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THE EFFECT OF ALCOHOLIC EXTRACT OF *Borage officinalis* ON MONOAMINE OXIDASE (MAO) AND ACETYLCHOLINESTERASE (AChE) IN HUMAN SERUM *IN VITRO.*

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ABSTRACT

This study was designed to show the inhibitory effect of different concentrations of alcoholic extract of *Borage officinalis* on the Monoamine oxidase (MAO) and Acetylcholinesterase (AChE) enzymes in human serum. The results obtained from the study exhibited that alcoholic extract of *Borage officinalis* caused inhibition to enzymes activity with all concentrations of the extract. The results also showed that when the concentration of the extract was (0.001 mg/ml), the percentage of inhibition was (4.3% with MAO and 15.2% with AChE) and this percentage increases until reaching up to (74.7% with MAO and 84.18% with AChE) when the concentration of the extract was (0.1 mg/ml). From the kinetic parameters, studies found that alcoholic extract of *Borage officinalis* is acting as competitive inhibitor with MAO enzyme and as uncompetitive inhibitor with AChE enzyme.

Key words: *Borage Officinalis* , Monoamine oxidase , Acetylcholine esterase

تأثير المستخلص الكحولي لنبات *Borage officinalis* (لسان الثور) على الفعالية الانزيمية للأنزيمات Monoamin oxidase و Acetylcholinesterase في مصل الدم البشري.

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الخلاصة

في هذا البحث تم تحضير المستخلص الكحولي لنبات *Borage officinalis* (لسان الثور) ودراسة تأثير تراكيز مختلفة من هذا المستخلص على الفعالية الانزيمية للانزيمات MAO و AChE في مصل الدم البشري. وقد اظهرت النتائج ان كافة تراكيز المستخلص الكحولي لنبات لسان الثور لها تأثير تثبيطي على الانزيمات وانه كلما زاد تركيز المستخلص زاد التأثير التثبيطي على الانزيم. كما اظهرت النتائج انه عندما يكون تركيز المستخلص الكحولي للنبات (0.001mg/ml) فأن النسبة المئوية للتثبيط للانزيمن MAO و AChE كانت (4.3%)، 15.2% على التوالي، كما وجد ان بزيادة تركيز المستخلص الكحولي للنبات تزداد النسبة المئوية للتثبيط لكلا الانزيمين قد تصل الى (74.70%، 84.18%) على التوالي عندما يكون تركيز المستخلص الكحولي (0.1 mg/ml). و وجد من خلال دراسة الخواص الحركية للمستخلص مع الانزيمات ان المستخلص الكحولي لنبات لسان الثور يسبب تثبيطا تنافسيا مع انزيم MAO وتثبيطا غير تنافسي مع أنزيم AChE.

الكلمات المفتاحية: نبات لسان الثور، أنزيم *Monoamine oxidase*، أنزيم *Acetylcholinesterase*

INTRODUCTION:

Borago officinalis (or *Borage)* is an herbaceous annual plant belonging to the family Boraginaceae. The high levels of gamma- lionlenic acid (GLA) in *Borage* seeds oil is responsible for *Borage* importance as a medical plant **(Samani *et al.*, 2014)**. Traditionally

Borage officinalis has been used in treatment of many disorders likes respiratory, cardiovascular and gastrointestinal disorders **(Wannes *et al*., 2009)**.

Monoamineoxidases (MAO-A and MAO-B) are Flavin Adenine Dinucleotide dependent enzymes located at the outer mitochondrial membranes in the liver, brain and other organs **(Vifia *et al.*, 2012)** They are responsible for inactivity of serotonin, dopamine and norepinphrine **(Abell & Kwan, 2011)**. MAO enzymes catalyzes the oxidation of amines to aldehyde lead to hydrogen peroxide formation which is cause some of disorders such as Alzheimers disease and Parkinson's **(Kaludercic *et al.*, 2014)**.

Acetylcholinesterase enzyme (ACh E) the prevalent cholinesterase in the brain, hydrolyses acetylcholine to acetate and choline. **(Wang, 2015)**. AChE enzyme regulates the transport of nerve impulse from nervous system **(Colovic *et al.*, 2013)**. In actuality the inhibition of AChE enzyme has been a handful approach for medication several diseases like Alzheimer disease **(Mukherjee *et al*., 2007)**.

