

ANTIMUTAGENIC EFFECT OF PHENOLIC ACIDS

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In the present study, the *Salmonella typhimurium* tester strain TA 100 was used in the plate-incorporation test to examine the antimutagenic potential of caffeic, ferulic and cichoric acids extracted from plant species of genera *Echinacea* (L) Moench, as well as of another phenolic acids, on 3-(5-nitro-2-furyl)acrylic acid (5NFAA) and sodium azide mutagenicity. All tested compounds possess antimutagenic activity. In the case of 5NFAA, the antimutagenic potency of tested compounds was in the order of gallic acid > ferulic acid > caffeic acid > syringic acid > vanillic acid. The mutagenic effect of sodium azide was inhibited by tested phenolic acids by about 20–35 %. The most effective compound, gallic acid inhibits this effect by 82 % in the concentration of 500 µg/plate. The only exception from favourable properties of tested phenolic acids is cichoric acid, which in the contrary significantly increased the mutagenic effect of 5NFAA.

INTRODUCTION

Phenolic compounds are ubiquitous in plant food, and have been associated with the sensory and nutritional quality of fresh and processed plant foods¹. In the last years, researchers and food manufacturers are increasingly interested in these compounds, which may be exploited for the development of functional foods or in the chemoprevention. Phenolic compounds have many biological activities; they can act as antioxidants², scavengers of active oxygen species and electrophiles³, blockers of nitration⁴ and chelators of metals⁵. They can undergo autooxidation to produce hydrogen peroxide in the presence of metals and are capable of modulating certain cellular enzyme activities⁶. Fortification of foods with materials rich in phenolic compounds has been shown to impart antimutagenic, antiinflammatory and antioxidant properties, which may be exploited for the development of health foods⁷.

Caffeic acid is found naturally in various agricultural products as coffee beans, fruits, vegetables, tobacco leaves, olive oils and wines⁸. This phenolic acid exhibit a cytoprotective effect on endothelial cells against oxidized low-density lipoprotein⁹, inhibits the oxidation of lipoprotein exposed to ferrylmyoglobin and recycles α -tocopherol from α -tocopherol radical¹⁰. Likewise as other phenolic acids (ferulic and gentisic acids) caffeic acid exhibits protective effect against the genotoxicity of acridin orange and ofloxacin in *Salmonella typhimurium*¹¹ as well as in *Euglena gracilis*¹². The therapeutic effect of *Echinacea* plants has been assigned to the presence of caffeic acid derivatives such as cichoric acid, echinacoside, chlorogenic acid beside of various other components found in the

hydroalcoholic extracts¹³. Cichoric acid has been shown to possess phagocytosis stimulatory activity *in vitro* and *in vivo*¹⁴, to inhibit hyaluronidase and to protect collagen type III from free radical-induced degradation¹⁴. Caffeic and chlorogenic acid possesses inhibitory effect on the mutagenicity of Trp-P-1 and Glu-P-2 (ref.¹⁵). The same phenolic acids may inhibit the formation of mutagenic and carcinogenic *N*-nitroso compounds because they are inhibitors of the *N*-nitrosation reaction *in vitro*⁴. Tanaka et al.¹⁶ described the inhibition of 4-nitroquinoline-1-oxide-induced rat tongue carcinogenesis by the caffeic, ellagic, chlorogenic and ferulic acid.

However, not all polyphenols and not all actions of individual polyphenols are necessarily beneficial. Some of them have mutagenic and/or pro-oxidant effect, and they may interfere with essential biochemical pathways¹⁷. A number of polyphenols, including quercetin, can bind to DNA¹⁸ and this direct interaction may be an important mechanism of bacterial mutagenicity. Cichoric acid showed mutagenic activity on *Salmonella typhimurium* TA98 and TA 100 strains¹⁹.

In the present study, the plate-incorporation test of the *Salmonella* mutagenicity assay was used to examine the effect of selected phenolic acids against 5NFAA and sodium azide mutagenicity.

MATERIALS AND METHODS

Histidin-dependent strain of *Salmonella typhimurium* TA100 was received from the Collection of Microorganisms, Masaryk University, Brno (Czech Republic).

