ISSN : 0974 - 7427 Volume 9 Issue 3

BIOCHEMISTRY
An Indian Journal

Regular Paper

BCAIJ, 9(3), 2015 [096-101]

Aquoeus extracts of rosmarinus officinalis, urtica diocia and soybean exert different effects on adenosine deaminase activity in cancerous and non cancerous human gastric and colon tissues

Zahide Esra Durak[1]*, Suleyman Buber[2], Hilmi Kocaoğlu[3], Bahadır Öztürk[4]

[1]Ordu University, Central Research Laboratory, ORDU, (TURKEY)

[2]Ankara University, Medical Faculty, Biochemistry Department, Ankara, (TURKEY)

[3]Ankara University, Medical Faculty, Surgical Oncology Department, Ankara, (TURKEY)

[4]Selçuk University Medical Faculty, Department of Biochemistry, Konya, (TURKEY)

E-mail : zaesrad@hotmail.com

ABSTRACT

This study aimed to investigate effects of aquoeus extracts of rosmarinus officinalis, urtica diocia and soybean on adenosine deaminase (ADA) activity in cancerous and non cancerous gastric and colon tissues.

In method, cancerous and non cancerous human gastric and colon tissues removed by surgical operations were studied. In the samples, adenosine deaminase activities were measured with and without plant extract incubated for 1 h.

As a result, it has been observed that rosemary extract inhibits ADA enzyme in cancerous and non cancerous gastric tissues, but not in colon tissues, and urtica extract inhibits the enzyme only in cancerous gastric tissue. On contrast, soybean extract activates ADA enzyme in colon tissues significantly.

Inhibition of ADA enzyme might play a part in the proposed anti-cancer properties of rosemary and urtica diocia. However, the finding of ADA activation in colon tissues by soybean extract is a new one which needs further verification.

INTRODUCTION

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 radiation therapy. In this regard, it has been observed that treatment of some types of cancers with plant sources may give rise to positive results.

There are many factors for cancer occurrence and development in humans. Most of the cancers are caused by environmental factors,[1] and of these, 30–40% of cancers are directly linked to the diet[1]. While many dietary 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 chemoterapeutic 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].

Rosmarinus officinalis (Rosemary) contains a number of potentially biologically active compounds, including antioxidants carnosic acid and rosmarinic acid. Other chemical compounds include camphor, caffeic acid, ursolic acid, betulinic acid, rosmaridiphenol and rosmanol. Rosemary antioxidants levels are found to be closely related to[9,19], some of which indicate a promising effect in controlling cancer development. This food has been shown to have significant antiproliferation activities against a variety of human cancer cell lines including breast, leukemia, prostate, lung and liver[11,12].

Urtica diocia, is a perennial plant growing in temperate and tropical wasteland areas around the world. The plant has been widely used for cancer treatment by people around the world for centuries. In the first century, Greek physicians Dioscorides and Galen reported that the leaf of urtica had diuretic and laxative properties and was useful for asthma, pleurisy and spleen illnesses. In fact, it is a herb that has a long tradition of use as an adjuvant remedy in the treatment of arthritis in Germany. Nettle leaf extract contains active compounds that reduce TNF-α and other inflammatory cytokines[13,14]. It has been demonstrated that nettle leaf lowers TNF-α levels by potently inhibiting the genetic transcription factor that activates TNF-α and IL-1B in the synovial tissue that lines the joint[15]. Nettle root extracts have been studied in human clinical trials as a treatment for symptoms of benign prostatic hyperplasia (BPH). These extracts have been shown to help relieve symptoms compared to placebo both by themselves[16] and when combined with other herbal medicines[17]. Urtica diocia is the most ferquently used herb in cancer therapy. Both roots and leaves of this plant were used traditionally[18]. In a study, it has been observed that adenosine deaminase (ADA) activity in prostate tissue is inhibited by aqueous extract of Urtica dioica. ADA inhibition by Urtica dioica extract has been suggested as one of the mechanisms in the observed beneficial effect of Urtica dioica in prostate cancer[18].

