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Cytotoxic, genotoxic and antimicrobial activity of caffeic and rosmarinic acids and their lithium, sodium and potassium salts as potential anticancer compounds

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ABSTRACT

Purpose: The aim of this study was to examine the cytotoxic, genotoxic, antioxidant and antimicrobial activity of caffeic and rosmarinic acids and their salts with Li, Na and K with use of *Escherichia coli* K-12 *recA:gfp* strain as a model organism.
Methods: Cytotoxic potency of tested chemicals were calculated on the basis on the dose that confers inhibition percentage such as 20% for each concentrations of analysed chemicals. Genotoxic properties were calculated on the basis of the fold increase (FI) of SFI values normalized with control. Antioxidant potencies were established on the base of DPPH assay. Antimicrobial activity of chemicals were established on the value of minimal inhibitory concentration (MIC).
Results: Obtained results indicated that lower concentrations of tested compounds exhibited stronger GFP fluorescence response after rosmarinic acids and their salts treatment. Genotoxic effects seemed to be independent of the salt ions. The caffeic acid salts with Li, Na and K showed reduced genotoxic effect in comparison to the caffeic acid while increased cytotoxic effect than that of caffeic acid. Moreover, caffeinate salts exhibited better antimicrobial activity against *E. coli* (MIC = 250 μg/mL) than K caffeinate salt (MIC > 500 μg/mL). The MIC values of Li, Na and K rosmarinate salts were above 500 μg/mL against all tested microorganisms.
Conclusion: The results of the experiment show that there is no clear positive correlation between the antioxidant potency of caffeic and rosmarinic acids and their Li, Na and K salts and their cytotoxic effect. Used salts ions Li, Na and K do not significantly affect the antioxidant effect of natural phenolic compounds and they do not have a significant impact on the biological parameters such as cyto- and genotoxicity. Perhaps it is connected with the reaction environment including polarity of the solvent (water).

1. Introduction

Natural polyphenols have potential health benefits in a number of oxidative stress-associated diseases (such as cancer). Due to their antioxidant properties, in *in vitro* and *in vivo* experiments, these natural compounds have ability to tumor cell death induction, interfere with carcinogenesis, tumor growth and dissemination. Some preclinical experiments showed, that polyphenols in combination with conventional chemoradiotherapy or with other polyphenols through the induction of apoptosis, the inhibition of coming out and spreading of cancer. In many experiments antioxidants, antithrombosis, antihypertensive, antifibrosis, antiviral, and anti-tumor properties of caffeic acid (3,4-dihydroxycinnamic acid) (CA) (Fig. 1) were proofed [1–4].

The previous studies revealed, that caffeic acid exerts protective effects against UVB-induced skin damage, by suppressing interleukin-10 and mitogen-activated protein kinase (MAPK) activation in mouse skin [1].

Koraneekit et al. [4], presented the synergistic effect of cisplatin and caffeic acid on cervical cancer cell lines. The simultaneous combination of caffeic acid-cisplatin allowed to decrease the dosage of cisplatin dose used, which could lead to a reduction of

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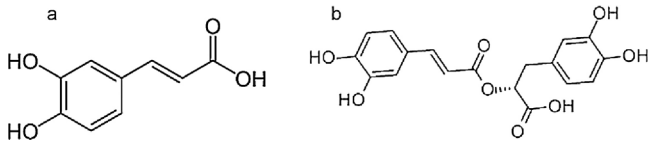

Fig. 1. Chemical structure of caffeic acid (3,4-dihydroksycinnamic acid) (a.) and rosmarinic acid (b).

side-effect of this drug. The addition of caffeic acid to the cell lines, treated with cisplatin, increased the efficiency of cisplatin anticancer activity. Caffeic acid has been predicted to act as a chemopreventive agent and emerging adjuvant for cancer chemotherapy [1–4].

Rosmarinic acid (RA) is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid (Fig. 1) and is known as (R)-α-[[3-(3,4-dihydroxyphenyl)-1-oxo-2E-propenyl]oxy]-3,4-dihydroxy-enzenepropanoic acid, with a molecular formula of $C_{18}H_{16}O_8$. RA is a phenolic compound found in *Rosmarinus officinalis*, *Thymus mastichina*, *Forsythia koreana*, *Ocimum sanctum*, *Hyptis pectinata* and some plants belonging to the *Agastache* genus of the *Lamiaceae* family. The antiviral, antibacterial, anticancer, antiinflammatory and antioxidant activities of rosmarinic acid have been reported [5–10].

