

# Aqueous Extracts of *Celandine*, *Red Clover*, *Flax Seed* and *Coriander* Inhibit Adenosine Deaminase Enzyme Activity in Cancerous Human Gastric Tissues

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## Abstract

**Background and Aim:** Cancer is the major problem for all people in the world. The scientists have long been looking to natural remedies for the treatment of cancer because of side effects of chemotherapy and radiotherapy. Therefore we aimed to investigate possible effects of aqueous extracts of *Celandine* (*Chelidonium majus*), *Red Clover* (*Trifolium pratense*), *Flax seed* (*Linum usitatissimum*) and *Coriander* (*Coriandrum sativum*) on adenosine deaminase (ADA) activity in cancerous and non cancerous human gastric and colon tissues. **Materials and Methods:** Cancerous and non cancerous human gastric (n=31) and colon tissues (n=19) removed by surgical operations were studied. The extracts were prepared in distilled water. Tissues were first homogenized in saline solution (20%, w/v), then centrifuged and supernatant fraction was used. In the tissue samples, adenosine deaminase activities were measured with and without plant extracts preincubated for 1 h. **Results:** It has been observed that aqueous extracts of *celandine*, *red clover*, *flax seed* and *coriander* inhibit ADA enzyme in cancerous gastric tissues significantly, but none affects the enzyme in noncancerous gastric tissue. In the colon tissues, none of those except *celandine* affects the ADA activity. *Celandine* however activates the enzyme in both colon tissues significantly. **Conclusion:** It has been suggested that inhibition of ADA enzyme by *celandine*, *red clover*, *flax seed* and *coriander* extracts might give selective advantage to the cancerous tissues to combat cancer process. Clarification of ADA activation in colon tissues by *celandine* extract however needs further studies.

## Keywords

Adenosine Deaminase, Cancer, Colon Tissue, Gastric Tissue, *Chelidonium majus*, *Trifolium pratense*, *Linum usitatissimum*, *Coriandrum sativum*

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## 1. Introduction

Cancer is the major problem for all people in the world. Therefore, it has long been investigated to find effective nontoxic remedies for the treatment of cancer because of side effects of chemotherapy and radiation therapy. Traditionally,

it has been observed that treatment of some types of cancers with plant sources may give rise to positive results.

In fact, there are many factors for cancer occurrence and development in humans. Most of the cancers are caused by environmental factors and of these; 30–40% of cancers are directly linked to the diet (1). While many dietary

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recommendations have been proposed to reduce the risk of cancer, unfortunately few have significant supporting scientific evidence (2).

Adenosine deaminase (ADA) is an enzyme (EC 3.5.4.4) involved in purine metabolism. It is needed for the breakdown of adenosine and for the turnover of nucleic acids. ADA is present virtually in all mammalian cells, and it is thought that its primary function in human beings is related to the immune system (3). However, the full physiological role of ADA is not completely understood (4). ADA association has also been observed with epithelial cell differentiation, neurotransmission, and gestation maintenance (3, 5). It has also been proposed that ADA, in addition to its role in adenosine breakdown, stimulates release of excitatory amino acids, and it is necessary to the coupling of A1 adenosine receptors and heterotrimeric G proteins (3, 4).

Some ADA inhibitors have been used for chemotherapeutic purposes in some types of cancers. From a scientific perspective of view, use of ADA inhibitors has helped much in understanding the mechanism of action of adenosine metabolites and analogs. ADA inhibitors have also led to the understanding of the regulatory processes associated with immunodeficiency characterized by a lack of ADA, and of maturation of the immune response (6). One of them, pentostatin (Nipent) is a nucleoside analog having potential to inhibit adenosine deaminase enzyme. Inhibition of ADA blocks the deamination reactions in the purine salvage pathway, result of which is the inhibition of ribonucleotide reductase. As a result, this process depletes the nucleotide pool and limits DNA synthesis (7).

