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The Effect Of Ginger Onmonoaminoxidase (MAO), And Acetylecholine Esterase(Ache) Enzyme Activity In Human Serum Invitro

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***Abstract*— This study was designed to show the effect of ethanol and methanol extract of ginger (juice and powder)on the ac-tivity of monoamioxidase(MAO) and acetylecholine esterase(AchE) enzyme in healthy human serum.Alcohol ex-tractionshow inhibitory effects on MAO and AchE activity and these effects increased with increasing the concentration of extraction Kinetic parameters were studied and the result show that all extraction caused competitive inhibition except AchE with methanol extract(juice) caused uncompetitive inhibition.**

Keywords—ginger, MAO, ACHE, medicinal plants, MAO-A, MAO-B

1. Introduction : In recent time, focus on plant research has been increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants in varioushabitual system [1]Ginger is the rhizome of the plant zingiberofficinate whichbelongs to the family zingiberaceae, it is known to contain a number of potentially bioactive phytochemicals such as gingerols,shagols ,and terpenes [2] .it has extensive medicinal history.It is used in traditional medicine as antipyretic rheumatism and bronchitis [3].its extracts have been extensively studied for a broad range of biological activities including antibacterial(4),anti-inflammatory and anti-ulcerogenic [5], anticancer [6], and reduce the risk of heart disease [7].

Monoamineoxidase(MAO)(EC 1.4.3.4) is a metabolically important enzyme which catalyzes the deaminating oxida-tion of amines to corresponding alde-hyde producing hydrogen peroxide and free amine [8].MAO is found in all tissues it is responsible for the major neurotransmitter degrading in the central nervous system(CNS).There are two major isoforms of MAO , MAO –A and MAO- B.[9].

MAO-A preferentially catalyzes the oxidative deamination of serotonin and adrenaline .An MAO-B mainly cata-lyzes the oxidative deamination of phenyl ethyl amine and benzyla-mine(8). Choline Esterase(AChE) (EC 3.1.1.7) hydrolyses acetylcholine to choline and acetate.Thus , AChE regulates nerve impulse transmission across cholinergic synapses[10].AChE inhibi-tors,that can increase the cholinergic transmission by stopping the hydrolyze of Ach ,are therefore used for alleviating symptoms of Alzhei-merdisease(AD)[11].

Inhibition of AchE is considered ap-proach for the treatment of (AD) and myasthenia gravis [12].

2.Materials and methods

Ginger was purchased from local market ,peeled ,washed and coarsely minced .this small pieces of ginger 20g macerated in 100ml alcohol (ethanol ,methanol) .then this mixture was stirred in a shaker for 48 hours . After that the contents of the flask were passed through filter paper and use the juice directly without evaporating the solvent.

3.Ginger powder extraction

Ginger was purchased from local mar-ket ,peeled,washed,coarsely minced, air dried and pulverized with a blender to afine powder.this powder 20g mac-erated in 100ml alcohol(ethanol ,methanol).then the mixture was stirred in a shaker for 48 hours. After that the contents of the flaskwere passed through filter paper and then were poured into a vacuum rotating vaporiz-er machine. The concentrated extract was then poured into Petri dishes and was oven dried .the dried powder was then collected .

MAO activity was assayed by Newen and Cohen method(13).

solutions	test	Control
serum	600 µl	600 µl
MAO buffer	750 µl	750 µl
benzyl amine	150 µl	-

Water bath shaking for 3 hrs. at 37c then,

benzyl amine	-	150 µl
Perchloric acid	150µl	150 µl
cyclohexane	1.5ml	1.5ml

Mix and centrifugation for 10 min . then measure absorbance of superna-tant at 242nm.

Different concentrations of crude extract of ginger was prepared (20,10,5,2.5,and 1)(mg/dl).MAO activ-ity was measured in human serum by using the same method with replace 750 µl of buffer solution with(500 µl buffer+250 µl extract of ginger).the inhibition percentage was calculated by using this equation:

%inhibition =100-(the activity in the presence of inhibitor/the activity in the absence of inhibitor) x 100A constant concentration of inhibitor (0.1)mg/dl was usedwith different concentration of substrate (0.008 ,0.006 ,0.004 ,0.002,0.001)M to determine type of inhibition and by using lineweaver-Burk Equation the following values were calculated:Vmap,Kmap, and inhi-bition type.

