Isolation and characterization of antimutagenic components of *Glycyrrhiza aspera* against *N*-methyl-*N*-nitrosourea

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**Abstract**

**Background:** A powdered ethanolic extract of *Glycyrrhiza aspera* root exhibits antimutagenic activity against *N*-methyl-*N*-nitrosourea (MNU) based on the Ames assay with *Salmonella typhimurium* TA1535. The aim of this study was to identify the antimutagenic components of the powdered ethanolic extract of *G. aspera* root.

**Results:** The powdered ethanolic extract of *G. aspera* root was sequentially suspended in *n*-hexane, carbon tetrachloride, dichloromethane, ethyl acetate, and ethanol, and each solvent soluble fraction and the residue were assayed for antimutagenic activity against MNU in *S. typhimurium* TA1535. The dichloromethane soluble fraction exhibited the highest antimutagenicity and was fractionated several times by silica gel chromatography. The fraction with the highest antimutagenic activity was further purified using HPLC, and the fractions were assayed for antimutagenicity against MNU in *S. typhimurium* TA1535. Finally, five components with antimutagenic activity against MNU were identified as glyurallin A, glyasperin B, licoricidin, 1-methoxyphaseollin, and licoisoflavone B.

**Conclusions:** The five components were demonstrated to possess an antigenotoxic effect against carcinogenic MNU for the first time. It is important to prevent DNA damage by *N*-nitrosamines for cancer chemoprevention.

**Keywords:** Glyurallin A, Glyasperin B, Licoricidin, 1-Methoxyphaseollin, Licoisoflavone B

**Background**

Humans are exposed to endogenous and exogenous *N*-nitroso compounds [1]. Approximately 45–75% of the total human exposure to *N*-nitroso compounds is estimated to be due to *in vivo* synthesis [2]. Almost all tested *N*-nitroso compounds have carcinogenic activity in experimental animals [1]. Therefore, exposure to *N*-nitroso compounds is suspected to induce human cancer. Several epidemiological studies have demonstrated that the endogenous formation of *N*-nitroso compounds is correlated with the cancer incidence in humans [3–9]. Recently, the International Agency for Research on Cancer (IARC) has reported that the consumption of red meat and processed meat is carcinogenic, and this may be caused by *N*-nitroso compounds that form during meat processing or cooking [10].

*N*-Methyl-*N*-nitrosourea (MNU) is a DNA alkylating carcinogen that induces cancer in various organs, particularly the forestomach, brain and nervous system, in rodents [11]. MNU is produced by the nitrosation of creatinine or fermented foods at the gastric pH [12–15]. Additionally, MNU is formed by the nitrosation of methylurea with nitrite in guinea pig stomachs [16]. Therefore, for cancer chemoprevention, it is important to identify compounds that can inhibit mutagenicity induced by MNU.

Short-term bacterial mutation assays, such as the Ames assay, are an effective screening tool for the identification of various mutagenic or antimutagenic compounds in complex materials [17]. The assay has advantages as an inexpensive and flexible screening method that provides preliminary information related to antimutagenesis. There are many reports about the
antimutagenicity of edible plants; however, the inhibitory effects against MNU mutagenesis are less well studied [18, 19].

*Glycyrrhiza* root has long been used worldwide as an herbal medicine and natural sweetener [20–22]. The genus *Glycyrrhiza* (Leguminosae) consists of about 30 species including *G. glabra*, *G. uralensis*, *G. inflata*, *G. aspera*, *G. korshinskyi* and *G. eurycarpa* [23]. In Japanese pharmacopoeia, only *G. glabra* and *G. uralensis* are permitted to be used as licorice and licorice powder, and the other *Glycyrrhiza* species can be used as raw materials of licorice extract [23]. *Glycyrrhiza* has reported chemopreventive effect based on its anticarcinogenesis and antimutagenesis toward both indirect-acting and direct-acting mutagens [24–29]; however, the inhibitory effects against MNU mutagenesis have not been studied in detail.

