ISSN: 0067-2904
GIF: 0.851

The Inhibitory Effect of Some Plant Extracts on Acetylcholinesterase Activity in Mice

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Abstract

The results of the phytochemical analysis of the crude aqueous and methanolicextracts of Myrtle (*Myrtuscommunis*), peppermint (*Menthapiperita*) and Sweet basil(*Ocimumbasilicum*) contain active compounds : Phenols, Flavonoids and Tannins and missing of Steroids and Coumarines in all extract but Saponins and Alkaloids found in methanolicextract only, while terpens were present in peppermint and basiland absent in Myrtle. Administratingto animals with different extracts showed no effect on serum Acetylcholinesterase (AchE) compared withthese fed on ethanol liquid diet, Methanolicand aqueous extracts of Myrtle, peppermint and basilin the serum of decreased Acetylcholinesterase level significantly($p \leq 0.05$)[(1.25 ΔpH/30 min, 1.23 ΔpH/30 min, 1.28 ΔpH/30 min, 1.20 ΔpH/30 min, 1.26 ΔpH/30 min, 1.28 ΔpH/30 min), liver(0.35 ΔpH/30 min, 0.34 ΔpH/30 min, 0.34ΔpH/30 min, 0.36ΔpH/30 min, 0.42ΔpH/30 min, 0.39ΔpH/30 min) and brain(0.32ΔpH/30 min, 0.37ΔpH/30 min, 0.39ΔpH/30 min, 0.36ΔpH/30 min, 0.34ΔpH/30 min, 0.37ΔpH/30 min)] respectively compared with animals fed on ethanol liquid diet [(1.37 ΔpH/30 min), (0.47 ΔpH/30 min), (0.45 ΔpH/30 min)] respectively.

Keywords: plants crude extracts, AchE**,** ethanol liquid diet, Albino mice.

التأثير المثبط لبعض المستخلصات النباتية في فعالية إنزيم اسيتل كولين استريز في الفئران

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الخلاصة

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نتائجالتحليلالكيميائيللمستخلصاتالمائيةوالمثانوليةللمستخلصات الخام للأوراقالأسوالنعناعوالريحانتحتويعلىالمركباتالنشطة: الفينولات،الفلافونويدات والعفصيات وفقدها الى الستيرويدات والكومارينات اما القلويدات والصابونين فانه موجود في المستخلص الكحولي فقط ومن ناحية أخرى فان التربينات موجودة في المستخلص الخام لأوراق النعناع والريحان ولا توجد في المستخلص الخام لأوراق الآس.أظهرت نتائج معاملة الحيوانات المختبريةبالمستخلصات المختلفة (مقارنة سالبة) عدم وجود فرق معنوي في التأثير على انزيم الاسيتل كولين استريز في الدم بالمقارنة مع النظام الغذائي الايثانولي السائل . أدت المعاملةبالمستخلصاتالكحولية والمائيةللأسوالنعناع والريحان إلى انخفاض معنوي بمستوى انزيم الاسيتل كولين استريز في مصل الدم [(30/1.25ΔpH دقيقة ، 30/1.23ΔpH دقيقة ، 30/1.28ΔpH دقيقة ، 30/1.20ΔpH دقيقة ، 30/1.26ΔpH دقيقة ،30/1.28ΔpH دقيقة) والكبد (30/0.35ΔpH دقيقة ، 30/0.34ΔpH دقيقة ، 30/0.34ΔpH دقيقة ، 30/0.36ΔpH دقيقة ، 30/0.42ΔpH دقيقة ، 30/0.39ΔpH دقيقة) والدماغ (30/0.32ΔpH دقيقة ، 30/0.37ΔpH دقيقة ، 30/0.39ΔpH دقيقة ، 30/0.36ΔpH دقيقة ، 30/0.34ΔpH دقيقة ، 30/0.37ΔpH دقيقة)] على التوالي ، بالمقارنة مع الحيوانات التي تغذت على النظام ألغذائي الايثانولي السائل [(30/1.37ΔpH دقيقة) ، (30/0.47ΔpH دقيقة) ، (30/0.45ΔpH دقيقة)] على التوالي .

