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ORIGINAL PAPER

Hepatic Protection by Noni Fruit Juice Against CCl_4-Induced Chronic Liver Damage in Female SD Rats

Mian-Ying Wang · Gary Anderson · Diane Nowicki · Jarakae Jensen

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Abstract *Morinda citrifolia* L. (noni) has been used throughout the Pacific, Southeast Asia, Central America, and the Caribbean for a variety of health conditions, including heart and liver ailments. In this study, we examined the hepatoprotective effects of TAHITIAN NONI® Juice (TNJ) against CCl_4-induced chronic liver damage in female Sprague Dawley (SD) rats. Twelve female SD rats were divided into control, placebo and TNJ (6 mL/rat/day) groups. On day 15, animals in the placebo and TNJ groups received 0.25 mL/kg CCl_4 in corn oil once a week for 12 successive weeks. All animals were sacrificed at week 16. Blood and liver were collected for liver function, lipid panel tests, and histological observation. Histopathological examination revealed that liver sections from the TNJ+CCl_4 appeared similar to controls, whereas typical hepatic steatosis was observed in the placebo+CCl_4 group. Serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT), total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) levels were increased in the placebo group compared with the TNJ group. In contrast, high-density lipoprotein (HDL) was increased in the TNJ group and decreased in the placebo group. Thus, TNJ juice appears to protect the liver from chronic exogenous CCl_4 exposures. Such protective mechanisms are supportive evidence for the utility of noni in traditional medicine for liver ailments.

Keywords *Morinda citrifolia* L. (Rubiaceae) (noni) · Carbon tetrachloride · Hepatic protection

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Introduction

Morinda citrifolia (noni) is a tropical plant grown in the South Pacific Islands and has been widely used in folk medicine by the Polynesians for over 2,000 years [1]. Ethnobotanical reports include a broad range of uses including the treatment of liver and heart conditions [2–4]. Many constituents have been identified in the fruit which have antioxidant activity and reported hepatoprotective and cardioprotective properties. These include 3, 3′-bisdemethylpinoresinol, americanol A, americanin A, morindolin, isoprincepin, neolignin, kaempferol, quercetin, rutin, catechin, and epicatechin [5–8].

Carbon-tetrachloride (CCl_4)-induced hepatic steatosis is a well known animal experimental model used to screen hepatoprotective agents, including nutritional supplements and liver-protective drugs [9, 10]. The CCl_4 metabolite, trichloromethyl free radical ($\cdot CCl_3$), initiates lipid peroxidation in cell membranes and leads to liver-injury processes such as steatosis [11, 12]. It is known that the liver plays a pivotal role in the detoxification and regulation of lipoprotein transport in plasma [13, 14]. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) appear to be particularly important in the lipoprotein transport [15]. LDL is a converted form of a very low-density lipoprotein (VLDL) which is considered "bad cholesterol". It contains cholesterol and cholesterol ester,

associated with an increased risk of atherosclerosis [16]. However, HDL contains relatively little cholesterol and is considered as "good cholesterol", associated with a decreased risk of atherosclerosis [17]. Hypercholesterolemia and hepatic steatosis will develop simultaneously after chronic CCl_4 exposure in animals and humans [18]. The accumulation of fat in the hepatocytic plasma has been demonstrated to be predominantly triglycerides (TGs). Hepatic steatosis is the result of a hepatocyte imbalance between the rate of synthesis and output of TGs into and out of the plasma [19]. The pathogenesis of this disease is a crucial public health problem [20]. Intervention studies have shown that total cholesterol (TC) and LDL levels correlate positively with the progression of atherosclerosis, while a low HDL level is a potent predictor of coronary heart disease in humans [21].

This investigation is to determine whether the hepatoprotective constituents of TAHITIAN NONI® Juice (TNJ) are able to mitigate liver injury effects in rats treated with multiple doses of CCl_4 over 12 weeks of exposure. Such determination would provide preliminary evidence and corroboration for its utility in folk medicine.

Materials and Methods

Materials

CCl_4 was purchased from Sigma Chemical Co. (St. Louis, MO, USA). *M. citrifolia* fruit juice (TAHITIAN NONI® brand noni juice) was made by Morinda Holdings, Inc. (Provo, UT, USA) from noni fruit originating in Tahiti. The placebo was prepared using the same procedures and ingredients for making TNJ, but without the noni component [22]. TNJ and placebo were kindly donated by Morinda Holdings, Inc.

Six-week-old female SD rats were purchased from Charles River Inc. (Wilmington, MA, USA). The animals were housed in a room maintained at 25 °C, and with a 12 h photoperiod. They were fed by a laboratory chow diet and water *ad libitum*. The experimental design for this study was approved by the Institutional Ethics Review Committee for animal experiments at UIC College of Medicine. Twelve rats were divided into three groups of four rats each: age-matched controls supplied with water only, placebo group supplied with a 10% placebo in drinking water, and TNJ group supplied with 10% TNJ in drinking water. At day 15, the placebo- and TNJ-treated animals started receiving weekly doses of 0.25 mL/kg CCl_4 in corn oil for 12 successive weeks. Placebo and TNJ treatments were continued for an additional 4 weeks. All animals were sacrificed at the 16th week after the initial CCl_4 administration. Blood was collected from each animal for liver function and lipid-profile tests, while the liver was removed for light microscope (LM) histological observation.

