Current trends and perspectives in nutrition and cancer prevention*

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There is an increasing evidence that dietary phytochemicals may play important roles as chemopreventive or chemotherapeutic agents in prevention of many diseases, including tumors. The purpose of this study was to examine antimutagenic effects and effect on the immune response of representative series of substances which commonly occur in human diet.

Using the Ames bacterial mutagenicity test and *in vivo* chemiluminescence test, we investigated antigenotoxic and immunomodulatory effects of juices and vegetable homogenates (carrot + cauliflower, cauliflower, red cabbage, broccoli, onion, garlic) on the genotoxicity of AFB_1 and pyrolysates of aminoacids. Using the Ames test and *in vivo* micronucleus, the chemiluminescence test, the blastic transformation test and the comet assay we examined antimutagenic effects of chemically identified chemoprotective substances in the pure form (resveratrol, diallylsulphide, phenethyl isothiocyanate, ellagic acid, epigallocatechin gallate, genistein and curcumine) on mutagenicity induced by three reference mutagens: aflatoxin B1 (AFB_1), 2-amino-3-metylimidazo[4,5,-f] chinolin (IQ) and N-nitroso-N-metylurea (MNU) and effect of phytochemicals on the immunosuppression caused by these mutagens.

All complete vegetable homogenates and substances of plant origin tested, showed a clear antimutagenic and immunomodulatory activities on mutagenicity and immunosuppression induced by reference mutagens. Only in the Ames test the effect of some phytochemicals against direct mutagen MNU was lower compared to indirect mutagens AFB_1 and IQ. Similarly, resveratrol and epigallocatechin gallate had no inhibitory effect on mutagenicity MNU in the Ames test.

Key words: phytochemicals, antimutagenic effects, effect on the immune response, Ames test, micronucleus test, chemiluminescence and blastic transformation tests

Tumors in man are largely the result of the action of environmental factors [1]. These environmental factors include chemical carcinogens and radiation almost exclusively originating from human activities. Besides carcinogenic substances, anthropogenic in character, a group of natural carcinogens exists.

Progress in understanding the biological basis of cancer revealed that damage to the genome or aberrant DNA methylation resulting in aberrant gene expression (suppression of tumor suppressor genes and inappropriate expression of oncogenes) is fundamental to tumorigenesis. The inter-individual variability in cancer expression is due to differences in the amount of DNA damage and capacity to repair that damage, both being influenced by genetic predisposition (gene polymorphism) and by dietary factors. Dietary factors play also important role in metabolization and detoxification of genotoxic chemicals. This situation offers us good oportunity for dietary intervention in cancer prevention.

It is generally known that high intake of fruits and vegetables can decrease the risk of cancer development [2]. Secondary metabolites presented in many plants as phenols, polyphenols, carotenoids, flavonoids, isoflavonoids, alkyl sulphides, isothiocyanates, alcaloids, phytoallexins and others are known for their anticancer properties. The mechanism of the protection includes a great variety of effects such as antioxidant activity against endogenous and exogenous oxidative damage to biomolecules, inhibition of metabolic activa-

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tion of carcinogens, increasing of enzyme activity participating in the detoxification of carcinogenic compounds, as glutathione-S-transpherase [3], blocking of nitrosamines, inhibition of synthesis of N-nitroso-compounds, altered estrogen metabolism, altered colonic milieu, effect on DNA methylation, DNA repair, effect on cell signaling cascades, increased apoptosis of cancer cells, decreased cell proliferation, positive effect on immune functions, etc. [4]. Carcinogenicity via the generation of free radicals may be modulated or prevented by scavenging free radicals with antioxidants, such as vitamins, curcumin, isoflavonoids, resveratrol and plant polysaccharides [5].

Consumption of certain fruit and vegetables is inversely associated with incidence of many cancers especially carcinoma of stomach, colon, esophagus, bladder, pancreas, lung, breast, cervix, endometrium, prostate and others as was proved in epidemiologic studies [6]. A low incidence of breast cancer in Asian women [7] is also attributed to the soya consumption. Several epidemiological studies have shown inverse association between increased green tea intake and cancers of many organs, especially of stomach, colorectum, liver and lung [8].

The biochemically and biologically active substances found in vegetables with regard to anticarcinogenic effects have been studied by many authors [9]. We have also demonstrated the antimutagenic and immunomodulatory effect of substances found in raw vegetables in mice conditioned with AFB_1, and pyrolysates of aminoacids [10, 11].

