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Antimutagenic Effect of the Ellagic Acid and Curcumin Combinations

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Abstract

Introduction: There is an evidence to support the health benefits of diets rich in fruits, vegetables, legumes, whole grains and nuts. Plant-based foods are complex mixtures of bioactive phytochemicals. The potential health effects of individual phytochemicals, their combinations or combinations of phytochemicals and others drugs is studied in detail. Ellagic acid belongs to the group of bioactive polyphenols in fruit: strawberries, raspberries, grapes, black currant and walnuts. Curcumin is a natural compound extracted from the root of *Curcuma longa* plant.

Methods: We perform *in vitro* Ames test and *in vivo* micronucleus test toward three mutagens/carcinogens, aflatoxin B1 and 2-amino-3-methylimidazo(4,5-f)quinoline and N-nitroso-N-methylurea to prove an antimutagenic effect of ellagic acid, curcumin and their combinations.

Results: We verified the dose dependent antimutagenic effect of ellagic acid, curcumin and their combinations in both tests. The significantly increased effect of some combinations on the mutagenicity of indirect mutagens in the Ames test and on the direct mutagenicity of MNU in the micronucleus test, as compared with effect of ellagic acid or curcumin used separately, was also ascertained.

Keywords: Phytochemical; Ames; Micronucleus; Carcinogenesis

Abbreviations: DNA: Deoxyribonucleic acid; NF-κB: Nuclear Factor kappa-light-chain-enhancer of activated B cells; VEGF: Vascular Endothelial Growth Factor; PDGF: Platelet-Derived Growth Factor; EA: Ellagic Acid; CRC: Curcumin; TNF: Tumor Necrosis Factor; AP-1: Activator Protein 1; IQ: 2-amino-3-methylimidazo(4,5-f)quinolone; AFB_1: Afflatoxin B_1; MNU: N-nitroso-N-methylurea; DMSO: Dimethyl Sulfoxide; b.w: Body Weight

Introduction

Exogenous factors, such as radiation and xenobiotics, can play an important role in carcinogenesis due to their mutagenic/promoting/co-carcinogenic effects [1]. They induce damage either directly by interacting with the macromolecules or indirectly by the creation of free radicals [2]. If oxidative stress is prolonged, reactive oxygen and nitrogen species are produced and carry out the process of damage. They exacerbate the oxidation of intracellular proteins, lipids, and nucleic acids [3-5]. DNA damage, if left unrepaired, can lead to base mutation, DNA cross-links, single and double-strand breaks, chromosomal breakage and rearrangement [6], genomic instability, neoplastic transformation and, ultimately, carcinogenesis [7]. The covalent interaction of carcinogen-induced reactive species with DNA may result in genotoxic damage during the initiation stage of chemical carcinogenesis [8-9].

This oxidative damage may be prevented or limited by dietary antioxidants (phytochemicals) found in fruits and vegetables [10]. A large number of phytochemicals possess antioxidant and free-radical scavenging properties and are known to modulate important cellular signaling pathways associated with carcinogenesis [7-8]. The epidemiologic studies evaluating associations between intake of a variety of plant-based foods indicate a protective effect, both on cardiovascular diseases and certain cancers. There is appreciable epidemiologic evidence that demonstrates a protective role in diets high in fruits and vegetable, legumes, whole grains and fish on different cancers and cardiovascular diseases [11].

Ellagic acid (EA) belongs to the group of bioactive polyphenols in fruit (strawberries, raspberries, grapes, black currant, walnuts). EA is found in plants in the form of hydrosable tannins called ellagitannins as the structural components of cell wall and cell membrane. EA demonstrates antimutagenic, antioxidant, anti-inflammatory and anticancer activity [12]. Anticancer activity is manifested by blocking initiation of carcinogenesis, suppressing progression and proliferation of tumors [13,14]. EA decreases the metabolic activation of carcinogenic substances by inhibition of cytochrome P450 and by induction of phase II enzymes of metabolic transformation [15]. EA also interferes with multiple cell signaling pathways, including the decrease of NF-κB, cyclooxygenase 2, cyclin D1, growth factors VEGF and PDGF and the increase of p21/WAF1 and p53 [14,16,17]. The antiproliferative and proapoptotic activities of EA are proved in cancer cell lines [18].