Many plants have been investigated with regard to their anticancer potentials and some of them were observed to have anticancer potency. However, there is still much work to be done. As to the subject, new plants need to be investigated with regard to their anticancer properties. Of them, *Celandine* (*Chelidonium Majus*) is a perennial herb. It has previously been established that celandine extract has strong antioxidant potential, specifically from the alkaloid and flavonoid components (8). Much alkaloid content has been found in the root, sometimes achieving 2-3% concentrations. This has prompted *celandine* to be included in some preparations designed to support the body components (9). *Celandine* also contains chelidonic acid, which has been found to relieve discomfort and be aggressive against certain harmful organisms (10). Traditionally, this herb has been recognized as a useful detoxifying agent (11). It was formerly used by gypsies as a foot refresher; modern herbalists use its purgative properties (12). Extracts of greater *celandine* have exhibited a broad spectrum of toxicity to harmful organisms as well as liver protecting activity and was used

for treatment of various inflammatory diseases (13). Its latex also contains proteolytic enzymes and the phytocystatin chelidostatin, a cysteine protease inhibitor (14).

Another one is *Red Clover* (*Trifolium Pratense*), which is an herbaceous, short-lived perennial plant, variable in size, growing to 20–80 cm tall. It is widely grown as a fodder crop, valued for its nitrogen fixation, which increases soil fertility. For these reasons it is used as a green manure crop. *Red clover* is commonly used to make a sweet-tasting herbal tea. It is an ingredient in some recipes for essiac tea. *Trifolium pratense* is used in traditional medicine of India as deobstruent, antispasmodic, expectorant, sedative, anti-inflammatory, and antidermatosis agent (15). In alternative medicine, *red clover* is promoted as a treatment for a variety of human maladies, including coughs, disorders of the lymphatic system and a variety of cancers. Due to its activity on estrogen receptors, *red clover* is contraindicated in people with a history of breast cancer, endometriosis, ovarian cancer, uterine cancer, uterine fibroids, or other estrogen-sensitive conditions but others have suggested the high isoflavone content counteracts this, and even provides benefits in these conditions (16).

The other plant is *Flax Seed* (*Linum usitatissimum*) which has unique nutritional characteristics. *Flax seeds* produce a vegetable oil known as flaxseed or linseed oil, which is one of the oldest commercial oils, and solvent-processed flax seed oil has been used for many centuries. *Flaxseed* raised  $\alpha$ -linolenic acid and long-chain n-3 fatty acids in both plasma and erythrocyte lipids, as well as raising urinary thiocyanate excretion 2.2- fold. Flaxseed also lowered serum total cholesterol and low-density-lipoprotein-cholesterol significantly (17). *Flaxseed* is a rich source of dietary lignan, and some researchers suggest that lignan has antimitotic, antiangiogenic, antioxidant, and phytoestrogenic effects (18).

*Coriandrum Sativum* (*Coriander*) is native to worldwide regions, southern Europe, North Africa, southwestern Asia etc. The leaves have a different taste from the seeds (19). *Coriander* is among such most commonly used spices, possessing the nutritional as well as medicinal properties, widely distributed and mainly cultivated for the seeds which contain an essential oil and the monoterpenoid-linalool. Many of healing properties of *coriander* can be attributed to its exceptional phytonutrients and hence, it is often referred to as store house for bioactive compounds (20). In a study, it has been observed that *coriander* plays a protective role against the deleterious effects in lipid metabolism in experimental colon cancer (21). In another study, Rafi M *et al* found that coriander root contains 8-methyl-dihydrobenzopyrone, which induces Bcl-2 phosphorylation, apoptosis, and G2/M cell cycle arrest in breast tumor cells

(22). In fact there are many studies indicating supportive potentials of *coriander* against several types of cancer (23).

Medicinal plants have therapeutic potential due to the presence of natural antioxidants functioning as reducing agents, free radical scavengers and quenchers of singlet oxygen. Majority of their antioxidant activity is due to bioactive compounds like flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins and isocatechins (24). In fact, apart from the traditional use, their beneficial physiological effects have been identified by extensive studies (25).

As summarized above, all of these plants need further studies to warrant their anticancer potentials if any. In this regard, investigation of their possible effects on the ADA activity in cancerous and non cancerous tissues seems of importance.