Introduction

Plants have been an important source of photochemical and this importance comes from their medical prevention of many diseases and increase the body's immunity. The World Health Organization estimates that up to 80 per cent of people still rely mainly on traditional remedies such as herbs for their medicines. Medicinal plants contain chemicals with great interest for its physiological effect with a medical activity, this is because they contain more than one active substance that synergy naturally available in the plant [1]. Pharmacological and therapeutic properties have been attributed to different chemical constituents isolated from plant crude extracts. In particular, chemical constituents with antioxidant activity can be found at high concentrations in plants and can be responsible for their preventive effects against various degenerative diseases, including cancer and neurological and cardiovascular diseases [2] and also respiratory, urinary, skin, gastrointestinal, liver disease, among others [3]. Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive degeneration of the hippocampal and cortical neurons that leads to impairment of memory and cognitive ability. The deficiency of acetylcholine (Ach) in AD has given rise to thegenesis of the symptoms of AD [4]. Cholinesterases (ChE) enzymes catalyze the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid. Cholinesterase inhibitors have been used in the treatment of human diseases such as Alzheimer's disease, senile dementia, myasthenia gravis, Parkinson's disease and ataxia [5]. These reports have identified natural compounds that have appreciable inhibitory potential against cholinesterase enzymes.Plantsecondary metabolites have been used as inhibitors of various classes of enzymes and several thousand plant extracts have been screened against AchE from different parts of the world [6].

Therefor, this study was aimed to assess the effect of crude extracts of Myrtle, peppermint and Sweet basilon AchE in blood, serum, liver and brain *in vivo* using experimental animals

Materials and Methods

This study was conducted in 2013 at Department of Biotechnology, College of Science, Baghdad University.

Preparation of leaf crudeextracts

Alcoholic extracts

Peppermintand Sweetbasilwere purchased from a local market and Myrtlefrom home garden. Leaves were dried and powdered using electrical grinder. The powdered materials (20 g) were extracted with 200 ml methanol (95%) for 24 hrs at room temperature. The suspensions were then filtered by filter paper and evaporated at room temperature. The powder extracts were stored at -4°C until use [7].

Aqueous extracts

The powdered materials (20 g) were extracted with 400 ml of D.W for 24 hrs at room temperature. The crude extract was evaporated at 60°C using oven and the concentrated crude extract was collected and stored at -4°C until use[8].

Barbital-phosphate buffer

The barbital-phosphate buffer solution (pH 8.1) was prepared by dissolving 1.237 g sodium barbital, 0.63 g potassium dihydrogen phosphate and 35.07 g sodium chloride in 900 ml of D.W then adjusted pH to 8.1 [9].

Acetylthiocholine iodide:

Acetylthiocholine iodide (7.5g) was dissolved in 100 ml of D.W; the solution was freshly prepared and usedevery day[10]**.**

Chemical analysis of crude extracts

The chemical analysis of leaves extracts was carried out to detect the following compounds:

1-Detection of alkaloidswere testedaccording to [11].

2-Detection of saponinswere testedaccording to [12].

3-Detection of glycosides,flavonoids, steroid, terpens,tannins and resinswere testedaccording to [13].

4-Detection of phenols and coumarineswere testedaccording to [14].

Experimental Design:

The experimentwas designedto evaluatethe effect ofcrude extract(methanolicand aqueous) of Myrtle, sweet basil and Peppermint leaves on theenzymeAchE and some biochemical parameters inalbino mice, their age was ranged (8-12) weeks and weighting (21-26) g.animals were grouped as follows:

Group I :- Not treated animals (control).

Group II :- The animals were administrated orally with methanolicextract of Myrtleonly at a concentration 0.7 g/kg of body weight for 24 hrs.

Group III :- The animals were administrated orally with aqueous extract of Myrtleonly at a concentration 0.4 g/kg of body weight for 24 hrs.

Group IV :- The animals were administrated orally with methanolicextract of Peppermintonly at a concentration 4 g/kg of body weight for 24 hrs.

Group V :- The animals were administrated orally with aqueous extract of Peppermintonly at a concentration 4 g/kg of body weight for 24 hrs.

Group VI :- The animals were administrated orally with methanolicextract of Sweetbasilonly at a concentration 1.5 g/kg of body weight for 24 hrs.