Curcumin (diferuloymethan) (CRC) is a natural compound extracted from the root of *Curcuma longa* plant. CRC is an anticancer, antioxidant, antiinflammatory and antiangiogenic agent, capable of inducing apoptosis of cancer cells [19-24]. The protective effect is detected in many *in vitro* and *in vivo* studies [22,25,26]. Prevalent evidence suggests that it may be useful for the chemoprevention of colon cancer in humans [27]. Preclinical studies of healthy individuals and patients with premalignant conditions or tumors are reviewed by Thomasset 2006 [28] and Von Löw 2007 [29]. The studies of molecular

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targets of curcumin reveal that curcumin modulates the expression of many transcription factors such as TNF-α, AP-1 and NF-κB, cell cycle proteins, signal transducting kinases [14,21,30-32]. CRC enhances the expression of cell cycle inhibitors p21 and p27 as well as tumor suppressor protein p53, but suppress expression of the Rb protein [33]. CRC also exerts the immunomodulatory effect [26,34].

We have already published the antimutagenic activity of ellagic acid and curcumin as single agents in Ames test, micronucleus test and comet assay [26,35]. To prove the presumption about synergism or antagonism of antimutagenic activity of ellagic acid and curcumin, we have studied the activity of their combinations in the Ames test and the micronucleus test and we compared it with the activity of single agents. We used two indirect mutagens/carcinogens aflatoxin B_1 and 2-amino-3-methylimidazo(4,5-f)quinoline and the direct mutagen/carcinogen N-nitroso-N-methylurea.

Material and Methods

Ames test

The antimutagenic activity of ellagic acid, curcumin and their combinations *in vitro* was detected using the Ames test with *Salmonella typhimurium* TA98 and TA100 strains [36-39]. Ellagic acid (Sigma-Aldrich) and curcumin (Sigma-Aldrich) were used at the following concentrations: 0.3 μg, 3 μg, 30 μg, and 300 μg/plate individually and in combinations. Mutagenic substances were used at the following concentrations: AFB_1 (Alexis Biochemicals, San Diego, CA, USA) in 10 μg and 1 μg per plate in both bacterial strains, IQ (ICN Biomedicals, Eschwege, Germany) in 0.1 μg and 0.01 μg per plate in the strain TA98, IQ in 10 μg and 1 μg per plate in the strain TA100. Direct mutagen MNU (Sigma-Aldrich, St. Louis, MO, USA) was used at concentrations of 100 μg and 10 μg per plate only in the strain TA100, as these concentrations had no effect in the strain TA98 [26,35]. Each concentration of mutagen was combined with four different concentrations (0.3 μg, 3 μg, 30 μg, and 300 μg/plate) of phytochemical substances individually and also in the following mixtures: 0.3 μg of EA+0.3 μg of CRC, 3 μg of EA+3 μg of CRC, 30 μg of EA+30 μg of CRC and 300 μg of EA+300 μg of CRC per plate. All chemicals were diluted in dimethyl sulfoxide (Sigma-Aldrich Co, Lousiana, USA). The S9 fraction of the liver homogenate from the laboratory rats induced by a mixture of polychlorinated biphenyls (Delor) was used for metabolic activation of indirect mutagens [38]. Percentage of inhibition of mutagenicity was calculated by the formula:

$$\frac{\text{No. of revertants of mutagen-No. of revertants of mixture of mutagen and antimutagen(s)}}{\text{No. of revertants of mutagen}} \times 100$$

Micronucleus test

The experiment *in vivo*, bone marrow micronucleus test, was carried out on male Balb/C mice each weighting 20-24 g (VELAZ s.r.o., Únětice, Czech Republic). The animals were housed under standard conditions and divided into groups of 10 mice for treatment. EA and CRC were tested individually and in combinations. They were applied to mice by gavage three days sequentially, ellagic acid at the doses of 1 and 2 g/kg b.w. and curcumin at the doses of 0.25 and 0.5 g/kg b.w. The combinations of them were used in concentrations: 1 g of EA/kg+0.25 g of CRC/kg and 2 g of EA/kg+0.5 g of CRC/kg b.w. Mutagens (AFB_1, IQ, and MNU) were applied individually and also in the mixtures with phytochemicals. AFB_1 was used in the concentration of 1 mg/kg b.w., IQ was used in the concentration of 20 mg/kg b.w. and MNU in the concentration of 50 mg/kg b.w. Mutagens were applied in the single dose on the third day. All substances (diluted in DMSO) were applied in volumes of 100 μl/10 g b.w. The control group of mice received 7% solution of DMSO orally. The micronucleus test on mouse bone marrow was carried out according to Schmid, 1975 [40]. A total number of 1000 polychromatophilic erythrocytes were scored per animal for an evaluation of frequencies of micronuclei.