Soybean is one of the few plants that provides a complete protein as it contains all eight amino acids essential for human health[19]. There is much evidence suggesting that compounds present in soybeans can prevent cancer in many different organ systems. The evidence for specific soybean-derived compounds having a suppressive effect on carcinogenesis in animal model systems is limited, however. There is evidence that some products derived from soybean suppress carcinogenesis in vivo: a protease inhibitor, the Bowman-Birk inhibitor, inositol hexaphosphate (phytic acid) and the sterol beta-sitosterol. Other compounds that may be able to suppress carcinogenesis in animals are the soybean isoflavones. Soybean compounds reported to have other types of anticarcinogenic activity include soybean trypsin inhibitor, saponins and genistein. There is much evidence to suggest that diets containing large amounts of soybean products are associated with overall low cancer mortality rates, particularly for cancers of the colon, breast and prostate. It is believed that supplementation of human diets with certain soybean products may markedly reduce human cancer mortality rates[20].

As discussed above briefly, all of these plants deserve further studies with regard to the properties of cancer prevention and therapy.

MATERIALS AND METHODS

Twenty two cancerous gastric tissues and 22 non cancerous adjacent gastric tissues were obtained from patients with gastric cancer by surgical operation. Eleven cancer and 11 non cancer colon tissues were similarly obtained from patients with colon cancer. Tissues were

Regular Paper

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[21].

The extracts were prepared by soaking plants 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.

Protein concentrations of the tissues were measured by Lowry method[22] and ADA activity was measured by the method of Guisti[23]. ADA activity measurements were performed with and without plant extract for 1 h. Statistical evaluations were made by using Wilcoxon test and values lower than 0.05 were evaluated significant.

RESULTS

Results are shown in the TABLE 1. As seen from the table, rosemary extract inhibits ADA enzyme in cancerous and noncancerous gastric tissue but not in colon tissue. Urtica extract inhibits the enzyme only in cancerous gastric tissue. On contrast, soybean extract activates ADA enzyme in colon tissue.

TABLE 1 : Effects of rosemary leaf, soy bean and urtica diocia extracts on ADA activities in gastric and colon tissues with and without cancer

Gastric tissue		Colon tissue	
Malign tisssue	**Benign Tissue**	**Malign tissue**	**Benign tissue**
A- 11.46±8.58	11.20±9.58	5.31±3.46	5.53±3.32
B- 5.61±3.67	9.10±8.07	4.18±2.26	6.18±4.42
C- 10.08±5.34	11.55±7.15	16.48±8.00	20.23±14.23
D- 7.02±3.81	12.55±9.28	7.40±3.00	8.52±7.88
Statistical evaluation (Wilcoxon test)			
A-B: 0.031	0.048	ns	ns
A-C: ns	ns	0.05	0.012
A-D: 0.039	ns	ns	ns

A :-ADA activity without extract; B :- ADA activity with rosemary leaf extract; C :- ADA activity with soy bean extract; D:- ADA activity with urtica diocia extract; p < 0.05 value was evaluated significant; n.s: Non significant

DISCUSSION

Nutritional foods are important sources for the treatment of some types of cancers, leading to the development of potential novel agents[21-23]. Several of the molecules available from foods have been shown to exert anticancer effects on cancer cells. These effects have been observed through in vitro and in vivo animal studies 6[24-26].

Rosemary (Rosmarinus officinalis L.) extract possesses antitumor properties against tumor cells from several organs. In a study, it has been observed that rosemary extract modulates estrogen and epidermal growth factor receptors in breast cancer cell lines[27]. Another study indicates that a standardized rosemary extract can disrupt the endoplasmic reticulum machinery to decrease the viability of prostate cancer cells and promote degradation of the androgen receptor. Two human prostate cancer cell lines, 22Rv1 and LNCaP, and prostate epithelial cells procured from two different patients undergoing radical prostatectomy were treated with standardized rosemary extract and evaluated by flow cytometry, MTT, BrdU, Western blot and fluorescent microscopy. A significant modulation of endoplasmic reticulum stress proteins was observed in cancer cells while normal prostate epithelial cells did not undergo endoplasmic reticulum stress. This biphasic response suggests that rosemary extract may preferentially target cancer cells as opposed to normal cells[28].

In a study with carnosol which is an active constituent of rosemary, it has been reported to possess anti-inflammatory and anticancer activities. However, the molecular mechanisms underlying the anticancer effects of carnosol remain poorly understood. It has been found that carnosol significantly reduced the viability of human colon cancer (HCT116) cells in a concentration- and time-dependent manner. Treatment of cells with carnosol induced apoptosis, which was associated with activation of caspase-9 and -3 and the cleavage of poly-(ADP-ribose) polymerase (PARP)[29].

