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Hepatoprotective Activity of Scopoletin, a Constituent of *Solanum lyratum*

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Scopoletin (7-hydroxy-6-methoxycoumarin), a coumarin, was isolated from the aerial part of *Solanum lyratum* Thunb. by the activity-guided fractionation employing carbon tetrachloride-intoxicated primary cultured rat hepatocytes as a screening system. Its hepatoprotective activity was first evaluated by measuring the release of glutamic pyruvic transaminase and sorbitol dehydrogenase from carbon tetrachloride-intoxicated rat hepatocytes into the culture medium. Scopoletin significantly reduced the releases of glutamic pyruvic transaminase and sorbitol dehydrogenase from the carbon tetrachloride-intoxicated primary cultured rat hepatocytes by 53% and 58%, respectively, from the toxicity in a dose-dependent manner over concentration ranges of 1 μM to 50 μM. Further studies revealed that at the concentration of 10 μM, scopoletin significantly preserved glutathione content by 50% and the activity of superoxide dismutase by 36% and also inhibited the production of malondialdehyde to the degree as seen in the control.

Key words : Hepatoprotective activity, Scopoletin, *Solanum lyratum*, Carbon tetrachloride, Primary cultured rat hepatocytes

INTRODUCTION

We have been searching for potential hepatoprotective compounds from natural products employing carbon tetrachloride (CCl_4)-intoxicated primary cultured rat hepatocytes as a screening system (Lee *et al.*, 1995; Sung *et al.*, 1997; Kim *et al.*, 1997). In searching for such compounds, we found that a methanolic extract of the aerial part of *Solanum lyratum* Thunb. (Solanaceae) exhibited a significant hepatoprotective activity against CCl_4-induced toxicity. The aerial part of *S. lyratum* has traditionally been used in therapies against malaria, jaundice, edema, hepatitis, cancers etc. (Shim *et al.*, 1995). A number of studies have been conducted to identify chemical constituents and pharmacological activities of the aerial part of this plant (Murakami *et al.*, 1985; Yahara *et al.*, 1989; Yu *et al.*, 1994; Kosuge *et al.*, 1985). However, heretofore, no note has been reported regarding hepatoprotective activity of constituents of this plant, even though the aerial part of *S. lyratum* has been used particularly as a therapeutic drug against jaundice and hepatitis in folk medicines.

In the present communication, we report the isolation of scopoletin based on the hepatoprotective activity -guided fractionation of *S. lyratum*. The hepatoprotective activity of scopoletin was determined by measuring activities of glutamic pyruvic transaminase (GPT) and sorbitol dehydrogenase (SDH) released from CCl_4-intoxicated primary cultured rat hepatocytes. To elucidate the mechanism of its hepatoprotective action, further studies were carried out by quantifying reduced glutathione (GSH) content, malondialdehyde (MDA) production, and the activities of glutathione *S*-transferase (GST), superoxide dismutase (SOD) and catalase in CCl_4-intoxicated primary cultured rat hepatocytes.

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MATERIALS AND METHODS

Plant material

Aerial parts of *S. lyratum* were collected from Unyang, a Kyungnam province of Korea and identified by Professor Jong-Hee Park at College of Pharmacy, Pusan National University. Voucher specimen documenting these collections has been deposited in the Herbarium of the Medicinal Plant Garden at College of Pharmacy, Seoul National University.

Isolation of compound 1

Aerial parts of *S. lyratum* were dried and the dried plant material (9.0 kg) was extracted 4 times with

80% MeOH in an ultrasonic apparatus, which yielded a methanolic extract (450 g) upon removal of the solvent *in vacuo*. This methanolic extract was then suspended in H_2O and partitioned with CH_2Cl_2 to give a CH_2Cl_2 fraction (70 g) and an aqueous fraction. The CH_2Cl_2 fraction was resuspended in 90% MeOH and partitioned with *n*-hexane to give an *n*-hexane fraction and a 90% MeOH fraction (50 g). The aqueous fraction was extracted with *n*-BuOH to give an *n*-BuOH fraction and an aqueous fraction. The 90% MeOH fraction, which showed the highest hepatoprotective activity among the fractions, was subjected to a step gradient vacuum silica gel column (400 g, TLC grade 15 μm, column size 9.0×40 cm, Merck) chromatography and eluted using the mixture of $CHCl_3$-MeOH increasing polarity (50:1→0:1) to give 11 subfractions (M-I~M-XI). The subfraction M-I (1.7 g) having the most significant hepatoprotective activity among 11 subfractions was chromatographed on the step gradient silica gel column using mixtures of *n*-hexane-EtOAc (5:1→0:1) and then EtOAc-MeOH (5:1→0:1) with increasing polarity, and resulted in 8 subfractions (m-1~m-8). The Sephadex LH-20 column (60 g, column size 2.0×20 cm, Pharmacia) chromatography using mixtures of *n*-hexane-CH_2Cl_2-MeOH (5:5:1) and then HPLC (Bondapak C_{18}, column size 7.8×300 mm, MeOH-H_2O [7:3]) of the subfraction m-7 (800 mg), yielded compound **1** (21 mg).