Group VII :- The animals were administrated orally with aqueous extract of Sweetbasilonly at a concentration 1.5 g/kg of body weight for 24 hrs.

Group VIII :- Animals were fed with ethanol liquid diet for 25 days, ata concentration 7.2% (v/v) of ethanol. The diet composed of: cows' milk 925 ml, ethanol 72 ml, vitamin A 5000 IU and sucrose 17 g [15] (positive control).

Group IX :- Animals were fed with ethanol liquid diet and administrated orallywith methanolicextract of Myrtleat a concentration 0.7 g/kg ofbody weight for 24 hrs.

Group X :- Animals were fed with ethanolliquid diet and administrated orallywith aqueous extract of Myrtleat a concentration 0.4 g/kg of body weight for 24 hrs.

Group XI :- Animals were fed with ethanolliquid diet and administrated orallywith methanolicextract of Peppermintat concentration 4 g/kg of body weight for 24 hrs.

Group XII :- Animals were fed with ethanolliquid diet and administrated orally with aqueous extract of Peppermintat a concentration 4 g/kg of body weight for 24 hrs.

Group XIII :- Animals were fed with ethanolliquid diet and administrated orallywith methanolicextract of Sweetbasilat a concentration 1.5 g/kg of body weight for 24 hrs.

Group XIV :- Animals were fed with ethanolliquid diet and administrated orally with aqueous extract of Sweetbasilat a concentration 1.5 g/kg of body weight for 24 hrs.

Electrometric method for determination ofCholinesterase activity in blood and serum

Venous blood samples were collected using heparinized test tubes, then serum was separated by centrifugation at 3000 rpm for 15 minutes.The reaction mixture composed of 3 ml D.W, 0.2 ml serum or whole blood and 3 ml of barbital-phosphate buffer solution (pH 8.1) [16]. The pH of the mixture (pH_1) was measured with a glass electrode using a pH meter, then 7.5% acetylthiocholine iodide was added to the mixture which is incubated at 37 ºC for 30 minutes. At the end of the incubation period, the pH of the reaction mixture (pH_2) was measured. The enzyme activity was calculated as follows:

Cholinesterase activity = ($pH_1 - pH_2$) - Δ pH of blank (Δ pH/incubation time)

ThepH of blank was measuredby adding all reagentwithout the blood sample. The unit of cholinesterase activity was expressed as Δ pH/incubation time, e.g. Δ pH/30 minutes.

Measurement of cholinesterase activity in liver and brain

Samples (0.5-1.5 g) of brain or liver were homogenized in the barbital-phosphate buffer solution (pH 8.1) about 100 mg of wet tissue weighthomogenizered with 3 ml of barbital-phosphate buffer solution using manual homogenizer [17]. Homogenization is performed on an ice bath, and all tissue homogenates were kept on ice before cholinesterase determination. For tissue cholinesterase activity, 0.2 ml of the tissue homogenate was used instead of the blood aliquot in the reaction mixture described above.

Statistical analysis

Data were analyzed using statistical software IBM (SPSS version 21). The values of the investigated parameters were given in terms of mean ± standard error, and Differences between means of all parameters were carried out using analysis of variance (ANOVA) . Differences were considered statistically significant at $p<0.05$.

Results and Discussion:

Chemical analysis of active compound in crude extracts of Myrtle,Peppermintand Sweetbasil

The results of chemical analysis of crude leaf extracts of Myrtle, Peppermint and Sweet basilrevealed that : phenols, flavonoids and tannins were present in both aqueous and methanolicextract, while steroids and coumarines were absent. On the other hand, alkaloids and saponins gave positive results in methanolicextract only. Other compounds like terpenes, steroids, glycosids and resins showed varying proportions in both extracts. Table 1.