Our results show that rosemary extract can significantly inhibit ADA enzyme in cancerous and noncancerous gastric tissues. This finding is 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].

In a study, anti-proliferative activity of urtica diocia extract on the human breast cancer cell line (MCF-7) and fibroblasts isolated from foreskin tissue was evaluated using MTT assay. Mechanisms leading to apoptosis were also investigated at the molecular level by measuring the amount of anti and pro-apoptotic proteins and at the cellular level by studying DNA fragmentation and annexin V staining by flow cytometry. The aqueous extract of Urtica dioica showed antioxidant and antiproliferative effects. The anti proliferative activity was found to be associated with an increase of apoptosis as demonstrated by DNA fragmentation. Study findings warrant further research on Urtica dioica as a potential chemotherapeutic agent for breast cancer[30].

A study investigated the hepatoprotective, nephroprotective, and antioxidant activity of Urtica dioica L methanolic extract (UDME) against cisplatin (CP) toxicity in Erhlich ascites tumor (EAT)-bearing mice. In this investigation, levels of serum hepatic enzymes, renal function markers, and oxidant/antioxidant parameters of liver tissue were measured. Mice were inoculated with EAT on day 0 and treated with nothing else for 24 hours. After a single dose of CP administration on day 1, the extract was given at the different doses daily during 6 days. Almost all doses of UDME performed a significant ($P < 0.05$) preventive role against CP toxicity. This suggests that UDME has a protective capacity and antioxidant activity against CP toxicity in EAT-bearing mice, probably by promoting antioxidative defense systems[31].

Our results show that urtica diocia extract inhibits ADA enzyme only in malign gastric tissue. This finding may be also of importance like action of rosemary leave extract on ADA enzyme relating to gastric cancer treatment.

In a soy food study, it has been reported that dietary soy consumption can lower the risk for breast cancer. Current human and animal data provide evidence for several anticancer properties of soy and its isoflavones. Although the specific quantities and constituents responsible for the observed anti-cancer effects have not been elucidated, it appears that soy isoflavones do not function as an estrogen, but rather exhibit anti-estrogenic properties. However, their metabolism differs between humans and animals and therefore the outcomes of animal studies may not be applicable to humans. The majority of breast cancer cases are hormone-receptor-positive; therefore, soy isoflavones should be considered as a potential anticancer therapeutic agent and warrant further investigation[32].

A study was conducted to examine the association between soy isoflavones consumption and risk of breast cancer incidence or recurrence. Soy isoflavones consumption was inversely associated with risk of breast cancer incidence. However, the protective effect of soy was only observed among studies conducted in Asian populations but not in Western populations. Soy isoflavones intake was also inversely associated with risk of breast cancer recurrence. Stratified analyses suggested that menopausal status may be an important effect modifier in these associations. They failed to identify a dose-response relationship between total isoflavones intake and risk of breast cancer incidence. This study suggests soy isoflavones intake is associated with a significant reduced risk of breast cancer incidence in Asian populations, but not in Western populations[33].

In our study, we have however observed that soybean extract significantly activates ADA enzyme in cancerous and non cancerous colon tissues. As far as we know, these results are first ones showing activating effects of soybean extract on ADA enzyme. For the time being, what is the importance of this finding is not clear for us and needs further studies.

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[34].

To sum up, rosemary leaf and urtica diocia extracts inhibit ADA enzyme in cancerous gastric tissues significantly but does not affect the enzyme in colon tissue. It seems quite possible that accumulated adenosine due to the inhibition of ADA enzyme might play an important function in the anti-cancer properties of rosemary and urtica diocia leaves, possibly through inhibition of ribonucleotide reductase and depletion of nucleotide pool for new DNA synthesis. However, soybean extract activates ADA enzyme in colon tissues, the signifi-

Regular Paper

cance of which is not known by us at the moment. Therefore, further researches including cell culture and animal studies are needed to obtain more information about the subject.

REFERENCES

[1] M.Abdulla et al.; Role of diet modification in cancer prevention. BioFactors **12(1–4)**, 45–51 **(2000)**.

[2] Food, nutrition, physical activity, and the prevention of cancer:a global perspective. World Cancer Research Fund & American Institute for Cancer Research, August 19, **(2009)**.

