Protective Effects of Components in Peanut Skins against D-Galactosamine-Induced Rat Hepatic Injury

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Abstract: The water extract powder (WEP) from normal oleic acid peanut skins at a dose of 200 mg/kg significantly inhibited the increases of serum bilirubin and the serum concentrations of AST, ALT, and GGT, marker enzymes for liver injury, in D-galactosamine (GalN)-induced liver injury rats. However, the WEP from high-oleic acid peanuts skins appeared to be less effective than that from normal ones at the same dose. *trans*-Resveratrol (0.67 mg/kg) and quercetin (0.67 mg/kg) were used for comparison. Their protective effects were as strong as that of the WEP from normal oleic acid peanut skins. These results indicated the ability of the WEP of peanut skins, especially from normal oleic acid peanut varieties, to ameliorate hepatic damage and suggested that they may contain the hepatoprotective agents, thought to be mainly polyphenolic compounds. Total polyphenolic content was higher in the WEP from normal oleic acid peanut skins, while the DPPH radical scavenging activity was higher in the WEP from high oleic acid peanut skins. Compared to high oleic acid peanut skins, normal oleic acid ones had a two-fold higher content of resveratrol, whereas no great differences were observed in the quercetin content analysed by reversed phase HPLC with UV detection.

Keywords: peanut skins; protective effects; polyphenols; HPLC; DPPH

INTRODUCTION

Polyphenolic compounds such as resveratrol (*trans*-3,4',5-trihydroxystilbene) and quercetin found in grapes, peanuts and their products were reported to have antioxidant activities, anticarcinogenic effects and play a role in the prevention of human cardiovascular disease and cancer [1–3]. Peanuts are widely used in western and oriental foods. Peanut skins are used to treat chronic haemorrhage and bronchitis in Chinese traditional medicine, but mainly as animal feed or waste. Karchesy and Hemingway [4] reported their high tannin content. Eight flavonoids, their glycosides, 2 novel indole alkaloids, and 6 A-type proanthocyanidins were isolated and identified from the water-soluble fraction of peanut skins [5, 6]. The liver is subject to acute and potentially lethal injury by viral hepatitis, drugs, and toxins including D-galactosamine, ethanol, CCl_4, and other compounds. In Japan, hepatitis viruses, mainly hepatitis A, B, and nonA-nonB viruses and drug intoxication caused fulminant hepatitis in 90% and 10% population of the patients, respectively [7]. Hepatotoxicity induced by D-galactosamine is a suitable experimental model of liver injury [8]. *trans*-Resveratrol and quercetin were shown to inhibit the hepatic stellate cell activation *in vitro* [9]. Therefore, this study was designed to investigate whether polyphenols of the water extract powders from peanut red skins have the protective effects against GalN-induced hepatitis in rats.

EXPERIMENTAL

Materials. Normal (Virginia) and high-oleic acid (Runner) peanuts were provided by Denroku Co. Ltd., Yamagata, Japan. The water extract powders (WEP) from peanut skins were prepared by extraction of separated red skins using distilled water

at 20°C for 24 hours, followed by freeze-drying. The resulted dry powders were kept at –20°C until use. The solvents and standards used were of analytical grade.

Methods. Total polyphenolic content was analysed spectrophotometrically by the Folin-Denis method [10]. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was measured following method of Cano *et al.* [11] expressed as nmol Trolox/60 μl equivalent. Resveratrol and quercetin contents were determined by a Waters 616 HPLC using an ODS Hypersil column (250 mm × 4.6 mm × 5 μm) and a UV detector with detection at 307 nm. Mobile phase was acetonitrile and water (75:25 v/v), adjusted to pH = 1.5.

Animals. The five-week old SPF male Wistar rats purchased from CLEA (Tokyo, Japan) were housed under standard conditions for 3 days. Rats were intraperitoneally injected with D-GalN at a dose of 700 mg/kg of body weight (Sigma Chemical Co., St. Louis, MO, USA). The suspensions of the water extract powders from peanut skins (200 mg/kg), *trans*-resveratrol (0.67 mg/kg), and quercetin (0.67 mg/kg) in filtered water were orally administered to rats 8, 24, and 32 h after GalN application. In the same way, control rats received filtered water.

Experimental protocol. The rats were anesthetised with nembutal 48 h after GalN intoxication and blood samples were collected from neck artery. After centrifugation, the serum was used for measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GGT) activities, and total bilirubin using an automatic clinical chemistry analyser (Spotchem^{TM}EZ SP-4430, Arkray Ltd., Kyoto, Japan) with a liver-2 kit. Data are given as means ± S.D. (n = 6). The statistical differences between individual groups were calculated by Student's t-test.

