Investigation of the genetic toxicology of dill essential oil and benzo(a)pyrene in mouse bone marrow by micronucleus test

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The genotoxic activity of dill (*Anethum graveolens* L.) essential oil extracted from seeds and of benzo(a)pyrene was studied by induction of micronuclei in mouse bone marrow cells. Dill essential oil at a dose of 1 g/kg did not increase the frequency of polychromatic erythrocytes (PCEs) containing micronuclei (MN). A double increase of micronucleated PCEs was found in mice treated with benzo(a)pyrene at a dose of 50 mg/kg. The third group of mice was treated with dill essential oil (1 g/kg) and benzo(a)pyrene (50 mg/kg). The frequency of PCEs containing MN in this mouse group was significantly lower than in mice treated only with benzo(a)pyrene. Thus, dill essential oil reduced the number of PCEs containing MN and in this case showed an antigenotoxic effect.

Key words: mouse bone marrow cells, polychromatic erythrocytes, micronuclei, essential oils

INTRODUCTION

Polycyclic aromatic hydrocarbons are widespread environmental pollutants due to intensive human activities during the last decades [1, 2]. Benzo(a)pyrene (BaP) is recognized as the prototype for this class of compounds and is used in investigations of their potential mutagenicity and carcinogenicity. BaP belongs to a large group of chemically inert compounds that exert their mutagenic and carcinogenic effects only after metabolic activation [3–5]. The primary group of enzymes involved in the activation and detoxication of chemicals is mono-oxygenases. Aromatic compounds are metabolized by microsomal monooxygenazes to electrophilically reactive epoxides. Such epoxides can bind covalently to cellular macromolecules [6–8].

BaP is mutagenic in different non-mammalian systems [9–13], as well as *in vivo* in the dominant lethal assay [14], in the SCE test in mammalian bone marrow, fetal liver cells and peripheral blood lymphocytes [15–19], and in the micronucleus test with mouse bone marrow [20–24].

Essential oils extracted from plants are widely used as fragrances in cosmetics and perfumes, flavouring additives of food beverages, scenting agents in a variety of household products and as constituents of some drugs. Investigation results of essential oils genotoxicity are rather contradictory. Some reports indicated that essential oils of various plants may be genotoxic *in vitro* as well as *in vivo*. Essential oils extracted from dill induced chromosome aberrations and sister chromatid exchanges in human lymphocytes *in vitro* as well as gene mutations in *Drosophila melanogaster* somatic cells *in vivo* [25, 26]. According to other authors, essential oil of such aromatic plants as *Mentha pulegium* L., *Origanum vulgare* subsp. *Hirtum* Ietswaart, *Coridothymus capitatus* Reichenb. and *Satureja thymbra* L. was not mutagenic in *D. melanogaster* somatic mutation and recombination (SMART) test *in vivo* [27, 28]. Aromatic sagebrush (*Artemisia dracunculus* L.) essential oil was not genotoxic in a Salmonella-microsome reversion assay [29].

Essential oil extracted from different parts of the same plant may show different gonotoxicity. Essential oil from dill herb induced a dose-dependent increase of somatic mutations and recombination in *D. melanogaster in vivo,* while essential oil from dill seeds was almost inactive in the same SMART test [26].

Thus, polycyclic aromatic hydrocarbons and essential oils are widespread in human environment and it is possible that these compounds can penetrate into the same organism and its tissues. There are reports indicating that some plant essential oil reduced the mutagenic effect of benzo(a)pyrene in mice [30, 31].

The mechanism of the antimutagenic effect of essential oil is still not quite clear. It is not established yet whether essential oil of all aromatic plants is able to prevent genetic damage induced by benzo(a)pyrene and which individual components of essential oil cause its antimutagenic properties.

The purpose of this study was to investigate the genotoxicity of essential oil isolated from the aromatic plant dill (*Anethum graveolens* L.) and its effect on the mutagenicity of benzo(a)pyrene using micronucleus test with mouse bone marrow *in vivo*.

MATERIALS AND METHODS

Test agents. Benzo(a)pyrene (Fluca 12780) and essential oil extracted from dill (*Anethum graveolens* L.) seeds were dissolved in sunflower oil and administered by a single intraperitoneal injection at a volume 5 and 10 ml/kg body weight, respectively.

Animals. C_{57}Bl/6 and CBA mice which are widely used in testing genotoxic agents [32] were distributed into four groups of 4–6 animals each. Male and female of both strains were 12–14 weeks old and weighed from 20 to 25 g.

Chemical treatment and slide preparation. Control animals (C) were treated with sunflower oil at a volume 10 ml/kg body weight (CBA mice) or treated neither with solutions nor solvent (C_{57}Bl/6 mice). Dill essential oil (EO) was injected into mice of the second group at a dose of 1g/kg, benzo(a)pyrene (BaP) – into the third mouse group at a dose of 50 mg/kg. The fourth group of mice was treated with dill essential oil and benzo(a)pyrene (EO+BaP) at the above-mentioned doses with one-hour interval between injections.

Bone marrow of all animal groups was sampled from the femur at 36 h after treatment, because at this time BaP exibits the maximal effect inducing micronuclei [21].