[3] D.K.Wilson, F.B.Rudolph, F.A.Quiocho; Atomic structure of adenosine deaminase complexed with a transition-state analog: understanding catalysis and immunodeficiency mutations. Science, **252(5010)**, 1278-84 **(1991)**.

[4] G.Cristalli, S.Costanzi, C.Lambertucci, G.Lupidi, S.Vittori, R.Volpini et al.; Adenosine deaminase: functional implications and different classes of inhibitors. Medicinal research reviews, **21(2)**, 105-28 **(2001)**.

[5] M.Aghaei, F.Karami-Tehrani, S.Salami, M.Atri; Adenosine deaminase activity in the serum and malignant tumors of breast cancer: the assessment of isoenzyme ADA1 and ADA2 activities. Clinical biochemistry, **38(10)**, 887-91 **(2005)**.

[6] R.I.Glazer; Adenosine deaminase inhibitors: their role in chemotherapy and immunosuppression. Cancer Chemother Pharmacol, **4(4)**, 227-35 **(1980)**.

[7] J.B.Brown, G.Lee, G.R.Grimm, T.A.Barrett; Therapeutic benefit of pentostatin in severe IL-10-/- colitis. Inflamm Bowel Dis, Jul, **14(7)**, 880-7, **(2008)**.

[8] NNFCC Project Factsheet: Assessment and Development of the Supply Chain to Deliver Rosemary Antioxidants to the Food and Pharmaceutical Industries (Defra). National Non-Food Crops Centre, NF0609.

[9] T.Nabekura, T.Yamaki, T.Hiroi, K.Ueno, S.Kitagawa; Inhibition of anticancer drug efflux transporter P-glycoprotein by rosemary phytochemicals. Pharmacological Research, 259–263 **(2010)**.

[10] M.R.al-Sereiti, K.M.Abu-Amer, P.Sen; Pharmacology of rosemary (Rosmarinus officinalis Linn.) and its therapeutic potentials. Indian Journal of Experimental Biology, 124–130 **(1999)**.

[11] S.Cheung, J.Tai; Anti-proliferative and antioxidant properties of rosemary Rosmarinus officinalis. Oncol. Rep, **1525–1531**, 17 **(2007)**.

[12] O.Yesil-Celiktas, C.Sevimli, E.Bedir, F.Vardar-Sukan; Inhibitory effects of rosemary extracts, carnosic acid and rosmarinic acid on the growth of various human cancer cell lines. Plant Foods Hum. Nutr., **158–163**, 65 **(2010)**.

[13] T.Teucher, B.Obertreis, T.Ruttkowski, H.Schmitz; Cytokine secretion in whole blood of healthy subjects following oral administration of Urtica dioica L. Plant extract. Arzneimittel-Forschung, **46(9)**, 906–10, **(1996)**.

[14] B.Obertreis, T.Ruttkowski, T.Teucher, B.Behnke, H.Schmitz; Ex-vivo in-vitro inhibition of lipopolysaccharide stimulated tumor necrosis factor-alpha and interleukin-1 beta secretion in human whole blood by extractum urticae dioicae foliorum. Arzneimittel-Forschung, **46(4)** 389–94 **(1996)**. PMID 8740085

[15] Riehemann, K; Behnke, B; Schulze-Osthoff, K: Plant extracts from stinging nettle (Urtica dioica), an antirheumatic remedy, inhibit the proinflammatory transcription factor NF-kappaB. FEBS letters, **442(1)**, 89–94, **(1999)**.

[16] M.R.Safarinejad; Urtica dioica for treatment of benign prostatic hyperplasia: A prospective, randomized, double-blind, placebo-controlled, crossover study. Journal of herbal pharmacotherapy, **5(4)**, 1–11, **(2005)**.

[17] N.Lopatkin, A.Sivkov, C.Walther, S.Schlafke, A.Medvedev, J.Avdeichuk, G.Golubev, K.Melnik, N.Elenberger, U.Engelmann; Long-term efficacy and safety of a combination of sabal and urtica extract for lower urinary tract symptoms—a placebo-controlled, double-blind, multicenter trial. World journal of urology, **23(2)**, 139–46 **(2005)**.

[18] I.Durak, H.Biri, E.Devrim, S.Sözen, A.Avci; Aqueous extract of Urtica dioica makes significant inhibition on adenosine deaminase activity in prostate tissue from patients with prostate cancer. Cancer Biol Ther., Sep, **3(9)**, 855-7 **(2004)**.