ACHE activity in human sera was as-sayed by using modified Ellmanmethod(14). 50 of DTNB solu-tion (0.001M) is added to 2.25ml of sodium phosphate buffer(PH 7.3, 0.2M),then 10 µl of serum was added, mixed well and (2ml)of the mixture transferred to a measuring cell (3mm),then 34 µl of acetylthiocholine iodide(ASCHI 0.06 M)is added ,the change in absorbency is measured be-fore and after adding the substrate at 430nm.

Different concentrations of crude ex-tract of ginger was prepared (20,10,5,2.5,and 1)(mg/dl). ACHE activity was measured in human serum by using the same method with replace 2.25ml of buffer solution with(2.00mlbuffer+0.25ml extract of ginger).the inhibition percentage was calcu-lated by using this equation:

%inhibition =100-(the activity in the presence of inhibitor/the activity in the absence of inhibitor) x 100

A constant concentration of inhibitor (10)mg/dl was used with different concentration of substrate (0.1, 0.06,0.04,0.02,0.01)M to determine type of inhibition and by using line weaver-Burk Equation the following values were calculated: Vmap, Kmap, and inhibition type.

4.Results and Discussion:

Herbal medicine has the advantage of being economical and readily available. The local practitioners also claim that these remedies frequently have fewer side effects(15).it was therefore considered to be of interest to evaluate and determine the effectiveness of some medicinal plants commonly used by traditional practitioners for their effects .the current study was taken on to screen medicinal plant ginger, for the invitro inhibition of MAO , and AchEas drug modelprovide against disease like cancer, liver and heart disease.

Different concentrations of the substrate were used to study the type of inhibition, the results obtained from line weaver-burke plots indicated that ginger extract acted as competitive inhibitor forMAO,AchE,, the kinetic parameters (km,vm,and type of inhibition) were also determined by usinglineweaver-Burk plot as shown in Table(3) and figure (1) .

The results obtained showed that different concentrations of alcoholic extract causes inhibitory effects on MAO and AchE activity as in Table(1)and(2). Ethanol extract jaice exhibited good enzyme inhibition activity MAO(94.7%), ACHE(80%), while methanol extract powder demonstrated the lower percent of inhibition MAO (69%) , ACHE (50.2%), as shown in figure (2)(3) and table(1)(2).

This extract reduced the MAO activity as compared to control ,hence exerted antidepressant- like action by inhibiting the metabolism of monoamines.MAO regulates the metabolic degradation of catecholamines, serotonin ,and other endogenous amines in central nervous system .inhibition of this enzyme causes a reduction in metabolism and subsequent increase in the concentration in biogenic amines. Ginger extract inhibit the activity of MAO because it is rich in poly phenolic compounds such as tannins, which is able to inhibit enzyme activity(16).

the results obtained showed that ginger powder and juice(ethanol and methanol) have inhibition effect on AChE activity this could be attributed to the antioxidant and free radical scavenging properties of ginger extract(17),the active ingredients of ginger such as terpenoids ,flavonoids ,phenols, and alkaloids have the ability to scavenge free radicals (18).phenols are able to remove free radicals ,active antioxidant enzyme ,and inhibit oxidases(4).the influence of flavonoids upon cellular pathway via inhibiting peroxidation of liposome and reducing malondialdehyde,indicates the benefits of these compounds in treatment of degenerative disorder(5).

