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Effects of garlic extract on adenosine deaminase, 5' nucleoditase, and xanthine oxidase enzymes in cancerous gastric tissues.

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Abstract

Introduction: Present study aims to investigate possible effect of garlic extract on the activities of purine metabolizing enzymes, namely ADA, 5'Nucleoditase and Xanthine Oxidase in cancerous and non-cancerous gastric tissue.
Methods: The study involved 14 cancerous and non-cancerous human gastric tissues, removed by surgical operations. The ADA, 5' Nucleoditase, and Xanthine Oxidase (XO) activities were measured in all the tissues treated and not treated with the garlic extract.
Results: The enzyme activities were found to be significantly lower in benign and malign human gastric tissues treated with the garlic extract as compared with those of non-treated tissues.
Conclusion: Lowered purine-metabolizing enzyme activities observed in the garlic extract-treated gastric tissues suggested that garlic might have anti-carcinogenic and cancer-preventive effects on gastric tissue.

Keywords: Garlic, Garlic extract, Gastric cancer, Adenosine deaminase, 5'Nucleoditase, Xanthine oxidase.

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Introduction

Cancer is one of the foremost health problems in the world with millions of fatalities each year. The carcinogenesis process has been extensively studied despite its complex and dynamic nature [1]. The classical treatment methods such as chemotherapy and radiotherapy may have very serious side effects and response to treatment is still insufficient. Therefore, scientists have long conducted studies involving the use of herbal products from the traditional treatments. It was found that various herbal treatments were associated with beneficial effects in certain types of cancer [2].

Garlic (*Allium Sativum*) species belong to the *Allium* genus. It has been used for the purpose of treatment for thousands of years. Garlic contains more than 200 chemical compounds. Some of the most important constituents include are volatile oils and enzymes composed of sulphur-containing compounds (allicin, alliin, ajoene), carbohydrates, minerals, amino acids, and vitamins [3]. Alliin is converted allicin by alliinaze enzyme. Allicin is rapidly broken down to variety of organosulfur compounds. The active components in garlic are the organosulfur compounds of DAS (Diallyl sulfide), DADS (Diallyl disulfide), and DATS (Diallyl trisulfide) [4].

Although the mechanism of the anti-carcinogenic action of garlic has not been exactly understood, it is most likely associated with its ability to block the nitrosamines considered as strong carcinogens in the digestive tract.

Unlike the normal cells, the cancer cells may have abnormal reproduction and stimulate its own reproduction. Moreover, cancer causes chronic inflammation, and the inflammation process leads to more rapid metastasis [5].

Changes in DNA and purine-pyrimidine enzyme activities have been observed in cancerous cells. Adenosine Deaminase (ADA), 5' Nucleoditase, and Xanthine Oxidase (XO) enzymes are involved in purine metabolism and catalyse important steps in DNA synthesis and degradation [6].

ADA (EC 3.5.4.4.) is involved in deamination of adenosines in purine metabolism. This reaction is very important for the turnover of nucleic acids, controlling the intracellular concentration of adenosine and deoxy-adenosine. ADA is considered as a purine synthesis pathway enzyme by some researchers, while others consider it as an enzyme of purine salvage pathway. ADA is the most important enzyme in lymphoid cell differentiation and is used for controlling many diseases with altered immunity [7]. However, ADA's physiological role is not completely understood.

5'-nucleoditase (5'NT, CD73, EC 3.1.3.5) is another key enzyme of adenine degradation. 5'NT separates the inorganic phosphates from ribo and deoxy ribo-nucleotide 5'

monophosphate and also ensures continuance of purine nucleotide pool. Thus alters the nucleoside triphosphate-monophosphate ratio and regulates the cellular energy homeostasis [8]. CD 73 is a glycosyl-phosphatidylinositol-linked enzyme, found in most tissues and it is known as ecto-5'-nucleotidase (ecto-5'-NT). CD73 high expression is correlated with tumor neovascularization, invasiveness, metastasis, as well as shorter patient survival. It was associated with that CD73 might play a significant role in controlling tumor progression [9].