[19] A.R.Kennedy; Soy consumption and cholesterol reduction: review of animal and human studies: The evidence for soybean products as cancer preventive agents. The Journal of Nutrition, **125(3 Suppl)**, 733-743 **(1995)**.

[20] I.Durak, H.Biri, I.Ergüder, E.Devrim, Ç.Senocak, A.Avcy; Effects of garlic and black grape extracts on the activity of adenosine deaminase from cancerous and noncancerous human urinary bladder tissues. Medicinal Chemistry Research, **16(6)**, 259-265 **(2007)**.

[21] O.H.Lowry, N.J.Rosebrough, A.L.Farr, R.J.Randall; Protein measurement with the Folin phenol reagent. The Journal of biological chemistry, **193(1)**, 265-275 **(1951)**.
[22] G.Guisti; Enzyme activities. Methods of enzymatic analysis. Weinheim Bergest: Verlag chemia, 1087-1091 **(1974)**.
[23] C.J.Chang, C.L.Ashendel, R.L.Geahlen, J.L.McLaughlin, J.Waters; Oncogene signal transduction inhibitors from medicinal plants. In vivo (Athens, Greece), **10(2)**, 185-190 **(1996)**.
[24] A.G.Desai, G.N.Qazi, R.K.Ganju, M.El-Tamer, J.Singh, A.K.Saxena, Y.S.Bedi, S.C.Taneja, H.K.Bhat; Medicinal plants and cancer chemoprevention. Current drug metabolism, **9(7)**, 581-591 **(2008)**.
[25] H.Lee, S.L.Hsu, M.C.Liu, C.H.Wu; Effects and mechanisms of aloe-emodin on cell death in human lung squamous cell carcinoma. European journal of pharmacology, **431(3)**, 287-295 **(2001)**.
[26] M.González-Vallinas, S.Molina, G.Vicente, R.Sánchez-Martínez, T.Vargas, M.R.García-Risco, T.Fornari, G.Reglero, A.R.de Molin; Modulation of Estrogen and Epidermal Growth Factor Receptors by Rosemary Extract in Breast Cancer Cells. Feb 25 **(2014)**.
[27] S.M.Petiwala, S.Berhe, G.Li, A.G.Puthenveetil, O.Rahman, L.Nonn, J.J.Johnson; Rosemary (Rosmarinus officinalis) Extract Modulates CHOP/GADD153 to Promote Androgen Receptor Degradation and Decreases Xenograft Tumor Growth., PLoS One, Mar, **9(3)**, **(2014)**.
[28] K.W.Park, J.Kundu, I.G.Chae, D.H.Kim, M.H.Yu, J.K.Kundu, K.S.Chun; Carnosol induces apoptosis through generation of ROS and inactivation of STAT3 signaling in human colon cancer HCT116 cells, Int J Oncol., Apr, **44(4)**, 1309-15 **(2014)**.
[29] S.Fattahi, A.M.Ardekani, E.Zabihi, Z.Abedian, A.Mostafazadeh, R.Pourbagher, H.Akhavan-Niaki; Antioxidant and apoptotic effects of an aqueous extract of Urtica dioica on the MCF-7 human breast cancer cell line. Asian Pac J Cancer Prev., **14(9)**, 5317-23 **(2013)**.
[30] H.Özkol, D.Musa, Y.Tuluce, I.Koyuncu; Ameliorative influence of Urtica dioica L against cisplatin-induced toxicity in mice bearing Ehrlich ascites carcinoma. Drug Chem Toxicol, Jul, Epub 2011 Sep 22, **35(3)**, 251-7 **(2012)**.
[31] C.C.Douglas, S.A.Johnson, B.H.Arjmandi; Soy and its isoflavones: the truth behind the science in breast cancer. Anticancer Agents Med Chem, Oct, **13(8)**, 1178-87 **(2013)**.
[32] J.Y.Dong, L.Q.Qin; Soy isoflavones consumption and risk of breast cancer incidence or recurrence: a meta-analysis of prospective studies. Breast Cancer Res Treat. Jan, **125(2)**, 315-23 **(2011)**.
[33] E.Kampman, A.Vrieling, F.J.van Duijnhoven, R.M.Winkels; Impact of Diet, Body Mass Index, and Physical Activity on Cancer Survival. Curr Nutr Rep., 2012 Jan 7, **1**, 30-36 **(2012)**.