SHORT COMMUNICATION

Antimutagenic components in *Glycyrrhiza* against *N*-methyl-*N*-nitrosourea in the Ames assay

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**ABSTRACT**

Antimutagenesis against *N*-nitroso compounds contribute to prevention of human cancer. We have found that *Glycyrrhiza aspera* ethanolic extract exhibits antimutagenic activity against *N*-methyl-*N*-nitrosourea (MNU) using the Ames assay with *Salmonella typhimurium* TA1535. In the present study, eight purified components from *Glycyrrhiza*, namely glabridin, glycyrrhetinic acid, glycyrrhizin, licochalcone A, licoricesaponin H2, licoricesaponin G2, liquiritigenin and liquiritin were evaluated for their antimutagenicity against MNU in the Ames assay with *S. typhimurium* TA1535. Glycyrrhetinic acid, glycyrrhizin, licoricesaponin G2, licoricesaponin H2 and liquiritin did not show the antimutagenicity against MNU in *S. typhimurium* TA1535. Glabridin, licochalcone A and liquiritigenin reduced revertant colonies derived from MNU in *S. typhimurium* TA1535. Glabridin, licochalcone A and liquiritigenin reduced revertant colonies derived from MNU in *S. typhimurium* TA1535 without showing cytotoxic effects, indicating that these compounds possess antimutagenic activity against MNU. The inhibitory activity of glabridin and licochalcone A was more effective than that of liquiritigenin. Thus, *Glycyrrhiza* contains antimutagenic components against DNA alkylating, direct-acting carcinogens.

1. Introduction

Almost all tested *N*-nitroso compounds have been shown to have carcinogenic activity in experimental animals; as a result, exposure to *N*-nitroso compounds is suspected to induce human cancer (Lijinsky 1992). Approximately, 45–75% of the total human exposure to *N*-nitroso compounds is estimated to be due to *in vivo* synthesis (Tricker 1997).
$N$-Methyl-$N$-nitroso urea (MNU) is a direct-acting alkylating agent (Preussmann & Eisenbrand 1984). MNU is formed in vivo and anticipated to be a human carcinogen (Deng et al. 1998; Sen et al. 2000, 2001; Engemann et al. 2013). Therefore, for cancer chemoprevention, it is important to find some compounds that can inhibit the mutagenicity induced by MNU.

We have reported on the inhibitory effect of plant extracts; Glycyrrhiza ethanolic extract, Glycine max extract with 40% isoflavone aglycone (ISOMAX AG40) and Zingiber officinale ethanolic extract, against MNU mutagenicity using the Ames assay with Salmonella typhimurium TA1535 among 43 extracts derived from medicinal and edible plant (Tatsuzaki et al. 2014). In addition, the Leguminosae family has been effective at inhibiting MNU-induced mutagenicity in the umu assay (Inami et al. 2014). Therefore, we focused on components in the Glycyrrhiza root, which belongs to the Leguminosae family. In the present study, we evaluated the antimutagenicity of eight purified components of Glycyrrhiza ethanolic extract, namely glabridin, glycyrrhetinic acid, glycyrrhizin, licochalcone A, licoricesaponin H2, licoricesaponin G2, liquiritigenin and liquiritin, against MNU in the Ames assay with S. typhimurium TA1535 (Figure 1).

2. Results and discussion

Glycyrrhiza root is one of the historical herbal medicines, however, it has been still found some novel bioactivity (Kao et al. 2014; Gou et al. 2015). Many components have been isolated from Glycyrrhiza root, including triterpene saponin, flavonoids, isoflavonoids and chalcones (Shibata 2000; Asl & Hosseinzadeh 2008; Tanaka et al. 2010). Many components have been isolated from licorice, including triterpene saponin, flavonoids, isoflavonoids and chalcones (Shibata 2000; Asl & Hosseinzadeh 2008; Tanaka et al. 2010; Kao et al. 2014). We chose the following compounds from each category: $\beta$-glycyrrhetinic acid, glycyrrhizin, licoricesaponin H2 and licoricesaponin G2 are pentacyclic triterpenoids; liquiritigenin and liquiritin

![Figure 1. Chemical structures of components isolated from the Glycyrrhiza root.](image)
are flavanones; glabridin is an isoflavone; and licochalcone A is a chalcone. These are the major components in each category, and they are commercially available.