In our previous study, a powdered ethanolic extract of *G. aspera* root decreased MNU-induced mutagenicity in a preliminary antimutagenic screen using the Ames assay [30]. The aim of this study was to identify the antimutagenic components of the powdered ethanolic extract of *G. aspera* root.

**Methods**

**General experimental procedures**

The reaction progress was monitored using thin-layer chromatography (TLC) on silica gel 60 F254 (0.25 mm, Merck, Darmstadt, Germany). Column chromatography was performed using silica gel 60 (0.04–0.063 mm, Merck). Melting points were determined using a Yanaco (Tokyo, Japan) micro-melting-point apparatus without correction. HPLC was performed using an EYELA Preparative LC system [VSP-3050 pump, UV-9000 spectrometric detector, LiChrosorb RP-18 column (10 μm, 25 mm × 300 mm)] (Tokyo Rikakikai Co. Ltd., Tokyo, Japan) and a Shimadzu LC system [LC-6 AD pump, SPD-20A UV spectrometric detector, Mightysil RP-18 column (5 μm, 20 mm × 250 mm)] (Kyoto, Japan). The NMR spectra were recorded with a JEOL JNM-LA400 spectrometer (Tokyo, Japan). The chemical shifts were expressed in ppm, downfield from TMS. The mass spectra were collected using a JEOL JMS-SX102A mass spectrometer (Tokyo, Japan).

**Reagents**

Sodium ammonium hydrogen phosphate tetrahydrate was purchased from Merck (Darmstadt, Germany). Bacto agar and Bacto nutrient broth were obtained from Becton Dickinson Microbiology Systems (Sparks, USA). MNU were obtained from Toshin Gousei (Tokyo, Japan). Other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan). A powdered ethanolic extract of *G. aspera* (China) root was kindly provided by Tokiwa Phytochemical Co. Ltd. (Chiba, Japan).

**Preparation of a powdered ethanolic extracts of *Glycyrrhiza aspera* root**

A root of *G. aspera* (100 g) was refluxed with 95% ethanolic aqueous solution (1000 mL) for 1 h, and the mixture was filtered with suction. The residue was refluxed again with 95% ethanolic aqueous solution (1000 mL) for 1 h, and the mixture was filtered with suction. The combined filtrates were concentrated under reduced pressure and vacuum dried to a constant weight, and finally a brown powder was obtained.

**Fractionation of the powdered ethanolic extract of *Glycyrrhiza aspera* root based on solubility in organic solvents**

The powdered ethanolic extract of *G. aspera* root (10 g) was added to hexane (100 mL) and stirred for 10 min. The supernatant was filtered with suction. The stirring and filtration of the residue was repeated twice. Sequentially, the residue was suspended in carbon tetrachloride (100 mL × 3), dichloromethane (100 mL × 3), ethyl acetate (100 mL × 3), and ethanol (100 mL × 3) following the same procedure. The organic solvent portions were removed organic solvent by rotary evaporator and the residue was dried in vacuo. The whole extraction procedure was repeated twice; the organic portions and residue were combined. Finally, hexane soluble fraction (62 mg), carbon tetrachloride soluble fraction (880 mg), dichloromethane soluble fraction (15.6 g), ethyl acetate soluble fraction (11.4 g), ethanol soluble fraction (700 mg) and the residue (1.7 g) were obtained from the powdered ethanolic extract of *G. aspera* root (30 g). Recovery of the weight was 101%.

**Isolation of antimutagenic compounds from the dichloromethane soluble fraction**

The dichloromethane soluble fraction was chromatographed on a silica gel, eluted with 5% methanol-CH2Cl2, 3% methanol-CH2Cl2, 1% methanol-CH2Cl2, 10% ethyl acetate-CH2Cl2, and later separated on an RP-18 column by preparative HPLC and eluted with 80% methanol in water (see the Additional file 1). Five peaks representing active components were purified using HPLC and characterized by comparing their spectroscopic (NMR and MS) properties with literature values.

