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Antigenotoxic effects of a bark extract from *Magnolia officinalis*

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Abstract: The aim was to detect antimutagenic and DNA protective effects of a plant extract from bark of *Magnolia officinalis* adversing oxidative DNA damage. The ability to inhibit mutagenicity induced by *tert*-butyl hydroperoxide (*t*-BOOH) and hydrogen peroxide (H_2O_2) was determined with Ames test using *Salmonella typhimurium* His$^-$ TA102 bacterial strain. Inhibition values of 72.8 and 98.7 % were detected for *t*-BOOH and H_2O_2, respectively. The protective effect of the extract against DNA strand scission induced by hydroxyl radicals was studied with plasmid pBluescript II SK(-). The analysis of DNA strand breaks in the plasmid DNA proved a significant inhibition of DNA damage.

Key words: Ames test, Antimutagenicity, DNA strand scission, *Magnolia officinalis*

Introduction

Plants are a rich source of various biologically active compounds. These compounds have been shown to possess a variety of biological activities: antioxidative, antimicrobial, antiviral, antiinflammatory, antimutagenic and anticarcinogenic (Boubaker et al. 2010, Carino-Cortés et al. 2007, Ho et al. 2001, Ramasamy & Agarwal 2008, Xia et al. 2010, Zhang & Rock 2004). Those biological activities play an important role in prevention of various diseases such as cancer or cardiovascular and neurodegenerative diseases (Kris-Etherton et al. 2002, Manach et al. 2004, Scalbert & Williamson 2000, Verhagen et al. 1997).

The aim was to detect the antigenotoxic potential of an extract from bark of *Magnolia officinalis*. These extracts have traditionally been used in Chinese and Japanese medicines and as components of dietary supplements and cosmetic products (Li et al. 2007). We investigated the antimutagenic and DNA protective effects of such an extract on the oxidative DNA damage. The protective activity of the analyzed extract was evaluated with the bacterial Ames test detection system employing *S. typhimurium* His$^-$ TA102 for detection of the mutagenicity of oxidative compounds. In the plasmid DNA test, the protective effect of the extract was determined on the basis of its capability to inhibit formation of DNA strand breaks in the plasmid DNA induced by hydroxyl radicals. The importance of these biological tests consists in the screening of the antigenotoxic potential of compounds isolated from plants.

Materials and methods

The plant extract from bark of *M. officinalis* was obtained from Favea, spol. s r.o. (Kopřivnice, Czech Republic). Plasmid pBluescript II SK(-) DNA was isolated from the bacterial strain *Escherichia coli* TOP10. The mutagenic activity of the extract was measured using the auxotrophic bacterial strain *S. typhimurium* His$^-$ TA102 (Ames test) with and without the *in vitro* metabolic activation obtained with a mixture of rat liver S9 microsomal fraction and the cofactor (Ames et al. 1975, Maron & Ames 1983, Mortelmans & Zeiger 2000) and was expressed as a number of revertant colonies found in the presence of the test agent compared to the control sample. The results were evaluated using the SALM software (Broekhoven & Nestmann 1991, Margolin et al. 1989). The antimutagenic activity of the extract against the DNA damage induced by reactive oxygen species (ROS) was investigated using the strain *S. typhimurium* His$^-$ TA102 with hydrogen peroxide and *tert*-butyl hydroperoxide as the model oxidative mutagens. Each mutagenicity and antimutagenicity test was repeated at least three times using two replica plates per sample. The protective effect of the extract sample on DNA damage induced by hydrogen peroxide and transition metal ions was studied using plasmid pBluescript II SK(-).

The principle of detection included the electrophoretic evaluation of changes in the topological state of plasmid DNA. In its native form the double strand DNA of the plasmid pBluescript II SK(-) is in a compact supercoiled conformation. After a single and double strand breaks occur, the supercoiled tertiary structure is impaired and results in the formation of an open circular and linear forms of DNA. The protective activity of the extract was evaluated as the ability to inhibit conversion of the supercoiled form to open circular and linear forms of DNA. The photos of gels were taken in UV light (transilluminator GeneGenius, SynGene, Cambridge, UK) and the bands were quantified using the software ImageJ (Abràmoff et al. 2004).

