

CLINICAL SCIENCE

Effects of aqueous soybean, mistletoe and red clover extracts on activities of adenosine deaminase and xanthine oxidase enzymes

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Abstract: Soybean (*Glycine max*), mistletoe (*Viscum album*) and red clover (*Trifolium pratense*) have been argued to have anti-cancer effects. In the present study it was aimed to investigate possible effects of these plant extracts on the activities of DNA turn-over enzymes, namely adenosine deaminase (ADA) and xanthine oxidase (XO) in cancerous and non-cancerous gastric and colon tissues. For this aim, 6 cancerous and 6 non-cancerous adjacent human gastric tissues, and 7 cancerous and 7 non-cancerous adjacent colon tissues were obtained by surgical operations. Our results suggest that aqueous soybean, mistletoe and red clover extracts may exhibit anti-tumoral activity by depleting hypoxanthine concentration in the cancer cells through XO activation, which may lead to lowered salvage pathway activity necessary for the cancer cells to proliferate in the cancerous colon tissue. Some foods like soybean, mistletoe and red clover may provide nutritional support to medical cancer therapy through inhibiting and/or activating key enzymes in cancer metabolism (Tab. 4, Ref. 33). Text in PDF www.elis.sk.

Key words: gastric cancer, colon cancer, soybean, mistletoe, red clover, adenosine deaminase, xanthine oxidase.

Colorectal cancer is the fourth most common cancer in men and third most common cancer in women worldwide (1). Environmental factors including dietary habits are important in the development of colorectal and gastric cancers (2). Many studies have been performed on this subject by various clinical centers. There is still no convincing therapeutic method in the colorectal and gastric cancers that is good enough for such a complex disease. Combination of surgery, chemotherapy and radiotherapy is the choice of therapy in these cancers, but side effects affect the performance of these methods.

Many epidemiological studies have revealed that there are positive correlations between colon cancer and ingestion of animal fat and inverse correlations between colon cancer and diets rich in vegetables (3–5). Nagata et al. have observed that there are inverse correlation between soy product intake and gastric cancer mortality rate in men, and inverse correlation between soy product intake and colorectal cancer in men and women. Soybean consists of several biologically active components including proteins, isofla-

vones, saponins, fiber, oil and fatty acids. Saponins and isoflavones have protective effects on colorectal cancers. Soybean contains high quantities of the isoflavones, namely genistein and daidzein. Genistein is capable of inhibiting cancer cell growth by blocking tyrosine protein kinases and DNA topoisomerases (6,7). Genistein has been shown to be anti-proliferative, to induce apoptosis and to inhibit angiogenesis, all of which are potential anti-cancer mechanisms (7). They have found that these correlations depend on the intake of soybean and isoflavones (8). They proposed that it is unknown yet whether soybean paste is protective or a risk factor for gastric cancer. Messina et al. suggested that risk for gastric cancer seemed to increase with the intake of fermented soybean foods and to decrease with the intake of non-fermented soybean foods (3). Mistletoe has beneficial properties such as anticancer (9, 10), antiviral (11), apoptosis-inducing (12) and immunomodulatory effects (13, 14). Mistletoe includes lectins which have cytotoxic effects on tumor cells and induction of apoptosis (15). Red clover is also a rich source of isoflavones. Genistein and daidzein are predominant isoflavones found in red clover (16).

ADA converts deoxyadenosine to deoxyinosine and ammonia and also converts adenosine to inosine and ammonia via hydrolytic deamination (17). XO, in the purine metabolism, catalyzes oxidation of xanthine and hypoxanthine, and it can produce superoxide radicals (18).

In this study it was aimed to investigate possible effects of soybean, mistletoe and red clover extracts on the activities of two DNA turn-over enzymes, namely adenosine deaminase (ADA) and xanthine oxidase (XO) in human colon and gastric cancer tissues

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Materials and methods

Patients

Six cancerous and 6 non-cancerous adjacent human gastric tissues and, 7 cancerous and 7 non-cancerous adjacent colon tissues were obtained from cancer patients by surgical operations. The work was approved by the Ethics Committee of Ankara Numune Teaching and Research Hospital.

Treatment of tissues

The tissues were treated with aqueous extracts of 3 different herbs (Soybean, mistletoe and red clover) *in vitro* at 3 different final concentrations (0.05 %, 0.5 % and 1.25 %) for 1 hour. Activity assays were performed in the same samples without the extracts as well. Protein level of the tissues was studied by the Lowry method (19), after adjusting to equal concentrations.