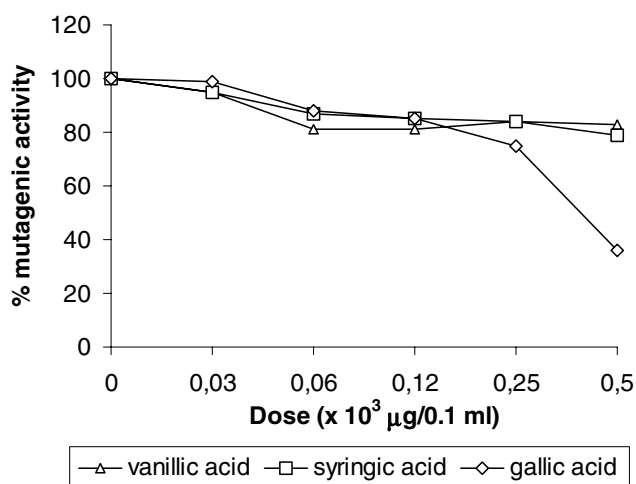


Fig. 1. Effect of derivatives of hydroxybenzoic acid on 5NFAA-induced mutagenicity in strain TA100 of *Salmonella typhimurium*. Percent of mutagenic activity = (the number of revertants per plate in the presence of phenolic acid / the number of revertants per plate in the absence of phenolic acid) \times 100.

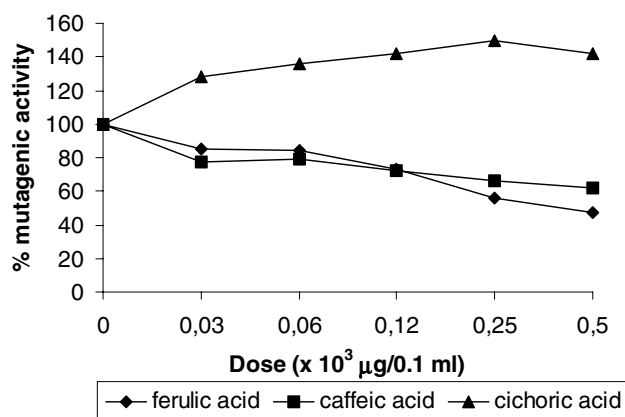


Fig. 2. Effect of derivatives of hydrocinnamic acid on 5NFAA-induced mutagenicity in strain TA100 of *Salmonella typhimurium*. Percent of mutagenic activity expressed as in Fig. 1.

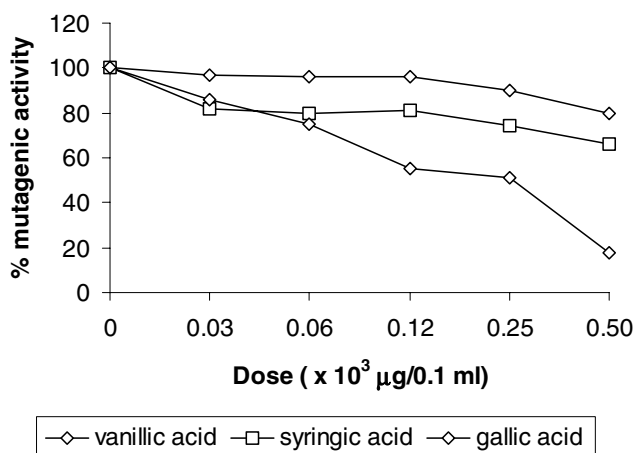


Fig. 3. Effect of derivatives of hydroxybenzoic acid on sodium azide-induced mutagenicity in strain TA100 of *Salmonella typhimurium*. Percent of mutagenic activity expressed as in Fig. 1.

