Chemoprotective Effects of Broccoli Juice Treated with High Pressure

LUCIE MANDELOVÁ and JIŘÍ TOTUŠEK

Institute of Preventive Medicine, Faculty of Medicine, Masaryk University in Brno, Brno, Czech Republic

Abstract

MANDELOVÁ L., TOTUŠEK J. (2006): **Chemoprotective effects of broccoli juice treated with high pressure**. Czech J. Food Sci., **24**: 19–25.

We investigated chemoprotective effects of broccoli juice, treated with high pressure method, using 500 MPa for a period of 10 minutes. By the use of this method, the conservation of nutritionally important substances occurs (for example vitamins, polyphenolic compounds, glucosinolates and other constituent substances). We followed the inhibition of mutagenicity of the model mutagen, N-nitroso-N-methylurea (MNU), by means of *in vivo* micronucleus test. After fourteen-day application of broccoli juice (0.2 ml/10 g of body weight of mouse) and after a single administration of MNU mutagen (50 mg/kg), a statistically significant decrease ($P < 0.01$) occurred of the number of micronuclei induced by the application of MNU. Broccoli juice treated with high pressure therefore seems to have a preventive potential against MNU-induced micronuclei formation in BALB/C mouse bone marrow cells.

Keywords: micronucleus test; broccoli; antimutagenicity; MNU

Tumourous diseases are becoming the second most common cause of death all over the world. Nutrition and the life style belong to their main causes. One third of all the tumourous diseases is caused by inadequate nutrition (SUGIMURA 1986). The incidence of colorectal carcinoma has an increasing tendency in the Czech Republic, which is number one in it all over the world.

Heterocyclic amines, polycyclic aromatic hydrocarbons or N-nitrosocompounds are formed during the thermal processing of typical western food with high contents of red meat and of animal fats (RAUSCHER *et al.* 1998).

Nitrosocompounds represent a large group of chemical substances in many components of the environment. Strong mutagen N-nitroso-N-methylurea (MNU) belongs to the well-known N-nitrosocompounds (STEPHANOU *et al.* 1996).

A great number of components, which are contained in food, can be nitrosated in the gastrointestinal tract, namely in the stomach. Stable nitrosamines, as well as less stable nitrosocompounds are formed there. Amines, aminoacids or other components of food can serve as precursors of those substances. With regard to the relatively high intake of these substances with food and with regard to the rate of nitrosating reaction, endogenically formed nitrosocompounds can represent the same risk as the intake of nitrosamines or of nitrosamides with food. Nitrosocompounds show genotoxic effects with cancerogenic potential (TUREK *et al.* 1994).

Supported by the Ministry of Agriculture of the Czech Republic, Project No. QF 3287.

Nitrosamides are direct mutagens which do not demand metabolic activation and which can thus act directly on mucosa of the stomach and can initiate the process of carcinogenesis (Luthy *et al.* 1984; Thorgeirsson *et al.* 1994; Turek *et al.* 1994). On the other hand, nitrosamines are subject to oxidation and by means of mediators, they form substances capable of the reaction with DNA resulting in the formation of adducts (Turek *et al.* 1994).

The exogenous formation is ensured by a high intake of red meat which increases intestinal nitrosation, this can explain the positive epidemiological relation between the intake of red meat and the risk of incidence of colorectal carcinoma (Donelly *et al.* 2004). Vegetables, tea, and soya block the formation of N-nitrosocompounds, thus being related to the protection against the emergence of colorectal carcinoma (Hughes *et al.* 2002).

Besides the exogenous supply of nitrosocompounds, more serious formation of N-nitrosocompounds can occur, for example by interaction of nitrates, eventually with nitrites formed of them, with substances suitable for nitrosation, coming mostly from the metabolism of proteins. Ascorbic acid, tocopherols, polyphenols and other substances can inhibit nitrosation (Turek *et al.* 1994). At present the possible inhibitory power of glucosinolates or of their degradation products – isothiocyanates contained in vegetables of kohlrabi family (*Brassicaceae*) – is considered.