**Bacterial mutation assay**

The antimutagenic effect of each plant extract was assayed according to the Ames method using the plate-incorporation protocol [31, 32]. Dr. T. Nohmi (National
Inami et al. Genes and Environment (2017) 39:5

Institute of Health Sciences, Tokyo, Japan) kindly provided the *S. typhimurium* TA1535.

A solution of MNU (1.5 μmol/50 μL DMSO) was added to a test tube and supplemented with 0.1 M sodium phosphate buffer (pH 7.4, 0.5 mL), a solution (50 μL) with various concentrations of fraction, and a culture of the *S. typhimurium* TA1535 (0.1 mL), and the solution was thoroughly mixed. Then, Top Agar (2 mL) was added, and the mixture was poured onto a minimal-glucose agar plate. The revertant colonies were counted after incubation at 37 °C for 44 h. Each sample was assayed using duplicate plates. The results are expressed as the mean number of revertant colonies per plate. Plates with neither MNU nor plant extract were considered negative controls. MNU (1.5 μmol/50 μL) resulted in 1470 ± 70 colonies. All of the tested plates were microscopically examined for thinning or the absence of a background lawn and/or presence of microcolonies, which are considered indicators of toxicity induced by the test material. Neither MNU nor plant extracts displayed toxicity to *S. typhimurium* TA1535 under the conditions of the antimutagenicity test.

Mutagenic activity in the presence of extracts is expressed as the percentage of mutagenicity (% = Rs/R × 100), where Rs is the number of his+ revertants/plate for plates exposed to MNU and plants extracts, and R is the number of his+ revertants/plate of MNU. The number of spontaneous revertants was subtracted beforehand to give Rs and R. Thus, the mutagenicity of MNU in the absence of plant extracts was defined as 100% MNU mutagenicity.

**Cytotoxicity test**

Toxicity assays under the same conditions as those used for the Ames test were performed to determine the maximum concentrations of plant extracts that could be added without exerting toxic effects on the bacteria used in the Ames test. A solution of MNU (1.5 μmol/50 μL DMSO) was added to a test tube, and supplemented with 0.1 M sodium phosphate buffer (pH 7.4, 0.5 mL), each solution of plant extract (50 μL), and a culture of *S. typhimurium* TA1535 (0.1 mL). A portion of the mixture was diluted 10^5 times in 1/15 M PB. The diluted solution (200 μL) was supplemented with histidine-free Top Agar (2 mL) and poured on a Nutrient Broth agar plate. The colonies were counted after incubation at 37 °C for 20 h. Each sample was assayed using duplicate plates. A substance was considered cytotoxic when the bacterial survival was less than 80% of that observed in the negative control. The mutation frequency was estimated as the number of mutants per 10^7 surviving bacterial cells [31, 32].

**Results and discussion**

Identification of antimutagenic components from a powdered ethanolic extract of *Glycyrrhiza aspera* root

A powdered ethanolic extract of *G. aspera* root was sequentially suspended in *n*-hexane, carbon tetrachloride, dichloromethane, ethyl acetate, and ethanol. Each soluble fraction and the residue were assayed for inhibitory effects against MNU mutagenesis in *Salmonella typhimurium* TA1535 (Fig. 1).

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The dichloromethane soluble fraction showed the highest antimutagenic activity, and was fractionated using a silica gel column and preparative high-performance liquid chromatography (HPLC), and its antimutagenic components were identified. The fractionation and mutagenicity assay were conducted repeatedly (Fig. 2 and the Additional file 1). In each fractionation step, the recoveries of weights were more than 89%.

Finally, the fraction (Fr.3-2-2-3-2) with the highest antimutagenic activity was separated using HPLC (Fig. 3a). Those fractions were tested for mutagenicity against MNU, and the compounds from peaks 4–8 each inhibited MNU-induced mutagenicity (Fig. 3b)
The compounds from fractions 4–8 were each analysed using mass spectrometry and 1H nuclear magnetic resonance spectroscopy, and five antimutagenic compounds were identified, i.e., glyurallin A [33], glyasperin B [34], licoricidin [35, 36], 1-methoxyphaseollin [37], and licoisoflavone B [38], by comparing their spectroscopic properties with literature values (Fig. 4).