Xanthine Oxidase (XO) is the last enzyme involved in the purine catabolism, which converts hypoxanthine and xanthine to uric acid with the production of superoxide radicals. XO attends the oxidation of very various endogenous and exogenous substrates. It is known as a rate limiting enzyme in nucleic acid degradation, through which all purines are conducted for the end stage of oxidation [10]. In this view, XO is a key enzyme between purine and free radicals metabolism. The end product of purine metabolism uric acid and superoxide radicals result in cellular structure damage and may lead to cancer [11]. The present study aimed to investigate the effects of garlic extract on DNA turn-over enzyme activities in cancerous and non-cancerous gastric tissues.

Materials and Methods

In this study, during surgical operations, 14 cancerous and adjacent non-cancerous stomach tissues were removed from patients with stomach cancer. Tissues were cleaned by saline solution and kept at -80°C until the analyses. The samples were homogenized in saline solution (20%, w/v) in DIAX 900 (Heidolph, Kelhaim, Germany) device for analyses. Homogenates were centrifuged at 5000 rpm for 30 minutes in the Harrier 18/80 (MSE, London, UK) device. Supernatants were removed for enzymatic analysis.

Aqueous extract of garlic were prepared at the concentration of 10% (w/v) into the distilled water and incubated 24 hours by continuous rotation. Activity measurements were carried out by incubating equal amounts of tissue samples with garlic extracts or physiologic saline solution for 1 hour at room temperature. Tissue homogenates were treated with aqueous extract of garlic for 1 hour.

Protein concentrations of the tissues were measured by the Lowry method [12]. Guisti [13], Hashimato [14], and Donald [15] methods were used for the ADA, XO and 5'NT activity measurement, respectively. The enzymatic activities were measured spectrophotometrically in the Helios Alpha Ultraviolet Spectrophotometer (Unicam, Cambridge, UK).

The statistical analyses between groups were performed using Mann-Whitney U test and p values lower than 0.05 were considered significant. All statistical analyses were performed by using SPSS statistical software (SPSS for Windows, version 15).

Results

ADA, 5' Nucleoditase, and Xanthine Oxidase enzyme activities in the tissues were presented in Table 1. In general, garlic extract inhibited key enzymes of purine nucleotide metabolism in cancerous gastric tissues significantly.

Table 1. *Effects of garlic extract on ADA, 5' NT, XO activities (mIU/mg protein) (in cancerous and non-cancerous gastric tissues (Mean ± SD).*

	Benign tissue		**Malign tissue**	
	Without extract	**With extract**	**Without extract**	**With extract**
ADA	38.58 ± 31.17	0.15 ± 0.51*	46.31 ± 22.45	3.35 ± 4.1*
5' NT	16.95 ± 14.32	5.16 ± 6.61*	27.71 ± 26.78	2.55 ± 2.25*
XO	0.296 ± 0.167	0.19 ± 0.081	0.277 ± 0.075	0.176 ± 0.097**

*Significantly low from without extract malign and benign tissue ($p<0.05$); **Significantly low from without extract malign tissue ($p=0.05$)

Discussion

Nutritional foods are effective sources for the treatment of certain types of cancer. Many relevant studies suggest that better understanding of the biological structures of the cancerous cells lead to the development of new potential agents [16]. Indeed, there are many studies on the protective roles of certain foods in cancer.

Garlic extract and several garlic constituents of garlic (DAS, DADS, DATS, Ajoene) are known to have cancer-preventive effects. The epidemiological studies clearly demonstrated that garlic extract and constituents altered the biological behaviour's and microenvironment of tumor, and protected tissues against the carcinogens that caused breast, esophagus, stomach, colon, and rectum cancers [17]. It was suggested for instance that diallyldisulfide, fat-soluble organic sulphur compound found in garlic, had anti-carcinogenic effects on hormone-dependent and non-hormone-dependent breast cancers [18]. It was also shown that garlic derivatives inhibited the proliferation of human prostate and breast cancer cell cultures [19]. Garlic was reported to have suppressed the proliferation of human colon, lung, and skin cancer cells, and induced apoptosis of human colon cells by increasing the intracellular calcium concentration [20]. Another study showed the cancer-preventive effect of a selenium (Se) compound (γ-glutamil-Se-methylselenocysteine) found in garlic [21]. A study on tissue cultures found that garlic had direct toxic effect on prostatic cell cultures and accordingly it was suggested that

this action might have been associated with its support to immune system [22]. Guyonnet et al., Balasenthil et al., Shukla et al., and Li et al., demonstrated the cancer-preventive effects of garlic ingredients through different mechanisms that inhibit the progression of cancer [23-26].

