# Effect of alcholic extract of *Lavendula multifida* and *Melissa officinalis* on monoaminooxidase(MAO) and acetylecholine esterase (AChE) in healthy human sera and mice brain tissue

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## Abstract:

In this work crud alcoholic extract of *Lavendula multifida* and *Melissa officinalis* were prepared .effects of different concentrations of these extract on the activity of MAO ,and AchE were studied in normal human sera and in mice brain . Kinetic constant (Kmap,Vmap,and type of inhibition) were calculated for the enzymes with extract of herbal . The results confirm that alcoholic extract of Melissa acted as competitive inhibitor with the two enzymes while alcoholic extract of lavender acted as uncompetitive inhibitor with the above enzymes .and there are significant differences in enzymes activity in mice before and after herbal dose. The aim of this study to show the effect of lavender and mellisa on the activity of MAO and AChE and using these herbal in treatment of any disease lead to increase these two enzymes .

## Introduction

*Lavender* (lavandula officinalis ) from labiates family is traditionally alleged to have a varity of therapeutic and curative properties ,including antibacterial[1] ,antiseptic[2] ,stomachache[3] ,sedative ,antifungal , and antioxidant[4] .chemical constituents of lavender are terpenes, camphor ,phenols ,and flavanoids[5] .

*Melissa (Melissa officinalis*) ( lamiaceae) ,is a perennial herbaceous plant ,it has been used extensively in traditional medicine .this plant has been used as antibacterial ,anti inflammatory ,antivirus[6] ,gall bladder

ailments[7] ,hepatic protector[8] ,analgesic ,antioxidant , and antispasmodic[9].

Monoamineoxidase (MAO) (EC 1.4.3.4) is FAD dependent enzyme which catalyzes the deaminating oxidation of amines to corresponding aldehyde producing hydrogen peroxide and free amine [10] . all tissues have MAO as essential component of it . in the central nervous system(CNS) MAO degrade neurotransmitter like dopamine, serotonine , and adrenaline. MAO present in the body in two form, A and B.[11].

The basic role of MAO-A is deamination of serotonin and adrenaline , while the principle action of MAO-B is oxidative deamination of special types of amines in the body like benzylamine [10].

Acetyle Choline Esterase (AChE) (EC 3.1.1.7) hydrolyses acetylcholine to produce choline and acetate. This enzyme increase with some disease like Alzheimer ,and decrease with some disorders like cancer and renal disease ,[12].AChE inhibitors are used for improve symptoms of Alzheimer's disease(AD)because it have the ability to stop hydrolyze of acetyl choline .[13]

## Materials and methods:

*Lavendula multifida* and *Melissa officinalis* flower powder was purchased from local market . soaking (50)gm flower powder in 500ml absolute ethanol with stirring for 48 hr. then filtrate the mixture by using multilayer gauze ,after that allowed filtrate to dry at temperature less than 40C to obtain crude extract of plants.

## Animals :

Swiss albino mice ( male) weight about 20-25gm were used .
mice were separated into 5 groups, 7 animals for each group.
Group 1:control
Group 2: dose 250mg Lavender extract / 1kg for mouse weight
Group 3: dose 450mg Lavender extract / 1kg for mouse weight
Group 4:dose 250 mg Melissa extract/ 1kg for mouse weight
Group 5:dose 450 mg Melissa extract/ 1kg for mouse weight

*Lavendula multifida* and *Melissa officinalis* administered to mice with drink water manually by syringe to be sure that each mouse swallow all dose for 14 days , after completing the last day mice sacrificed, and collect the brain samples .

Brain samples were homogenized by using schurr and livne method[ 14 ]

MAO assay in mice brain tissue and human sera : [15]

Test tube : Add 200µl (serum or brain fraction solution) and 250 µl phosphate buffer (PH 7.4) to 50 µl benzyl amine substrate

Blank tube : the same components of test tube except benzyl amine substrate , incubate test and blank tube 3hr.at 37 C, then add substrate to blank tube only , 50 µl perchloric acid and 0.5ml cyclohexan to each tube , read absorbance of test against blank at wave length =242nm.