Table 1- Chemical analysis of ofMyrtle, Peppermintand Sweet basilcrude extract

	Myrtle		Peppermint		Sweet basil	
Chemical compounds	Aqueous extract	methanolic extract	Aqueous extract	methanolic extract	Aqueous extract	methanolic extract
Tannins	(+)	(+)	(+)	(+)	(+)	(+)
Glycosides	(+)	(+)	(-)	(-)	(-)	(+)
Flavonoids	(+)	(+)	(+)	(+)	(+)	(+)
Saponins	(-)	(+)	(-)	(+)	(-)	(+)
Alkaloids	(-)	(+)	(-)	(+)	(-)	(+)
Terpens	(-)	(-)	(+)	(+)	(+)	(+)
Steroids	(-)	(-)	(-)	(-)	(-)	(-)
Phenols	(+)	(+)	(+)	(+)	(+)	(+)
Resins	(+)	(+)	(-)	(+)	(-)	(-)
Coumarines	(-)	(-)	(-)	(-)	(-)	(-)

+ presence of the compounds, - absence of the compounds

Phytochemical studies have revealed that crude extracts of Myrtle leaves contain several compounds, such as flavonoids, tannins, polyphenolic compounds and several volatile compounds. These results agree with [18] who mentioned that Peppermintplant contains such compounds in varying proportions. Flores *et al*. [19] mentioned that Leaves ofBasil contain phenolic compoundsandaromatic, alkaloids, saponins, terpenoids and glycosides. These differences in the existences of active secondary metabolites in leaf crude extracts of the plants under study dueto the degree ofpolarity between water and alcoholic, also the environment and growth conditions have an important effect on accumulation of secondary metabolites in different parts of the plants [20].

Effect of crude plant extractson acetylcholinesterase

After feeding animals with ethanol liquid diet, the AchE in whole blood has decreased significantly reached (1.07 ΔpH/30 min) (table 2). While in serum, liver, and brain increased significantly reached (1.37 ΔpH/30 min, 0.47 ΔpH/30 min and 0.45 ΔpH/30 min) respectively in comparison with control (blood 1.15 ΔpH/30 min, serum 1.07 ΔpH/30 min, liver 0.34 ΔpH/30 min and brain 0.35 ΔpH/30 min). Treated animals with Myrtle and Peppermint methanolic and aqueous extract and methanolic extracts of Sweet basil showed no significant differences of AchE in whole blood (1.13ΔpH/30 min, 1.11ΔpH/30 min, 1.04ΔpH/30 min, 1.11ΔpH/30 min and 1.12ΔpH/30 min) respectively while aqueous extract of Sweet basil showed a significant increase (1.17ΔpH/30 min) in comparison with positive control (1.07 ΔpH/30 min). Treated animals with Myrtle, Peppermint and Sweet basil methanolic and aqueous extract have reported a significant decrease of AchE in serum (1.25 ΔpH/30 min, 1.23 ΔpH/30 min, 1.28 ΔpH/30 min, 1.20 ΔpH/30 min, 1.26 ΔpH/30 min and 1.28 ΔpH/30 min) respectively in comparison with positive control (1.37 ΔpH/30 min). On the other hand, the level of AchE in the liver decreased significantly when animals treated with Myrtle, Peppermint and Sweet

basil methanolic and aqueous extracts (0.35 ΔpH/30 min, 0.34 ΔpH/30 min, 0.34ΔpH/30 min, 0.36ΔpH/30 min, 0.42ΔpH/30 min and 0.39ΔpH/30 min) respectively in comparison with that of positive control (0.47 ΔpH/30 min). The level of AchE in the brain of the treated animals with Myrtle, Peppermint and Sweet basilmethanolic and aqueous extracts also decreased significantly and reached (0.32ΔpH/30 min, 0.37ΔpH/30 min, 0.39ΔpH/30 min, 0.36ΔpH/30 min, 0.34ΔpH/30 min and 0.37ΔpH/30 min) respectively in comparison with animals administrated with ethanol liquid diet (0.45 ΔpH/30 min).