Because *Glycyrrhiza* has well-known antimicrobial and antiviral activities (Shibata 2000; Nomura et al. 2002; Asl & Hosseinzadeh 2008; Kao et al. 2014), the doses of the components for the Ames assay that lacked cytotoxicity were determined to evaluate the precise antimutagenicity. The results were the following maximum concentrations; 1.0 mg/mL for glycyrrhizin, β-glycyrrhetinic acid, licoricesaponin H2, licoricesaponin G2, liquiritigenin and liquiritin and 0.1 mg/plate for glabridin and licochalcone A. These components were examined for their ability to inhibit the mutagenicity of MNU in the Ames assay.

β-Glycyrrhetinic acid, glycyrrhizin, licoricesaponin H2, licoricesaponin G2 and liquiritin did not decrease the revertant colonies derived from MNU in *S. typhimurium* TA1535 at a concentration of 0.05–1.0 mg/plate (Supplemental Tables S1–S5). Glabridin, liquiritigenin and licochalcone A reduced the revertant colonies derived from MNU in *S. typhimurium* TA1535 without showing cytotoxicity, indicating that flavonoids and chalcones possessed antimutagenic activity against MNU (Figure 2, Supplemental Tables S6–S8). The inhibitory activities of glabridin and licochalcone A were more effective than that of liquiritigenin. Glabridin and licochalcone A had a similar extent of inhibition.

Those components are classified into two groups, aglycons (β-glycyrrhetinic acid, glabridin, liquiritigenin and licochalcone A) and glycosides (glycyrrhizin, licoricesaponin H2, licoricesaponin G2 and liquiritin). None of the tested glycosides influenced the MNU-induced mutagenicity in *S. typhimurium* TA1535. It can be difficult for the glycosides to pass through

![Figure 2. Antimutagenicity of glabridin (●), licochalcone A (▲) and liquiritigenin (■) against MNU in *Salmonella typhimurium* TA1535.](image-url)
the bacterial membrane because of their higher water solubility (Kiyosawa et al. 1995). It is important to evaluate the antimutagenicity of aglycons using the Ames assay.

To compare the antimutagenic activity of the *G. aspera* ethanolic extract and the components, the same maximum concentration (1.0 mg/plate) were used. In a previous study, the *G. aspera* ethanolic extract, at a concentration of 1.0 mg/plate, decreased the MNU-induced mutagenicity to 5.4% in *S. typhimurium* TA1535 without any cytotoxicity (Tatsuzaki et al. 2014). Licorittigenin inhibited the mutagenicity of MNU to 31.2% at a concentration of 1.0 mg/plate (Figure 2, supplemental Table S8). Glabridin and licochalcone A inhibited the mutagenicity of MNU between 5.9 and 31.0%, respectively, at a concentration of 0.1 mg/plate (Figure 2, supplemental Tables S6 and S7). At the concentration used, glabridin and licochalcone A may contribute to the antimutagenic activity of the *G. aspera* ethanolic extract. We are working on isolating the antimutagenic components from the *G. aspera* ethanolic extract.

For effective cancer chemoprevention, it is important to identify compounds that can inhibit the mutagenicity induced by MNU. Flavonoids and isoflavonoids are already known as chemopreventive agents (Birt et al. 2001). Glabridin, licochalcone A and licorittigenin were found to inhibit the mutagenicity of MNU for the first time. Further investigation is required to identify the antimutagens in the *Glycyrrhiza* root.

**Acknowledgement**

We thank J. Tatsuzaki, Tokiwa Phytochemical Co. Ltd. (Chiba, Japan), for providing components from *Glycyrrhiza*.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan; Grant-in-Aid for the Science Research Promotion Fund from the Japan Private School Promotion Foundation.

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