

# Antimutagenic Activities Extracts from Leaves of the *Morus alba*, *Morus nigra* and Their Mixtures

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## Abstract

The influence of *Morus alba* and *Morus nigra* leaf extracts, and their mixture on the structure and functions of plants and animals cells were studied. The absence of the genotoxicity of the extracts and their mixture has been established. All active biological compounds studied demonstrate genoprotective properties. The studied extracts and their mixtures decreased the mutability level induced with chemical mutagens, gamma-rays and ageing in the plants (*Vicia faba*, *Arabidopsis thaliana*) and animals (rats) cells.

**Keywords:** Antimutagens, Genoprotectors, Plant extracts, Gamma-Rays, Ageing, *Morus alba*, *Morus nigra*

## 1. Introduction

Biologically active compounds obtained from the different plant species have a potential to positively influence health and longevity and can be used as food additives (Surh et al., 1995; Cardaror-Martinez et al., 2002; Romina et al., 2006, Agabeyli, 2008; Weisburger, 2002). A use of biologically active compounds as nutritional supplements/food additives allows enhancing quality of food and provides food products with new features. This approach increases competitiveness among food industry products and results in higher sales and expansion of markets.

Nutritional supplements are different in nature and belong to different classes of natural and synthetic-chemical compounds and their complexes. The most promising food additives and supplements usually originate from plants. While additives of herbal origins are normally most suitable for the use in food industry, a thorough assessment is necessary in order to rule out substances with possible side effects and confirm health benefits. The results of numerous past studies, aimed at assessment of genetic activity of composite antimutagens or food additives with genoprotective properties, demonstrate that these qualities are manifested best by the mixtures of several agents, rather than by the individual components (Alakbarov, 2002; Agabeyli et al., 2005; Alakbarov et al., 2002).

The aim of this study was to conduct a comprehensive assessment of the influence of herbal extracts obtained from the leaves of species on the levels of chromosome mutations induced by chemical products and radiation. *Morus alba* and *Morus nigra* species are widely distributed throughout the territory of Azerbaijan Republic, and may be considered as a source of promising and effective nutritional supplement/food additive for various food industries.

## 2. Materials and Methods

### 2.1 Research objects

#### 2.1.1 Plants

1) Beans - (*Vicia faba* L.), 2) *Arabidopsis* (*Arabidopsis thaliana*)

#### 2.1.2 Animals

Wistar rats, aged 10-12 weeks (weighing 120-130 g).

## 2.2 Inductors of mutation process

1). Gamma-rays (GR), the seeds of *V. faba* were exposed to installing K-25, Co<sup>60</sup>, in a dose 60Gy; animals in a dose of 3 Gy. 2). Chemical mutagens: N-methyl<sup>1</sup>-nitro-N-nitrozoguanidin (MNNG), the firm “Serva”-direct-type action on DNA, in concentrations -1 mM; 3mM, 3). Sodium fluoride - NaF, in concentrations -20 mg fluorine per 100g weight of the animal.

## 2.3 Modifiers of mutation process

Water-ethanol extracts from leaves of *M. alba* – variety Bidan (E1) and black mulberry *M. nigra* – variety Har here (E2), and their mixture - composition (CP) with a high content of amino acids, antioxidants, and vitamin C.

## 2.4 Methods

Analysis of gene mutations was performed on a Mueller’s embryonic test (*Arabidopsis thaliana*) (Müller, 1963). Analyses of chromosome aberrations in the anaphase in plants cells and statistics data processing have been done according standard procedures [Agabeyli et al., 2011].

Plant extracts (E1, E2 and CP) were obtained by vacuum distillation, consisting of the stages of extraction, purification of ballast substances and evaporation (Muraviev, 1978) at the Department of Pharmacognosy and Botany of the Azerbaijan State Medical University.

## 3. Results and Discussion

Results of comparative evaluation of the influence of extracts - E1, E2, and their mixture (CP) on spontaneous mutations levels and mutations induced by aging and environmental xenobiotics in the cells of plant and animal test systems are summarized below.