Quantification of adenosine deaminase and xanthine oxidase activity

After and before incubation period, XO and ADA enzyme activities were measured by the spectrophotometry. Xanthine oxidase activity was determined by measuring the optical density of uric acid, which is formed from xanthine (catalog number of xanthine X-0626, Sigma) by xanthine oxidase, at 293 nm as described

(20). Adenosine deaminase activity was studied by the method of Guisti (21). ADA converts adenosine (catalog number of adenosine A-9251, Sigma) into inosine and ammonia. The resulting ammonia reacts with phenol and sodium hypochloride to create indophenol with blue color. The optical density of indophenol is measured at 628 nm by using spectrophotometry (Unicom Helios- α UV-vis spectrophotometer). Enzyme activities were expressed as μ IU/mg-protein and mIU/mg-protein for XO and ADA, respectively. IU is the amount of an enzyme that will catalyze the transformation of 10^{-6} mol (μ mol) of substrate per minute. mIU is the amount of an enzyme that will catalyze the transformation of 10^{-9} mol (nmol) of substrate per minute.

Statistical analysis

The Friedman and Wilcoxon Signed Ranks tests were used for statistical evaluation of the results. Values of p lower than 0.05 were considered as significant.

Results

The results are presented in the Tables 1–4. In both cancerous and non-cancerous tissues, XO activities were found to elevate in correlation with increased extract concentrations in both cancer

Tab. 1. ADA activities in cancerous and noncancerous gastric tissues with and without extracts.

Extracts	Concentration	Non-cancerous Tissue		Cancerous Tissue	
		No Incubation	Incubation	No Incubation	Incubation
Red clover	No extract	72.87±65.90	63.72±56.02	40.60±33.64	35.27±20.10
	0.1%	70.36±63.69	66.80±55.84	34.50±31.11	31.35±23.28
	1%	72.82±64.81	65.10±57.10	34.96±34.61	31.27±18.41
	2.5%	73.01±69.53	67.20±60.45	35.23±30.11	36.30±29.45
Mistletoe	No extract	72.87±65.90	63.72±56.02	40.60±33.64	35.27±20.10
	0.1%	75.44±70.51	64.23±58.49	39.90±38.06	32.87±19.80
	1%	70.92±65.88	67.29±60.63	40.23±28.38	40.62±22.95
	2.5%	73.50±68.64	64.17±56.00	42.29±31.91	37.12±15.97
Soy bean	No extract	72.87±65.90	63.72±56.02	40.46±33.64	35.27±20.10
	0.1%	68.66±63.38	63.42±57.46	38.33±27.45	35.20±22.78
	1%	72.44±68.06	72.70±60.20	37.39±24.99	28.76±26.51 ¹
	2.5%	68.00±61.11	69.24±58.55 ^c	35.14±28.16	34.35±24.80

^c0.1% vs. %2.5, ¹incubated and non-cancerous tissue vs. incubated and cancerous tissue; $p < 0.05$.

Tab. 2. XO activities in cancerous and noncancerous gastric tissues with 3 different extracts and no extracts.

Extracts	Concentration	Non-cancerous Tissue		Cancerous Tissue	
		No Incubation	Incubation	No Incubation	Incubation
Red clover	No extract	84.73±50.36	76.79±57.47	58.28±30.53	43.16±23.35
	0.1%	73.77±49.19	79.90±37.21	61.94±27.69	50.95±24.85 ²
	1%	74.06±33.45	71.74±24.06	68.99±29.07	69.99±27.43 ^{b,d}
	2.5%	97.59±33.44	106.76±39.60	86.91±37.12 ^{c,e,f}	76.58±28.31 ^{c,e,f}
Mistletoe	No extract	84.73±50.36	76.79±57.47	58.28±30.53	43.16±23.35
	0.1%	71.65±43.67	84.68±37.86	57.89±25.49	70.18±49.97
	1%	90.68±45.83	80.39±34.15	78.16±39.77 ¹	65.68±40.36
	2.5%	84.64±32.46	80.36±39.73	85.16±26.8 ^{c,e}	64.63±38.60
Soy bean	No extract	84.73±50.36	76.79±57.47	58.28±30.53	43.16±23.35
	0.1%	66.65±46.15	81.50±48.69	66.90±23.17	54.42±25.04
	1%	59.55±35.46	60.66±31.73	60.26±27.07	52.56±21.33
	2.5%	54.55±26.20	52.50±36.30	61.57±23.28	57.37±38.40

^b 1% vs. null, ^c 2.5% vs. null, ^d 0.1% vs. 1%, ^e 0.1% vs. 2.5%, ^f 1% vs. 2.5%, ¹ no-incubated and non-cancerous tissue vs. no-incubated and cancerous tissue, ² incubated and non-cancerous tissue vs. incubated and cancerous tissue; $p < 0.05$.