AChE detected by using this method [17]

| solution | Test |
|---|---|
| Dithiobis-2-nitro benzoic acid (0.001M) | 25 µl |
| Phosphate buffer PH=7.3,0.2M | 1.125ml |
| Serum or brain solution | 5 µl |

Read absorbance A1 after 3 minute at 430nm ,then add

| | |
|---|---|
| Acetylthiocholineiodide(0.06M) | 15 µl |

Then read absorbance A2 ,and calculate the difference between A1 and A2

The effect of lavender and Melissa on MAO and AChE activity measured by preparation of different herbals concentrations (0.1, 0.05, 0.01, 0.005) (mg/mL) from crud alcoholic extract of and *Lavendula* and *Melissa* , add these concentrations with buffer (200µl buffer +50 µl herbal samples) in MAO and(1.00ml buffer with 0.125ml herbal samples)in AChE then complete the same steps as above in MAO and AChE assay .Lineweaver burk equation was used to detect (inhibition type,Vmap, and Kmap) [16].

the percentage of inhibition detected by dividing enzyme activity with lavender and Melissa over activity without these herbals [16].

## Statistical analysis :

MAO and AChE activities in mice brain were expressed as (Mean ± SD ) using SPSS program .the statistical analysis between two groups were detected by t-test,P – Value ≤ 0.05 accepted as significant .

## Results and Discussion:

The result obtained in this study showed that different concentration of alcoholic extract of Melissa causes inhibitory effect in healthy human serum with AchE as in table (1) and MAO as in table (2) ,high percentage of inhibition 84.74% and 79.45% respectively at alcoholic extract of Melissa 0.1 mg/ml . Also lavender has inhibition effect with MAO and AChE , (0.1 mg/ml ) of lavender give ( 70.21% MAO) and (62.58 % AchE ) as in table (3),(4) .

Saraydin et.al showed that Melissa extract inhibit malignant cell volume [18 ],another study found that melissa extract elevated T3 and T4 hormone and reduce TSH hormone[19]. A different studies found that Melissa extract decrease lipid levels, ALP and ALT [20 ].

Most studies on lavender concentrated on the use of lavender in aromatherapy[21], neroprotective , and treatment hypertension disorder[22].

Different concentrations of the substrate were used to study the type of inhibition, the results obtained from line weaver-burke plots indicated that alcoholic extract of lavender acted as un competitive inhibitor for MAO and AChE, while with alcoholic extract of mellisa act as competitive inhibitor with both enzymes. the kinetic parameters (km,Vm) were also determined by using line weaver- Burk plot as shown in Table(7) and figure (1) .

MAO and AChE has been inhibited in mice brain with 250mg and 450 mg of these herbals and there are significant difference in enzymes activity between control mice and mice administered herbal ,as in table 5 and 6 respectively ,the main reason of this inhibition is that alcoholic extract or lavender and Melissa rich in carbonyl group in flavanoids compounds ,so the hydroxyl group of amino acid residue of enzyme attack the carbonyl group of flavanoids in Melissa and lavender instead of attack carbonyl group of acetyl choline and forms inhibitor _enzyme complex instead of substrate _enzyme complex , the formation of this complex lead to inhibition of enzyme in brain and serum .

Alcoholic extract of this plant inhibit MAO because the active site of this enzyme bind to amine group of substrate (benzyl amine) to form substrate _ enzyme complex while in the presence of *lavender and Melissa* which contain amine group and carboxyl group [2] ,amine group of extract react with active site of enzyme to form inhibitor –enzyme complex so this complex decrease the activity of enzyme in serum and brain .

