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Original Article

In vitro adenosine deaminase inhibitory activity of some selected plant extracts and chemical compounds

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ABSTRACT

Background and Aims: Adenosine deaminase (EC 3.5.4.4, ADA) is distributed in all human tissues. It catalyses the deamination of adenosine (deoxyadenosine) to inosine (deoxyinosine) via removal of an amino group. The determination of ADA-inhibition is of extreme significance in the field of fundamental research and clinical diagnosis. In our study, the effects of various plant extracts and chemicals on ADA activity were investigated.
Methods: Adenosine deaminase activity was determined spectrophotometrically at 625 nm.
Results: The inhibitory activities of the extracts and chemical compounds increased in a dose-dependent manner. Among the plant extracts used, lemon extract was observed to exhibit the highest ADA inhibition activity with an IC_{50} value of 0.05 ± 0.001 mg/mL, while quercetin had the highest ADA inhibition (IC_{50} value of 0.004±0.0005 mg/mL) among the chemical compounds. All plant extracts and chemical compounds showed ADA inhibition activities.
Conclusion: The obtained results indicate that plant extracts and some chemical compounds examined in this study can be a potential source of novel ADA-inhibitors for therapeutics.

Keywords: Adenosine deaminase, enzyme inhibition, plant extracts, chemical compounds

INTRODUCTION

Cancer is a complicated disease that varies from one patient to another in terms of manifestation, development and outcomes. It is a multi-step process whereby cells undergo metabolic and behavioural changes leading to extreme and timeless proliferation, which escapes from the observation of the immune system, and which consequently leads to the invasion of distant tissues to form metastases (Markman & Shiao, 2015). Due to the increasing frequency of cancer and cancer related deaths, in addition to complications in cancer treatment, the presence of cancer-causing factors, and the need for social and psychological support, cancer is regarded as a major public health problem worldwide and its significance is increasing day by day.

The most important factors in the development of cancer include the use of tobacco and tobacco products, alcohol consumption, malnutrition, obesity, viruses, exposure to ionizing radiation, occupational diseases and environmental pollution (Soerjomataram et al., 2018). Different treatment modalities such as chemotherapy, radiotherapy, surgical methods, hormone therapy and biological methods have been the main methods of cancer treatment. (Zaigham & Sakina, 2018). Adenosine deaminase (EC 3.5.4.4, ADA), is distributed in all human tissues. It is a vital enzyme in intracellular and extracellular purine metabolism, and

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its primary function is the deamination of deoxyadenosine into deoxyinosine by removing an amino group (Dolezelova, Zurovec, Dolezal, Simek, & Bryant, 2005). It also plays an essential role in the maturation, function and maintenance of immunology response (Van der Weyden & Kelley, 1976; Fleischman et al., 1998). Accumulating evidences have proven that ADA deregulation or altered expression is closely related to many malignant diseases. For example, about 20% of severe combined immunodeficiency disease (SCID) cases are linked to ADA deficiency (Aiuti et al., 2009; Aldrich, Blackburn, & Kellems, 2000). By contrast , over-expression of ADA is a great potential cause of liver cancer, acute leukaemia, oesophagus tumors and so on (Hoffbrand & Janossy, 1981; Ibiş et al., 2007). Moreover, increased serum ADA activity is reported in laryngeal cancer (Canbolat, Akyol, Kavutcu, Isik, & Durak, 1994), head and neck cancer (Lal, Munjal, Wig, & Saini, 1987), breast cancer (Walia, Mahajan, & Singh, 1995) and lung cancer (Nishihara, Akedo, Okada, & Hattori, 1970). Plants have been used for therapeutic purposes for centuries. Their extracts exhibit protective effects due to abundance of secondary metabolites, therefore are potential sources of novel compounds for treatment of various diseases. As a consequence, the determination of ADA-inhibition potentials of plant extract is of extreme significance in fundamental research and clinical cancer therapy (Wang, Chen, Su, Wang, & Su, 2019).

In this study, we investigated adenosine deaminase activity of some selected plant extracts and chemical compounds.

MATERIALS AND METHODS

Preparation of aqueous extracts

Plants were collected from local markets in Istanbul. The plant materials were washed with distilled water and dried at room temperature. In the study, aqueous extracts of edible parts of plants were prepared. Dried plants (5 g) were extracted by adding distilled water and then they were boiled for 7 hours. The extracts were filtered and the filtrates were evaporated under reduced pressure using a rotary evaporator. These extracts were used in the experiment.