Many epidemiological studies indicate that the consumption of kohlrabi vegetables (for example broccoli, cabbage, cauliflower, kale, and Brussels sprouts) is connected with a reduced incidence of tumourous diseases, especially of colorectal carcinoma (Steinmetz & Potter 1996; Weisburger 2000; Kassie *et al.* 2003; Kim *et al.* 2003; Papapolychroniadis 2004; Schonhof *et al.* 2004).

Broccoli, as a main representative of kohlrabi family (*Brassicaceae*), recorded recently an increased consumption. While in 1980 its consumption in the Czech Republic was almost imperceptible, in 1998 it amounted to 0.8 kg per head and year. Broccoli has a high content of vitamin C (100–120 mg/100 g) and also of vitamin E (0.8–1.6 mg/100 g), furthermore its contents of potassium (427–644 mg/100 g), magnesium (66 mg/100 g), calcium (90 mg/100 g), iron (7.3 mg/100 g) and of trace amounts of iodine and of selenium are worthy of notice. Besides nutrients mentioned above, broccoli contains the highest amount of glucosinolates of all cabbage stalk vegetables (Malý 2000).

These substances induce biotransformational enzymes of the second phase (for example glutathione transferase, epoxid hydrolase, NAD(P)H, quinone reductase and glucuronyl transferase) and support the antioxidative activity on mammalian level. These substances are mostly ascribed to sulforaphane, belonging to isothiocyanates. Sulforaphane and other isothiocyanates are stored in plants as ineffective precursors called glucosinolates. Isothiocyanates are released by hydrolysis by means of enzyme myrosinase which is found in kohlrabi – type vegetables, together with glucosinolates (Fahey *et al.* 1997; Shapiro *et al.* 2001; Talalay & Fahey 2001; Rambousková 2002; Paolini *et al.* 2004).

In this study we investigated the antimutagenic efficiency of broccoli juice treated with high pressure a process causing the inactivation of microorganisms but maintaining simultaneously the nutritionally important substances (for example vitamins, polyphenolic compounds and other constituent substances).

MATERIALS AND METHODS

Chemicals. N-nitroso-N-methylurea from Sigma-Aldrich, s. r. o., Prague, Czech Republic was used as a model mutagen. Foetal Bovine serum and other chemicals were obtained from Sigma-Aldrich, s. r. o. (Prague, Czech Republic).

Preparation of broccoli juice. Broccoli was cleaned, washed and cut into pieces. Juice was obtained by pressing out in the juicer Green power (USA). After pressing out, juice was allowed to stand at rest for a period of 100 minutes. Due to the mechanical breakdown, the contact of the enzyme myrosinase with inactive glucosinolates occurs, with the formation of biologically active isothiocyanates, namely of sulforaphane. After allowing the juice to stand, it was strained through a coarse sieve (diameter of opening 0.5 mm). After this, the juice was filled into PET bottles of the volume of 1.5 l and treated with the pressure of 250 MPa for a period of 2 min to remove air. Subsequently, the juice was acidified to pH 4 (with citric acid) and filled into PET bottles of the volume of 100 ml. The filled bottles were subjected to the pressure of 500 MPa for a period of 10 min in a high pressure press CYX 6/0103 (ŽĎAS, a. s., Czech Republic). The temperature of the samples before the treatment was 21°C, the starting temperature in the chamber was 10°C, the temperature after the interrption of the pressure was 12°C (room temperature 17°C). After marking the samples with labels, these were stored in an ice-box and on 5th day they were transported in a thermo-box to the laboratory for another testing, where they were stored in an ice-box at the temperature of 4–6°C.

Experimental animals. The experiment was performed on 7–10 weeks old males of BALB-C mice of a body mass 15–21 g, which were obtained from breeding and supplier's facilities of Faculty of Medicine of Masaryk University (Brno, Czech Republic). The animals were kept under standard laboratory conditions at 12-hour cycle light/darkness, at the temperature of 22 ± 2°C. They were fed the complete laboratory mixture for laboratory mice and for brown rats in SPF breeds, water was served *ad libitum*. One week before the experiment, the animals were left at a standstill, for the purpose of setting down.