Table 1: The relation between AChE activity and different concentrations of *Melissa*

| Conc. of *Melissa*(mg/ml) | AChE activity(µmol/ml) | %Inhibition |
|---|---|---|
| NIL | 6.52 | |
| 0.005 | 6.01 | 7.82 |
| 0.01 | 5.12 | 21.47 |
| 0.05 | 3.41 | 47.70 |
| 0.1 | 1.34 | 79.45 |

Table 2: The relation between MAO activity and different concentrations of *Melissa*

| Con. Of *Melissa*(mg/ml) | MAO activity(µmol/3min/ml) | %Inhibition |
|---|---|---|
| NIL | 33.61 | |
| 0.005 | 24.10 | 28.30 |
| 0.01 | 12.88 | 61.67 |
| 0.05 | 9.46 | 71.85 |
| 0.1 | 5.13 | 84.74 |

Table 3: The relation between AChE activity and different concentrations of lavender

| Con.of lavender(mg/ml) | AChE activity(µmol/ml) | %Inhibition |
|---|---|---|
| NIL | 6.52 | |
| 0.005 | 5.14 | 21.16 |
| 0.01 | 4.68 | 28.22 |
| 0.05 | 3.27 | 49.84 |
| 0.1 | 2.44 | 62.58 |

Table 4 : The relation between MAO activity and different conc. of lavender

| Conc. of lavender(mg/ml) | MAO activity(µmol/3min/ml) | %Inhibition |
|---|---|---|
| NIL | 33.61 | |
| 0.005 | 29.02 | 13.65 |
| 0.01 | 21.67 | 35.52 |
| 0.05 | 15.13 | 54.98 |
| 0.1 | 10.01 | 70.21 |

Table (5):the effect of Melissa on MAO and AChE in mice brain

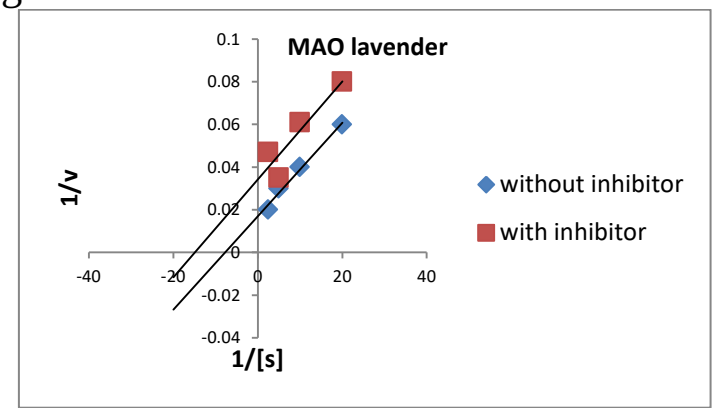
| Dose(mg/kg) | MAO(Mean±SD) (U/g) | AChE(Mean±SD) µmole/g)( |
|---|---|---|
| Control | 102±7 | 140±15 |
| 250 | 70±13 | 82±17* |
| 450 | 61±10 | 45±6* |