Identification of compound 1

Colorless needles (21 mg), m.p. 203~204°C. Compound **1** was identified as scopoletin (7-hydroxy-6-methoxycoumarin) by spectral (UV, IR, NMR, MS) data comparisons with those of published values (Bohlmann and Jakupovic, 1979).

Culture of hepatocytes and exposure to carbon tetrachloride

Isolated hepatocytes were prepared from a male Wistar rat (200~250 g) by the collagenase perfusion technique of Berry and Friend (1969) with minor modifications (Lee *et al.*, 1995; Sung *et al.*, 1997; Kim *et al.*, 1997). One day after the rat hepatocytes were plated, the cultured cells were exposed to medium containing 6.5 mM CCl_4/ethanol (in a final concentration of 0.07%) for 1 h to induce hepatotoxicity (Lee *et al.*, 1995; Sung *et al.*, 1997; Kim *et al.*, 1997; Kiso *et al.*, 1983).

Screening for hepatoprotective activity

In order to screen the hepatoprotective activity of each fraction of *S. lyratum*, aliquots of each fraction were lyophilized and dissolved in dimethyl sulphoxide (DMSO, in a final concentration at most 0.1%). Compound **1** was also dissolved in ethanol (in a final concentration at most 0.5%). Hepatoprotective activity of *S. lyratum* was determined by a standardized protocol: One day after the isolated rat hepatocytes were plated, the cultured cells were exposed to medium containing 6.5 mM CCl_4 with or without samples being tested for hepatoprotective activity. One hour after the CCl_4 challenge, the culture medium was collected and used to measure the activities of GPT and SDH, and the cells were harvested for the determination of GSH content, MDA production and the activities of GST, SOD, and catalase.

Measurement of GPT and SDH activities

The activities of GPT and SDH in the culture medium were determined by the method of Reitman and Frankel (1957) using an assay kit (Yeong Dong Pharmaceutical. Corp., Seoul, Korea), and by the method of Gerlach (1965), respectively.

Measurement of GSH and MDA levels

GSH and MDA levels were measured by the method of Griffith (1980) and by the method of Recknagel (1966), respectively.

Measurement of SOD and catalase activities

SOD and catalase activities were determined by the method of Yoshihiko (1984) and by the method of Fridovich (1986), respectively.

Measurement of GST activity

The activity of GST was determined by the method of Habig *et al.* (1974)

Measurement of protein content

The protein content was measured by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard.

Statistical analysis

The statistical significance was determined by an "ANOVA" test using a computerized statistical package. The data were considered to be statistically significant if the probability had a value of 0.05 or less.

RESULTS AND DISCUSSION

A methanolic extract of aerial parts of *S. lyratum* was found to exhibit hepatoprotective activity in CCl_4-intoxicated primary cultured rat hepatocytes. The methanolic extract gave an *n*-hexane, a 90% MeOH, an *n*-BuOH, and an aqueous fraction by a series of subsequent fractionations, and among which the 90%

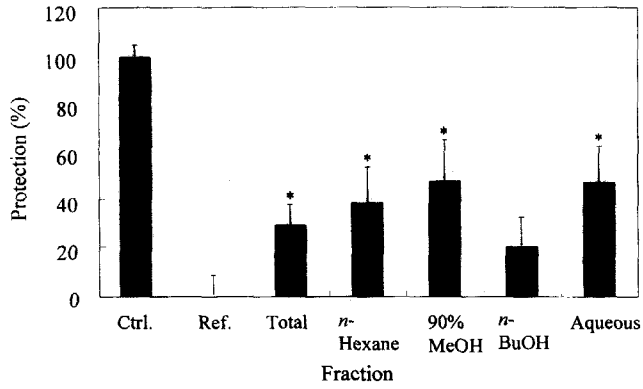

Fig. 1. The effect of each fraction of *S. lyratum* on the activity of GPT released from CCl_4-intoxicated primary cultured rat hepatocytes. Each value represents the mean±SD (n=4). The concentration of each fraction was 100 μg/ml. Ctrl. is the value for hepatocytes which were not challenged with CCl_4. Ctrl. value of GPT was 16.24±13.50 IU/L. Ref. is the value for hepatocytes which were challenged with 6.5 mM CCl_4. Ref. value of GPT was 87.4±20.7 IU/L. The % value of protection was calculated as 100×(value of Ref.-value of sample)/(value of Ref.-value of Ctrl.). Significantly different from the value of Ref. (*$p<0.05$).

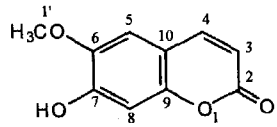

Fig. 2. The chemical structure of compound **1** (scopoletin).