For these effects the named substances may be used as effective chemopreventive and chemoprotective agents. While mutagenic and co-mutagenic substances may occur in foodstuffs as well as in ambient air, antimutagens can act only through their intake in foodstuffs [12].

Diallyl sulphide belongs to the group of thiolic substances, (found mostly in garlic and onions), and these have been particularly assessed for their antibacterial and antiatherogenic activity; however, also their antioncogenic effects have been observed [13]. Diallyl sulphide and similar substances also influence the endogenic formation of nitrosamines and decrease the risk of gastric carcinoma. They inhibit carcinogens activation and accelerate carcinogens detoxification, influence signal transduction pathways and induce apoptosis [14]. Ellagic acid belongs to the polyphenols and is found in fruit (strawberries, raspberries, grapes, black currant, walnuts). It decreases the metabolic activation of carcinogenic substances by its anti-oxidative effect and by decreasing the activity of cytochrome P450 [15]. It also increases the activity of glutathione-S-transferase and protects the DNA against damage [16]. It markedly inhibits the mutagenic activity of aflatoxin B_1 [17].

Phytoallexin resveratrol, found mainly in the skin of grapes and in derived products such as red wine, has many important effects (anti-inflammatory, anti-oxidative, anti-carcinogenic) and influences activity of the immune system [18]. It can prevent oxidative damage to DNA which plays an important role in activity of many genotoxic substances [19]. Many authors have described the effect of resveratrol in the prevention of cardiovascular diseases (so-called French paradox). Resveratrol modulates lipid metabolism, inhibits oxidation of LDL and thrombocyte aggregation [20]. Molecular mechanisms of chemopreventive effects of resveratrol were described by BODE and DONG [21].

Phenethyl-isothiocyanate belongs to the group of aryl-isothiocyanates which decrease the activity of cytochrome P450 1A2 and influence the metabolic activity of carcinogenic substances [22]. Like several other aryl-isothiocyanates it increases the activity of glutathione-S-transferase, NAD(P)H, chinone oxidoreductase, and UDP-glucuronosyl transferase as was proved in laboratory mice, rats, and humans [23]. Possible targets for chemoprevention by PEITC and other phytochemicals are discussed by LEE and SURH [24].

Isoflavone genistein, a phenolic compound, present in high concentration in soybeans as a natural isoflavonoid, phytoestrogen [25], has anticancer activities, including influence on differentiation, apoptosis, inhibition of cell growth and inhibition of angiogenesis [8]. Two antibody-genistein conjugates, B43-genistein and EGF-genistein, are in clinical development for the treatment of acute lymphoblastic leukemia and breast cancer. Genistein is considered as effective chemotherapeutic agent against carcinoma with antioxidative and anti-inflammatory effects [26].

The therapeutic potential of genistein and epigallocatechin gallate (polyphenolic compound and major ingredient in green tea) was described by WANG [26], PARK and SURH [8]. Chemopreventive effects of green tea against human tumors were also confirmed by CONNEY et al [27]. Mechanisms of its anticarcinogenic activity on molecular level were discussed by BODE and DONG [21].

Yellow pigment curcumin – diferuloyl methane, isolated from the root of turmeric (*Curcuma longa)*, commonly used as a spice and food coloring material (E 100), is a chemopreventive agent with multiple mechanism of action [28]. Very important role of curcumin is in altering metabolic activation or detoxification of mutagens and carcinogens. It inhibits cytochrome P450 enzymes involved in activation of mutagens and carcinogens [28] and has effect on detoxifications enzymes [29]. Supressive effects on human brest carcinoma cells were also described [34] by inhibiting genes through influence of estrogen receptors. Curcumin exerts a variety of immunomodulatory effects [30].

For our study we chose two different representants of mutagenes which commonly occur in the human diet – mycotoxin AFB_1, food mutagen IQ (promutagens) and third MNU, direct mutagen with endogenous origin.

Aflatoxin B_1 (AFB_1) – contaminating foodstuffs is one of the most thoroughly studied and the well known mycotoxin with carcinogenic activity. In the present study it is used as a reference mutagen which exerts mutagenic activity in all prokaryotic and eukaryotic testing systems [31].

Another reference mutagen 2-amino-3-methylimida-

zo[4,5-f]quinoline (IQ), one of toxic substances contaminating foods, is formed by heat processing of foodstuffs. It is one of the most serious amino acid pyrolysates (heterocyclic amines) with mutagenic, carcinogenic and immunosuppressive activity [32].