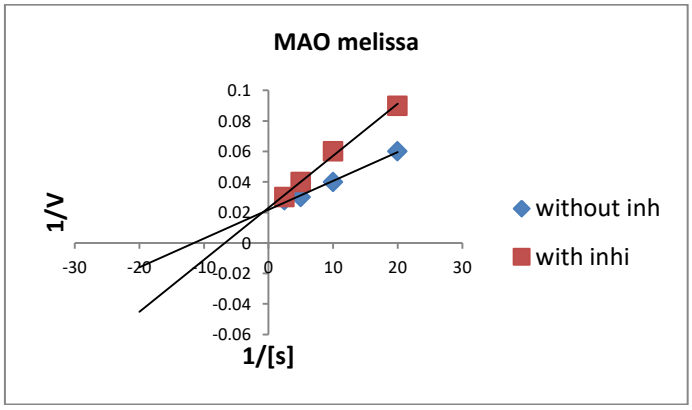
*:P<0.05:Significance

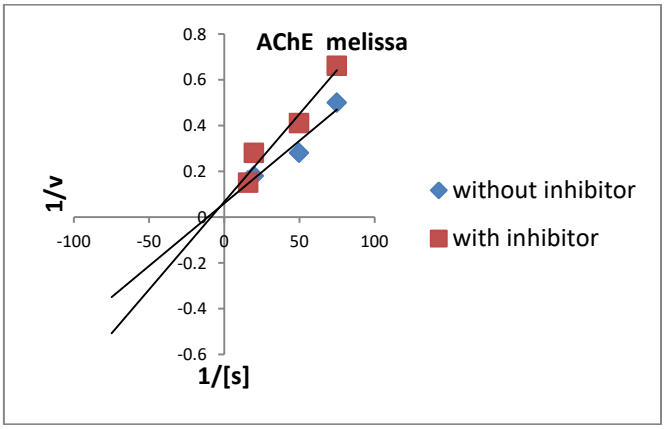
Table (6):The effect of lavender on MAO and AChE in mice brain

| Dose(mg/kg) | MAO(Mean±SD) (U/g) | AChE(Mean±SD) µmole/g)( |
|---|---|---|
| Control | 99±12 | 151±13 |
| 250 | 87±8 | 110±9* |
| 450 | 40±5 | 87±11* |

*:P<0.05:Significance



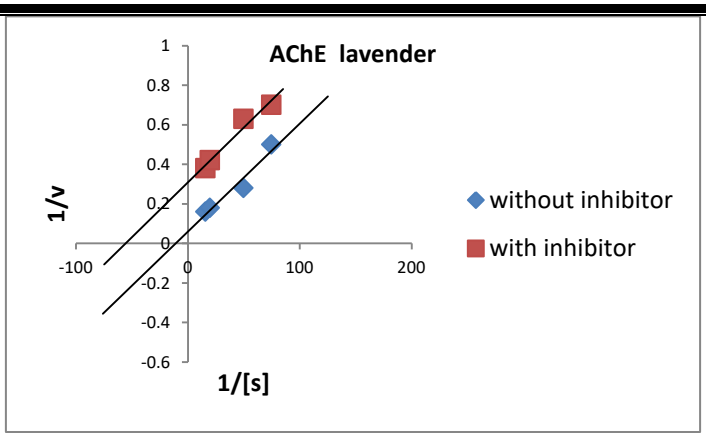



Figure (1):kinetic properties for MAO and AChE with ***lavender*** and ***Melissa***

Table( 7): kinetic properties for *Melissa* and *lavender* with MAO and AChE

| Herbal with enzyme | | inhibition | Vmap | Kmap |
|---|---|---|---|---|
| lavender | MAO | uncompetitive | 28.61 | 0.067 |
| | AChE | uncompetitive | 3.33 | 0.018 |
| Melissa | MAO | competitive | 47.62 | 0.083 |
| | AChE | competitive | 25.0 | 0.125 |

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## تأثير المستخلص الكحولي للخزامي والترنجان على الفعالية الانزيمية للمونواميناوكسيديز والاسيتيل كولين استيريز في مصل الدم البشري للاصحاء وانسجة دماغ الفئران

### الخلاصة :

تم في هذا البحث تحضير المستخلص الكحولي لنبات الخزامي والترنجان ودراسه تأثير تراكيز مختلفه من هذه المستخلصات على الفعاليه الانزيميه للانزيمات التاليه : (مونواميناوكسيديز ,اسيتيل كولين استيريز) في مصل الدم البشري ودماغ الفئران .تم دراسة الخواص الحركيه للانزيمات ( قيمة ثابت ميكالس منتن والسرعة القصوى ونوع التثبيط) مع المستخلص النباتي . ظهرت النتائج ان جميع تراكيز المستخلص الكحولي لنبات الترنجان لها تأثير تثبيط تنافسي على الانزيمات بينما مستخلص الخزامي له تاثير لاتنافسي على الانزيمات المذكوره اعلاه ويوجد اختلاف معنوي للفعاليه الانزيميه في دماغ الفئران قبل وبعد الجرعة المعطاة من الخزامي والترنجان . الهدف من هذه الدراسه هو بيان تاثير عشبه الخزامي والترنجان على الأنزيمات ألمذكوره اعلاه واستخدام هذه الاعشاب كعلاج للحالات المرضيه التي ترتفع فيها نسبه هذه الانزيمات .