In spite of most microorganisms have similar biochemical pathways as higher organisms, there is no complete compatibility in genotoxicity tests using bacteria and human assays. *Salmonella* Ames assay is most widely used bacterial assay for mutagenic and carcinogenic screening of compounds. There is a correlation between mutagenicity as measured by the Ames assay and carcinogenicity in mammals [11,12]. Kirkland et al., [13] demonstrated that this correlation is not perfect because mutations are only one of many stages in tumor development. Moreover, it could be possible to meet with specific mutagenic response to the bacteria or the test protocol, e.g., bacterial specific metabolism, exceeding a detoxification threshold, or the induction of oxidative damage to which bacteria may be more sensitive than mammalian cells *in vitro* or tissues *in vivo*, or an *in vitro* metabolic activation preparation that does not mimic the *in vivo* situation [13]. Additionally, the Ames assay is simple to perform but requires high replication, a wide range of controls, extensive culturing and time-consuming enumeration. The Ames test actually measures mutagenicity. On the other hand, activation of SOS repair system following DNA damage has been used to measure the mutagenic and genotoxic effects of various chemicals and physical treatments. These microbial bioassays – also called as microbial biosensors – used for cyto- and genotoxic assessment offer some advantages; they are inexpensive, have short life cycles, simple to apply, sensitive and easy to measure. In addition, the most of microorganisms have similar biochemical pathways as the higher organisms and respond rapidly to environmental changes [13,14].

The aim of this study was the assessment of cytotoxic, genotoxic effects, antioxidant potency and antimicrobial activity of caffeic and rosmarinic acid and their salts with Li, Na and K toward *Escherichia coli* K-12 *recA:gfp* as microbial biosensor strain.

The alkali metals constitute the logical series, where in the order: Li, Na, K atomic radius is steadily increasing and the degree of oxidation is constant. To select the alkali metals to this study following criteria (which are important from the point of view of further possible application) were taken into account: (1) as small as possible harmfulness of the human body and the environment; (2) the possibility of the practical application because of the good solubility of the alkali metal compounds in water and polar solvents; (3) the availability, ease of preparation and stability.

Escherichia coli K-12 contained plasmid transcriptional fusion between *recA* promoter which belongs to DNA-damage genotoxin-inducible group of bacteria promoters, involved in the SOS regulon response was selected as a testing model. In this genetic construct fast folding variant of *gfp* gene – *gfpmut2* was used [13]. Bacterial biosensors, detect the genotoxic mode of action of some chemicals and drugs, are based on fluorescence measurements. This leads to an increase concentration of GFP protein and as a consequence – its fluorescence [14–16]. *RecA* promoter-operator, as it was previously shown, is useful to detect genotoxicants, environmental chemicals, anticancer drugs and some new candida the drugs [12,14–21]. So far, many authors have presented the cytotoxicity analysis of caffeic and rosmarinic acids. There is no literature presented some correlation among cyto-, genotoxic properties of tested chemicals and their antioxidant potency using the *recA:gfp* genetic system in *E. coli*. Do salts ions (Li, Na and K) affect the antioxidant effect of natural phenolic compounds?

Cytotoxic potency of tested chemicals were calculated on the basis on the dose that confers at certain inhibition percentage such as 20% for each concentrations of analysed chemicals. Genotoxic properties were calculated on the basis of the fold increase (FI) of SFI values normalized with control. Antioxidant potencies were established on the base of DPPH assay. Antimicrobial activity of chemicals was established on the value of minimal inhibitory concentration (MIC).

Our team is involved in examination of chemical reaction and electron structure of natural ligands for many years. In our previous papers we have studied the influence of metals on the molecular structure and electronic charge distribution of biologically important ligands such as nicotinic, picolinic, salicylic, benzoic acids [22–27]. We have showed that toxic metals such as Hg(I), Hg(II), Ag(I), Pb(II), Cd(II) and alkali metals disturb uniform electronic system of examined ligands, while 3-d transition metals, lanthanides and aluminum stabilized electronic systems by bonds delocalization effect. We have showed that a degree of perturbation of the uniform distribution of electronic system of ligands and its aromatic properties depended on an ionic potential of metals. This studies have been realised using FT-IR, FT-Raman, NMR, X-ray methods and theoretical calculations. In the next works we showed also in several cases that the molecular structure and electronic charge distribution determine the biological activity of ligand and complexes [25–27].

2. Materials and methods

2.1. Chemicals preparation

Caffeic and rosmarinic acids were commercially obtained (Sigma Aldrich, UK). Chemicals were dissolved in Milli-Q® water and were transferred to phosphate buffered saline – PBS buffer (1.44 g Na_2HPO_4, 0.24 g KH_2PO_4, 0.2 g KCl, 8 g NaCl in 1000 mL distilled water, pH = 7.0) with bacteria strains.

Lithium, sodium and potassium, caffeinates or rosmarinates were prepared by dissolving the powder of acids in a water solution of the appropriate alkali metal hydroxide in a stoichiometric ratio (1:1). All reagents were Aldrich analytical chemicals, with the exception of LiOH (Sigma). The solutions were than heated in a shaker to ca 70 °C for to completely dissolve the acid (somewhere 1 h). The pH of the aqueous solutions of the acids was about 4, while the pH of the saline solutions was about 6.The products precipitated by slow evaporation. Then the salts were dissolved and crystallized from demineralised water. After drying, the IR spectra were recorded. The correctness of the synthesis was determined by the disappearance of characteristic bands in the acid spectrum.