Preparation of ADA homogenate

Mature male bovine liver was used as the source of ADA in the study. The collected liver was homogenized in phosphate buffer (pH=8.8) to make up a 10% (w/v) homogenate. The homogenate was employed in the ADA inhibitory assay.

Adenosine deaminase inhibitory activity assay

Adenosine deaminase inhibition was performed according to the method of Blum and Schwedt (Blum & Schwedt, 1998). 1 mL of acetate buffer (pH=5.6) was added to each tube. Then 0.5 mL of inhibitor's solutions was added to the respective tube, followed by 0.1 mL of homogenate, and incubation at 25°C for 60 min. A 0.1 mL of adenosine as substrate was thereafter added to each tube, incubated for 30 min at 25°C, before addition of 1mL of 1 M sodium hydroxide solution, 1mL of phenol solution and 50 µL of hypochloric acid (HOCl). After incubation for 45 minutes at room temperature, absorbance was taken spectrophotometrically at 625 nm. In the control solution, distilled water was added in place of the inhibitor. *Erythro*-9-(2-Hydroxy-3-nonyl)adenine hydrochloride EHNA was used as a standard compound. The inhibition ADA was calculated according to the following formula

$$\text{Inhibition (\%)} = \left[\frac{A_c - A_n}{A_c}\right] \text{x } 100$$

Where: A_c= Absorbance of control and A_n = Absorbance of test.

For ADA inhibitor activities, the results are given as half maximal inhibitory concentrations (IC_{50} values) calculated regression prepared from the concentrations of samples.

RESULTS

The ADA inhibitor activities of plant extracts and chemical compounds were found to increase in a dose dependent manner. A higher ADA inhibitor activity is associated with a lower IC_{50} value. The IC_{50} values of plant extracts and chemical compounds used in the ADA inhibition studies are presented in Table 1 and 2 respectively. For ADA, plant extract showed IC_{50} values between 0.05±0.001 mg/mL 0.05±0.001 mg/mL and 22.02±0.40 mg/mL.

Among the plant extracts, lemon had the highest ADA inhibition activity resulting from its lowest IC_{50} value of 0.05±0.001 mg/mL, followed by black grape (3.55±0.03 mg/mL), pomegranate (4.43±0.55 mg/mL), kiwi (5.11±0.02 mg/mL) and quince (6.37±0.17 mg/mL) respectively. The lowest ADA inhibition was observed in extract of grapefruit and red apple with an IC_{50} of 22.02±0.40 mg/mL and 16.84±0.57 mg/mL respectively.

Among the chemical compounds used, quercetin exhibited the highest ADA inhibitor effect, with the lowest IC_{50} value of 0.004±0.0005 mg/mL. This is followed by kaempferol, myrisitine and xanthine with IC_{50} values of 0.06±0.003 mg/mL, 0.081±0.002 mg/mL and 0.14±0.005 mg/mL respectively. EHNA which was employed as the standard inhibitor had an IC_{50} value of 6.38±1.13 mg/mL. Adenosine deaminase inhibitor activities of chemical compounds and standard compounds decreased in following order: Quercetin> kaempferol> myristicin > xanthine> guanine> adenine> $AgNO_3$> $CuSO_4.7H_2O$> EHNA> biotin> vitamin U> guanosine> cytosine> uracil> allopurinol> nicotinamide> $VOSO_4.H_2O$> $ZnSO_4.7H_2O$.

DISCUSSION

Inadequate physical activity, obesity, unbalanced nutrition, and alcohol and tobacco consumption are among the most common risk factors for cancer. These factors are considered to cause more than half of all cancers (Colditz & Wei, 2012). In addition, environment factors such as UV radiation, smoke and radon exposure increase the risk of cancer development. Some dietary foods may be protective, while others may increase the risk of cancer (Ozdemir, Serin, & Savas, 2018). Reports have shown that 70% of all cancers are linked or associated with nutritional habits, and an estimated 40% of cancer-related deaths (Willett, 2000). The leading methods in cancer treatment are chemotherapy,

Table 1. Adenosine deaminase inhibitory activity of some selected aqueous plant extracts.

