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The Effect of Aloe Vera Extract on Acetylcholinesterase and Monoamine Oxidase

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Abstract
The aim of this study is to evaluate the inhibition effect of ethanol extract for Aloe vera plant on Acetylcholinesterase AchE and Monoamine Oxidase MAO enzymes activity in vitro. The types of inhibitions were known by using the (Michaelis-Menten and Lineweaver-Burk) equations. The Aloe vera extract showed a highly significant percentage of inhibition. In this study some of the traditional claims for using the Aloe vera as a functional food and the using as a treatment for many diseases that are related to the nervous system have been validated.

Keywords: Aloe vera extract, Acetylcholinesterase, Monoamine Oxidase.

الخلاصة
الهدف من هذه الدراسة تقييم تاثيرمستخلص الإيثانول من نبات الاوليفيرا على نشاط الانزيمات أسيتيل كولين استريز و أوكسيديز احادي الامين . تم معرفه نوع التثبيط وذلك باستخدام معادلات (ميكايليس- مينتين و لينويفر –بورك). وأظهرت مستخلصات الاوليفيرا أعلى نسبة تثبيط بشكل ملحوظ. ان النشاط الذي لوحظ في هذه الدراسه يميل الى التحقق من صحه بعض الاستعمالات التقليديه لنبات الاوليفيرا كنبات دوائي واستخدامه كعلاج لكثير من الأمراض التي ترتبط بالجهاز العصبي.

Introduction

Aloe vera is considered to be a plant that resists drought, permanent plant, succulent and belong to the esophodalase family. The word (aloe) is came from the Arabic (alloeh) and this word means a shiny material[1]. This plant located in south and east Africa, in the Mediterranean region and it is grown in Europe, India, Africa, America and Venezuela, for commercial use[2]. The extract of aloe vera extracted from plant leaves contains many useful substances used in the pharmaceutical industry and cosmetics such as enzymes, minerals, vitamins, sugars, anti-oxidants, anti-inflammatories, antifungal and anti-helmenthic[3]. Aloe vera is used in many medicinal uses, It reduces and control the level of glucose in the blood when taken by eating by mixing it with yogurt or drinking in the form of herbal tea. It is also used in the manufacture of cosmetics, creams, anti-aging[4] and wrinkles, and it can be used directly to treat wounds, burns, and for the treatment of ulcers in the stomach[5] where it is taken in the form of juice or with any food. It is also an anti-bacterial used to treat infections[6], which is used to treat mouth and gums diseases[7]. It is used in Thailand as a basic herb to treat burns and wounds[8]. Traditional medicine plays an important role in recent scientific research in the treatment of diabetes worldwide[9]. Because of the efforts made in the scientific research has been revealed and access to a large number of new antibiotics extracted from plants and introduced for medical uses[10]. Acetylcholinesterase (AchE) plays a major biological role in the transmission at cholinergic synapses[11], this enzyme belongs to the carboxyl esterase family of enzymes, and it is a massive and complex protein[12][13]. It is found in humans mainly in the blood, tissues and central nervous system[14]. Monoamine oxidase (MAO) is an enzyme found in the outer mitochondrial membrane[15]. In most

cell types in the body, an enzyme was discovered by Mary Bernheim in the liver and was called the name of the tyramine oxide[16]. MAO plays a crucial role in some mental and neurological disorders, including depression disease[17].

Preparation of extract

The leaves of Aloe vera were cut and washed with tap water and then patted using clean filter papers. The lower leaf base, the tapering point at the leafy top and the short spines located along the leaf margins are removed by sharp blades. The epidermis of the leaves were peeled off. The parenchymatous tissue was collected. The colourless, solid mucilaginous gel was cut into pieces and crushed using an electric blender. The result gel was extracted with ethanol for 4 hrs. The ethanol containing of the extract is filtered and concentrated using the rotary evaporator and stored at 4 ˚C.

Materials and methods

All methods are manual, MAO activity was assayed by Newen and Cohen method[18], Phosphate buffer solution (0.2 M) prepared by : (a) dissolving (10.999 gm) NaH_2PO_4 in distill water and the volume was completed to 500 ml with distill water. (b) the (14.08 gm) of Na_2HPO_4 was dissolved in distill water and the volume was completed to 500 ml with distill water, then the 28 ml from solution (a) was added to 72 ml from solution (b) and then the volume was completed to (200 ml) by distill water to give (pH=7.2).

solutions	Test	Control
serum	0.6 ml	0.6 ml
MAO buffer	0.75ml	0.75ml
benzyl amine	0.15ml	-

Water bath shaking for 3 hrs. at 37 ˚C then,

benzyl amine	-	0.15ml
Perchloric acid	0.15ml	0.15ml
cyclohexane	1.5ml	1.5ml

It mixed and centrifuged for 10 min, then the absorbance was measure of supernatant at 242 nm. The different concentrations of Aloe vera extract was prepared as shown in the Table 1. The MAO activity was measured in human serum with replace 0.75ml of buffer solution with (0.25ml extract mixed with 0.5ml buffer). A constant concentration of inhibitor (0.1) M has been used with different concentration of substrate (0.008, 0.006, 0.004, 0.002, 0.001)M. AchE activity in human serum was evaluate by using Ellman's method[19]. A 0.05mL of (Ellman's Reagent) (5,5-dithio-bis-(2-nitrobenzoic acid 0.001M) DTNB solution was added to the buffer (sodium phosphate 0.2M, 2.25 ml), then the serum was added (0.01ml), mixed well and the (2ml) of the mixture has been put in to a measuring cell, then the (0.034ml) of (ASCHI 0.06 M) acetylthiocholine iodide was added, the absorbance was measured before and after adding the substrate at 430 nm. Different concentrations of extract was prepared (Table 2). The AchE activity was measured in human serum with replace 2.25 ml of buffer solution with (0.25ml extract mixed with 2.00ml buffer. A constant concentration of inhibitor (0.1M) has been used with different concentration of substrate (0.1, 0.06, 0.04, 0.02, 0.01 M). The inhibition percentage of both enzymes were calculated according to the equation[20]:

$$\%\text{inhibition} = 100 - (\text{the activity in the presence of inhibitor} / \text{the activity in the absence of inhibitor}) \times 100$$

by using the Lineweaver–Burk equation, the activity of both enzymes was determined with and without the inhibitor. The values of Vmax maximal velocity and km Michael constant were calculated.

Results and Discussion

In this study, The enzyme activity was determined as shown in Figure 1 and 2. The effect of the Aloe vera extract showed an inhibitory effect of both enzyme activity as shown in Tables 1 and 2. The result showed that Aloe vera extract exhibited a significantly higher percentage of inhibition and it increased with increasing the concentration of the extract. Under the same conditions and at different concentrations of Aloe vera extract, the type of inhibition was determined (by using Lineweaver-Burk equation) and evaluated

values: Vmax, Km, and the type of inhibition. The results have revealed that MAO enzyme have the type of inhibition (uncompetitive inhibition), but AchE enzyme have the type of inhibition (competitive inhibition) as shown in Table 3.

Table 1: The effect of different concentrations of Aloe vera extract on the activity of MAO enzyme in human serum.

Aloe vera extract con. (M)	Enzyme MAO activity IU/ml	Inhibition %
0.00	34.12	0.00
0.001	32.60	4.46
0.005	27.27	20.08
0.01	19.14	43.91
0.05	15.98	53.17
0.1	6.44	81.13

Table 2: The effect of different concentrations of Aloe vera extract on the activity of AchE enzyme in human serum.

Aloe vera extract con. (M)	Enzyme AchE activity IU/ml	Inhibition %
0.00	6.24	0.00
0.001	5.19	16.83
0.005	3.76	39.98
0.01	3.02	51.61
0.05	1.98	68.27
0.1	1.01	89.82

Table 3: The kinetic properties of MAO and AchE with Aloe vera extract

Enzyme	Km (M)	Vmax (*mol/ml*/min)	Type of inhibition
MAO	0.083	٤٠	uncompetitive
AchE	0.٢٥	14.2	competitive

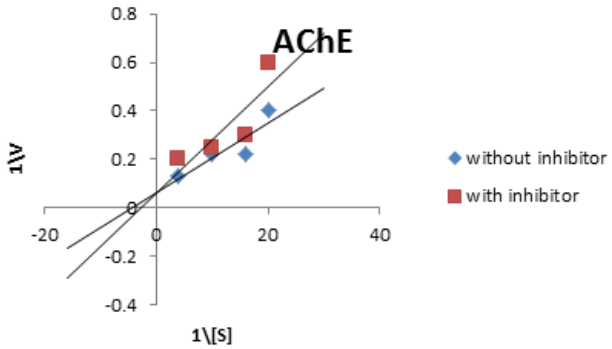

Figure 1: The Lineweaver – Burk plot in the presence and absence of Aloe vera extract with AchE enzyme.

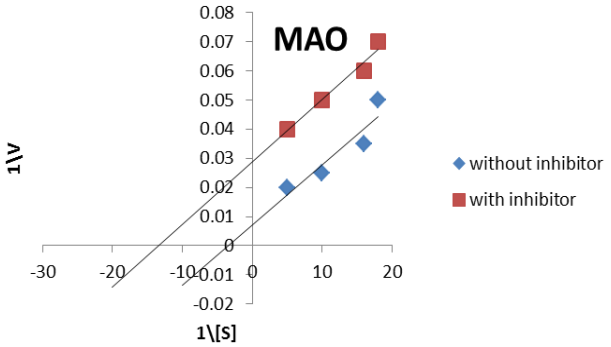

Figure 2: The Lineweaver – Burk plot in the presence and absence of Aloe vera extract with MAO enzyme

The results showed that different concentrations of alcohol extracts cause inhibitory effects on MAO and AchE activity. The extract showed a good enzyme inhibition with increased of the concentration (for MAO (81.13%) and for ACHE (89.82%) in 0.1 M for Aloe vera extract) as shown in Tables 1 and 2.

Conclusion

There are an unique benefits of Aloe vera plant to the body, Aloe vera has a clearing effect on the body‘s nervous system (Settles nerves)[21]. Some of medications work to inhibit both enzymes AMO and AchE treat the depression disease, parkinson's disease, hallucinations, and the cognitive (memory and learning deficits mostly) symptoms of dementia, but there are side effects with using drugs like hypertensive crisis or bradycardia[22][23]. There are studies shows that Aloe vera showes some improvement for treat and reduces Alzheimer's disease symptoms and increases cognition scores[24]. Because it contains a lot of substances that affect the effective enzymes such as vitamins (A,B12,C,E, and folic acid), enzymes, and minerals which are necessary for the proper functioning of enzymes systems in metabolic pathway[25], we recommend to use Aloe vera extract as a dietary supplement for treatment of some disease such as depression and Alzheimer's disease because it has an effect on inhibiting of enzymes AMO and AchE.

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