The *Morus* leaves are rich in amino acids, vitamin C and antioxidants (Luo, 1994), which have known with antimutagenic activities (Agabeyli, 2008). The results of the evaluation of the genetic activity of E1, E2 and CP in experiments on *V. faba* seeds having a high level of spontaneous mutations of chromosomes revealed a high level of antimutagenic activity (AA) in all ranges of studied concentrations (Table 1). With increasing concentration of the studied extracts within the range of 1-100 mkg/ml their effectiveness in preventing frequency of chromosomes aberrations exceeded 50% of control level. The same effect was observed during the assessment of E2 effectiveness. However, the antimutagenic effect of CP on aging-induced frequency of chromosome aberration was even more prominent as compared to control. In high concentrations of E1, E2, CP did not demonstrate any mutagenic or other toxic activity.

Results of experiments demonstrate higher antimutagenic and gene-protective activities of CP as demonstrated by the modification of ageing induced chromosome aberrations levels in the cells of *V. faba* where mixture of extracts (CP) demonstrated reduction of the level of the aberrations of chromosomes by 84-86%, exceeding the antimutagenic efficiency of the components used separately (Table 2). Both studied extracts demonstrated gene protection activity in modification of chromosome aberrations level induced by chemical mutagen MNNG in cells of *V. faba*. Antimutagen efficiency of CP was confirmed for studied concentrations and ranged from 70% to 86% as compares to control levels. Similar to E1 and E2, efficiency of CP increased in higher concentrations (1-100mkg/ml) and reached 84-86%.

To examine universality of the effects of plants extracts and their mixture, different test systems were used. Test-object *A. thaliana* has a short life cycle (30 days), it is easy to cultivate in the laboratory and allows analyzing the influence of various factors on the frequency of gene mutations (Ivanov, 1974). The results of comparative evaluation of antimutagenic activity of E1, E2 and CP on the frequency of dominant embryonic chlorophyll mutations induced by MNNG in *A. thaliana*, revealed higher efficiency of mixture of extracts (CP) as compares to the separate use of the composites (Table 3).

Antimutagen’s assessment standards require comprehensive examination of universality and specificity of their effectiveness in reducing chromosome instability induced by genotoxicants with different mechanism of action.

Therefore ionizing irradiation (gamma-rays) and environmental chemical mutagen (NaF) were used as mutagenic factors in the experiments. Ionizing radiation is a potential DNA damaging agent. Comparative analysis of successful reduction of radiation-induced chromosome aberrations rate by the plant extracts E1 and E2 and their mixture CP is presented in the Table 4. Under the influence of GR frequency of chromosome aberrations in bone marrow cells of rats increases relatively to control more than 9 times (Table 4).

Prior to exposure (12 days), prophylactic injection of herbal extracts decreases cellular chromosomes aberrations rate induced by GR and efficiency of antimutagenic effects exceeded 50% level in all variations of the

experiment. With increasing concentration of extracts E1, E2 and CP their antimutagenic efficiency respectively varied from 58 to 68%, 64-70% and 77-83%.

Fluorine mutagens are known environmental genotoxicants (Aaderma et al., 1989; Tsebyujinskiy et al., 1981). Sodium fluoride NaF is widely used in various branches of ferrous metallurgy, medicine and everyday life. NaF is known to impact DNA repair mechanisms (10, Aaderma et al., 1989; Tsebyujinskiy et al., 1981). Therefore, in a series of experiments antimutagen efficiency of plant extracts and their mixtures was studied on NaF induced mutations process in rat's bone marrow cells. A preliminary two-week introduction of plant extracts to the standard diet of rats did not lead to the manifestation of mutagenic and other side effects (Table 5). In animals with NaF - induced high levels of chromosome mutations use of E1, E2 and CP effectively reduced levels of chromosome aberrations, E1 and E2 reduced these levels by to 53-59% and CP by more than 66-78% as compares to control.

Comparative assessment of antimutagen efficiency in variations of experiment shows higher efficiency of extracts mixture (CP) in prevention of NaF - induced mutations of chromosomes (Table 5).

The majority of the known plant antimutagens demonstrated the effectiveness in decreasing the level of generational and regulation disorders induced by different environmental xenobiotics, including the mutagens and carcinogens with different mechanisms of action. The experiments shown that studied extracts demonstrated effectiveness against the mutability induced also by ageing processes. The antimutagenic effectiveness is associated with the multicomponent nature of the studied extracts and their mixtures. Therefore the creation of the plants extracts compositions and their mixtures with is a real way for increasing of their effectiveness in prevention of the genetically disorders induced by environmental xenobiotics (Alakbarov, 2002).