Results

The antimutagenic effect of the extract was detected with the Ames test using *S. typhimurium* His$^-$ TA102. H_2O_2 and *tert*-butyl hydroperoxide (*t*-BOOH) were used as the model oxidative mutagens at a concentration of 100 µg per plate. The extract was tested in a range of 50-300 µg sample per plate. A significant antimutagenic effect of the extract was detected with both oxidative mutagens used (Figure 1). The number of induced revertants was obtained by subtracting the number of spontaneous revertants from the number of revertants on the plates containing the mutagen and the extract. The maximal decrease in revertant numbers was found when the highest concentration of the extract was used.

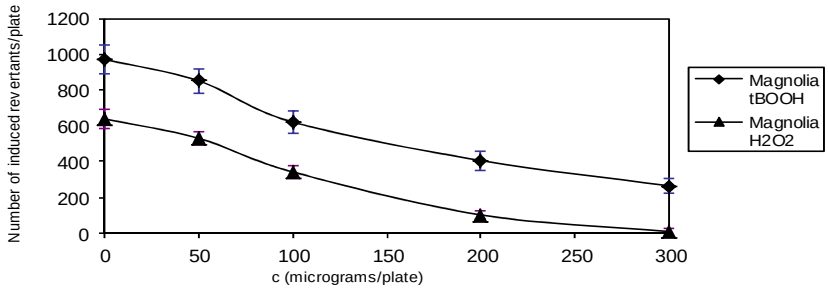

Fig 1: Antimutagenic effect of the extract in Ames test (strain TA102) without metabolic activation. Mutations were induced by *t*-BOOH or H_2O_2 (100 µg/plate).

The respective percentages of the inhibition of mutagenicity with *t*-BOOH and H_2O_2, when the extract was used at a concentration of 300 µg per plate, were 72.8 and 98.7 % (Table 1). The inhibition rate (%) was calculated as follows: percent inhibiton (%) = [1 – (number of revertants on plates with the oxidant and the test compound – number of spontaneous revertants)/(number of revertants on plates with the oxidant alone – number of spontaneous revertants)] x 100 (Stagos et al. 2006). No mutagenic effect of the extract (50-300 µg/plate) was detected with the strain *S. typhimurium* His$^-$ TA102 in the tests with and without the *in vitro* metabolic activation.

Tab 1: Antimutagenic activity of the extract measured with Ames test using *S. typhimurium* His$^-$ TA102 with *t*-BOOH or H_2O_2 as the mutagenic compounds.

Mutagen (100 µg/plate)	Extract			
	50 µg/plate [a]	100 µg/plate [a]	200 µg/plate [a]	300 µg/plate [a]
tert-butyl hydroperoxide	12.4	36.0	58.3	72.8
Hydrogen peroxide	16.9	46.4	84.0	98.7

[a] The numbers represent the percentage of mutagenicity inhibition.

Figure 2 shows the protective activity of the extract inhibiting the formation of DNA strand breaks in the plasmid pBluescript II SK(-) induced by H_2O_2 in the presence of $FeSO_4$. The sample was tested in a concentration range of 0.6-3 µg in a volume of 10 µl of the reaction mixture. When the smallest dose of 0.6 µg was applied, no inhibition of the formation of DNA strand breaks was detected. The protective effect of the analyzed extract was observed starting from a dose of 1.0 µg. The strongest effect of inhibition of DNA strand breaks formation was found when 1.5-3 µg sample were used.

As to all the extract concentrations tested, the content of supercoiled DNA form was quantified using software ImageJ (Abràmoff et al. 2004). The value obtained for the supercoiled DNA form was related to that of the control. In a concentration range of 0.6-3 µg extract applied an increasing rate of the protective effect was found, expressed by per cent value of the presence of supercoiled form plasmid DNA. The experiments showed no potential genotoxicity of the extract in this plasmid DNA test.