Application of tested substances. The animals were divided into 4 groups, with 8 mice in each. The substance tested (broccoli juice) was applied to first (I.) and second (II.) groups of mice for a period of 14 days in a dose of 0.2 ml/10 g of body weight of mouse. Second group, fed the complete mixture for laboratory mice, served as control. On 14th day, mutagen N-nitroso-N-methylurea (MNU) in the dose of 50 mg/kg (0.2 ml/10 g) was administered in a single dose to first (I.) and third (III.) groups, one hour after the application of broccoli juice. 7%-dimethylsulfoxide (DMSO) in the dose of 0.2 ml/10 g, which was used for dissolution of mutagen, was administered to fourth (IV.) group. All substances administered were applied *per os* by the gavage.

Micronucleus test. *In vivo* mouse bone marrow micronucleus test is a well established, reliable, and short-term test for the detection of chemicals with clastogenic effect (ability of producing chromosomal breaks). The test was carried out according to Schmid (1975). An increased number of micronuclei in polychromatic erythrocytes, in comparison with the control group, indicate that the chemical compound tested produces chromosomal damage in erythrocytes of marrow. Chromosomal damage is associated with the appearance and/or progression of tumours and with adverse reproductive and developmental outcomes (Krishna & Hayashi 2000).

24 hours after the application of substances, all animals were killed and subsequently treir both femurs were dissected. The marrow from both femurs was rinsed into 2 ml of foetal bovine serum and incubated in the thermostat at the temperature of 37°C. The test tubes with the rinsed marrow were subsequently centrifuged at 1000 revolutions per minute for a period of 5 minutes. After centrifugation, the supernatant was gently sucked of and the sediment with blood cells was mixed and spread on microscopic slides. After 24 hours the coatings were stained with May-Grünwald and Giemsa as described by Schmid (1975). Three preparations were made from every animal tested. 1000 of polychromatic erythrocytes from two preparations (500 cells from every preparation) were evaluated on the whole for determining the micronucleated polychromatic erythrocytes (MNPCE).

Statistical processing of data. Programme Statistika 7 was used for the statistical analysis. The differences in the numbers of micronuclei per 1000 of polychromatic erythrocytes were evaluated by means of non-parametric Mann Whitney U test. Body weights were compared using the ANOVA test.

Table 1. Effect of broccoli juice on the formation of micronuclei, produced by MNU

Group	Applied substances	MNPCE/1000 PCE	Number of animals	Mean ± SD
I.	broccoli juice (14 × 0.2 ml/10 g) + MNU (1 × 50 mg/kg)	8, 7, 7, 6 ,8, 8, 8, 7	8	7.38 ± 0.74*
II.	broccoli juice (14 × 0.2 ml/10 g)	2, 2, 3, 3, 3, 0, 2, 3	8	2.25 ± 1.04
III.	MNU (1 × 50 mg/kg)	17, 16, 20, 21, 20, 16, 16, 18	8	18.0 ± 2.07**
IV.	7% DMSO (1 × 0.2 ml/10 g)	1, 2, 3, 2, 0, 2, 3, 2	8	1.88 ± 0.99

MNPCE – number of micronuclei in polychromatic erythrocytes; PCE – polychromatic erythrocytes; SD – standard deviation; *$P < 0.01$ – significantly lower number of micronuclei in relation to MNU; **$P < 0.01$ – significantly higher number of micronuclei in relation to control (7% DMSO)