The results of the research also indicate that introduction of the extracts into the standard diet of the animals two weeks prior to the experiments did not lead to manifestation of mutagenic or any other side effects (Table 5). Extracts from the fruits of Black *Morus nigra* L., White mulberry *Morus alba* L. and Red mulberry *Morus rubra* L. mulberry have high content of ascorbic acid, amino acids, glucose, minerals, tannins and triterpenes and demonstrate antioxidant properties (Ercizli et al., 2007, 2008, Ozgen et al, 2009; Soni et al., 1999). Experiments with three flavonoids from *Morus alba* L. - Morusine (morusin), kuanon H (Kuanon H) and morusin 4'-glucoside (morusin 4' - glycoside) and their derivatives revealed their sensitivity to photooxidation and the manifestation of anti - HIV activity in vitro. The authors are the first to report anti - HIV activity and cytotoxicity of morusin - 2' - glucoside, morusin - 4' - glucoside, kuanon H-6' - octare kuanon H-7-acetate and triacetate malberroforan D (Luo, 1994). There is now ample evidence of the antimutagenic effect of phenolic compounds (Agabeyli, 2008, Ercisli et al, 2007, 2008), including the protective effect of dietary supplementation with phenolic compounds against aflatoxin-induced mutagenicity and hepatocarcinogenicity (Soni et al., 1999); antimutagenic effect of natural phenolic compounds of beans (De Mejia et al., 1999) and others [Smerak et al., 2002; Masuda et al., 1999; Rice-Evans et al., 1997; Eman et al., 2008; Kutlu et al. 2011; Owen et al., 2000; Kaur et al, 2002; Cai et al., 2004).

Research in biologically active compounds obtained from the different plant species shows that they do not exhibit genotoxic and cytotoxic properties and are of interest for further studies for the prospective use as dietary supplements/food additives that have antioxidant and antimutagenic activity. This study suggests that these compounds exhibit considerable gene protective properties, which have a potential to greatly enhance their future use in the food industry (Surh et al., 1995; Weisburger, 2002; Agabeyli et al., 2005; Alakbarov et al., 2002). The practical application of plant composition to neutralize the mutagenic effect of environmental genotoxicants may be one of the practical ways to improve the nutritional value and competitiveness of food products. Studied extracts demonstrate strong gene protection properties, which significantly increase value of their possible use in food industry. Use of herbal extracts in food industry to provide valuable source of antimutagenic protection is a promising way to increase nutritional value and competitiveness of food industry products.

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Table 1. Effect of extracts from leaves *Morus alba* (E1), *Morus nigra* (E2) and their composition (CP) on the frequency of chromosome aberrations in meristematic cells of roots of the *V. faba*

Experiment variations	Concentration, mkg/ml, NG, mM	Number of cells studied	Cells with CA, %		Td	AE*, %
			N	M±m		
O (control)	-	899	63	7.19±0.86	-	-
E1	100	840	24	2.85±0.32	4.6	60
	10	820	19	2.31±0.27	4.5	67
	1	930	22	2.36±0.24	3.5	67
	0.1	994	26	2.61±0.25	4.0	63
	0.01	954	25	2.62±0.26	2.5	63
	0.001	1125	49	4.35±0.36	2.8	39
E2	100	1009	29	2.87±0.52	4.9	60
	10	1100	31	2.81±0.49	4.4	60
	1	1007	30	2.97±0.53	4.1	58
	0.1	949	32	3.37±0.58	3.7	53
	0.01	1020	37	3.62±0.55	3.4	49
	0.001	1038	39	3.75±0.58	3.3	47
CP	100	940	16	1.70±0.17	4.8	76
	10	1058	13	1.22±0.11	6.3	83
	1	936	14	1.49±0.15	5.9	79
	0.1	996	18	1.80±0.31	6.1	74
	0.01	620	20	3.22±0.70	3.5	55
	0.001	750	25	3.33±0.42	6.1	53

AE\*- antimutagenic efficiency.