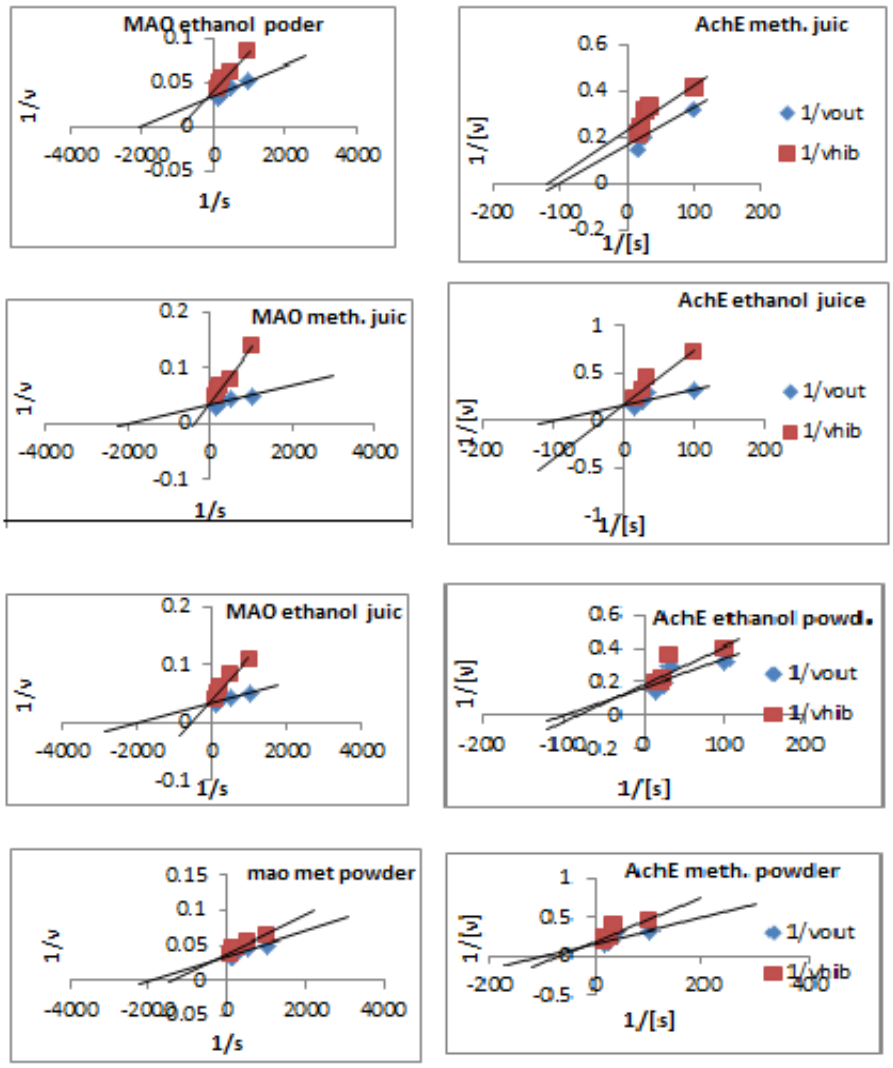
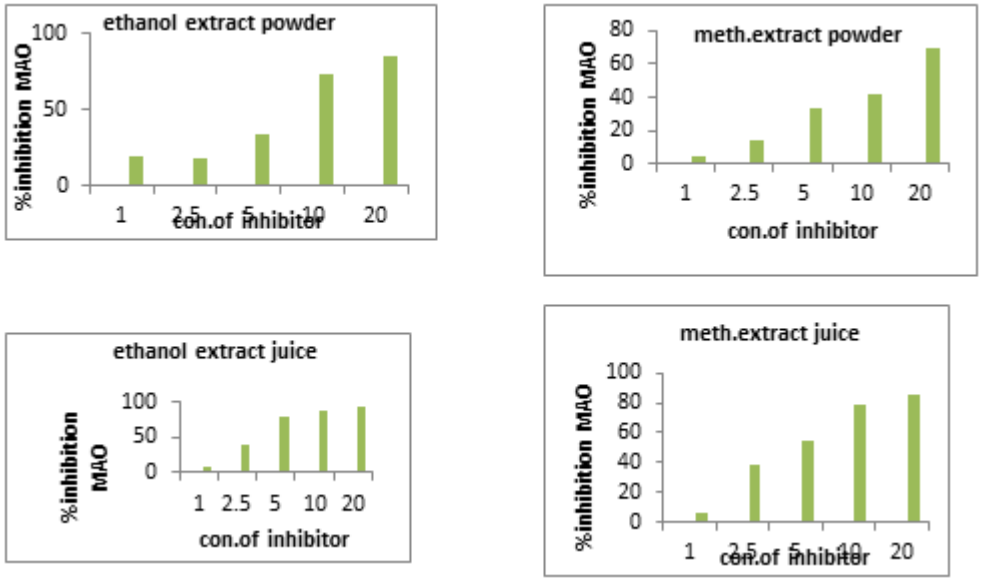
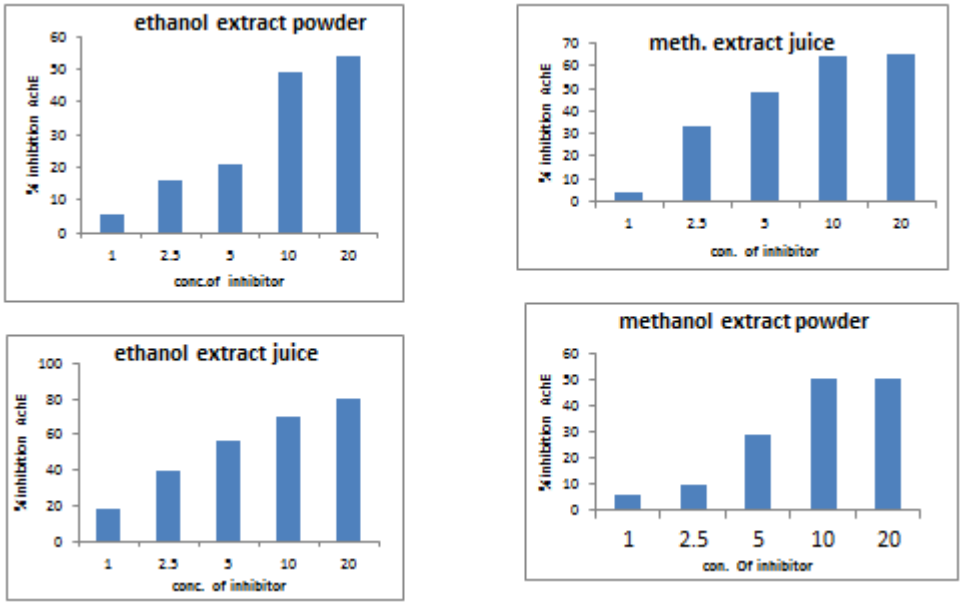