Plant extract	Collected of plants	Concentration (mg/mL)	Inhibition (%)*	IC_{50} (mg/mL)*
Black grape	**Fruit**	1	11.47±0.93	3.55±0.03
		1.5	21.64±2.32	
		2	28.17±0.70	
		2.5	33.58±0.09	
Black radish	**Root**	5	26.77±2.11	10.73±0.32
		10	43.94±1.62	
		15	70.72±0.38	
		20	88.63±0.75	
Cabbage	**Vegetables**	10	51.78±2.74	9.48±1.10
		12.5	56.49±3.42	
		15	65.81±1.01	
		20	79.56±1.55	
Grapefruit	**Fruit**	10	16.84±0.66	22.02±0.40
		15	25.22±0.80	
		20	42.89±4.81	
		25	60.87±1.41	
Green apple	**Fruit**	5	19.59±0.43	12.28±0.19
		10	37.93±1.56	
		15	54.10±1.26	
		20	92.71±0.43	
Kiwi	**Fruit**	1	19.46±1.47	5.11±0.02
		2	27.27±1.85	
		3	34.49±0.55	
		4	41.60±0.30	
Lemon	**Fruit**	0.0001	8.73±1.25	0.049±0.001
		0.001	18.20±1.47	
		0.01	36.04±4.33	
		0.5	47.60±0.34	
Pear	**Fruit**	5	12.65±2.16	14.66±0.51
		10	29.82±4.32	
		15	56.68±1.16	
		20	67.58±1.41	
Persimmon	**Fruit**	5	39.75±3.25	7.89±0.93
		10	56.28±4.07	
		15	77.18±1.59	
		20	89.54±0.61	
Pomegranate	**Fruit**	0.5	16.05±8.30	4.43±0.55
		1.5	24.47±3.20	
		2	33.95±4.95	
		5	53.64±4.35	
Quince	**Fruit**	2.5	17.28±0.71	6.37±0.17
		5	31.92±2.11	
		7.5	50.33±0.79	
		10	54.55±2.08	
Red apple	**Fruit**	5	18.98±0.38	16.84±0.57
		10	33.00±1.22	
		15	50.33±0.79	
		20	54.55±2.08	
Red radish	**Root**	5	5.80±1.76	12.50±0.36
		10	30.51±3.10	
		15	70.97±3.68	
		20	92.58±0.17	
White grape	**Fruit**	5	31.33±0.28	7.35±0.17
		7.5	53.36±2.69	
		10	71.67±0.37	
		15	90.77±0.25	

*Mean ± SD

Table 2. Adenosine deaminase inhibitory activities of some chemical compounds.

Chemical compounds and standard	Concentration (mg/mL)	Inhibition (%)*	IC_{50} (mg/mL)*
Allopurinol	12.5	19.86±1.75	42.14±2.25
	25	44.25±7.19	
	50	67.99±2.85	
	100	79.68±1.88	
Adenine	25	40.05±0.74	4.43±3.01
	50	56.86±1.68	
	75	64.33±1.44	
	100	70.11±3.39	
$AgNO_3$	2	5.09±0.71	4.55±0.12
	3	21.73±1.41	
	4	40.70±4.44	
	5	57.86±0.90	
Biotin	1	9.85±1.22	7.75±0.24
	5	33.56±2.15	
	7.5	53.60±0.95	
	10	59.69±1.56	
$CuSO_4.7H_2O$	1	11.71±0.69	5.49±0.13
	2	25.17±1.44	
	3	36.63±1.77	
	6	51.30±0.90	
Cytosine	1	22.25±1.30	14.60±0.75
	5	30.28±2.78	
	10	44.33±1.06	
	25	69.20±1.22	
Guanine	0.1	8.23±2.18	4.29±0.28
	1	33.01±0.89	
	5	62.79±2.49	
	10	88.05±0.56	
Guanosine	0.1	10.50±1.59	11.08±0.99
	1	18.61±1.97	
	5	34.03±2.41	
	10	44.34±3.24	
Kaempferol	0.001	13.70±1.83	0.06±0.003
	0.01	32.99±2.65	
	0.05	52.87±0.81	
	0.1	60.49±1.30	
Myrisitine	0.001	5.23±1.72	0.081±0.002
	0.01	20.31±1.17	
	0.05	40.15±1.77	
	0.1	55.63±0.77	
Nicotinamide	2.5	4.92±1.47	92.45±4.43
	5	20.88±1.47	
	10	37.38±5.24	
	100	51.38±1.30	
Quercetin	0.05	57.71±1.07	0.004±0.0005
	0.1	65.84±0.94	
	0.5	74.38±1.88	
	1	85.90±0.47	
Uracil	5	31.95±3.18	16.17±2.17
	10	48.07±0.53	
	25	66.31±3.03	
	50	76.71±0.77	
Xanthine	0.001	4.04±0.64	
	0.01	9.64±0.45	0.14±0.005
	0.05	25.67±0.68	
	0.1	34.82±2.33	