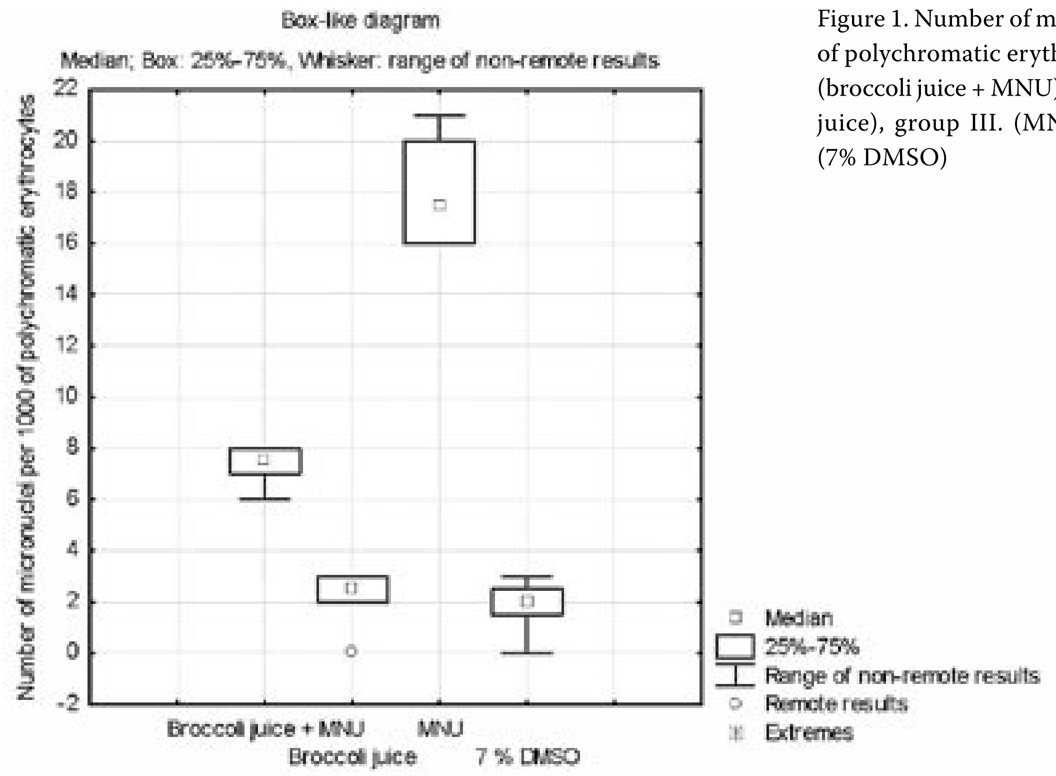

Figure 1. Number of micronuclei per 1000 of polychromatic erythrocytes in group I. (broccoli juice + MNU), group II. (broccoli juice), group III. (MNU) and group IV. (7% DMSO)

RESULTS

Male BALB/C mice were treated with broccoli juice for the period of fourteen days with a dose of 0.2 ml/10 g of the body weight of mouse.

The average percentage gains in the body weight during the first week of the 14-day period were 8.15%, 7.94%, 6.59%, and 5.95% in the I., II., III. and IV. groups, respectively. After fourteen days, these percentage gains in the body weight were 0.71%, 2.79%, 5.84%, and 10.15%, respectively. The effect of the diet supplemented with broccoli juice on the weight gain was not statistically significant ($P > 0.05$).

It is evident from Figure 1 that broccoli juice administered *per os* by the gavage reduced the number of micronuclei in group I. that was given broccoli juice and mutagen MNU, in comparison with group II., which was administered MNU mutagen only.

Statistically significant differences ($P < 0.01$) in the number of micronuclei were proved between group I. (MNU 1 × 50 mg/kg + broccoli juice 14 × 0.2 ml/10 g) and group III. given mutagen only (MNU 1 × 50 mg/kg). These results were proved on the level of significance $P < 0.01$ (Table 1). With group III., where only mutagen (1 × 50 mg/kg) was applied in a single dose, a statistically significant difference was found in comparison with group IV. (7% DMSO), on the level of significance $P < 0.01$ (Table 1).

The numbers of micronuclei did not differ statistically significantly between group II., where broccoli juice only (14 × 0.2 ml/10 g) was applied, and group IV., where 7% DMSO (1 × 0.2 ml/10 g) was administered in a single dose.

The results obtained here prove the suppressive role of broccoli juice on the mutagenic effect of MNU in this test system.

DISCUSSION

The results obtained in this study prove that the application of MNU, acting as a direct mutagen, leads to statistically higher numbers of micronuclei, thus causing chromosomal damage of immature erythrocytes of marrow. The broccoli juice applied, treated with high pressure shows chemoprotective effects against MNU.

Similarly, Sedmíková *et al.* (1999) monitored antimutagenic activity of cauliflower juice treated with high pressure and pasteurised. It is evident from the results of Ames test that the treatment with high pressure preserved nutritionally important substances and antimutagenic activities towards strong IQ mutagen.

