reviewed and approved by the National Taiwan University Animal Care and Use Committee (IACUC No. 100-101).