## 2. Materials and Methods

The study protocol was approved by the local Ethical Committee of Clinical Research. Thirty one cancerous gastric tissues and 31 non cancerous adjacent gastric tissues were obtained from patients with gastric cancer by surgical operation. Nineteen cancer and 19 non cancer colon tissues were similarly obtained from patients with colon cancer. Non cancerous tissue adjacent to cancer tissue was determined by the surgeon. Tissues were first cleaned by saline solution and stored at -80 °C until analysis not to exceed 6 months. In the analysis process, they were first homogenized in 0.9% saline solution (20%, w/v). After homogenization, samples were centrifuged at 5000 rpm for 30 min to remove debris and to obtain clear supernatant fraction. Analyses were performed in this fraction (26).

The extracts were prepared by soaking ground powder into the distilled water at the concentration of 5 % (w/v) and waiting for 24 h at room temperature by continuously rotating. After the debris was removed, supernatants were centrifuged at 10.000 rpm for 20 min and upper clear part was removed to be used in the assays. In the assays performed with the extracts, supernatants were incubated with the extracts at the ratio of 1/1 (v/v) for one h before ADA activity measurement.

Protein concentrations of the tissues were measured by Lowry method (27) and ADA activity by the method of Guisti (28). ADA activity measurements were performed with and without plant extract for 1 h.

Data were analyzed by using statistical software and presented as Mean  $\pm$  Standard deviation. The distribution of the variables was analyzed with the Kolmogorov-Smirnov test. Due to nonparametric values, Mann–Whitney U-test was used.  $P < 0.05$  was considered as statistically significant.

## 3. Results

Results are shown in the Table 1. As seen from the table, aqueous extracts of *celandine*, *red clover*, *flax seed* and *coriander* inhibit ADA enzyme in cancerous gastric tissues significantly, but none of them affect the enzyme in noncancerous gastric tissue. In the colon tissues, none except *celandine* affect the ADA enzyme. *Celandine* however activates the enzyme in both colon tissues meaningfully.

**Table 1.** Effects of aqueous extracts of *Celandine* (*Chelidonium majus*), *Red Clover* (*Trifolium pratense*), *Flax seed* (*Linum usitatissimum*) and *Coriander* (*Coriandrum sativum*) on adenosine deaminase enzyme activities (IU/mg protein, Mean $\pm$ SD) in gastric and colon tissues with and without cancer

	Gastric tissue (n=31)		Colon tissue (n=19)	
	Malign	Benign	Malign	Benign
A-	12.30 $\pm$ 7.42	10.98 $\pm$ 8.64	5.80 $\pm$ 3.06	5.60 $\pm$ 3.05
B-	6.60 $\pm$ 2.62	12.10 $\pm$ 6.80	8.60 $\pm$ 3.02	9.40 $\pm$ 6.80
C-	6.70 $\pm$ 3.30	10.05 $\pm$ 7.02	6.03 $\pm$ 3.02	6.08 $\pm$ 4.20
D-	6.02 $\pm$ 2.80	10.55 $\pm$ 7.20	6.40 $\pm$ 3.02	7.62 $\pm$ 5.80
E-	7.60 $\pm$ 3.50	10.06 $\pm$ 7.06	5.50 $\pm$ 3.02	5.72 $\pm$ 3.25
Statistical evaluation (Mann–Whitney U-test)				
A-B:	0.025	n.s	0.035	0.028
A-C:	0.030	n.s	n.s	n.s
A-D:	0.029	n.s	n.s	n.s
A-E	0.050	n. s	n. s	n. s

A- ADA activity without extract

B- ADA activity with celandine extract

C- ADA activity with red clover extract

D- ADA activity with flax seed extract

E- ADA activity with coriander

$p < 0.05$  value was evaluated significant, n.s: Non significant

## 4. Discussion

*Chelidonium majus* has been used in complementary herbal medicine for the treatment of benign and malignant tumors. NSC-631570 (Ukrain) is a semisynthetic compound of thiophosphoric acid and the alkaloid chelidonine from the plant *Chelidonium majus*. In unresectable advanced pancreatic cancer, it has been observed that Ukrain alone and in combination with gemcitabine nearly doubled the median survival times in patients suffering from advanced pancreatic cancer (29). Another study suggests that constituents of *celandine* may also have cancer preventive and antimicrobial properties. *Celandine* has also traditionally been advocated as a topical treatment for warts (30). The synergistic biological action of five celandine alkaloids in normal and cancer cells was investigated by capillary electrophoresis with light-emitting diode-induced native fluorescence detection. Their results showed a differential ability of celandine alkaloids to penetrate into the normal and cancer cell interior, which was inversely proportional to their cytotoxic activity (31, 32).