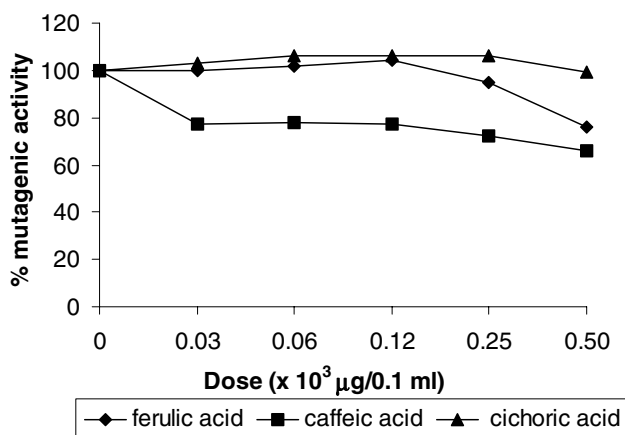


Fig. 4. Effect of derivatives of hydroxycinnamic acid on sodium azide-induced mutagenicity in strain TA100 of *Salmonella typhimurium*. Percent of mutagenic activity expressed as in Fig. 1.

Caffeic acid (3,4-dihydroxycinnamic acid), ferulic acid (4-hydroxy-3-methoxycinnamic acid) and cichoric acid (2,3-*O*-dicafeoyltartaric acid) were extracted, isolated and identified according to the methods of Perry et al.²⁰ and Wills and Stuart²¹. Vanillic acid (4-hydroxy-3-methoxybenzoic acid), syringic acid (3,5-dimethoxy-4-hydroxybenzoic acid) and gallic acid (3,4,5-trihydroxybenzoic acid) were purchased from Sigma-Aldrich (Germany). The positive mutagens: 3-(5-nitro-2-furyl)acrylic acid (5NFAA) was from Slovakofarma Hlohovec (Slovakia) and sodium azide was purchased from Sigma-Aldrich (Germany).

The inhibitory effect of phenolic acids on mutation induction by several positive mutagens was investigated

with *Salmonella typhimurium* TA100 using pre-incubation method²². 0.1 ml of the positive mutagen, 0.1 ml of phenolic acid and 0.1 ml of bacterial culture (cultivation for 16 h at 37 °C, approximate cell density $2-5 \times 10^8$ cells/ml) were mixed and preincubated at 37 °C for 30 min. Soft agar (2 ml) was added and the mixture was poured onto minimal agar plates. After 48 h of incubation at 37 °C the number of revertants was counted. The results from the antimutagenicity studies represent the mean of three separate experiments, each run in triplicate, and they were statistically evaluated using the Student's *t*-test. Antimutagenicity was expressed as percentage of mutagenicity inhibition following the formula:

$$\% \text{ mutagenicity} = 100 - [(X1/X2) \times 100]$$

where X1 = number of revertants per plate in the presence of mutagen and antimutagen, X2 = number of revertants per plate in the absence of antimutagen

RESULTS AND DISCUSSION

Doses of 10 µg/plate of 5NFAA and 5 µg/plate of sodium azide were chosen for the antimutagenicity studies since these doses were not toxic and induced 1835 ± 178 (5NFAA) and 1263 ± 153 (sodium azide) revertants in *Salmonella typhimurium* TA100. In selecting the concentration of phenolic acids we used the amounts where cell viability was found to be above 90 %.

The inhibitory effects of derivatives of hydroxybenzoic acid on the mutagenicity of 5NFAA are illustrated in Fig. 1. The positive control of mutagen in each case was considered as 100 % mutagenicity. Gallic acid was the only phenolic acid successful in inhibiting the mutagenicity of 5NFAA by more than 50 % at the concentration of 500 µg/plate. Vanillic and syringic acids decreased the number of revertants starting from 60 µg/plate by about 20 %.

It is evident from the Fig. 2 that cichoric acid increased the number of revertants induced by 5NFAA. Ferulic and caffeic acids decreased the mutagenic effect of 5NFAA by about 50 %.

Figs. 3 and 4 show the results of inhibitory effect of phenolic acids on the mutagenic effect of sodium azide. With the exception of cichoric acid which has no antimutagenic effect, all tested compounds decreased the number of revertants induced by sodium azide by about 20–35 %. Similarly as in the case of 5NFAA, the effect of sodium azide was significantly reduced by gallic acid and within the concentration tested a marked dose-dependence was found. At the highest used concentration this phenolic acid inhibits mutagenic activity of sodium azide by 82 %.

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