All groups of samples were tested in two separate experiments and each sample was tested in three plates in the Ames test. In the micronucleus test, all samples were tested in three separate experiments. For statistical analysis we used Student's t-test.

Results

Results of Ames test

The results of the Ames test are presented in Tables 1-4. They are expressed as a number of revertants and also as a percentage of inhibition of mutagenic activity. The samples of EA, CRC and their combinations were tested separately without mutagen (Table 1), and in combination with mutagen AFB_1 (Table 2), with mutagen IQ (Table 3) and with mutagen MNU (Table 4). An activity of mixtures of EA and CRC were compared to the results of phytochemicals used separately all in combination with mutagens (Tables 2-4). Neither ellagic acid and curcumin, nor their combinations revealed any mutagenicity in both bacterial strains TA98 and TA100 (Table 1). Significant dose dependent antimutagenic activity was detected at two highest concentrations (30 and 300 μg/plate) of EA, CRC and their combinations on mutagenicity of both concentrations of AFB_1 (10 and 1 μg/plate) (Table 2). The only exception of significance was in the decrease of mutagenicity of the combinations of 30 μg of EA/plate mixed with 10 μg of AFB_1 in both bacterial strains and 30 μg of EA mixed with 1 μg of AFB_1 in the strain TA 100 (Table 2). In both strains, the antimutagenic activity of two combinations (3 μg of EA+3 μg of CRC and 30 μg of EA+30 μg of CRC mixed with 1 μg of AFB_1) was significantly higher than the activity of the same concentrations of EA or CRC used separately.

The dose dependent inhibition effect of phytochemicals and their combinations were detected on mutagenicity of both concentrations of IQ (0.1 and 0.01 μg/plate in the strain TA98 and 10 and 1 μg/plate in the strain TA100) (Table 3). There was significant difference between combinations with concentrations of 30 μg of EA+30 μg of CRC combined with 0.1 μg or 0.01 μg of IQ in the strain TA98 and in the concentrations of 3 μg of EA+3 μg of CRC and 30 μg of EA+30 μg of CRC both combined with 1 μg of IQ in the strain TA100 than EA or CRC of the same concentrations used separately (Table 3).

The activity of phytochemicals against the direct mutagen MNU, used at concentration of 100 μg/plate was significant only in the combinations of phytochemicals with higher concentrations (30 μg of EA+30 μg of CRC, 300 μg of EA+300 μg of CRC) (Table 4). The differences between the activity of combinations and separate phytochemicals were not significant. The antimutagenic activity against lower concentration of MNU (10 μg/plate) was more obvious, but the effect of phytochemicals combinations similarly did not differ from the effect of EA or CRC used separately (Table 4).

Results of micronucleus test

All three mutagens revealed significant mutagenic activity in the micronucleus test. The number of micronuclei in animals, which received phytochemicals and their combinations without mutagen, did not differ from those of the control group (Table 5). EA, CRC and their combinations significantly reduced the number of micronuclei, which was high by the mutagenic activity of AFB_1, IQ and MNU. The decrease of micronuclei numbers was dose dependent (Figures 1-3). The activity

EA+CRC dose (µg/plate)	*S. typhimurium* TA98+S9		*S. typhimurium* TA100+S9	
	No of revertants	± *SD*	No of revertants	± *SD*
0.3EA	20	5	79	3
3EA	24	5	78	6
30EA	20	3	69	10
300EA	17	2	67	7
0.3CRC	18	5	81	9
3CRC	22	4	71	6
30CRC	19	5	70	7
300CRC	17	5	68	5
0.3EA+0.3CRC	18	2	74	8
3EA+3CRC	20	6	73	9
30EA+30CRC	16	4	70	8
300EA+300CRC	20	7	68	3
control - DMSO	22	4	79	9

SD: standard deviation

Table 1: Ellagic acid, curcumin and their combinations in Ames test.

of the combinations of EA and CRC on the mutagenicity of indirect mutagens AFB_1 and IQ did not differ from the activity of phytochemicals used separately (Figures 1 and 2). Only the mutagenicity of 50 mg/kg of MNU was significantly more reduced by the combination of EA and CRC at concentrations of 2 g/kg of EA+0.5 mg/kg of CRC in three daily doses in comparison with the same doses of individual phytochemicals (Figure 3).