Tab. 3. ADA activities in cancerous and noncancerous colon tissues with 3 different extracts and no extracts.

Extracts	Concentration	Non-cancerous Tissue		Cancerous Tissue	
		No Incubation	Incubation	No Incubation	Incubation
Red clover	No extract	16.51±16.31	17.38±16.97	21.32±9.47	19.58±8.21
	0.1	15.86±16.06	20.21±18.29	20.40±8.15	21.40±6.06
	1	17.23±17.56	23.30±23.14	21.02±8.02	22.30±5.65
	2.5	18.17±19.19	17.84±19.86	50.21±77.55	21.05±6.66
Mistletoe	No extract	16.51±16.31	17.38±16.97	21.32±9.47	19.58±8.21
	0.1	10.07±5.20	19.43±20.26	17.33±10.58 ²	21.16±8.16
	1	13.28±7.15	15.47±10.05	20.82±8.16 ²	18.61±7.02 ³
	2.5	18.66±22.64	15.53±10.05	21.12±9.27	21.56±7.68
Soy bean	No extract	16.51±16.31	17.38±16.97	21.32±9.47	19.58±8.21
	0.1	16.95±16.02	24.68±19.51 ^{a,1}	22.43±7.6	20.27±6.85 ³
	1	17.66±14.44	14.99±19.20 ^b	21.02±6.55	23.34±10.88
	2.5	16.92±17.46	16.92±17.46 ^c	23.48±7.49	20.59±6.80

^a0.1% vs. null, ^b0.1% vs. 1%, ^c0.1% vs. 2.5%, ¹ no-incubated and non-cancerous tissue vs. Incubated and non-cancerous tissue, ² no-incubated and non-cancerous tissue vs. no-incubated and cancerous tissue, ³ no-incubated and cancerous tissue vs. incubated and cancerous tissue; p<0.05.

Tab. 4. XO activities in cancerous and noncancerous colon tissues with 3 different extracts and no extracts.

Extracts	Concentration	Non-cancerous Tissue		Cancerous Tissue	
		No Incubation	Incubation	No Incubation	Incubation
Red clover	No extract	37.56±11.08	38.62±3.77	45.69±24.06	41.16±24.02
	0.1%	49.95±14.74 ^a	45.67±14.40	67.97±62.10	66.50±21.99 ^{a,2}
	1%	39.81±17.67	44.98±13.92	58.69±22.98 ^{b,1}	62.74±24.97 ^b
	2.5%	65.46±39.80	67.76±23.68 ^{c, e, f}	68.86±21.34 ^{c, f}	74.77±22.40 ^{c, f}
Mistletoe	No extract	37.56±11.08	38.62±3.77	45.69±24.06	41.16±24.02
	0.1%	37.79±8.64	33.91±11.93	45.38±23.04 ¹	55.17±26.06
	1%	29.64±14.16 ^b	30.74±7.49	43.11±19.44 ¹	51.35±22.19
	2.5%	22.82±12.24 ^{c, d, e, f}	16.41±8.57	48.29±23.47 ¹	47.15±11.71
Soy bean	No extract	37.56±11.08	38.62±3.77	45.69±24.06	41.16±24.02
	0.1%	40.28±12.46	35.10±10.09	48.27±24.99	46.01±15.59
	1%	36.08±19.00	44.68±15.00	61.24±10.90 ¹	52.82±9.00
	2.5%	49.33±17.10	55.37±27.81	70.35±12.04 ¹	69.26±14.68

^a0.1% vs. null, ^b1% vs. null, ^c2.5% vs. null, ^d0.1% vs. 1%, ^e0.1% vs. 2.5%, ^f1% vs. 2.5%, ¹ no-incubated and non-cancerous tissue vs. no- incubated and cancerous tissue, ² incubated and non-cancerous tissue vs. incubated and cancerous tissue; p<0.05.

types. However, no change was found between ADA activities in the tissues.

It was found that soybean extract increased XO activity in cancerous colon tissue without preincubation. However, it inhibited the activity of XO in non-cancerous gastric tissue with and without preincubation.

It was observed that aqueous mistletoe extract significantly inhibited XO activity in non-cancerous colon tissue. However, it caused increases in the activity of XO in cancerous gastric tissue. Additionally, red clover caused significant increases in the XO activities of both cancerous tissues.