N-nitroso-N-methylurea (MNU) is an important carcinogenic N-nitroso compound; and contrary to nitrosamines and AFB_1 it is a directly acting carcinogen requiring no metabolic activation [33].

In our study we combined methods of study of antimutagenic effects of chemopreventive agents with methods of testing of their effect on immune response.

Material and methods

Material. We studied chemopreventive effects of carrote with cauliflower, cauliflower, red cabbage, broccoli, onion, garlic as juices and complete vegetable homogenates, using the Ames test and chemiluminescence test, The effect of resveratrol, diallylsulphide, phenethyl isothiocyanate, ellagic acid, epigallocatechin gallate, genistein and curcumine as individual chemically identified chemoprotective substances in the pure form (Sigma-Aldrich Co, Lousiana, USA) on the mutagenicity or immunosuppression caused by three known mutagens AFB_1 (Alexis Corporation, USA), IQ (ICN Biomedicals, Inc., Germany) and MNU (Sigma-Aldrich Co, Lousiana, USA), was studied using the Ames bacterial mutagenicity test and *in vivo* micronucleus test, chemiluminescence and blastic transformation tests. Chemicals were diluted in DMSO (Sigma-Aldrich Co, Lousiana, USA).

Ames bacterial mutagenicity test was performed on auxotrophic his- bacterial strains of *Salmonella typhimurium* TA98 and TA100 [35].

Mutagenic substances were applied in the following concentrations: IQ in concentrations of 0.1 mg, 0.01 mg and 0.001 mg per plate in strain TA98; in concentrations of 10 mg, 1 mg and 0.1 mg in strain TA100. AFB1 in concentrations of 10 mg, 1 mg and 0.1 mg per plate in both strains, TA98 and TA100. MNU in concentrations of 1000 mg, 100 mg and 10 mg only in strain TA100 as these concentrations had no effect in strain TA98. Each concentration of any mutagen was combined with four different concentrations of antimutagen. For metabolic activation of indirect mutagens (AFB1 and IQ) the S9 fraction of liver homogenate from laboratory rats induced by a mixture of polychlorinated biphenyls Delor was used.

Each combination of mutagen and antimutagen was tested in two separate experiments with three plates in each experiment.

Percentage of inhibition of mutagenity was calculated as follows: (No of revertants of mutagen – No of revertants of mixture of mutagen and curcumin /No of revertants of mutagen) x 100. For statistical analysis Student's t-test was used.

Experimental animals. All *in vivo* experiments (bone marrow micronucleus test, chemiluminescence test, blastic transformation method and commet assay) were carried out on ten-week-old male Balb C mice, of weight 22–26 g (BIOTEST, Konárovice, CR). For each group 7–10 mice were used. All experiments were performed on the same animals.

Statistical evaluation of all methods was carried out by the t-test.

Methods. The mouse bone marrow micronucleus test was carried out according to SCHMID [36]. A total of 1000 polychromatophilic erythrocytes were scored per animal for evaluating the frequencies of micronucleated polychromatophilic erythrocytes. Each experiment was run three times.

The chemiluminescence test was performed according to the modification of ŠESTÁKOVÁ [11]. In the chemiluminescence test the degree to which phagocytes are capable of liquidating ingested material is determined. Well known are the mechanisms in which hydrogen peroxide participates in killing. H2O2 is synthesized in phagocytes upon receiving a signal by their membrane. The finding of a higher or lower activity of the complex H2O2-MPO-Cl- (I-) speaks of the readiness of the first defense line against bacteria, tumor cells as well as carcinogen-altered cells. Results are presented in maximum values (mV) of the chemiluminescence response of polymorphonuclear leukocytes in the dependence on time.

For the study of the readiness of cells in acquired immunity we have selected a functional test assessing T-lymphocytes, the blastic transformation method [37]. This test of lymphocyte activation determines the functional capacity of T-lymphocytes to react to a mitogen by proliferation, and is a more direct examination of immune competence than just determining the numbers of various lymphocyte populations [38].

Results

Using the Ames test all vegetable homogenates revealed significant antimutagenic activity as they decreased mutagenicity of AFB_1. Juice of cauliflower showed a clear antimutagenic activity against mutagenicity of 2-amino-3-metylimidazo[4,5,-f] chinolin (IQ). Vegetable juice of carrot with cauliflower effectively prevented the negative effect of AFB_1 and aminoacids pyrolysates in one of the phases of phagocytosis as measured by chemiluminescence (Tab. 1).