In vivo micronucleus test can be a useful short term test the serving explanation of the mechanisms and the identification of chemical compounds responsible for anticlastogenic effect. Edenharder *et al.* (1998) proved anticlastogenic effects of fruits and vegetables, commonly consumed in Germany, against benzo(a)pyrene and cyclophosphamide. Similar effects were confirmed in the subsequent study (Edenharder *et al.* 2003).

Fimognari *et al.* (2005) showed that sulforaphane, butylisothiocyanate and the mixture of isothiocyanates do not increase the number of micronuclei. In addition to that, sulforaphane, in the dependence on the dose, induces an increase of apoptic cells. The authors also pointed out that kohlrabi vegetables contain not only substances with chemoprotective effects, but also compounds with potential genotoxic and carcinogenic effects (benzylisothiocyanates and phenylisothiocyanates).

Besides those of vegetables, the positive effects of some sorts of fruits, soya, and tea are also documented; these can block the formation of N-nitrosocompounds and are connected with the chemoprotective effect against colorectal carcinoma. Hughes *et al.* (2002) investigated the effect of the consumption of 400 g of vegetables daily on the decrease of N-nitrosocompounds levels in stools. This effect was not confirmed, nevertheless foods rich in vegetables accelerate the passage through the intestine, thus reducing the contact of intestinal mucosa with these substances.

Another study confirms a decreased incidence in brown rats of the cancer of breast, induced by the application of MNU, due to the subsequently served food enriched with cabbage (Bresnick *et al.* 1990). Similar results were proved with brown rats whose food was enriched with garlic powder (Schaffer *et al.* 1996), or with brown rats to which genistein and daidzein, two main representatives of isoflavanones, were administered in the form of injections (Constantinou *et al.* 1996).

Besides glucosinolates, kohlrabi vegetables also contain indoles. Indole-3-carbinol shows protective effects against cyclophosphamide, causing the formation of micronuclei (Agrawal & Kumar 1998).

The consumption of kohlrabi vegetables (broccoli, cauliflower, cabbage, kale, Brussels sprouts, radish, watercress, and kohlrabi) is relatively high in comparison with other sorts of vegetables. But the amounts are dependent on the geographic region. In Singapore, for example, the consumption of kohlrabi vegetables is estimated to 40 g daily, representing three times the amount consumed in USA (Park & Pezzuto 2002).

The results of studies prove that kohlrabi vegetables and their juices can protect against various carcinogens. These properties are connected with the induction of biotransformational enzymes which take part in detoxication. It is questionable whether the protective effects of kohlrabi vegetables can be ascribed to individual compounds suche as isothiocyanates or indoles, or to other compounds contained (carotenoids, folic acid, selenium, fiber, flavonoids etc.) (Steinkellner *et al.* 2001; Conaway *et al.* 2002).

Kohlrabi vegetables and bioactive compounds present in vegetables of this family such as isothiocyanates and indole-3-carbinol, are extensively studied on experimental *in vitro* and *in vivo* models. The results suggest that chemoprotective compounds from kohlrabi vegetables can influence the process of carcinogenesis during the initiating and promoting phases. The conclusions of epidemiological and of clinical studies support this idea and are similar. But there is no complex evaluation of all aspects concerning the relations between the consumption of kohlrabi vegetables and the prevention of tumourous disease. We are still far from understanding the effects of phytochemicals present in kohlrabi vegetables and acting in chemoprotective way and their overall mechanism of actions, leading to the protective effects (Murillo & Mehta 2001).

In the present study, evidence is presented that broccoli juice treated with high pressure, containing glucosinolates, polyphenols, fiber, vitamins, minerals, and other bioactive compounds, can inhibit clastogenic activity induced by MNU and thereby interfering with the process of cancer initiation. Consequently, using this method (high pressure) is very useful for the conservation of the nutritionally important substances and the maintenance of the antimutagenic activity, and therefore for the interference with the process of carcinogenesis.

However, further investigations are needed to find whether broccoli juice treated with high pressure can fulfill its promise as a functional food.

Acknowledgement. The authors are much obliged to Mrs. K. Sedlářová and Mrs. M. Pokludová for their selfless help.