The current study was attempted to estimate the inhibitory effects of *Borage officinalis* on the activity of MAO and AChE enzymes.

MATERIALS AND METHODS

Borage officinalis flower was purchased from local market. Alcoholic extract was obtained by macerated 100g of flower powder in 500 ml ethanol (96%) at room temperature, then mixing it by incubator shaker for 48 hours, and then filtered by Whatman filter paper NO. 1. The extract was concentrated by used rotating vaporizer apparatus at the temperature 40C°. then dried in oven at 37C° and refrigeration until used **(Nagappan, 2012)**.

Chemical detection of active compounds in *Borago officinalis*:

Detection of Tannins: Used the method in **Hossain *et al*., (2013)**.

Detection of Saponines: Used the method in **Saleh (2015)**.

Detection of Alkaloides: Used the method in **Pandey & Tripathi (2014)**.

Detection of Flavonoides: Used the method in **Hossain *et al.*, (2013)**.

Detection of phenols: Used the method in **Pandey & Tripathi (2014)**.

Determination of Monoaminoxidase enzyme activity:

MAO enzyme activity was assayed by using Mcwen and Cohen method **Mcwen & Cohen (1963)**. Different concentrations (0.1, 0.05, 0.01 and 0.001 mg/ml) of extract of *Borage officinalis* was prepared. The inhibition percentage was determined by using the method in **Salma *et al.*, (2011)**.

0.1 mg/ml of extract of *Borage officinalis* were applied with various concentrations of Benzylamine substrate (0.008, 0.006, 0.004, 0.002 and 0.001M) to established type of inhibition. The values of Vmap, Kmap and inhibition type were determined by applying lineweaver –Burk plot equation .

Determination of Acetylcholinesterase enzyme activity:

AChE enzyme activity was assayed by using modified Ellman method **Ellman & Courtne (1961)**. Different concentrations (0.1, 0.05, 0.01and 0.001 mg /ml) of extract of *Borage officinalis* were prepared. The inhibition percentage of AChE enzyme activity was calculated by using the method in **Salma *et al*., (2011)**. 0.1 mg /ml of extract of *Borage*

officinalis was used with different concentrations of Acetylthiocholine iodide substrate (0.1, 0.006, 0.004, 0.002 and 0.001M) to assayed type of inhibition. The values of Vmax , Km and inhibition type were determined by applying lineweaver – Burk plot equation.

RESULTS AND DISCUSSION

From the (Table, 1) results showed that the flower of *Borage officinalis* contains active compounds like tannins, saponins, alkaloid, flavonoids and phenols. These results agree with many studies **(Gupta & Singh, 2010, Giri *et al.*, 2012)**.

Table (1): Chemical detection of active compounds in *Borage officinalis.*

Test	Result
Tannins	+ve
Saponins	+ve
Alkaloids	+ve
Flavanoids	+ve
Phenols	+ve

The results from (Table 2 and 3) showed that different concentrations of alcoholic extract (0.1 , 0.05 , 0.01 and 0.001 mg/ml) cause inhibitory effects on enzyme activity MAO and AChE , Which is agree with other studies **(Giri *et al.*, 2012, Jager *et al.*, 2013)** .

Table (2): The effect of different concentrations of *Borage officinalis* on the activity of monoaminoxidase (MAO) enzyme in healthy human serum.

Alcohol extract of *Borage officinalis* (mg/ml)	MAO activity (µmol/ml/min)	% inhibition
Nil	39.87	
0.001	38.16	4.3
0.01	30.22	24.20
0.05	20.14	49.50
0.1	10.09	74.70

Table (3): The effect of different concentrations of *Borage officinalis* on the activity of acetylcholinesterase (AChE) enzyme in healthy human serum.

Alcohol extract of *Borage officinalis* (mg/ml)	AChE activity (µmol/ml/min)	(%) inhibition
Nil	6.70	
0.001	5.68	15.20
0.01	4.09	35.95
0.05	3.14	53.13
0.1	1.06	84.18

The results also showed that in the low concentration of extract (0.001)mg/ml , the percentage of inhibition was (4.3%) with MAO enzyme and (15.2 %) with AChE enzyme , But this percentage will be increase with elevating concentration of extract, until it is reached to (74.7%) with MAO enzyme and (84.18%) with AChE enzyme when the concentration of extract was (0.1)mg/ml as shown in (Figure, 1) .