Discussion

Antimutagenesis, a prevention of genotoxic damage is a part of chemoprevention and could be considered as a major mechanism to inhibit carcinogenesis in the initiation stage [9]. Chemoprevention, as a defense anti-cancer mechanism provided by phytochemicals, was defined in 1966 by Wattenberg [41]. In our department, we studied the antimutagenic and immuno-modulatory effects of individual phytochemicals of natural origin both *in vitro* and in *vivo* conditions. We confirmed that the phytochemicals in the pure forms

AFB_1+antimutagen(s) dose (µg/plate)	*S. typhimurium* TA98+S9			*S. typhimurium* TA100+S9		
	No of revertants	± *SD*	% of inhibition	No of revertants	± *SD*	% of inhibition
10+0	471	94		762	90	
1+0	559	100		703	72	
10+0.3EA	446	67	-5	729	114	-4
10+3EA	467	81	-1	701	129	-8
10+30EA	406	119	-14	685	131	-10
10+300EA	127**	42	-73	501**	134	-34
10+0.3CRC	446	117	-5	666	141	-13
10+3CRC	456	99	-3	641	138	-16
10+30CRC	67**	25	-86	522**	212	-32
10+300CRC	9**	5	-98	226**	108	-70
10+0.3EA+0.3CRC	434	41	-8	605*	168	-21
10+3EA+3CRC	474	38	+1	601*	174	-21
10+30EA+30CRC	73**	30	-85	480**	193	-37
10+300EA+300CRC	12**	7	-98	146**	67	-81
1+0.3EA	548	56	-2	703	100	0
1+3EA	573	26	+3	681	88	-3
1+30EA	449*	88	-20	634	158	-10
1+300EA	54**	18	-90	244**	109	-65
1+0.3CRC	591	39	+6	644	108	-8
1+3CRC	575	22	+3	663	80	-6
1+30CRC	103**	31	-82	532**	129	-24
1+300CRC	36**	22	-94	147**	41	-79

1+0.3EA+0.3CRC	511	81	-9	581	166	-17
1+3EA+3CRC	481**▼	73	-14	516**▼	104	-27
1+30EA+30CRC	57**▼	8	-90	193**▼	89	-73
1+300EA+300CRC	23**	13	-96	107**	33	-85
control-DMSO	22	6		101	14	

SD: Standard Deviation

*Statistically significant difference between the sample with mutagen and antimutagen(s) and the sample with specific mutagen (1) $p \leq 0.05$

**Statistically significant difference between the sample with mutagen and antimutagen(s) and the sample with specific mutagen (1) $p \leq 0.01$

▼Statistically significant difference between the sample with combination of antimutagens and samples with specific antimutagen

Table 2: Effect of EA, CRC and their combinations on mutagenicity of AFB_1 in Ames test.

***S. typhimurium* TA98+S9**				***S. typhimurium* TA100+S9**			
IQ+antimutagen(s) dose (µg/plate)	**No of revertants**	**± *SD***	**% of inhibition**	**IQ+antimutagen(s) dose (µg/plate)**	**No of revertans**	**± *SD***	**% of inhibition**
0.1+0	1365	145		10+0	586	104	
0.01+0	497	166		1+0	711	79	
0.1+0.3EA	1211	194	-11	10+0.3EA	577	71	-2
0.1+3EA	1141**	157	-16	10+3 EA	579	97	-1
0.1+30EA	244**	97	-82	10+30 EA	161**	25	-73
0.1+300EA	32**	8	-98	10+300 EA	98**	17	-83
0.1+0.3CRC	1267	197	-7	10+ 0.3CRC	588	100	0
0.1+3CRC	1060**	189	-22	10+3CRC	571	128	-3
0.1+ 30CRC	528**	140	-61	10+30CRC	446*	92	-24
0.1+300CRC	18**	4	-99	10+300CRC	346**	109	-41
0.1+0.3EA+0.3CRC	1195*	180	-13	10+0.3EA+0.3CRC	595	100	+2
0.1+3EA+3CRC	1032**	192	-24	10+3EA+3CRC	465*	96	-21
0.1+30EA+30CRC	53**▼	31	-96	10+30EA+30CRC	151**	69	-74
0.1+300EA+300CRC	23**	5	-98	10+300EA+300CRC	83**	12	-86
0.01+0.3EA	419	179	-16	1+0.3EA	619*	104	-13
0.01+3EA	416	125	-16	1+3 EA	609**	45	-14
0.01+30EA	66**	36	-87	1+ 30 EA	240**	79	-66
0.01+300EA	29**	5	-94	1+300 EA	96**	19	-87
0.01+0.3CRC	480	149	-3	1+0.3CRC	681	114	-4
0.01+3CRC	424	127	-15	1+3CRC	604**	113	-15
0.01+30CRC	187**	85	-62	1+ 30CRC	415**	79	-42
0.01+300CRC	25**	8	-95	1+300CRC	163**	45	-77
0.01+0.3EA+0.3CRC	363	175	-27	1+0.3EA+0.3CRC	585**	129	-18
0.01+3EA+3CRC	337*	148	-32	1+3EA+3CRC	514**▼	69	-28
0.01+30EA+30CRC	32**▼	10	-94	1+30EA+30CRC	120**▼	22	-83
0.01+300EA+300CRC	24**	8	-95	1+300EA+300CRC	84**	9	-88
control-DMSO	28	6		control-DMSO	83	6	