Glyurallin A was a pterocarpene and 1-methoxyphaseollin was a pterocarpan. Glyasperin B, licoricidin, and licoisoflavone B were an isoflavanone, an isoflavan, and an isoflavone, respectively. A phenolic hydroxyl group is a common in five isolated compounds with antimutagenicity.

Flavonoids are well-known antimutagens based on the results of Ames assays [39, 40], whereas pterocarpenes and pterocarpans are not well-known for their antimutagenicity.

Inhibitory effect of licorici din from the powdered ethanolic extract of Glycyrrhiza aspera root on MNU-induced mutagenicity

Licorici din (peak 6) did not show any revertant colonies by cytotoxicity (Fig. 3b). For the antimutagenicity assay, it was necessary to determine the concentration at which the viability of the tester strain did not decrease. Licorici din reduced revertant colonies induced by MNU in S. typhimurium TA1535 (Fig. 5a) without cytotoxicity at the maximum concentration of 100 μg/plate (Fig. 5b). To assess the precise antimutagenic potency of licorici din, mutation frequency was calculated by dividing the number of mutants with the surviving fraction of bacteria (Fig. 5c). These data clearly showed that licorici din possessed antimutagenic activity against MNU in S. typhimurium TA1535.

The antimutagenic activity of the G. glabra extract against ethyl methanesulfonate (EMS) is reportedly attributed to glabrene [41]. In our study, glabrene was not isolated from the fraction with the highest mutagenic activity. The difference between the isolated compounds in antimutagenic activity for the powdered ethanolic extract of G. aspera root probably reflects differences in the mechanism of action between MNU and EMS. MNU reacts mainly via an S_N1 mechanism and efficiently alkylated both nitrogens and oxygens in DNA. EMS, which reacts predominantly via an S_N2 mechanism, alkylates the nitrogens at the DNA bases and produced little alkylation of the oxygens in DNA bases [42, 43]. Thus, the MNU is far more mutagenic than
EMS. Additionally, the main bioactive components were different between *G. glabra* and *G. aspera* [23].

In vitro, *N*-nitrosamine formation is inhibited by phenolic compounds, ascorbic acid, thiols, and metals [18, 19]. Mutagenesis by direct-acting mutagens can be reduced or prevented in several ways; MNU can be decomposed to non-mutagenic products via antimutagens, or reactive mutagenic products from MNU can react with antimutagens before reaching DNA. It is also possible to induce repair enzymes in the *Salmonella* strain [44].

A number of known flavonoids (such as genistein etc) possess significant antimutagenic activity [40, 45, 46]; however, the detailed mechanism for the antimutagenicity has not been completely established. The inhibitory effect on the mutagenicity of direct-acting mutagens is probably caused by a chemical reaction between the mutagen and antimutagen. The inhibitory effect of phenolic acid results from the scavenging action on an electrophilic decomposition product of MNU [47]. Hung et al have reported that hydroxylated flavonoids showed antimutagenic activity toward benzo[a]pyrene 7,8-diol-9,10-epoxide by direct interaction with the 7,8-diol-9,10-epoxide [48]. Therefore, we assumed that the antimutagenic mechanisms of the isolated compounds were similar to that of phenolic acid or hydroxylated flavonoids. Furthermore, MNU treatments are reported to induce not only DNA alkylation but also increase intracellular ROS level [49–51], and then the antimutagenicity was partly attributed to its radical scavenging potency of flavonoids [52–54]. We are working to elucidate the antimutagenic mechanisms for the isolated compounds against MNU.

Antimutagens and anticarcinogens in the diet are suggested to be highly effective for cancer prevention [44, 55, 56]. The intake of medicinal and edible plants that include antimutagens may play a role in improving human health.

**Conclusions**

It is important to prevent DNA damage by *N*-nitrosamines for cancer chemoprevention. In this study, five components with antimutagenic activity against MNU from a powdered ethanolic extract of *G. aspera* root were identified as glycurallin A, glyasperin B, licoricidin, 1-methoxyphaseollin, and licoisoflavone B. To the best
of our knowledge, this report describes the first demonstration of the antigenotoxic effects of these components against carcinogenic MNU.