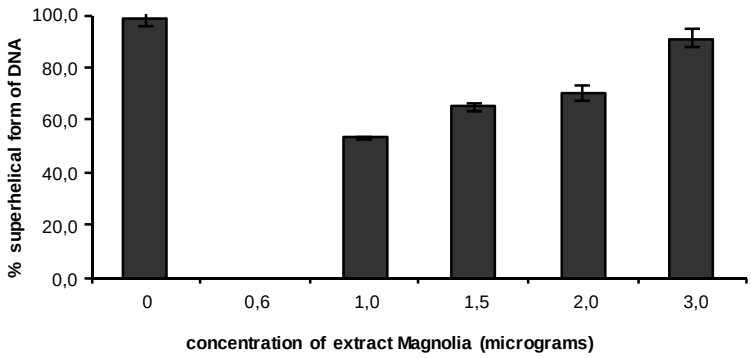

Fig 2: Analysis of supercoiled form of the plasmid pBluescript II SK(-) after oxidative DNA damage in the presence of extract (0.6-3 µg).

Discussion

Bark extracts from *M. officinalis* have been studied using biological tests to look for the presence of biological active compounds magnolol and honokiol (Bang et al. 2000, Ikeda & Nagase 2002, Ho et al. 2001, Li et al. 2007, Nagase et al. 2001, Park et al. 2003, Saito et al., 2006). These compounds were reported to exhibit various biological activities such as antimicrobial effect (Chang et al. 1998, Clark et al. 1981, Ho et al. 2001), antioxidative activity (Chen et al. 2009, Haraguchi et al. 1997, Lo et al. 1994), antifungal activity (Bang et al. 2000), anti-inflammatory effect (Wang et al. 1995), and anticarcinogenic and antimutagenic activities (Fujita & Taira 1994, Nagase et al. 2001, Ikeda & Nagase 2002, Saito et al. 2006). Honokiol was found to have a strong antioxidative activity in biological systems (Park et al. 2003) that was reported to be 1000-fold greater than that of Vitamin E (Chiu et al. 1997).

Antimutagenic activity of compounds isolated from plants can be evaluated on the basis of their ability to inhibit mutagenicity (Horn & Vargas 2008, Makena & Chung 2007). In this study, the antimutagenic effect of an extract from *M. officinalis* against the mutagenicity induced by *tert*-butyl hydroperoxide and hydrogen peroxide mutagens was investigated using the *S. typhimurium* His^- TA102 Ames test. A significant inhibition of mutagenicity caused by *tert*-butyl hydroperoxide and hydrogen peroxide resulting from the application of the tested extract was demonstrated. Similarly, Fujita & Taira (1994) detected antimutagenic effects of magnolol and honokiol using the strain *S. typhimurium* His^- TA102 where the mutagenicity

was generated by UV irradiation. In their previous study, Taira et al. (1993) found that magnolol and honokiol were effective hydroxyl radical scavengers.

We also checked whether the extract itself did not exhibit a mutagenic effect. No potential genotoxicity of the sample was found. Our results thus extended the findings of Saito et al. (2006) and Li et al. (2007) who brought an evidence of non-existence of the mutagenic effect of magnolol using the strains *S. typhimurium* His$^-$ TA98 and TA100 and, also, of Li et al. (2007) who evaluated the mutagenicity of the Magnolia bark extract using the strains TA98, TA100, TA1535 and TA1537. In the latter case no mutagenic activity of the extract was demonstrated.

The ability of the extract to inhibit oxidative damage of DNA was further investigated using the plasmid DNA test where the protective activity of the extract was evaluated on the basis of its capability to inhibit formation of DNA strand breaks. The protective effect measured probably reflected the presence of biological active compounds in the extract tested. The results obtained with the Ames- and plasmid DNA tests extended the knowledge of the beneficial biological potential of the bark extract and suggested its possible applicability in the food- and pharmaceutical industry.

Conclusions

The Ames test was used for the assessment of mutagenicity and antimutagenicity of an extract from bark of *Magnolia officinalis* and demonstrated a significant inhibition of mutagenicity induced by oxidative mutagens. The use of the plasmid DNA test documented an ability of the extract to efficiently inhibit the oxidative damage of DNA. No potential genotoxicity of the extract was detected. These results brought an evidence of a beneficial biological effect of the analyzed extract and also demonstrated a possibility of using the Ames test and the analysis of DNA strand breaks for the investigation of protective activities of plant compounds.

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