Figure 1: lineweaver_burk plots of MAO and AchE enzymes with ethanol and methanol ginger extraction (powder and juice)



Figure(2):%inhibition of MAO enzymedifferent extract of ginger



Figure(3):% inhibition of AchE enzyme with different extract of ginger

Table (1): the effect of different concentrations of methanol and ethanol ginger extract (juice and powder) on cholinesterase (AchE) activity enzyme in human sera

Inh. Con . mg/dl	Activity Of enzyme	Inhibition %
Methanol extract powder		
Nil	6.1	
20	3.04	50.2
10	3.01	50.6
5	4.36	28.5
2.5	5.5	9.6
1	5.7	5.9
Methanol ext.jauce		
Nil	6.1	
20	2.1	64.9
10	2.2	64.4
5	3.1	48.5
2.5	4.1	33.4
1	5.8	4.4
Ethanol extact powder		
Nil	6.1	
20	2.8	54
10	3.1	49
5	4.8	21
2.5	5.1	16
1	5.7	5.9
Ethanol extract jauce		
Nil	6.1	
20	1.2	80
10	1.8	70
5	2.7	56
2.5	3.7	40
1	5.01	18

Table (2) the effect of different concentrations of methanol and ethanol ginger extract (powder and juice) on monoaminoxidase (MAO) activity enzyme in human sera.

Inhibitor Con . mg/dl	Activity Of enzyme	Inhibition %
Methanol extract powder		
Nil	26.4	
20	8.0	69
10	15.2	42
5	17.6	33
2.5	22.5	14
1	25.2	4.5
Methanol ext. juice		
Nil	26.4	
20	3.5	86
10	5.7	78
5	14.5	55
2.5	16.4	38
1	24.8	6
Ethanol extact powder		
Nil	26.4	
20	3.7	85.9
10	6.9	73.9
5	17.4	34.1
2.5	21.6	18.2
1	21.2	19.7
Ethanol extract juice		
Nil	26.4	
20	1.4	94.7
10	3.3	87.5
5	5.3	79.9
2.5	16.1	39
1	24.1	9.1

Table(3):the kinetic properties of MAO and AchE with methanol and ethanol extract of ginger (powder and juice)

	Kmap(mg\dl)	Vmap	Type of inhibition
Ethanol ext.powder(AchE)	0.0125	5	competitive
Ethanol ext.juice(AchE)	0.018	5.5	competitive
Methanol ext.powder(AchE)	0.011	4.5	competitive
Methanol ext.juice(AchE)	0.009	4	uncompetitive
Ethanol ext.powder(MAO)	0.001	33.3	competitive
Ethanol ext.juice(MAO)	0.0016	33.3	competitive
Methanol ext.powder(MAO)	0.0008	28.5	competitive
Methanol ext.juice(MAO)	0.002	25	competitive

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