**Additional file**

**Additional file 1**: Fractionations of antimutagenic compounds from dichloromethane soluble fraction. (DOCX 3675 kb)

**Abbreviation**

MNU: N-Methyl-N-nitrosourea

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**Authors’ contributions**

KI coordinated the study, the data analysis, the statistical analysis, and the writing the manuscript. YM, JT and CM performed the experiment and the data analysis. MM conceived of the study, participated in designing the study, and helped to draft the manuscript. All authors read and approved the final manuscript.

**Competing interests**

The authors declare that they have no competing interests.

**Ethics approval and consent to participate**

Not applicable.

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**References**

1. Lijinsky W. Chemistry and biology of N-nitroso compounds. Cambridge Monographs on Cancer Research. Cambridge: Cambridge University Press; 1992.
2. Tricker AR. N-Nitroso compounds and man: sources of exposure, endogenous formation and occurrence in body fluids. Eur J Cancer Prev. 1997;6:226–68.
3. Keszei AP, Goldbohm RA, Schouten LJ, Jakszyn P, van den Brandt PA. Dietary N-nitroso compounds, endogenous nitrosation, and the risk of esophageal and gastric cancer subtypes in the Netherlands cohort study. Am J Clin Nutr. 2013;97:135–46.
4. Catsburg CE, Gago-Dominguez M, Yuan JM, Castelao JE, Cortessis VK, Pike MC, Stern MC. Dietary sources of N-nitroso compounds and bladder cancer risk findings from the Los Angeles bladder cancer study. Int J Cancer. 2014;134:125–35.
5. Dellavalle CT, Daniel CR, Aschebrook-Kilfoy B, Hollenbeck AR, Cross AJ, Sinha R, Ward MH. Dietary intake of nitrate and nitrite and risk of renal cell carcinoma in the NIH-AARP diet and health study. Br J Cancer. 2013;108:205–12.
6. Loh YH, Jakszyn P, Luben RN, Mulligan AA, Mitrou PN, Khaw KT. N-Nitroso compounds and cancer incidence: The European prospective investigation into cancer and nutrition (EPIC)-Norfolk study. Am J Clin Nutr. 2011;93:1053–61.
7. Kaminaga F, Chow WH, Abnet CC, Dawsey SM. Environmental causes of esophageal cancer. Gastroenterol Clin North Am. 2009;38:27–57.
8. Santarelli RL, Pierre F, Corpet DE. Processed meat and colorectal cancer: a review of epidemiologic and experimental evidence. Nutr Cancer. 2008;60:131–44.
9. Jakszyn P, Gonzalez CA. Nitrosamine and related food intake and gastric and oesophageal cancer risk: a systematic review of the epidemiological evidence. World J Gastroenterol. 2006;12:4296–303.
10. International agency for research on cancer (IARC). Evaluate consumption of red meat and processed meat. 2015. https://www.iarc.fr/en/media-centre/pr/2015/pdfs/pr204_E.pdf. Accessed 16 Dec 2016.
11. Preussmann R, Eisenbrand G. N-Nitroso carcinogens in the environment. In: Searle CE, editor. Chemical carcinogens, ACS Monograph No. 182. Washington DC: American Chemical Society; 1984. p. 829–68.
12. Sen NP, Seaman SW, Burgess C, Baddoo PA, Weber D. Investigation on the possible formation of N-nitroso-N-methyleurea by nitrosation of creatin in model systems and in cured meats at gastric pH. J Agric Food Chem. 2000;48:5088–96.