Liver samples were collected from each rat and preserved in 10% neutral buffered formalin and dehydrated in a graded alcohol series. Following xylene treatment, the specimens were embedded in paraffin blocks and cut into 5-μm-thick sections. Consecutive sections were stained with hematoxylin and eosin (H & E) and examined under an Olympus BH2 light microscope [23] by an experienced pathologist.

Methods

Blood serum was separated by centrifugation at 1,600 ×*g* for 10 min at 4 °C. TC, TG, LDL, VLDL, HDL, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine transaminase (ALT), direct bilirubin (DBIL), total bilirubin (TBIL), total protein (TP), and albumin (ALB) were measured with an automatic biochemical analyzer in the Family Medicine Clinical Laboratory, UIC College of Medicine.

Statistical analysis was performed by a Student two-tail T test [24]. Differences were considered significant at $P<0.05$ and very significant at $P<0.01$. Results are expressed as mean±standard deviation (SD).

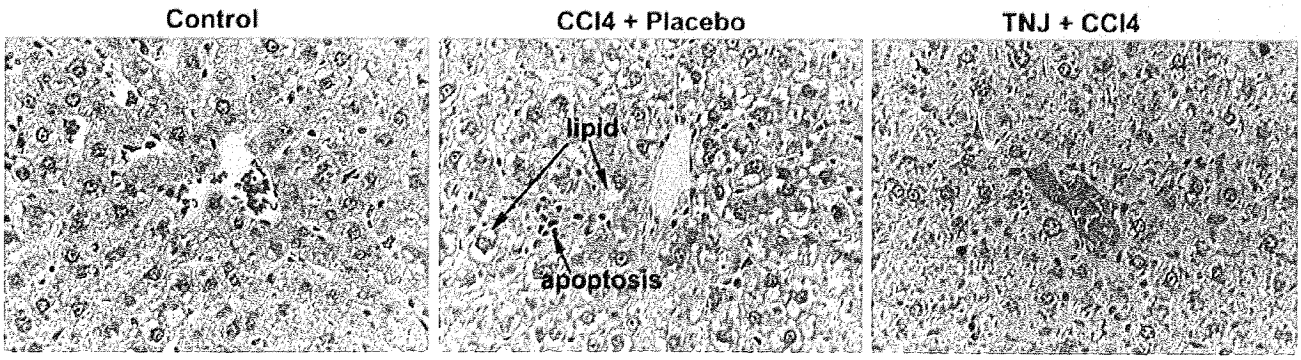

Fig. 1 H & E stained sections (200×) of the liver of the control (*left panel*), placebo+CCl_4 (*middle panel*), and TNJ+CCl_4 (*above right panel*)

Results and Discussion

Typical chronic liver injury (hepatic steatosis) was observed at the 16th week after CCl_4 administration in the placebo+CCl_4 group (center panel, Fig. 1), compared with the normal liver lobules of the controls (left panel, Fig. 1). The injury included increased cellular swelling with cytoplasmic lipid accumulation, and increased apoptosis with lymphoid inflammation (arrow head, center panel, Fig. 1). In contrast, sections of the liver from rats in the TNJ+CCl_4 group appears similar to the control group, with no apoptotic cells present (left panel for control, right panel for TNJ+CCl_4, Fig. 1).

Data from the serological markers for liver function, including AST, ALT, ALP, TP, ALB, DBIL, and TBIL, are shown in Table 1. These serum-marker results are a clear indication of TNJ's protective effects against CCl_4-induced liver damage. The AST and ALT levels of the control and TNJ+CCl_4 groups are similar. Additionally, a comparison of the AST, ALT, and TP, from the TNJ+CCl_4 versus placebo+CCl_4 groups, shows a significant ($P<0.05$) protective effect from the addition of TNJ. Also, except for the TBIL, all of the liver-function levels in the placebo+CCl_4 group showed significant ($P<0.05$) increase over the control group.

Elevated serum AST and ALT levels have been reported as a sign of liver-cell damage [25]. The placebo+CCl_4 results in Table 1 confirm this report. In contrast, the TNJ+CCl_4 results indicate a significant protective effect. The normalization of serum AST and ALT levels in TNJ-treated animals indicates that TNJ may stabilize the cell membrane and prevent a leakage of intracellular enzymes into the blood. Thus, a long-term TNJ treatment would reduce the CCl_4-induced elevated biomarkers such as serum AST and ALT, and improve the liver functions.

TNJ was supplied for an additional month after the final CCl_4 administration. Therefore, the animals had a month to heal the damaged liver in the placebo, as well as in the TNJ groups. The histological results (Fig. 1), data from the liver functions (Table 1), and lipid-panel changes (Table 2), of this study showed that TNJ enhances the healing power compared with that in the placebo+CCl_4 group. TNJ thus protects against CCl_4-induced liver damage at the initial stage, and enhances healing after chronic CCl_4 exposure.