Table 2. Continued.

Vitamin U	5	29.80±1.88	
	10	58.64±1.50	10.44±1.34
	25	77.04±0.80	
	50	88.00±1.71	
$VOSO_4.H_2O$	250	26.88±4.79	628.14±48.50
	500	49.34±3.61	
	750	58.67±0.84	
	1000	64.55±0.92	
$ZnSO_4.7H_2O$	500	12.39±1.15	5074.60±70.00
	1000	23.24±1.53	
	2000	27.78±0.47	
	3000	33.33±0.35	
EHNA (Standard)	0.01	9.33±0.59	6.38±1.13
	0.1	32.31±0.89	
	1	47.52±1.31	
	10	69.20±1.22	

*Mean ± SD

radiotherapy and surgical operations (Arruebo et al., 2011). Nevertheless, hormone therapy as well as biological methods can be employed as a support to these main methods (Portenoy & Ahmed, 2018). Despite advances in cancer treatment and use of these methods, challenges still exist. These challenges are due to individual variations, existence of variant forms of a cancer, requirements for modified treatment protocols, and nonexistence of an established single clear-cut standard procedure for treatment of all cancers types (Russo & Sundaramurthi, 2019). For this reason, complementary and alternative treatment methods are necessary and significant besides main treatment methods. In this research, the effects of some plant extracts and chemical compounds on the activity of adenosine deaminase, which is found in virtually all the human tissues and plays a vital role in purine metabolism as well as development and maintenance of the immune system, were investigated.

Citruses are rich sources of vitamin C. In addition they are cheap sources of folic acid, potassium, pectin and a wide range of active phytochemical substances that can protect cells or tissues and improve wellbeing. Lemon has an important place among citrus fruits owing to its high content of vitamin C, its antioxidant activity and phenolic contents (Proteggente, Saija, De Pasquale, & Rice-Evans, 2003; Gorinstein et al., 2004; Anagnostopoulou, Kefalas, Papageorgiou, Assimopoulou, & Boskou, 2006; Guimarães et al., 2009). Citrus fruits are reported to exhibit a wide range of pharmacological properties including antiatherogenic, antiinflammatory, antitumor, antithrombotic as well as antioxidant activity (Alu'datt et al., 2017; Asencio et al., 2018; Musumeci et al., 2020). It is suggested that flavonoid compounds in citrus fruits (such as quercetin, myricetin and kaempferol and their derivatives) are responsible for an inhibitory effect on ADA activity, as well as *in vivo* modulation of hepatic lipid metabolism (Cha et al., 2001). A study by Arun et al., reported that hibifolin molecule (a flavonoid type substance) inhibited ADA activity (Arun et al., 2016). The high ADA inhibitory potential of lemon (IC_{50} value of 0.050±0.001 mg/mL) observed in this study may be attributed to the aforementioned flavonoids present in lemon fruit extract. On the other hand, quercetin followed by kaempferol (IC_{50}=0.004±0.0005 mg/mL and 0.06±0.003 mg/mL respectively) which have been previously reported to be present in citrus extract had the highest inhibitory effect on ADA activity when compared to other chemical substances used in this study. Therefore, a strong correlation exists between these flavonoids and the high ADA inhibitory action of lemon extract.

CONCLUSION

It can be concluded that the consumption of quercetin and kaempferol rich fruits/foods such as lemons may serve as a potential source of ADA inhibitor and ultimately affect purine metabolism as well as the maintenance of the immune system in cancer patients.

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