Table 2- Effect Myrtle, Peppermintand Sweetbasilmethanolicand aqueous extractson the level of AchEin whole blood , serum ,liver and brain in mice treated with ethanol liquid diet

Treatment	The level of AChEΔpH/30 min			
	AChE(blood) ΔpH/30 min Mean ± SE	AChE(serum) ΔpH/30 min Mean ± SE	AChE(liver) ΔpH/30 min Mean ± SE	AChE(brain) ΔpH/30 min Mean ± SE
Control	1.15 ± 0.00577 A	1.07 ± 0.01A	0.34 ± 0.00577 A	0.35 ± 0.00577 A
Myrtle: methanolic (positivecontrol)	1.03 ± 0.02309 B	1.09 ± 0.03A	0.31 ± 0.00577 A	0.33 ± 0.01155 A
Myrtle: aqueous (positivecontrol)	1.07 ± 0.04163 C	1.08 ± 0.01528 A	0.30 ± 0.00577 B	0.35 ± 0.00577 A
Peppermint: methanolic (positivecontrol)	1.06 ± 0.04619 D	1.07 ± 0.02309 A	0.34 ± 0.01528 A	0.35 ± 0.00577 A
Peppermint: aqueous (positivecontrol)	1.14 ± 0.01A	1.07 ± 0.01732 A	0.32 ± 0.02309 A	0.31 ± 0.01155 B
Sweet basil: methanolic (positivecontrol)	0.98 ± 0.01155 E	1.16 ± 0.00577 B	0.34 ± 0.02646 A	0.34 ± 0.02082 A
Sweet basil: aqueous (positivecontrol)	1.06 ± 0.01732 F	1.10 ± 0.01732 A	0.33 ± 0.01732 A	0.35 ± 0.00577 A
Ethanol	1.07 ± 0.01155 GI	1.37 ± 0.01155 CJ	0.47 ± 0.01528 CF	0.45 ± 0.00577 CE
Myrtle: alcoholic (negativecontrol)	1.13 ± 0.01155 AI	1.25 ± 0.02082 DK	0.35 ± 0.01155 AI	0.32 ± 0.01 DF
Myrtle: aqueous (negativecontrol)	1.11 ± 0.01155 AI	1.23 ± 0.04041 EL	0.34 ± 0.02082 AJ	0.37 ± 0.01528 AG
Peppermint: alcoholic (negativecontrol)	1.04 ± 0.03786 HI	1.28 ± 0.03512 FM	0.34 ± 0.01155 AK	0.39 ± 0.00577 AH
Peppermint: aqueous (negativecontrol)	1.11 ± 0.01528 A,I	1.20 ± 0.02 GN	0.34 ± 0.00577 AL	0.36 ± 0.00577 AI
Sweet basil: methanolic (negativecontrol)	1.12 ± 0.01 AI	1.26 ± 0.02517 HO	0.42 ± 0.00577 DM	0.34 ± 0.03 AJ
Sweet basil: aqueous (negativecontrol)	1.17 ± 0.00577 AJ	1.28 ± 0.01155 IP	0.39 ± 0.00577 EN	0.37 ± 0.00577 AK

Different letters refer to a significant ($p \leq 0.05$) differences between treatments.

The results have showed that animals treated with ethanol liquid diet led to decreasing AchE in blood significantly. It is reported that increasing levels of alcohol in blood affected on the connection of the AchE with Erythrocytes membrane leading to separation of the enzyme and increasing its level in serum [21].

The significant increase in AchE enzyme in the animals liver treated with ethanol liquid diet, could be due tothe fact that the liveris amain organresponsible formetabolismanddetoxificationin the body andthisresult is in consistent withwhatemergedfrom the results ofblood analysis. Long-term alcohol consumption causes alcoholic liver disease in susceptible people. Alcohol consumption induces lipid

peroxidation in rats and that the degree of lipid peroxidation is related to the extent of liver injury [22]. The level of AchE increased in thebrain significantly after treatment of animals with ethanol liquid diet and these results agree with [23].

Natural active constituents in plant extracts such as flavonoids andterpenoids have a strong inhibitory activity to retain the increasing concentration ofAchE to normal level. It was mentioned that flavonoids are new promising potential natural compounds for treating Alzheimer's disease through inhibiting AchE [24]. Besides researches revealed that specific compounds like rosmarinic acid, eriocitrin and eriodictyol have inhibitory effect against AchEactivity and they are predominant constituents in the Peppermint[25].

Conclusions

Methanolic and aqueous leaves extracts of Myrtle , Peppermintand Sweet basilshowd a significant decrease of AchE in the serum, liver and brain, in comparison with mice fed ethanol liquid diet and that is due to the effect of active compounds exist in extracts.

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