SD standard deviation

*Statistically significant difference between the sample with mutagen and antimutagen(s) and the sample with specific mutagen $p \leq 0.05$

**Statistically significant difference between the sample with mutagen and antimutagen(s) and the sample with specific mutagen (1) $p \leq 0.01$

▼Statistically significant difference between the sample with combination of antimutagens and samples with specific antimutagens

Table 3: Effect of EA, CRC and their combinations on mutagenicity of IQ in Ames test.

and also in the form of juices of natural plants might have an important role in the prevention of carcinogenesis by their antimutagenic effect [26,35,42-47]. In adition, it was presented by other research groups that CRC and EA were able to activate or inhibit many cellular molecules of signaling pathways and became involved in the regulation of cancer cell division [14].

Food phytochemicals provided complex interactions in biological systems [48]. The combination of natural phytochemicals in fruits and vegetables, which provided health benefits, might not be replaced by the effect of single phytochemicals [10,49]. Also, combinations of phytochemicals might have result in significant effect at concentrations, in which the single agents were inactive [50].

MNU+antimutagen(s) dose (µg/plate)	*S. typhimurium* TA100		
	No of revertants	± *SD*	% of inhibition
100+0	1643	166	
10+0	392	26	
100+0.3EA	1624	164	-1
100+3EA	1658	145	+1
100+30EA	1567	166	-5
100+300EA	1359	223	-17
100+0.3CRC	1619	209	-2
100+3CRC	1536	263	-7
100+30CRC	1490	212	-9
100+300CRC	1243	228	-24
100+0.3EA+0.3CRC	1569	205	-5
100+3EA+3CRC	1481	236	-10
100+30EA+30CRC	1288*	240	-22
100+300EA+300CRC	1015**	243	-38
10+0.3EA	414	35	+6
10+3EA	407	69	+4
10+30EA	327	126	-17
10+300EA	254**	32	-35
10+0.3CRC	385	47	-2
10+3CRC	422	90	+8
10+30CRC	211**	21	-46
10+300CRC	154**	9	-61
10+0.3EA+0.3CRC	406	89	+4
10+ 3EA+3CRC	364	69	-7
10+30EA+30CRC	187**	29	-52
10+300EA+300CRC	144**	16	-63
control-DMSO	112	9	

SD standard deviation

*Statistically significant difference between the sample with mutagen and antimutagen(s) and the sample with specific mutagen $p \leq 0.05$

**Statistically significant difference between the sample with mutagen and antimutagen(s) and the sample with specific mutagen $p \leq 0.01$

Table 4: Effect of EA, CRC and their combinations on mutagenicity of MNU in Ames test.