Also plant substances (resveratrol, diallylsulphide, phenetyl isothiocyanate, ellagic acid, epigalocatechin galate, genistein and curcumine) showed a clear antimutagenic activity.

In the Ames test all phytochemicals revealed dose dependent antimutagenic effect against all concentrations of two indirect mutagen AFB_1 and IQ in both strains TA98 and TA100 (Tab. 2, 3). The most effective concentrations were 30 and 300 μg/plate. Only effect of DAS against all concentrations of AFB_1 and against some concentrations of IQ was not detected in TA100.

The effect of all phytochemicals against direct mutagen MNU was lower in comparison with indirect mutagen and

Table 1. Effects of juices and vegetable homogenates

Effects / tests	Antimutagenic (mutagen) Ames test	Effect on immune response chemiluminescence	blastic transformation
Carrot +cauliflower	+ AFB_1	+ AFB_1 + pyrolysate	+ AFB_1 + pyrolysate
Cauliflower	+ AFB_1 + IQ	N	N
Red cabbage	+ AFB_1	N	N
Broccoli	+ AFB_1	N	N
Onion	+ AFB_1	N	N
Garlic	+ AFB_1	N	N

+: antimutagenic and immunostimulatory or immunomodulatory effects, N: not tested

Table 2. Ames test – effects of phytochemicals on mutagenicity of AFB_1, IQ and MNU on TA98 strain

		mutagen bacterial strain								
		AFB_1 TA98			IQ TA98			MNU TA98		
	µg/plate	10	1	0.1	0.1/10	0.01/1	0.001/0.1	1 000	100	10
Res	0.3	–	–	–	–	–	–	–	–	–
	3	–	–	–	+	+	+	–	–	–
	30	+	+	+	+	+	+	–	–	–
	300	+	+	+	+	+	+	–	–	–
DAS	0.3	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–
	30	–	+	+	+	+	–	–	–	–
	300	+	+	+	+	+	+	–	–	–
PEITC	0.3	–	+	–	+	+	+	–	–	–
	3	+	+	+	+	+	+	–	–	–
	30	+	+	+	+	+	+	–	–	–
	300	t	t	t	t	t	t	–	–	–
EA	0.3	–	–	–	–	–	–	–	–	–
	3	+	–	–	–	–	–	–	–	–
	30	+	+	+	+	+	+	–	–	–
	300	+	+	+	+	+	+	–	–	–
EGCG	0.3	+	+	–	–	–	–	–	–	–
	3	+	+	–	–	–	–	–	–	–
	30	+	+	+	+	+	+	–	–	–
	300	+	+	+	+	+	+	–	–	–
Gen	0.3	–	–	–	–	–	–	–	–	–
	3	–	+	+	–	–	+	–	–	–
	30	+	+	+	+	+	+	–	–	–
	300	+	+	+	+	+	+	–	–	–
Cur	0.3	–	–	–	–	–	–	–	–	–
	3	–	–	–	+	–	+	–	–	–
	30	+	+	+	+	+	+	–	–	–
	300	+	+	+	+	+	+	–	–	–

+: significant decrease of mutagenic activity, –: without significant decrease of mutagenic activity, t: toxicity

resveratrol and EGCG was not antimutagenic at all. EA decreased mutagenic activity only in the lowest concentration of MNU (10 µg/plate), no effect against higher concentrations of MNU was detected. On the other hand PEITC and curcumine was antimutagenic against MNU. PEITC was toxic in the highest concentration (300 µg/plate), and was a very potent antimutagen also in lower concentrations against indirect mutagens. Concentration 30 µg/plate of PEITC was effective against all concentrations of MNU. The effect of curcumine against MNU was significant in concentrations 30 and 300 µg/plate but the level of inhibition of mutagenity was lower in comparison with the effect against indirect mutagens.

In the micronucleus test all plant substances had inhibitory effect on mutagenicity induced by reference mutagens, (IQ, AFB1, MNU), and reduced its mutagenic effect to a statistically significant degree (Tab. 4).

All tested substances of plant origin in combination with mutagens repaired the degree of blastic transformation after their administration.

The results show that all tested substances effectively prevented the negative effect of mutagens in one of the phases of phagocytosis as measured by chemiluminescence (Tab. 4).