Table 2. Effects of extracts from *Morus alba* (E1), *Morus nigra* (E2) and their compositions (CP) on and MNNG-induced (1mM) frequency of chromosome aberrations in the root meristem cells of *Vicia faba*

Experiment variations	Concentration, mkg/ml, NG, mM	Number of cells Studied	Cells with CA, %		t <sub>d</sub>	AE,%
			N	M±m		
O (control)	-	899	63	7.19±0.86	-	
MNNG	1	959	145	15.98±1.21	-	
E1	100	703	50	7.82±1.05	4.6	70
	10	1008	82	8.13±0.86	5.2	64
	1	1010	89	9.71±1.11	4.7	57
	0.1	893	79	8.84±0.94	5.0	61
	0.01	906	72	7.94±0.89	5.3	70
	0.001	1099	79	7.18±0.81	8.8	74
E2	100	954	40	4.19±0.42	9.2	73
	10	749	30	4.00±0.71	8.5	74
	1	895	42	4.69±0.49	8.6	70
	0.1	948	51	5.37±0.73	7.5	66
	0.01	999	60	6.0±0.28	8.0	77
	0.001	1000	60	6.0±0.28	8.0	77
CP	100	516	13	2.51±0.71	9.4	84
	10	1614	34	2.10±0.40	10.3	86
	1	735	18	2.44±0.35	10.7	84
	0.1	849	22	2.59±0.54	10.1	80
	0.01	985	30	3.04±0.54	9.7	80
	0.001	857	27	3.15±0.58	3.0	80

AE\*- antimutagen efficiency.

Table 3. Analysis of frequency of dominant embryonic chlorophyll mutations induced by (MNNG, 3 mM) in *Arabidopsis thaliana* and their modification of extracts from *Morus alba* (E1) and *Morus nigra* (E2) and their composition (CP)

Experiment variations	Number of cells studied	Cells with CA, %		td	AE,%
		N	M±m		
0(control)	-	-	0.02	-	-
MNNG	240	62	25.83±2.82	5.6	-
MNNG +E1,30mkg/ml	390	35	8.97±1.44	5.3	62
MNNG +E1 60 mkg/ml	435	27	6.20±1.15	5.6	75
MNNG +E2,30 mkg/ml	460	30	6.52±1.15	6.7	74
MNNG +E2,60 mkg/ml	460	28	6.08±1.11	6.6	74
MNNG +CP,30 mkg/ml	374	25	6.68±1.29	6.2	74
MNNG +CP,60 mkg/ml	420	24	5.71±1.13	6.6	77

P<0.001.

Table 4. Effect of extracts from *Morus alba* (E1) and *Morus nigra* (E2) and their composition (CP) on the gamma-rays (GR) induced mutation process in bone marrow cells of rats

Experiment Variants	Concentration, mkg/ml; mkg/100gr of weight	Number of cells Studied	Cells with CA, %		td	AE,%
			N	M±m		
O (control)	-	1034	13	1.29±0.32	-	-
GR	3	1119	110	9.83±0.89	-	-
E1+ GR	30	1005	44	4.37±0.64	5.3	58
	60	1130	37	3.27±0.52	6.5	66
E2+ GR	30	1050	37	3.52±0.32	5.9	64
	60	1100	32	2.90±0.50	6.7	70
CP+ GR	30	1825	41	2.24±0.34	7.9	77
	60	1810	29	1.60±0.08	8.8	83

Table 5. Effect of extracts from *Morus alba* (E1) and *Morus nigra* (E2) and its composition (CP) on the frequency of spontaneous and NaF induced mutation process in bone marrow cells of rats

Experiment Variants	Concentration, mkg/ml, mg/100g of weight	Number of cells Studied	Cells with CA, % rate,		td	AE,%
			N	Percent		
O (control)	-	1835	29	1.58±0.28	-	-
E1	30	1235	15	1.21±0.31	-	-
	60	1040	11	1.05±0.31	-	-
E2	30	1325	11	1.05±0.28	-	-
	60	1200	10	0.83±0.28	-	47
CP	-	1089	7	0.64±0.24	2.5	59
NaF	20	2009	140	6.96±0.56	-	-
E1+ NaF	30	1850	60	3.24±0.41	5.3	53
	60	1860	60	3.22±0.40	5.4	53
E2+ NaF	30	1147	38	3.31±0.27	5.8	52
	60	1234	35	2.83±0.47	5.6	59
CP+ NaF	30	1780	41	2.30±0.35	7.0	66
	60	2010	30	1.49±0.27	8.7	78