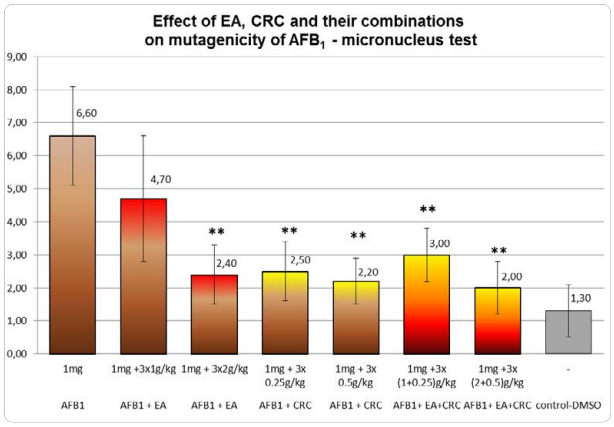
Substances tested	Dose	No of micronuclei	SD
EA	3 × 1 g/kg	0.4	0.5
EA	3 × 2 g/kg	0.2	0.4
CRC	3 × 0.25 g/kg	0.6	0.9
CRC	3 × 0.5 g/kg	0.4	0.5
EA+CRC	3 × (1+0.25) g/kg	0.4	0.5
EA+CRC	3 × (2+0.5) g/kg	0.2	0.4
control-DMSO	-	0.2	0.4

SD: Standard Deviation

Table 5: Ellagic acid, curcumin and their combinations in micronucleus test.

Interactions of phytochemicals might be antagonistic, additive and/or synergistic depending on the certain experimental conditions and concentrations [48,49]. The additive or synergistic effect of combinations of phytochemicals or phytochemicals and synthetic drugs was previously detected in many research projects under both *in vitro* and *in vivo* conditions [51-58]. For instance, Verma et al. (1997) described a synergistic inhibition effect of curcumin and genistein on proliferation of MCF-7 breast cells induced by estrogenic pesticides [51]. Lev-Ari et al. (2005) provided that curcumin synergistically potentiated the growth inhibition and the pro-apoptotic effect of celecoxib in pancreatic adenocarcinoma cells [52] or colorectal cancer cells [53]. Also ellagic acid and guercetin interacted synergistically with resveratrol in the induction of apoptosis in human leukemia cells [54]. Resveratrol combinations with ellagic acid and other phytochemicals were very potent inhibitors of skin tumorgenesis [55].

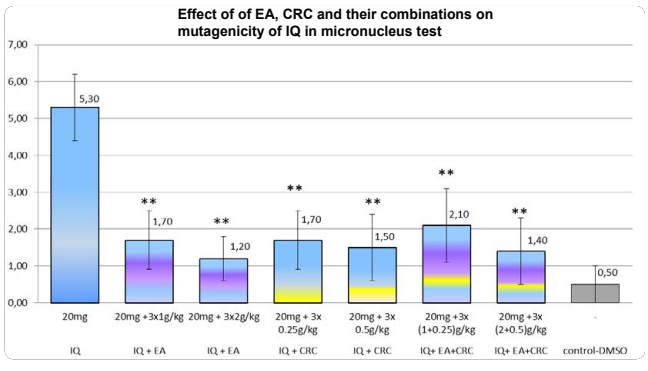
In our research the antimutagenic effect of the combinations of phytochemicals and individual phytochemicals of the same high concentrations was detected on mutagenicity of both concentrations of indirect mutagens, AFB_1 and IQ, in the Ames test. The increased antimutagenic effect of the combinations was mostly detected in two middle concentrations of phytochemicals (3 and 30 µg of EA and CRC) in the comparision to the effect of individual phytochemicals of the same concentration. The increased significant antimutagenic effect of combinations was limited by concentration. Considering these results, we could not confirm the presumption about effective low-concentration combinations of tested phytochemicals [50]. The combination of the highest concentration (300 µg of EA and CRC) did not show an



*Statistically significant difference between the sample with mutagen and antimutagen(s) and the sample with specific mutagen $p \leq 0.05$.

**Statistically significant difference between the sample with mutagen and antimutagen(s) and the sample with specific mutagen $p \leq 0.01$.

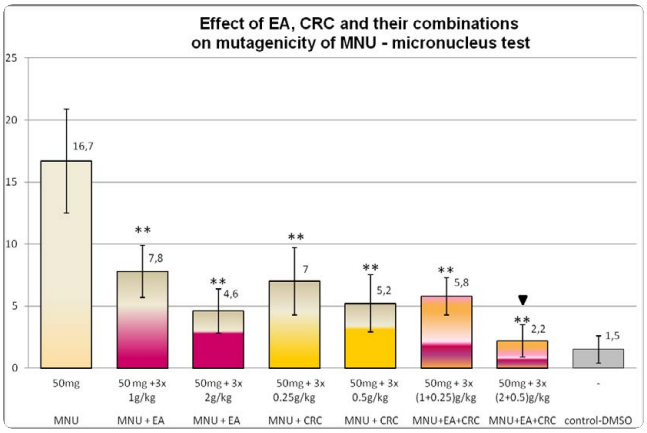
Figure 1: Effect of EA, CRC and their combinations on mutagenicity of AFB_1 in micronucleus test.