It was suggested in relevant studies that garlic triggered many cancer-preventive mechanisms. A study by National Cancer Institute found that people residing in China and Italy that consume great quantities of garlic, is protected against the gastric cancer [27]. In the present study garlic extract was administered to tissue with and without stomach cancer with an aim to demonstrate its anti-tumor and anti-cancer by examining its activity on DNA turno-over enzymes (ADA, 5'- NT, XO), which are significant as regards cancer.

The purine and pyrimidine metabolism is important for cancer. There is an increased need for purine nucleotides in the cancerous tissue to stimulate the cell cycles [28]. We observed in the present study that increased ADA, 5'- NT, and XO activity in malign gastric tissues decreased upon administration of garlic extract.

There is a major need for purine nucleotides in cancerous tissues due especially to an increase in the cell cycle. Decrease in ADA and 5' NT activity in tissues administered with garlic extract is important for controlling the intracellular levels of adenosine and deoxy-adenosine. These two purine nucleosides are considered as the substrates of the degradation and salvage pathways. Furthermore the decrease in ADA and 5' NT enzyme activities in benign tissues suggest that it may prevent from carcinogenesis.

Although Gocmen et al. found no significant differences in ADA levels in malign and benign gastric tissues, 5' NT levels were high similar to the present study. [29]. Durak et al. observed high ADA activity in gastric cancerous tissues [30].

There is no agreement in the studies about the ADA activity. It was considered that the garlic extract was associated with an increase in adenosine and deoxyadenosine by inhibiting the ADA activity. This preventive action in benign tissues and anti-carcinogenic action in malign tissues may result from the chemical constituents in the garlic extract. These constituents are suggested to have apoptotic, anti-inflammatory, antioxidant, and immune stimulatory activities [31-33].

5' Nucleotidase catalyses dephosphorylation of AMP to produce adenosine. This production also prevents synthesis of nucleic acids by draining the mononucleotide pool. One type of 5' NT is Ecto 5'-Nucleoditase (as known CD73) that forms adenosine by ensuring extracellular mononucleotide cycle. Increased 5'NT activities are found in colon, lung, pancreas, ovary, and gastric tumors. Lu et al. found increased CD73 levels similar to the present study. Elevated Ecto-5'NT expression and activity are associated with increased neovascularization, invasion, and metastasis [34]. The decrease in 5'-NT activity upon administration of garlic extract suggests that it may have effects that reduce the progression of tumor.

Extracellular adenosine which is generated by Ecto 5'-NT, induces immunosuppressive effects through four adenosine-binding G protein-coupled receptors: A1, A2A, A2B and A3 [35]. Adenosine through A2A receptor inhibits antitumor T cells and protects tumor cells [36]. This is similar to the study by Jin et al., where anti-tumor T cell immunity and anti-inflammatory actions of adenosine were induced [37].

XO is an important enzyme involved in purine metabolism. It catalyses the last two reactions of the purine metabolism leading to uric acid formation. XO is also involved in the free radical metabolism. XO reaction is one of the superoxide anion production ways in the body; it produces large amounts of O_2 and hydrogen peroxide under some certain conditions [38]. In a previous study, Chung et al. found that allicin, one of the functional constituents of garlic extract decreased the XO activity [39]. It was suggested that it had cancer-preventive effects associated with the prevention of superoxide formation by XO inhibition. The present study also demonstrated that the XO activity in malign tissues treated with garlic extract was decreased compared to the untreated tissues.

Conclusion

Findings of the present study demonstrated that aqueous garlic extract inhibited the activities of enzymes playing a significant role in the purine and DNA metabolisms in cancerous gastric tissues. Therefore, it was concluded that garlic might have cancer-preventive and therapeutic actions for certain types of cancers, such as gastric cancer, through suppression of the nucleotide pool necessary for new DNA synthesis, induction of apoptosis by accumulation of adenosine, and anti-inflammatory effects.

These results show importance of further investigation of the anti-carcinogenic actions of the garlic extract in malign tissues through *in vitro* and *in vivo* studies.

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