Reference

Agrawal R.C., Kumar S. (1998): Prevention of cyclophosphamide-induced micronucleus formation in mouse bone marrow by indol-3-carbinol. Food and Chemical Toxicology, **36**: 975–977.

Bresnick E., Birt D.F., Wolterman K., Wheeler M., Markin R.S. (1990): Reduction in mammary tumorogenesis in the rat by cabbage and cabbage residue. Carcinogenesis, **11**: 1159–1163.

Conaway C.C., Yang Y., Chung F. (2002): Isothiocyanates as cancer chemopreventive agents: Their biological activities and metabolism in rodents and humans. Current Drug Metabolism, **3**: 233–255.

Constantinou A.I., Mehta R.G., Vaughan A. (1996): Inhibition of N-methyl-N-nitrosourea-induced mammary tumors in rats by the soybean isoflavones. Anticancer Research, **16**: 3293–3298.

Donelly E.T., Bardwell H., Thomas G.A., Williams E.D., Hoper M., Crowe P., McCluggage W.G., Stevenson M., Phillips D.H., Hewer A., Osborne M.R., Campbell F.C. (2004): Modulation of N-methyl-N-nitrosourea-induced crypt restricted metallothionein immunopositivity in mouse colon by a non-genotoxic diet-related chemical. Carcinogenesis, **25**: 847–855.

Edenharder R., Frangart J., Hager M., Hofmann P., Rauscher R. (1998): Protective effects of fruits and vegetables against *in vivo* clastogenicity of cyclophosphamide or benzo[a]pyrene in mice. Food and Chemical Toxicology, **36**: 637–645.

Edenharder R., Krieg H., Köttgen V., Platt K.L. (2003): Inhibition of clastogenicity of benzo[a]pyrene and of its *trans*-7,8-dihydrodiol in mice *in vivo* by fruits, vegetables, and flavonoids. Mutation Research, **537**: 169–181.

Fahey J.W., Zhang Y., Talalay P. (1997): Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proceedings of the National Academy of Sciences of the United States of America, **94**: 10367–10372.

Fimognari C., Berti F., Iori R., Cantelli-Forti G., Hrelia P. (2005): Micronucleus formation and induction of apoptosis by different isothiocyanates and a mixture of isothiocyanates in human lymphocyte cultures. Mutation Research, **582**: 1–10.

Hughes R., Pollock J.R., Bingham S. (2002): Effect of vegetables, tea, and soy on endogenous N-nitrosation, fecal ammonia, and fecal water genotoxicity during a high red meat diet in humans. Nutrition and Cancer, **42**: 70–77.

Kassie F., Uhl M., Rabot S., Grasl-Kraupp B., Verkerk R., Kundi M., Chabicovsky M., Schulte-Hermann R., Knasmüller S. (2003): Chemoprevention of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-induced colonic and hepatic preneoplastic lesions in the F344 rat by cruciferous vegetables administered simultaneously with the carcinogen. Carcinogenesis, **24**: 255–261.

Kim D.J., Shin D.H., Ahn B., Kang J.S., Nam K.T., Park Ch.B., Kim Ch.K., Hong J.T., Kim Y., Yun Y.W., Jang D.D., Yang K. (2003): Chemoprevention of colon cancer by Korean food plat components. Mutation Research, **523–524**: 99–107.

Krishna G., Hayashi M. (2000): *In vivo* rodent micronucleus assay: protocol, conduct and data interpretation. Mutation Research, **455**: 155–166.

Luthy J., Carden B., Friederich U., Bachmann M. (1984): Goitrin-a nitrosatable constituent of plant foodstuffs. Experientia, **15**: 452–453.

Malý I. (2000): Brokolice – nutričně hodnotná zelenina. Výživa a potraviny, **55**: 44–46.

Murillo G., Mehta R.G. (2001): Cruciferous vegetables and cancer prevention. Nutrition and Cancer, **41**: 17–28.