The following procedures of slides preparation were as described by Schmid [33]. Polychromatic erythrocytes (PCEs), 1000–2000 per animal, were analysed for the presence of micronuclei.

Statistical analysis. All statistical analyses were performed using InStat V2.02 (GraphPad Software, USA) statistical package. The percentage of micronucleated polychromatic erythrocytes of different mice groups were compared applying Student's t criteria. The difference was considered as significant at $P < 0.05$.

RESULTS AND DISCUSSION

Effects of the above-mentioned chemical compounds on the frequency of micronucleated polychromatic erythrocytes (MN-PCEs) in the bone marrow of both mouse strains are shown in Table. Dill essential oil did not increase the frequency of PCEs containing MN, while benzo(a)pyrene induced a considerable increase of micronucleated PCEs. This increase was statistically significant in C_{57}Bl/6 mice. No statistically significant increase of MN-PCEs was found in mice of both strains treated with dill essential oil and benzo(a)pyrene.

As mentioned above, results of essential oil genotoxicity investigations are rather contradictory. Essential oil extracted from some aromatic plant species was able to induce genetic damages in various test-systems [25, 26, 34], while essential oil from other plant species did not show any genotoxic effect [27–29]. It is even more interesting that essential oil extracted from different parts of the same plant might show different genotoxicity. For example, essential oil from dill herb demonstrated stronger genotoxic properties than essential oil from dill seeds, which was almost inactive in a *D. melanogaster* SMART test [26]. Essential oil from dill seeds in the present investigation was not active in mouse bone marrow and did not increase the frequency of micronuclei. This phenomenon probably could be explained by a different concentration of individual components in essential oil from different parts of a plant. Furthermore, a seasonal variation in the chemical composition of essential oils of aromatic plants was indicated [35]. Thus, the genotoxic properties of essential oil of the same plant may vary during seasons of the year.

The protective role of essential oil of various plants against mutagenicity of benzo(a)pyrene was indicated in some re-

Table. **Effects of dill essential oil and benzo(a)pyrene on the frequency of micronucleated polychromatic erythrocytes (MN-PCEs) in C_{57}Bl/6 and CBA mouse bone marrow**

Treatment group	Number of animals	Analysed PCEs	Total MN-PCEs	MN-PCEs, % ± S. E. M
		C_{57}Bl/6 mice		
C	4	4098	7	0.17 ± 0.07
EO	5	5225	9	0.17 ± 0.06
BaP	4	4100	22	0.54 ± 0.05*
EO+BaP	4	4107	11	0.27 ± 0.07
		CBA mice		
C	4	8071	16	0.19 ± 0.05
EO	5	10049	27	0.27 ± 0.05
BaP	6	12447	50	0.40 ± 0.06
EO+BaP	5	10072	26	0.26 ± 0.05

*P < 0.05, Student's t test.

ports. Essential oil extracted from aqueous turmeric (*Curcuma* L.) and evening primrouse (*Oenothera* L) inhibited the ability of benzo(a)pyrene to induce micronuclei in mouse bone marrow [30, 31]. Results of the present investigation demonstrated that essential oil isolated from dill (*Anethum graveolens* L.) was able to inhibit micronuclei formation induced by benzo(a)pyrene in mouse bone marrow as well. This antimutagenic effect essential oil of dill and other plants *in vivo* probably could be caused by some of its compounds, for example, β-myrcene. According to some reports, β-myrcene, terpinol, menthol and some other compounds of essential oil are able to inhibit liver mono-oxygenases responsible for activation of polycyclic aromatic hydrocarbons promutagenes [36, 37]. The protective role against the effect of benzo(a)pyrene was indicated also for such a compound of essential oil as cumarin [38].

Thus, results of the investigation showed that essential oil extracted from the aromatic plant dill (*Anethum graveolens* L.) was not genotoxic for mouse bone marrow cells *in vivo* and even reduced the mutagenic effect of benzo(a)pyrene inducing micronuclei. This antimutagenic effect of dill essential oil can be caused by some of its individual components able to inhibit enzymes responsible for activation of promutagene.

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KRAPŲ (*ANETHUM GRAVEOLENS* L.) ETERINIO ALIEJAUS IR BENZ(A)PIRENO GENOTOKSIŠKUMO PELIŲ KAULŲ ČIULPŲ LĄSTELĖMS TYRIMAI MIKROBRANDUOLIŲ TESTU

S a n t r a u k a

Tirtas krapų (*Anethum graveolens* L.) eterinio aliejaus ir benz(a)pireno genotoksiškumas pelių kaulų čiulpų ląstelėms. Krapų eterinis aliejus nepadidino polichromatinių eritocitų su mikrobranduoliais dažnio, o veikiant abiem minėtoms medžiagoms kartu, sumažino benz(a)pireno genotoksinį aktyvumą indukuojant mikrobranduolius. Antimutageninis krapų eterinio aliejaus poveikis greičiausiai sietinas su kai kurių jo komponentų gebėjimu inhibuoti promutageną aktyvinančius fermentus.

Raktažodžiai: pelių kaulų čiulpai, polichromatiniai eritrocitai, mikrobranduoliai, eteriniai aliejai