13. Sen NP, Seaman SW, Baddoo PA, Burgess C, Weber D. Formation of N-nitroso-N-methyleurea in various samples of smoked/dried fish, fish sauce, seafoods, and ethnic fermented/pickled vegetables following incubation with nitrite under acidic conditions. J Agric Food Chem. 2001;49:2096–103.
14. Deng D, Li T, Ma H, Wang R, Gu L, Zhou J. Characterization of N-nitrosomethyleurea in nitrosated fermented fish products. J Agric Food Chem. 1998;46:202–5.
15. Haorah J, Zhou L, Wang X, Xu G, Minish SS. Determination of total N-nitroso compounds and their precursors in frankfurters, fresh meat, dried salted fish, sauces, tobacco, and tobacco smoke particulates. J Agric Food Chem. 2001;49:6068–78.
16. Engemann A, Focke C, Humpf HU. Intestinal formation of N-nitroso compounds in the pig cecum model. J Agric Food Chem. 2013;61:998–1005.
17. Neresyan A, Milik M, Knasmüller S. Methods used for detection of antimutagens: an overview. In: Knasmüller S, DeMarini DM, Johnson I, Gerhäuser C, editors. Chemoprevention of cancer and DNA damage by dietary factors. Weinheim: Wiley-VCH; 2009. p. 211–27.
18. Gichner T, Veliminsky J. Inhibitors of N-nitroso compounds-induced mutagenicity. Mutat Res. 1988;202:325–34.
19. Asl MN, Hosseinzadeh H. The Ames test on some medicinal and edible plant extracts. J Agric Food Chem. 2001;49:709–24.
20. Shibata S. A drug over the millennia: pharmacognosy, chemistry, and pharmacology of licorice. Yakugaku Zassi. 2000;120:849–62.
21. Kao TC, Wu CH, Yen GC. Bioactivity and potential health benefits of licorice. J Agric Food Chem. 2014;62:542–53.
22. Nomura T, Tsuchiya T, Akimasa T. Chemistry of phenolic compounds of licorice (Glycyrrhiza spp) and their estrogenic and cytotoxic activities. Pure Appl Chem. 2002;74:1199–206.
23. Wang ZF, Nixon DW. Licorice and cancer. Nutr Cancer. 2001;39:1–11.
24. Nishino H, Tokuda H, Satomi Y, Masuda M, Onozuka M, Yamaguchi S, Takayasu J, Tsuturu J, Takeamura M, II T, Ishiihi E, Kuchide S, Okuda M, Murakoshi M. Cancer chemoprevention by phytochemicals and their related compounds. Asian Pac J Cancer Prev. 2000;1:49–55.
25. Wall ME. Antimutagenic agents from natural products. J Nat Prod. 1992;55:1–18.
26. Noguera R, Teel RW, Lau BH. Modulation of mutagenesis, DNA-binding, and metabolism of aflatoxin B1 by licorice compounds. Nutr Res. 1992;12:247–57.
27. Agabelli RA. Genetic effects of root extracts of Glycyrrhiza glabra L. in different test systems. Cytol Genet. 2012;46:297–301.
28. Zani F, Cuzzoni MT, Daglia M, Benvenuti S, Vampa G, Mazza P. Inhibition of mutagenicity in Salmonella typhimurium by Glycyrrhiza glabra extract, glycyrrhizinic acid, 18β- and 18γ-glycyrrhetinic acids. Planta Med. 1993;59:502–7.
29. Tatsuzaki J, Jinwei Y, Kojo Y, Mine Y, Ishikawa S, Mogchizuki M, Inami K. Genotoxicity screening of extracts from medicinal and edible plants against N-methyl-N-nitrosourea by the Ames assay. Genes Environ. 2014;6:39–46.
30. Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. Mutat Res. 1983;113:173–215.
31. Merteltmann K, Zeiger E. The Ames Salmonella/microsome mutagenicity assay. Mutat Res. 2000;465:29–60.
32. Shibano M, Hermsi A, Matsumoto Y, Kusano G, Miyase T, Hatakeyama Y. Pharmaceutical botanical studies on some Glycyrrhiza species. Heterocycles. 1997;45:203–60.