Discussion

It has been estimated that some human cancers could be prevented by modification of lifestyle including dietary modification [39].

Epidemiological studies have indicated a significant difference in the incidence of cancers among ethnic groups who have different lifestyles and have been exposed to different environmental factors [40]. The consumption of fruits, soybean and vegetables has been associated with reduced risk of several types of cancers [41].

Many authors focused their attention on the study of anticarcinogenic and antimutagenic effects of phytochemicals in *in vivo* and *in vitro* tests [8].

Our studies focused on substances of plant origin, and on effects of the complex of substances contained in uncooked vegetables (carrot, cauliflower, garlic). We have proven antimutagenic and immunomodulatory properties of the selected compounds of plant origin in the form of chemically defined substances (resveratrol, diallyl sulphide, phenethyl isothiocyanate, ellagic acid, epigallocatechin gallate, genistein, curcumin) examined in combination with three selected strong carcinogens with proven mutagenic effect (aflatoxin B_1, 2-amino-3-methylinidazol[4,5] quinoline and N-nitroso-N-methylurea) [42].

Table 3. Ames test – effects of phytochemicals on mutagenicity of AFB_1, IQ and MNU on TA100 strain

		mutagen bacterial strain								
		AFB_1 TA100			IQ TA100			MNU TA100		
	µg/plate	10	1	0.1	0.1/10	0.01/1	0.001/0.1	1 000	100	10
Res	0.3	–	–	–	–	+	+	–	–	–
	3	–	+	–	+	+	+	–	–	–
	30	–	+	+	+	+	+	–	–	–
	300	+	+	+	+	+	+	–	–	–
DAS	0.3	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–
	30	–	–	–	–	+	–	–	–	–
	300	–	–	–	–	+	–	+	+	+
PEITC	0.3	–	+	+	–	+	+	–	–	–
	3	+	+	+	+	+	+	–	–	–
	30	+	+	+	+	+	+	+	+	+
	300	t	t	t	t	t	t	t	t	t
EA	0.3	–	+	–	–	–	–	–	–	–
	3	–	+	–	–	–	–	–	–	+
	30	+	+	+	+	+	+	–	–	+
	300	+	+	+	+	+	+	–	–	+
EGCG	0.3	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–
	30	+	+	+	+	+	+	–	–	–
	300	+	+	+	+	+	+	–	–	–
Gen	0.3	–	–	–	–	–	–	–	–	–
	3	–	+	+	–	–	–	–	–	–
	30	+	+	+	–	–	–	–	–	–
	300	+	+	+	+	+	–	–	+	+
Cur	0.3	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	+	–	–	–	–
	30	+	+	+	–	+	+	+	+	+
	300	+	+	+	+	+	+	+	+	+

+: significant decrease of mutagenic activity, –: without significant decrease of mutagenic activity, t: toxicity

Our results of *in vitro* Ames test discovered that antimutagenic effect of phytochemicals tested was less pronouncied against direct mutagen MNU than against indirect mutagens AFB_1 and IQ, both requiring metabolic activation. This was detected in all chemicals, and resveratrol and epigallocatechin galate were even ineffective against MNU.

Curcumin is a strong inhibitor of mutagenicity of indirect mutagens AFB_1 and IQ, and the effect on mutagenicity of MNU was also significant but somewhat lower than against indirect mutagens.

Similar results were achieved by several authors using the Ames test. NAGABUSHAN [43] described inhibition of mutagenicity of several indirect mutagens by curcumin, but inhibition of mutagenicity of direct mutagens sodium azide, monoacetylhydrazine, streptozocin and 4-nitrophenylendiamine was not observed. SONI [44] proved decrease of mutagenicity of AFB_1 and SHISHU [45] inhibition of mutagenicity of several indirect cooked food mutagens including IQ by curcumin in the Ames assay.

It is in agreement with the fact that the dominant reason for antimutagenicity of phytochemicals in *in vitro* tests is the interference with metabolic activation of mutagens, and another reason can be the cytotoxic effect of highest concentrations detected in our experiment in the Ames test using the highest concentration of phenetyl isothiocyanate. Also curcumin inhibits metabolic activation of mutagens especially by inhibiting phase I enzymes [28] or exerts effect on detoxifications enzymes [29], and this effect may be more extensive. Significant inhibitory activity of the tea catechins, ECG and EGCG, against the mutagenicity of Trp-P-2 and *N*-OH-Trp-P-2 has been found by OKUDA [46] using *Salmonella typhimurium* TA98 and TA100.