Paolini M., Perocco P., Canistro D., Valgimigli L., Pedulli G.F., Iori R., Croce C.D., Cantelli-Forti G., Legator M.S., Abdel-Rahman S.Z. (2004): Induction of cytochrome P450, generation of oxidative stress and *in vitro* cell-transforming and DNA-damaging activities by glucoraphanin, the bioprecursor of the chemopreventive agent sulforaphane found in broccoli. Carcinogenesis, **25**: 61–67.

Papapolychroniadis C. (2004): Environmental and other risk factors for colorectal carcinogenesis. Techniques in Coloproctology, **8**: 7–9.

Park E.J., Pezzuto J.M. (2002): Botanicals in cancer chemoprevention. Cancer Metastasis Review, **21**: 231–255.

Rambousková J. (2002): Brokolice – nutriční význam a účinky podporující zdraví. Diabetologie, metabolismus, endokrinologie, výživa, **5**: 38–42.

Rauscher R., Edenharder R., Platt K.L. (1998): *In vitro* antimutagenic and *in vivo* anticlastogenic effects of carotenoids and solvent extracts from fruits and vegetables rich in carotenoids. Mutation Research, **413**: 129–142.

Schaffer E.M., Liu J.Z., Green J., Dangler C.A., Milner J.A. (1996): Garlic and associated alyl sulfur components inhibit N-methyl-N-nitrosourea induced rat mammary carcinogenesis. Cancer Letters, **102**: 199–204.

Schmid W. (1975): The micronucleus test. Mutation Research, **31**: 9–15.

Schonhof I., Krumbein A., Brückner B. (2004): Genotypic effects on glucosinolates and sensory properties of broccoli and cauliflower. Nahrung, **48**: 25–33.

Sedmíková M., Turek B., Bárta I., Strohalm J., Šmerák P., Houška M., Müllerová J. (1999): Hodnocení antimutagenní aktivity tlakově ošetřené (paskalizované) a tepelně ošetřené (pasterované) květákové šťávy. Czech Journal of Food Sciences, **17**: 149–152.

Shapiro T.A., Fahey J.W., Wade K.L., Stephenson K., Talalay P. (2001): Chemoprotective glucosinolates and isothiocyanates of broccoli sprouts: Metabolism and excretion in humans. Cancer Epidemiology Biomarkers & Prevention, **10**: 501–508.

Steinkellner H., Rabot S., Freywald Ch., Nobis E., Scharf G., Chabicovsky M., Knasmüller S., Kassie F. (2001): Effects of cruciferous vegetables and their constituents on drug metabolizing enzymes involved in the bioactivation of DNA-reactive dietary carcinogens. Mutation Research/Molecular Mechanisms of Anticarcinogenesis and Antimutagenesis, **480–481**: 285–297.

Steinmetz K.A., Potter J.D. (1996): Vegetables, fruit, and cancer prevention: a review. Journal of American Dietery Association, **96**: 1027–1039.

Stephanou G., Vlastos D., Vlachodimitropoulos D., Demopoulos N.A. (1996): A comparative study on the effect of MNU on human cultures *in vitro* evaluated by O6-mdG formation, micronuclei and sister chromatid exchange induction. Cancer Letters, **109**: 109–114.

Sugimura T. (1986): Studies on environmental chemical carcinogenesis in Japan. Science, **233**: 312–318.

Talalay P., Fahey J.W. (2001): Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. Journal of Nutrition, **131**: 3027–033.

Thorgeirsson U.P., Dalgard D.W., Reeves J., Adamson R.H. (1994): Tumor incidence in a chemical carcinogenesis study of nonhuman primates. Regulatory Toxicology and Pharmacology, **19**: 130–151.

Turek B., Hrubý S., Černá M. (1994): Nutriční toxikologie. IDVPZ, Brno: 123.

Weisburger J.H. (2000): Approaches for chronic disease prevention based on current understanding of underlying mechanisms. American Journal of Clinical Nutrition, **71**: 1710–1714.

Received for publication June 10, 2005
Accepted after corrections October 20, 2005

Corresponding author:

Mgr. Lucie Mandelová, Masarykova Univerzita v Brně, Fakulta sportovních studií, Sladkého 13, 617 00 Brno, Česká republika
tel.: + 420 549 498 664, fax: + 420 777 675 013, e-mail: mandelova@fsps.muni.cz