*Statistically significant difference between the sample with mutagen and antimutagen(s) and the sample with specific mutagen $p \leq 0.05$.

**Statistically significant difference between the sample with mutagen and antimutagen(s) and the sample with specific mutagen $p \leq 0.01$.

Figure 2: Effect of EA, CRC and their combinations on mutagenicity of IQ in micronucleus test.



*Statistically significant difference between the sample with mutagen and antimutagen(s) and the sample with specific mutagen (1) $p \leq 0.05$.

**Statistically significant difference between the sample with mutagen and antimutagen(s) and the sample with specific mutagen (1) $p \leq 0.01$.

▼Statistically significant difference between the sample with combination of antimutagens and samples with specific antimutagen.

Figure 3: Effect of EA, CRC and their combinations on mutagenicity of MNU in micronucleus test.

increased effect against indirect mutagens in the Ames test. The results support the presumption of the "saturation effect" by Abraham et al. (2012) [1]. The effect of phytochemicals against the direct mutagen MNU was proven mainly in the combinations of phytochemicals of high concentration. If we compared the effect of phytochemicals against the direct and indirect mutagens, the antimutagenic effect was stronger against the indirect mutagens. It might be indicative of importance of a biotransformation process of indirect mutagens in metabolism. The effect of CRC and EA on metabolic activation or detoxication of carcinogens was previously proven. They have an inhibitory effect towards cytochrome P450 enzymes [59-61] and *in vivo* induce GST enzymes of rat liver [62,63].

In micronucleus test, both phytochemicals and their combinations provided a significant decrease in the number of micronuclei induced by mutagens. The increased significant antimutagenic effect of the combinations of EA and CRC in comparison with the effect of the phytochemicals used individually of the same concentration was detected in the highest concentration (2 g of EA/kg+0.5 g of CRC/kg b.w) of phytochemicals against direct mutagen MNU. The interaction of phytochemicals might indicate the potentiation, additivity or synergism, if the compounds act via different mechanisms and/or on different targets [64]. The potentiation of effects of phytochemicals was usually verified, if the effect included many molecular targets [49]. EA and CRC differ slightly in molecular size and solubility, which might affect the bioavailability and distribution in different macromolecules, subcellular organelles, cells, organs and tissues [64]. The other possible mechanism of increasing protective effect of the EA and CRC combination might be the mutual stabilization of their molecules, because phytochemicals tend to increase the therapeutic effect by increasing the bioavailability of the other drug or, by stabilizing the other drug in the system [50]. A possible explanation of the increased antimutagenic effect the EA and CRC combinations might be the combination of the above mentioned mechanisms.

Recent research on protective phytochemicals contributed to understanding their chemical and biological functions and their beneficial effects on human health [65]. There was growing evidence that the combinations of two or more compounds might be more efficacious. Many phytochemicals were reported to act synergistically, which might explain why some food items or diets show cancer preventive effects, which could not be explained based on individual bioactive ingredients [49]. The synergistic effects of dietary phytochemicals should be further explored for additional beneficial and reliable outcomes in the field of cancer prevention [66]. Because of pharmacological safety, some chemoprotective agents of natural origin could be used not only to prevent cancer, but also to treat cancer in combination with chemotherapy. In adition, natural products had the potential to provide the pharmacologist with a source of novel structures, on the basis of which most current cancer drugs were synthesized [50]. Recent studies showed that phytochemicals were also able to reverse the chemoresistance or radioresistance of tumor cells [66,67]. More information about the combined effects of phytochemicals is needed to avoid the possible unfavorable effects of their unbalanced combinations.

Conclusion

In Ames test (*in vitro*) and in micronucleus test *(in vivo)*, ellagic acid and curcumin did not show any mutagenic effect to bacteria and mice. We verified the antimutagenic effect of ellagic acid and curcumin against the indirect mutagens IQ and AFB_1 and against the direct mutagen MNU in the Ames test and in the micronucleus test. The antimutagenic effect of ellagic acid and curcumin was dose dependent. An increased antimutagenic effect of the ellagic acid and curcumin combinations was proved in the Ames test against the indirect mutagens IQ and AFB_1 and in the micronucleus test against the direct mutagen MNU as compared with effect of ellagic acid or curcumin used separately.

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Declaration of Interest Statement

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