MeOH fraction exhibited the highest activity (Fig. 1). The 90% MeOH fraction was further fractionated and screened using the same system. This resulted in the isolation of compound **1** with various chromatographic techniques. Compound **1** was identified as scopoletin (7-hyrdoxy-6-methoxycoumarin) from its physical attributes and from its spectral data in comparison with those of published values (Fig. 2). The hepatoprotective activity of scopoletin was evaluated by measuring its reducing effect on the release of GPT and SDH into the culture medium of CCl_4-intoxicated primary cultured rat hepatocytes. Scopoletin significantly reduced the release of both GPT and SDH from CCl_4-intoxicated primary cultured rat hepatocytes in a dose-dependent manner over concentration ranges of 1 μM to 50 μM (Fig. 3). This hepatoprotective effect of scopoletin is consistent with the fact that some coumarins (not including scopoletin) with a 7-hydroxyl group reduced the activities of GPT released in CCl_4 and galactosamine-intoxicated primary cultured rat hepatocytes (Kiso *et al.*, 1984). Moreover, scopoletin has reactive oxygen-scavenging properties (Martin-Aragon *et al.*, 1996; Foti *et al.*, 1996). So, further studies focused on the antioxidative activity of scopoletin were carried out at the concentration of 10 μM scopoletin showing the

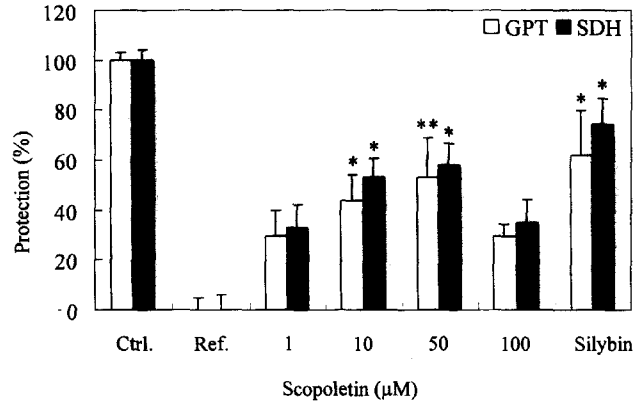

Fig. 3. Effects of scopoletin on activities of GPT and SDH released from CCl_4-intoxicated primary cultured rat hepatocytes. Ctrl. is the value for hepatocytes which were not challenged with CCl_4. values of GPT and SDH were 16.24±13.50 IU/L and 2.8±0.2 IU/L, respectively. Ref. is the value for hepatocytes which were challenged with 6.5 mM CCl_4. Ref. values of GPT and SDH were 87.4±20.7 IU/L and 48.7±0.2 IU/L, respectively. The concentration of silybin was 100 μM. The % value of protection was calculated as 100×(value of Ref.-value of sample)/(value of Ref.-value of Ctrl.). Significantly different from the value of Ref.: *$p<0.05$ and **$p<0.01$.

significant activity. GSH is a substance which plays an important role in quenching free radical species generated by oxidative stress. In CCl_4-intoxicated primary cultured rat hepatocytes, the content of GSH drastically decreased and the ratio of the oxidized glutathione (GSSG) to the total GSH (GSH+GSSG) also significantly increased. As expected, scopoletin, at the concentration of 10 μM both preserved the content of GSH and decreased the ratio of GSSG/total GSH significantly. Moreover, scopoletin preserved the activity of an antioxidant enzyme, SOD in CCl_4-intoxicated primary cultured rat hepatocytes (Table I). However, scopoletin exerted little effect on the activity of GST, an enzyme which has been known to play a central role in the detoxification and excretion of xenobiotics (Jakoby W. B., 1978) (data not shown). In addition, scopoletin almost completely suppressed the production of MDA, the measure of lipid peroxidation (Table I).

Based upon our results, we can conclude that scopoletin protects hepatocytes from CCl_4-induced toxicity by maintaining the GSH content, the activity of SOD, and inhibiting the production of MDA as a result of its antioxidation and free radical-scavenging effect. Scopoletin is a well-known, simple coumarin which is widely distributed in the various families of the Angiosperms, especially Solanaceae, Convolvulaceae, Compositae, etc. (Murray *et al.*, 1982) but has never been previously isolated from *S. lyratum*. Its hepatoprotective activity and the mechanism of action are for the first time reported in the present communication.

Table I. The antioxidative activity of scopoletin

	GSH (nmol/mg protein)	GSSG/Total GSH	SOD (units/mg protein)	Catalase (μmol/ mg protein)	MDA (nmol/mg protein)
Ctrl.[a]	87.6±21.5	0.205±0.047	33.6±4.0	1592.6±120.5	6.70±0.23
Ref.[b]	41.9±6.9	0.490±0.008	15.1±3.0	849.0±57.0	9.16±1.06
Scopoletin-treated[c] (10 μM)	64.9±0.5*	0.360±0.043*	21.7±3.6*	964.6±110.6	6.35±0.43*

Each value represents the mean±SD (n=3).
[a]Ctrl. is the value for hepatocytes which were not challenged with CCl_4.
[b]Ref. is the value for hepatocytes which were challenged with 6.5 mM CCl_4.
[c]Scopoletin-treated is the values for hepatocytes which were challenged with 6.5 mM CCl_4 and then treated with 10 μM scopoletin.
Significantly different from the value of Ref.[b] (*$p<0.05$).

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