Our results show that aqueous extracts of celandine can significantly inhibit ADA enzyme in cancerous gastric tissue, and this may play part in the anticancer potential of celandine.

The results showing activating potency of celandine on ADA enzyme in colon tissues needs further studies in order to make any evaluation.

In a study done with red clover, investigators saw that 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 (33). A study reports that red clover-derived dietary isoflavones may halt the progression of prostate cancer by inducing apoptosis in low to moderate-grade tumors, potentially contributing to the lower incidence of clinically significant disease in Asian men. (34). Another study says that, long-term use of nonsteroidal anti-inflammatory drugs is associated with a reduction in the incidence of a range of cancers, the mechanism of which is thought to be cyclooxygenase (COX) inhibition. Because some legumes rich in isoflavones (beans, peas, lentils etc.) have been observed to associate with reduced cancer incidence, it was considered useful to examine the COX-inhibitory activities of individual isoflavones. Red clover dietary supplements also contain varying ratios of the 4 isoflavones commonly found in legume-based diets, namely, daidzein, genistein, formononetin, and biochanin. Using 2 separate cell assays, this study examined the ability of the isoflavones found in red clover to inhibit COX enzyme activity in both the murine macrophage cell line and human monocytes. Thus, it is possible that the lower rates of some cancers in populations with a high intake of dietary isoflavones are linked to their inhibition of COX activity (35).

In our study, we established that red clover extract inhibits ADA enzyme only in malign gastric tissue. This finding might be one of the factors leading to anticancer activity of red clover in some types of cancers.

Initial studies on flax seed suggest that flax seeds in the diet may benefit individuals with certain types of breast cancer (18, 36). It has been established that flaxseed supplementation reduces prostate cancer proliferation rates in men presurgery (37) and flaxseed-derived enterolactone is inversely associated with tumor cell proliferation in men with localized prostate cancer (38). Indeed, some studies indicate that diets rich in phytoestrogens and n-3 fatty acid have anticancer potential. The study performed by Jianmin C et al shows that dietary flaxseed inhibits human breast cancer growth and metastasis and downregulates expression of insulin-like growth factor and epidermal growth factor receptor. Investigators mark that flaxseed inhibited the established human breast cancer growth and metastasis in a nude mice model, and this effect is partly due to its down regulation of insulin-like growth factor I and epidermal growth factor receptor expression (39).

The results of the present study also indicate that components in flax seed extract significantly inhibit ADA enzyme in cancerous gastric tissue, which may play part in its anticancer potency as proposed previously.

In a study performed on anticancer potential of coriander, it has been observed that coriander plays a protective role against experimental colon cancer (21). In another study, Rafi M et al found that some constituents of coriander induce Bcl-2 phosphorylation, apoptosis, and G2/M cell cycle arrest in breast tumor cells (22). It is possible that some other factors as observed in the present study might be also possible in the anticancer potency of coriander at least for some tissues like gastric one.

Studies in patients with breast, colorectal, or prostate cancer show that the influence of dietary factors on survival remains to be determined. Adiposity and a lack of physical activity, however, appear to influence cancer outcome negatively (40).

Inhibition of ADA enzymes in cancerous gastric tissue by celandine, red clover, flax seed and coriander are of significance because of the fact that inhibition of adenosine deaminase blocks the deamination of adenosine to inosine, and deoxyadenosine to deoxyinosine in the purine salvage pathway. This accumulation of metabolites inhibits ribonucleotide reductase, which depletes the nucleotide pool and limits DNA synthesis (7). It seems quite possible that accumulated adenosine due to the inhibition of ADA enzyme might play an important function possibly through inhibition of ribonucleotide reductase and depletion of nucleotide pool for new DNA synthesis.

Inhibition of some key enzymes like ADA by plant components may give advantage in the therapy of gastric cancer, and they may be evaluated as nutritional support for the gastric cancer patients after medical therapy. However, it is obvious that further researches including cell culture and animal studies are needed to obtain more information and to make concise evaluation on the subject.

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