Table 1 Liver function changes before and after exposure to the test materials

	Control	Placebo+CCl_4	TNJ+CCl_4
AST (IU/L)	232±65	353±86*	239±18**
ALT (IU/L)	70±20	207±83*	67±8**
ALP (IU/L)	76.5±12	101±10*	94.7±2.1
TP (g/dL)	6.5±0.5	7.2±0.2*	6.8±0.1**
ALB (g/dL)	1.8±0.1	2.1±0.1*	2.0±0.1
DBIL (mg/dL)	0.2±0.0	0.24±0.1*	0.24±0.01
TBIL (mg/dL)	0.7±0.05	0.7±0.1	0.7±0.01

*$P<0.05$, placebo+CCl_4 vs. control; **$P<0.05$, TNJ+CCl_4 vs. placebo+CCl_4

Table 2 Serum lipid panel changes before and after experiment in different groups

	Control	Placebo+CCl_4	TNJ+CCl_4
TG (mg/dL)	130±30	183±39*	111±43**
TC (mg/dL)	42±4	82±24*	55±7.4**
LDL (mg/dL)	25±5	33±5*	21±7**
VLDL (mg/dL)	26±6	42±6.4*	30±8.6**
HDL (mg/dL)	41±5.5	41±21	55±8.1

* $P<0.05$, placebo+CCl_4 vs. control; **$P<0.05$, TNJ+CCl_4 vs. placebo+CCl_4

Table 2 shows the changes in TG, TC, LDL, VLDL, and HDL for the three groups of rats. Except for the HDL (unchanged), all other lipids showed significant ($P<0.05$) treatment effects. In the placebo+CCl_4 group, the lipids were increased, when compared with the control. Conversely, those of the TNJ+CCl_4 group decreased when compared with the placebo+CCl_4 group.

Oxidative stress and lipid peroxidation are relevant in hepatic steatosis induced by CCl_4. An accompanying decline of anti-oxidative ability plays an important role in pathogenesis of chronic liver diseases [26]. An antioxidant therapy might protect hepatocytes from elevated oxidative stress induced by CCl_4, and prevent or relieve chronic liver-damaging processes [27]. Our previous study demonstrated that TNJ is a strong liver-protective nutritional supplement in acute liver damage induced by CCl_4. The protective process involves scavenging of reactive oxygen free radicals, quenching lipid hydroperoxides, protecting liver function, and exhibiting anti-inflammatory properties [28]. In this study, we demonstrated that TNJ in drinking water prevents hepatic steatosis. CCl_4-induced chronic-hepatic steatosis is reported to impair the hepatic secretion of VLDLs and lipoglycoproteins, occurring soon after CCl_4 poisoning [29]. Other investigators have indicated that CCl_4 may selectively and precociously impair the total microsomes and Golgi bodies, where synthesis, maturation, and release of hepatic lipoglycoproteins occur. Additionally, hepatic steatosis results from the hepatocyte imbalance between the rate of synthesis and output of triglycerides into the plasma [30]. Triglycerides are not secreted by combining with a glycoprotein moiety, particularly the VLDLs. This fraction is involved in the transport of hepatic triglycerides to extrahepatic tissues [31].

The present study provides additional scientific evidence verifying that TNJ is a strong antioxidant, able to protect the liver from CCl_4-caused chronic-liver injury in female

SD rats. We hypothesize that this protective effect of TNJ is through the regulation, secretion, and metabolism of VLDLs and lipoglycoproteins. When rats were pre-treated with TNJ in drinking water for 15 days, during the experiment period, as well as an additional month after CCl_4 administration, the liver appeared normal under LM observation. Furthermore, the liver functions of the animals in the TNJ group showed significant protection, compared with the placebo+CCl_4 group. The lipoprotein profiles were also improved in the TNJ-treated animals compared with those in the placebo+CCl_4 group.

In summary, the hepatohistological structure, serological markers, and lipid-panel profiles were significantly protected by TNJ. Our results indicate that a long-term supply of TNJ in drinking water protects the rat liver against chronic CCl_4 induced liver damage. Thus, drinking TNJ on a daily basis may protect the liver from a highly polluted environment. The scavenging of free radicals, quenching of lipid hydroperoxides, and strong anti-inflammatory activity of TNJ may result in the protective effect of the chronic liver damage model induced by CCl_4. The improved lipoprotein profiles in the TNJ group may relate to the protective effect of TNJ on liver microsomes and Golgi complexes, as shown in our earlier study [32]. A further mechanistic study is needed for the hepatic protection of *M. citrifolia* on CCl_4-induced hepatic steatosis.

These findings are consistent with previous studies where chemical constituents identified in TNJ, such as catechin, epicatechin, quercetin, and rutin, demonstrated hepatoprotective properties [33—35]. The protective and healing properties of TNJ observed in this study provide some mechanistic evidence for why indigenous people of the Pacific Islands, Southeast Asia, and others in the Americas found it useful for treating liver ailments.

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