RESULTS AND DISCUSSION

The WEP from normal oleic acid peanut skins had a 1.5-fold higher content of total polyphenolics determined by the Folin-Denis method, but showed a 1.3-fold lower DPPH radical scavenging activity than that in the WEP from high oleic acid ones (Table 1).

GalN administration increased serum levels of hepatic bilirubin and liver enzymes AST, ALT,

Table 1. Total polyphenolic (TP) content and DPPH radical scavenging activity of the water extract powders (WEP) from peanut skins

TP and DPPH activity	WEP from high oleic acid peanut skins	WEP from normal oleic acid peanut skins
Total polyphenolics (mg/100 g)	332.78 ± 12.51	484.53 ± 13.52
DPPH radical scavenging activity (nmol Trolox/60 μl equivalent)	231.94 ± 2.06	177.26 ± 4.79

Table 2. Changes of serum bilirubin, AST, ALT and GGT activity 48 h after D-GalN administration

Treatment	T-Bil (mg/dl)	AST (IU/L)	ALT (IU/L)	GGT (IU/L)
Control	0.47 ± 0.06**	84.33 ± 10.21**	16.67 ± 0.58**	0.00 ± 0.00**
D-GalN	3.1 ± 0.06	1000.00 ± 0.00	1000.00 ± 0.00	15.33 ± 2.08
D-GalN+N	0.43 ± 0.06**	200.00 ± 192.35**	80.00 ± 99.59**	1.33 ± 1.15**
D-GalN+H	0.43 ± 0.12**	363.67 ± 265.39*	488.33 ± 250.41*	2.33 ± 1.15**
D-GalN+R	0.47 ± 0.06**	170.33 ± 134.92**	97.00 ± 66.73**	1.00 ± 0.00**
D-GalN+Q	0.33 ± 0.06**	165.67 ± 135.43**	47.33 ± 56.01**	1.33 ± 0.58**

*P < 0.05; **P < 0.01: indicate significant differences between groups with and without the WEP treatment

N – water extract powder from normal oleic acid peanut skins (200 mg/kg)

H – water extract powder from high oleic acid peanut skins (200 mg/kg)

R – *trans*-resveratrol (0.67 mg/kg)

Q – quercetin (0.67 mg/kg)

and GGT by more than 6.6, 2.7, 2, and 6.6-fold, respectively, at 48 h in the GalN group compared with those in the control and GalN+extract powder treated rat groups (Table 2). The treatment with the WEP from normal oleic acid peanut skins significantly inhibited induced elevation of these parameters in rats at a dose of 200 mg/kg. However, the protective effect of the WEP from high-oleic acid peanuts skins was rather moderate at the same dose. By comparison of the hepatoprotective effects between the WEP from peanut skins and their components alone, both *trans*-resveratrol and quercetin were found to be effective at the dose of 0.67 mg/kg.

Resveratrol, quercetin, isorhamnetin, 3′,5,7-trihydroxy-4′-methoxyisoflavone and their glycosides have been reported in peanut skins [6, 12], and confirmed by GC/MS-SIM in our study. These flavonoids from peanut skins were shown to have a significant DPPH radical scavenging activity as well as the protein glycation inhibitory effects. Resveratrol and quercetin contents in peanut skins were determined by using HPLC. Normal oleic acid peanut skins contained a 2.1-fold higher content of total resveratrol than that of high oleic acid ones, the 2.7 and 1.7-fold higher content for *cis*- and *trans*-resveratrol, respectively (Table 3). No great differences were observed in the quercetin content between skins of normal and high oleic acid peanut varieties. GalN causes lipid peroxidation and tumor necrosis factor-α (TNF-α) production mediated liver injury in rats. The protective effect of the WEP from normal oleic acid peanut skins was shown to correlate with total polyphenolic content analysed spectrophotometrically and resveratrol content determined by HPLC. The underlying mechanism of action is thought to be provided by the synergistic effects of its components related to their antioxidant activities to scavenge free radicals as well as by an unknown mechanism based on inhibition of TNF-α production and a inducible NO synthase level, thus can protect liver damage. Further isolation and purification of the WEP components from peanut skins is necessary to elucidate the mechanism, by which they exert the hepatoprotective effects.

Table 3. Resveratrol and quercetin content in peanut skins

	High oleic acid peanut skins	Normal oleic acid peanut skins
Resveratrol (mg/kg) – total	1.15	2.40
trans-Resveratrol	0.71	1.20
cis-Resveratrol	0.44	1.20
Quercetin (mg/kg)	5.00	5.30

CONCLUSIONS

The WEP from normal oleic acid peanut skins appeared to be more effective than that from high oleic ones in protecting against GalN induced liver injury. It is probable that its stronger protective effect is due to, in part at least, a higher content of resveratrol.

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