Effects of aqueous extract from *Silybum marianum* on adenosine deaminase activity in cancerous and noncancerous human gastric and colon tissues

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ABSTRACT

Objective: Investigation of possible effects of *Silybum marianum* extract (SME) on adenosine deaminase (ADA) activity in cancerous and noncancerous human gastric and colon tissues to obtain information about possible mechanism of anticancer action of *S. marianum*. **Materials and Methods:** Cancerous and noncancerous human gastric and colon tissues removed from patients by surgical operations were used in the studies. The extract was prepared in distilled water. Before and after treatment with the extract, ADA activities in the samples were measured. **Results:** ADA activity was found to be lowered significantly in cancerous gastric tissues but not in noncancerous gastric tissues after treatment with the SME. In the colon tissues, ADA activities were however found to increase after the treatment of SME. **Conclusion:** Our results suggest that the aqueous extract from *S. marianum* inhibits ADA activity in cancerous gastric tissues significantly. It is suggested that in addition to other proposed mechanisms, accumulated adenosine due to the inhibition of ADA might also play a part in the anticancer properties of the *S. marianum*.

Key words: Adenosine deaminase, cancer, colon tissue, gastric tissue, *Silybum marianum*

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INTRODUCTION

Cancer is a leading health problem worldwide. Plants have long been used in the treatment of cancer,[1] and the search on this subject was started in late 1960s.[2]

Silybum marianum is a traditional plant,[3] used for several purposes like hepatoprotectant during and after chemotherapy, or potentiating chemotherapy and radiation therapy as an adjunctive treatment as well as to treat liver and biliary disorders.[4] *S. marianum* seed is highly enriched in silymarin, a complex mixture of polyphenolic molecules.[5] *S. marianum*'s active ingredients were investigated for the prevention and treatment of cancer and it has been observed that they have anticarcinogenic effects for cancers of the colon, breast, prostate, bladder, and skin.[6-12]

Adenosine deaminase (ADA) is an enzyme (EC 3.5.4.4) of purine metabolism. It catalyzes the breakdown of adenosine to inosine in the tissues. ADA is present almost in all mammalian cells. Although its full physiological role is not completely understood, its primary function in human beings is proposed to play part in the immune system.[13,14] ADA may also function in the epithelial cell differentiation, neurotransmission, and gestation maintenance.[13-15] It has also been proposed that in addition to its role in adenosine breakdown, ADA stimulates release of excitatory amino acids and catalyzes the coupling reactions of A1 adenosine receptors and heterotrimeric G proteins.[13,14]

As discussed briefly above, although some mechanisms are supposed for the action of *S. marianum* in the cancer, it is obvious that in addition to known mechanisms, there should be some others unknown in detail yet. Therefore, it is clear that further studies are needed. As to the subject, investigation of the effects of aqueous extract from *S. marianum* on ADA activity in cancerous and noncancerous human tissues might give useful results since ADA is a key enzyme in purine nucleotide metabolism, thereby in cancer process.

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MATERIALS AND METHODS

Twenty-two cancerous gastric tissues and 22 noncancerous adjacent gastric tissues were obtained from patients with gastric cancer by surgical operation. Eleven cancer and 11 noncancer colon tissues were similarly obtained from patients with colon cancer. The protocol was approved by local Ethics Committee. All subjects volunteered for the trial and written consent was obtained according to the Declaration of Helsinki. Tissues were first cleaned by saline solution and stored at −80°C until analysis. In the analysis process, they were first homogenized in 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.[16]

The extracts were prepared by soaking ground powder (*S. marianum*) into the distilled water at the concentration of 10% (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 *et al.* method[17] and ADA activity was measured by the method of Guisti.[18]

Data were analyzed by using statistical software and presented as Mean ± 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.

RESULTS

As we could be inferred from Table 1, *S. marianum* extract (SME) significantly inhibits ADA enzyme activity in cancerous gastric tissues. In the colon tissues, ADA activities were however found to increase after the treatment with SME.

