Hepatoprotective Effects of Acerola Cherry Extract Powder Against D-Galactosamine-Induced Liver Injury in Rats and its Bioactive Compounds

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Abstract: Treatment with the water and tropical lemon juice extract powders from acerola fruit purees and leaves (100 mg/kg) significantly ameliorates the hepatic inflammatory responses such as increased serum levels of AST, ALT, and GGT in rats subjected to acute D-galactosamine (GalN) intoxication. The protective effects of their constituents could be related to their antioxidant activities to neutralise free radicals to attenuate hepatic lipid peroxidation and thus can protect liver damage. The effect of the water extract powder from fruit purees (100 mg/kg) was moderately stronger than that of ascorbic acid (10 mg/kg), but weaker than that of cyanidin-3-*O*-rhamnoglucoside (13.3 mg/kg). The water and lemon juice extract powders from Acerola fruit purees possess the 18.6 and 24.1-fold higher DPPH radical scavenging activities, respectively, than those from leaves, the higher so for those extracted with lemon juice than for those extracted with water. The vitamin C contents were much more higher in the extract powders from fruit purees compared with those from leaves. γ-Tocopherol predominated in the extract powders from fruit purees and α-tocopherol in those from leaves. Polyphenolic compounds were identified and analysed by GC/MS-SIM after acid hydrolysis, extraction and derivatisation to trimethylsilyl ethers.

Keywords: acerola; hepatoprotective effects; vitamin C; anthocyanins; DPPH

INTRODUCTION

Acerola fruit (*Malpighia emarginata* D.C.), also known as the Barbados cherry or West-Indian cherry, grown in subtropical countries, is known for its high concentration of natural vitamin C and potentially an excellent dietary source of polyphenolic compounds such as phenolic acids, flavonoids, their glycosides and anthocyanins. Therefore, the use of acerola fruit has long held sustained attention in conventional and alternative health practices as a great antioxidant. Recent studies have demonstrated the ability of acerola cherry extract to inhibit chemically induced lung tumorigenesis in mice [1] and NO production in mouse macrophage-like cells [2]. Hexane extract of Acerola fruits had both tumor-specific cytotoxic and multidrug resistance reversal activities, which could be used in application for cancer therapy [3]. In its presence, soy and alfalfa phytoestrogen extracts prevent the oxidation of low-density lipoprotein (LDL) associated with a risk of coronary heart disease [4]. Galactosamine (GalN)-induced hepatitis in rats is a well-established animal model for studying the mechanisms of liver injury during human viral hepatitis and fulminant hepatic failure [5]. The objective of the present study was to investigate the protective effects of components in the water and lemon juice extract powders of acerola fruit purees and leaves in a D-GalN induced model of acute hepatic injury in rats.

EXPERIMENTAL

Materials. Acerola fruits and leaves were obtained from Acerola Fresh Ltd., Okinawa, Japan.

Fruit purees were prepared after washing fruits by grinding and removing seeds. Acerola leaves were washed, dried and crushed. Dry extract powders of fruit purees and grind leaves were prepared by an extraction using water and % lemon juice (1:1 w/w), followed by freeze-drying and spray-drying, respectively, and kept at –20°C until use. The solvents and standards used were of analytical grade.

Methods. The vitamin C content was analysed spectrophotometrically following the hydrazine method [6]. Vitamin E content was determined using GC/FID after saponification and hexane extraction [7]. Polyphenolic compounds were identified and quantified by GC/MS-SIM using a HP-5MS column (30 m × 0.32 mm i.d. × 0.25 μm film thickness) after acid hydrolysis, extraction with ethyl acetate, followed by the derivatisation with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) at 70°C for 4 h [8]. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was measured by a method of Fujie *et al.* [9] expressed as mg of ascorbic acid/100 ml equivalent.

Animals. The six-week old SPF male Wistar rats (CLEA, Tokyo, Japan) housed under standard conditions were administered the suspension of the extract powders in physiological salt solution (100 mg/kg) by oral gavage at 8, 24, and 32 h after the intoxication induced by intraperitoneal injection of D-GalN (700 mg/kg of body weight; Sigma Chemical Co., St. Louis, MO, USA). Control rats received 0.9% physiological saline solution in the same manner.

Experimental protocol. At the beginning (0 h) and the end of the treatment (48 h), the rats were anesthetised with nembutal (pentobarbital sodium) and blood samples were collected from neck artery. After centrifugation, the serum was used for measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (GGT) activities. The measurements were performed on an automatic clinical chemistry analyser (Spotchem^{TM}EZ SP-4430, Arkray Ltd., Kyoto, Japan) using a liver-2 kit. The values represented means ± S.D. from 6 animals. The data were statistically analysed using Student's *t*-test.

RESULTS AND DISCUSSION

The water and lemon juice extract powders from fruit purees contained much more ascorbic acid, showed a 54.9 and 60-fold higher content of total vitamin C, and had a 18.6 and 24.1-fold stronger DPPH radical scavenging activity, respectively, compared with those from leaves (Table 1). γ-Tocopherol predominated in the extract powders from fruit purees, while α-tocopherol in those from leaves. However, both extract powders contained α-tocopherol within the same level.