Discussion

Red clover has long been used to treat some cancer types among people. The use of red clover as an anticancer agent can be traced back to the 1940s when herbalists suggested the use of herbs as an alternative choice to surgery and radiation therapy (22, 23). Red clover contains high amounts of isoflavone compounds such as genistein, daidzein, formononetin and biochanin A (24). Several researchers have shown that these isoflavones may help prevent cancer. In a study, it has been demonstrated that isoflavone derivatives inhibit the cell growth of gastric can-

cer lines in vitro and supposed that this might occur through activation of a signal transduction pathway for apoptosis. In another study, biochanin A, one of the isoflavones in red clover has been found to inhibit carcinogen activation in cells in culture medium (25). Biochanin A and formononetin are potent aryl hydrocarbon receptor (AhR) agonist, activation of which affects cell cycle and drives cells to apoptosis in vitro. Daidzein is another isoflavone in red clover. It is metabolized to equol in mammals which is associated with reduced incidence of breast cancer. In another study, the researchers found that, consumption of red clover before prostatectomy increased apoptosis in malignant tissue (26). However, the precise mechanism of action and responsible constituents for the proposed benefits of red clover in the cancer process are not clearly known yet.

Mistletoe has been used for the treatment of many diseases like solid cancers. Extracts from viscum album were used as complementary cancer treatments. It was found to reduce side effects during chemotherapy and adjuvant therapy (27). Mistletoe extracts also could reduce harmful and mutagenic effects of the oxygen free radicals generated during radiotherapy and chemotherapy (28, 29). Lectins are among the active constituents of mistletoe extracts. Lectins caused strong cytotoxic effects on tumor cells by apoptosis. Viscum album coloratum agglutinin (VCA) is

a lectin and isolated from Korean mistletoe. VCA drives cells to apoptosis by inhibiting telomerase activity. Khil et al. found that VCA selectively killed colon cancer cells but not normal human cells in vitro and in vivo. They found that VCA induced apoptosis in colon cancer cells due to activation of caspases and inhibition of anti-apoptotic proteins such as tumor necrosis factor (15). However, the precise mechanism of action and responsible constituents are also not known for the proposed benefits of Mistletoe in the cancer process.

Soybean contains several active components that may contribute individually or synergistically to the health benefits of this plant. It has been used for gastrointestinal cancers, especially for gastric and colon cancers (30, 31). Isoflavones, saponins, fiber, oils and fatty acids are the components of soybean that have been hypothesized to provide health benefits. Although several biological activities of this plant have been reported in many studies, Hong-Mei Nan suggested that no study was performed to examine soybean effects on DNA turn-over enzymes in vitro. Hong-Mei Nan et al. observed that soybean pastes were risk factors of gastric cancer (32).

Salted, smoked, pickled, and preserved foods rich in salt, nitrite, and preformed N-nitroso compounds have been reported to be associated with an increased risk of gastric cancer. In contrast, high intake of fresh fruit, raw vegetables, and antioxidants significantly reduced the risk of gastric cancer (33). Soybean was established to have protective role against gastric cancer in a study (9).

In the present study, we have found that soybean extract increases XO activity in cancerous colon tissue without preincubation. However, it inhibits the activity of XO in non-cancerous gastric tissue with and without preincubation. Additionally, it has been observed that aqueous mistletoe extract significantly inhibits XO activity in non-cancerous colon tissue. However, it has caused increases in the activity of XO in cancerous gastric tissue. Red clover causes significant increases in the XO activities of both cancerous tissues, namely gastric and colon tissues. Our results suggest that aqueous extracts of soybean, mistletoe and red clover may exhibit anti-tumoral activity by depleting hypoxanthine concentration through XO activation, which may lead to lowered salvage pathway activity necessary for the cancer cells to proliferate in the cancerous colon and gastric tissues. Hypoxanthine has been converted to inosine mono phosphate (IMP) by Hypoxanthine-Guanine Phosphoribosyl Transferase enzyme in this salvage pathway. As known, IMP is used in DNA synthesis. For this reason, ADA and XO enzyme activities were investigated in the present study. The validity of ancient medicine is now being evaluated and tested critically in some tissue models. Preventive and therapeutic trials of some plants such as soybean, red clover, mistletoe, etc. are still in the early stage, although there are many lines of research suggesting the potential effects of these plants on cancer as emphasized above. The current knowledge does not recognize these plants as a true alternative drug. However, we think that it will certainly find a place in future for their role in the cancer prevention and treatment regimens.

In our conclusion t soybean, mistletoe and red clover may provide nutritional support and can be combined for medical cancer treatment. Additionally, XO enzym activity may be used in assessment of anti-cancer treatment in the future. However, the subject needs future investigations.

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