Furthermore, ADA enzyme activities were found to be higher in gastric tissues compared with colon tissues. There were however no significant differences between enzyme activities of cancerous and noncancerous samples of both tissues.

DISCUSSION

It has long been searched for selective anticancer agents without the side effects having potential to target "cancer-specific" molecules to eliminate only cancer cells.[19,20] For this purpose, many drugs have been synthesized, from natural sources or through structural modification of natural products.[21] One of these natural products is *S. marianum*. It has some functional constituents with anticancer potential as well as to treat some other disorders.[4] Of them, silymarin, which is a mixture composed of silibinin, a flavanone from *S. marianum* and flavonolignan have drawn special antitumor activity.[9,11,22,23] These effects have been shown through *in vitro* and *in vivo* animal studies.

Table 1: Mean±SD values of adenosine deaminase enzyme activities (IU/mg protein) in the tissues incubated with and without SME

Groups	Group A (without extract)	Group B (with extract)
I-Noncancerous colon tissues (*n*=11)	5.53±3.32	9.69±7.63
II-Cancerous colon tissues (*n*=11)	5.30±3.46	9.32±5.26[a]
III-Noncancerous gastric tissues (*n*=22)	11.19±9.57	10.02±6.20
IV-Cancerous gastric tissues (*n*=22)	11.45±8.57	6.28±5.10[b]

[a]Significantly higher than Group II-A (P=0.04); [b]Significantly lower than Group IV-A (P=0.027). SD: Standard deviation; SME: *Silybum marianum* extract

A previous experiment showed that oral consumption of silibinin inhibited the development and growth of primary lung tumors. It also leads to a decrease in cell proliferation and inhibits angiogenesis in mice.[24] In a study, it has been established that silibinin inhibited advanced human prostate carcinoma growth in an *in vivo* human prostate cancer model through IGFBP-3 induction.[9] In addition, a protective effect of silibinin has been shown on ultraviolet B-induced skin carcinogenesis in mice, which occurs through inhibition of DNA synthesis and cell proliferation and induction of apoptosis.[23] In another study, it has been reported that dietary administration of silymarin significantly suppressed the development of azoxymethane-induced rat colonic carcinoma by modulation of cell growth and induction of the phase II enzymes quinone reductase and glutathione *S*-transferase in the liver and large intestine, and reduced the levels of β-glucuronidase and prostaglandins 2 in the colorectal mucosa.[11]

In a study, Ri *et al.* reported that ADA is involved in the regulatory system of gastric cancer risk.[25]

Therefore, it seemed valuable to us to investigate possible effects of SME on some critical components having function in the living cells in the body. In this regard, ADA is of importance since it is a key enzyme

in the purine metabolism, inhibition of which may give a selective advantage to combat with cancer. Therefore, investigation of possible effects of some natural foods may give useful information about their anticancer potential mechanisms. From a scientific perspective, the use of ADA inhibitors is important in the understanding the mechanism of action of such metabolites and analogs from natural sources. These inhibitors have also enabled us to understand the regulatory processes associated with immunodeficiency that is characterized by lack of ADA, and to understand the maturation of the immune response.[26]

As observed in the present study, aqueous extract of *S. marianum* inhibits ADA activity in cancerous gastric tissue significantly but does not affect the enzyme activity in adjacent gastric tissue. We think that this might give a selective advantage for the cancerous gastric tissues to combat cancer process. Unfortunately, we have observed no inhibition in the ADA activities of colon tissues. On the contrast, we observed some activation in the ADA activities of colon tissues after treatment with the extract. Whether or not this result is of importance with regard to the cancer process in colon tissue is unknown by us yet. Similarly, the reason and mechanism of the different effects created by SME on the ADA enzymes in the gastric and colon tissues are not clear at the moment. This subject also seems open for further researches.

CONCLUSION

The results of this study demonstrate that further studies are needed to obtain clear information about the action of *S. marianum* in the cancer process in general.

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