GalN administration caused an increase of the serum levels of three liver enzymes AST, ALT, and GGT by more than 2.2, 3.8, and 1.9-fold, respectively, at 48 h compared to those in the control and GalN+extract powder treated rat groups (Table 2). The treatment with ethanol, water and lemon juice extract powders significantly suppressed GalN-induced elevation of these parameters in rats by > 75, > 74, and > 61%, respectively, at 48 h. These data support the view that the components of the extract powders ameliorated the hepatic inflammatory response, decreased hepatocellular injury, and improve liver function in rats subjected to acute GalN intoxication. The water extract powder from fruit purees at a dose of 100 mg/kg was found to be

Table 1. Vitamin C, vitamin E content and DPPH radical scavenging activity of Acerola extract powders

Vitamins (mg/100 g)	A	B	C	D
Vitamin C total	10 865.4	10 865.4	197.9	181.1
Dehydroascorbic acid	94.7	102.9	194.7	115.4
Ascorbic acid	10 770.7	10 762.5	3.2	65.7
Tocopherols				
α-Tocopherol	0.272	0.280	0.208	0.228
γ-Tocopherol	2.376	2.056	0.064	0.067
δ-Tocopherol	0.186	0.158	0.048	0.075
DPPH radical scavenging activity (mg ascorbic acid/100 ml)	12 309.5	16 466.2	660.9	683.1

Table 2. Changes of serum AST, ALT and GGT activity after D-GalN administration

Treatment	AST (IU/L)		ALT (IU/L)		GGT (IU/L)	
	0 h	48 h	0 h	48 h	0 h	48 h
Control	45.0 ± 2.8	32.0 ± 4.8*	10.0 ± 0.0	11.1 ± 0.4*	2.3 ± 0.5	1.3 ± 0.5*
D-GalN	21.2 ± 8.2	125.0 ± 2.8*	10.2 ± 0.4	53.0 ± 4.2*	1.3 ± 0.5	4.7 ± 0.6*
D-GalN + A	39.5 ± 7.5	40.3 ± 8.1*	10.4 ± 1.1	10.3 ± 0.6*	2.2 ± 0.4	1.7 ± 0.9*
D-GalN + B	40.6 ± 8.0	42.0 ± 11.8*	10.0 ± 0.0	11.2 ± 0.5*	1.6 ± 0.5	2.0 ± 0.8*
D-GalN + C	43.8 ± 9.3	53.7 ± 21.2*	10.3 ± 0.8	13.8 ± 7.5*	1.7 ± 0.5	2.2 ± 0.8*
D-GalN + D	38.2 ± 4.0	57.3 ± 28.4*	10.0 ± 0.0	12.2 ± 4.4*	1.6 ± 0.5	1.8 ± 0.4*
D-GalN + E	37.3 ± 5.9	44.0 ± 16.0*	10.0 ± 0.0	10.4 ± 0.9*	1.7 ± 0.5	2.4 ± 0.5*

*$P < 0.01$ indicate significant differences between groups without and with the extract powder treatment

A – water extract powder of Acerola fruit purees

B – lemon juice extract powder of Acerola fruit purees

C – water extract powder of Acerola leaves

D – lemon juice extract powder of Acerola leaves

E – ethanol extract powder of Acerola fruit purees

slightly more effective than the calculated ascorbic acid dose (10 mg/kg), but less active than cyanidin-3-*O*-rhamnoglucoside (13.3 mg/kg) (results not shown). Malvidin-3,5-diglucoside, cyanidin-3-rhamnoside, pelargonidin-3-rhamnoside, and quercetin-3-rhamnoside were reported in acerola fruits [10]. In this study, polyphenolic compounds were identified and analysed by use of GC/MS-SIM. The extract powders from fruit purees contained not only mentioned anthocyanins and quercetin, but also phenolic acids such as ferulic acid, *p*-coumaric acid, and *p*-hydroxybenzoic acid, which are found also in the extract powders from leaves. Compared to the water and lemon juice extract powders from fruit purees, those from leaves had a significantly higher content of kaempferol and isorhamnetin, which is no doubt one important factor that contributes to the preventive function of the leaf extract powders. However, only a slight difference was found between the concentrations of quercetin in the extract powders from fruit purees and leaves. Lipid peroxidation, tumour necrosis factor alpha (TNF-α) secretion, and its inducible nitric oxide mediate damage in a GalN liver injury model [11]. The proposed mechanism was considered that the extract powder constituents like vitamin C, anthocyanins and flavonoids, which may act synergistically, exert a membrane-stabilising action by scavenging potentially deleterious radicals such as NO and superoxide anion (O_2^-), thus inhibit lipid peroxidation and prevent hepatotoxicity. Further animal studies are needed to be carried out for better understanding the mechanism of action and to evaluate hepatoprotective effects of other isolated components from the extract powders.

CONCLUSIONS

The following may thus be concluded from the results of this research:

(1) The hepatoprotective effect exerted by Acerola extract powders from fruit purees is attributed to the combination of high vitamin C content, phenolic acids, anthocyanins and flavonoids while that from leaves is due to the presence of phenolic acids and a fairly large amount of flavonoids.

(2) The components of the ethanol, water and lemon juice extract powders from Acerola fruit purees and leaves could be of potential benefit in protection of hepatotoxic, viral and alcohol-related liver diseases.

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