In micronucleus test we detected antimutagenic effect of the tested phytochemicals against all three mutagens. Similar effects of curcumine were described by TRESHIAMA [47] on chromosomal aberrations or micronuclei induced by irradiation. Induction of micronuclei and chromosomal aberrations produced by whole body exposure to ionizing radiation in mice was found to be significantly inhibited by oral administration of ellagic acid [47]. Hot water extracts of green tea effectively suppressed AFB_1-induced chromosome aberrations in bone marrow cells in rats [48].

Plant phytochemicals have a wide variety of effects. Their activity is pleiotropic and it concerns not only metabolic activation or detoxification of mutagen and carcinogens or free radical scavenging effects, but also exerts effects on DNA methylation, DNA repair, cell signalling cascade and apoptosis etc. [4]. That is why chemoprevetive agents work in all stages of carcinogenic processes as blocking agents in the stage of initiation or as supressing agents of promotion or progression stages [1, 8].

Immune system plays significant role in combination with prooxidative and antioxidative processes in organism, especially in the initial stages of oncogenesis. Many substances, for example the all above mentioned mycotoxins, are important immunosupressors and play important and critical role in initial stages of cancer progession. In the final stage of oncogenesis in which it is decided whether a tumor cell shall progress or shall be eliminated, the controlling and liquidating action of the immune system is namely decisive.

According to our experimental experiences a significant biological phenomenon is that the behavior of many substances depends on various conditions, particularly on dos-

Table 4. Effects of chemoprotective agents on mice

Effects	Antimutagenic effect	Effect on immune response	
Tests	Micronucleus	Chemilumine-scence	Blastic transformation
Resveratrol	+ AFB_1 + IQ + MNU	+ AFB_1 + IQ + MNU	+ AFB_1 + IQ + MNU
Dialylsulphide	+ AFB_1 + IQ + MNU	+ AFB_1 + IQ + MNU	+ AFB_1 + IQ + MNU
Phenetyl isothiocyanate	+ AFB_1 + IQ + MNU	+ AFB_1 + IQ + MNU	+ AFB_1 + IQ + MNU
Ellagic acid	+ AFB_1 + IQ + MNU	+ AFB_1 + IQ + MNU	+ AFB_1 + IQ + MNU
Epigallocatechin gallate	+ AFB_1 + IQ + MNU	+ AFB_1 + IQ + MNU	+ AFB_1 + IQ + MNU
Genistein	+ AFB_1 + IQ + MNU	+ AFB_1 + IQ + MNU	+ AFB_1 + IQ + MNU
Curcumin	+ AFB_1 + IQ + MNU	+ AFB_1 + IQ + MNU	+ AFB_1 + IQ + MNU

+: antimutagenic, immunostimulatory or immunomodulatory effects

age. Such substances may either act as mutagenic or antimutagenic agents, or show no provable positive or negative mutagenic activity. In this case, the effect depends not only on a combined action of individual substances but also on the time. The time factor is therefore important not only in respect to the duration of the action of a particular substance but also in respect to the dosing regimen, i.e. the time at which the particular substance starts to act. This is analogous to the subsequent action of initiators and promotors in a multistage carcinogenic process in which the sequence of actions is critical [12].

The differences between the activities of extracts obtained from the particular plant (whole plant, roots, leaves, seeds) and separated substances were observed in some cases. In such cases the resultant effect depended on the amount of additional substances in the particular extract or leachate and on the activity of additional substances which may either show an antimutagenic effect by inhibiting the activity of a mutagenic substance (particularly if the mutagenic substance is present at a low concentration), or enhance the mutagenic effect of other substances. Quercetin in combination with hypericin may serve as an example [49]. Study of possible synergistic effects or other interactions of phytochemicals is necessary for understanding their effects [50].

Phytochemicals with chemopreventive effects can be used as dietary supplements, functional foods or even drugs. Interaction between phytochemical components can modify the effect on human health and can explain the health effects of regional differences in diets, unexpected and side effects of drugs or dietary supplements [50].

Functional foods and nutraceuticals constitute a great promise in efforts to improve health and prevent aging-related chronic diseases. Study of phytochemicals with chemopreventive effects and better understanding of their health-related interactions should lead to better use of dietary intervention in cancer prevention.

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