33. Zeng L, Fukai T, Nomura T, Zhang RY, Lou ZC. Four new prenylated flavonoids, Glyasperins A, B, C, and D from the roots of Glycyrrhiza aspera. Heterocycles. 1992;34:575–87.
35. Fukui T, Toyono M, Nomura T. On the structure of licoricidin. Heterocycles. 1988;27:2309–13.
36. Park SY, Lim SS, Kim JK, Kang JJ, Kim JS, Lee C, Kim J, Park JH. Hexane-
ethanol extract of Glycyrrhiza uralensis containing licoricidin inhibits the
metastatic capacity of DU145 human prostate cancer cells. Br J Nutr. 2010;
104:1272–82.
37. Kitagawa I, Chen WZ, Hori K, Harada E, Yasuda N, Yoshikawa M, Ren J.
Chemical studies of Chinese licorice-roots. I. Elucidation of five new
flavonoid constituents from the roots of Glycyrrhiza glabra L. collected in
Xinjiang. Chem Pharm Bull. 1994;42:1056–62.
38. Teng SC, Tsai HJ, Tsai MG, Lee WM, Chen IC, Lin CC. Using both chemical
and biological fingerprints for the quality study of estrogenic licorice
(Glycyrrhiza uralensis). J Food Science. 2003;68:2372–7.
39. Birt DF, Hendrich S, Wang W. Dietary agents in cancer prevention:
flavonoids and isoflavonoids. Pharmacol Ther. 2001;90:157–77.
40. Resende FA, da Silva Almeida CP, Vilegas W, Varanda EA. Differences in the
hydroxylation pattern of flavonoids alter their chemoprotective effect
against direct- and indirect-acting mutagens. Food Chem. 2014;155:251–5.
41. Mitscher LA, Drake S, Gollapudi SR, Harris JA, Shankel DM. Isolation and
identification of higher plant agents active in antimutagenic assay systems:
Glycyrrhiza Glabra. In: Shankel DM, Hartman PE, Kada T, editors. Antimutagenesis and anticarcinogenesis mechanisms. New York: Springer;
2013. p. 153–65.
42. Singer B. Sites in nucleic acids reacting with alkylating agents of differing
carcinogenicity or mutagenicity. J Toxicol Environ Health. 1977;2:1279–95.
43. Eder E, Wiedenmann M, Deininger C, Kütt W. The relationship between
mutagenicity in His G46 Salmonella and the O6-guanine alkylation in
bacterial DNA by monofunctional methane sulphonates. Toxicol in Vitro.
1990;4(3):167–74.
44. Bhattacharya S. Natural Antimutagens. Res J Med Plant. 2011;5:116
– 2013. p. 153–65.
45. Cassady JM, Baird WM, Chang CJ. Natural products as a source of potential
antimutagenic agents for chemoprevention. Expert Opin Drug Discov.
2007;2(3):229–42.
46. Huang MT, Chang RL, Wood AW, Newmark HL, Sayer JM, Yagi H, Jerina DM,
Hiscox AS. Antimutagenic effect of flavonoids and isoflavonoids. Pharmacol Ther. 2001;90:157
– 167.
47. Gichner T, Pospíšil F, Velemínský J, Volkeová V, Volke J. Two types of
antimutagenic effects of gallic and tannic acids towards N-nitroso-
compounds-induced mutagenicity in the Ames Salmonella assay. Folia
Microbiol (Praha). 1987;32:55–62.
48. Huang MT, Chang RL, Wood AW, Newmark HL, Sayer JM, Yagi H, Jerina DM,
Conney AH. Inhibition of the mutagenicity of bay-region diol-epoxides of
polycyclic aromatic hydrocarbons by tannic acid, hydroxylated
anthraquinones and hydroxylated cinnamic acid derivatives. Carcinogenesis.
1985;6(2):237–42.
49. Hebels DG, Briedé JJ, Khampang R, Kleinjans JC, de Kok TM. Radical
expression modulations in Caco-2 cells. Toxicol Sci. 2010;116:194–205.
50. Toda S, Shirataki Y. Inhibitory effects of licoisoflavones A and B on the
metastatic capacity of DU145 human prostate cancer cells. Br J Nutr. 2010;
104:1272–82.