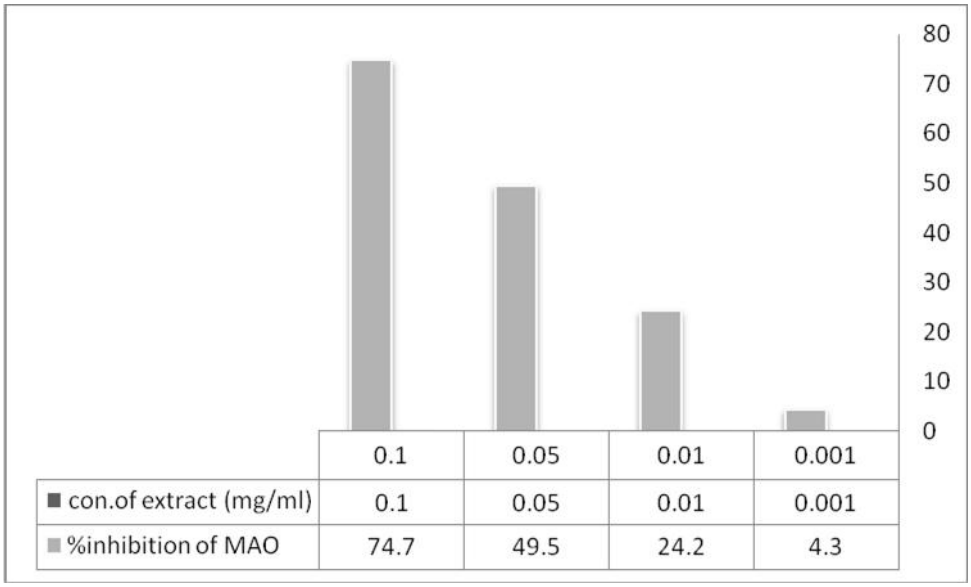

Figure (1): A- : % inhibition MAO enzyme with different concentrations of alcoholic extract of *Borage officinalis*

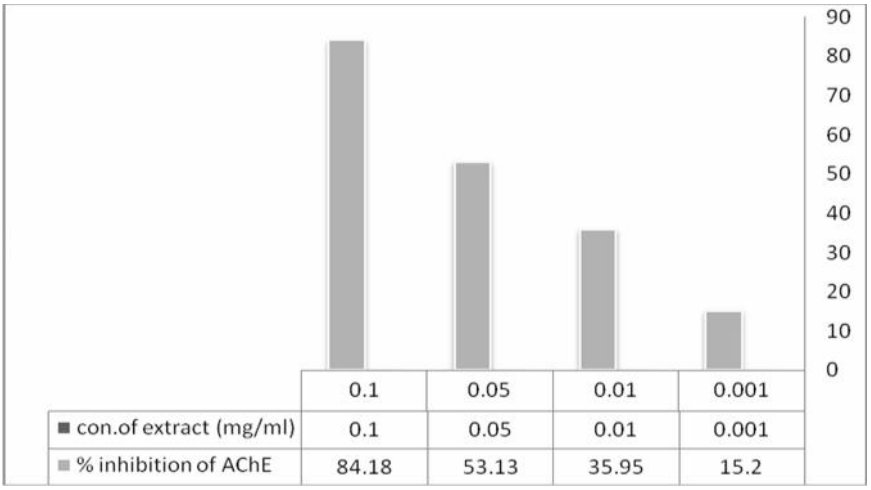

Figure (1): B- : % inhibition AChE enzyme with different concentrations of alcoholic extract of *Borage officinalis.*

Different concentrations of substrate were used to determined the type of inhibition, the results calculated by using line Weaver– Burke plots and showed that *Borage officinalies* extract acting competitive inhibitor for MAO enzyme and uncompetitive inhibitor for AChE enzyme , the Kinetic parameters (Km , Vm and type of inhibition) were calculated by using line Weaver-Burk plots as exhibited in (Table, 4) and (Figure, 2).

Table (4): The kinetic characteristic of MAO enzyme and AChE enzyme with alcoholic extract of *Borage officinalis*.

Enzyme	Vmax	Km	Type of inhibition
MAO	0.2	20	competitive
AChE	0.09	5	uncompetitive

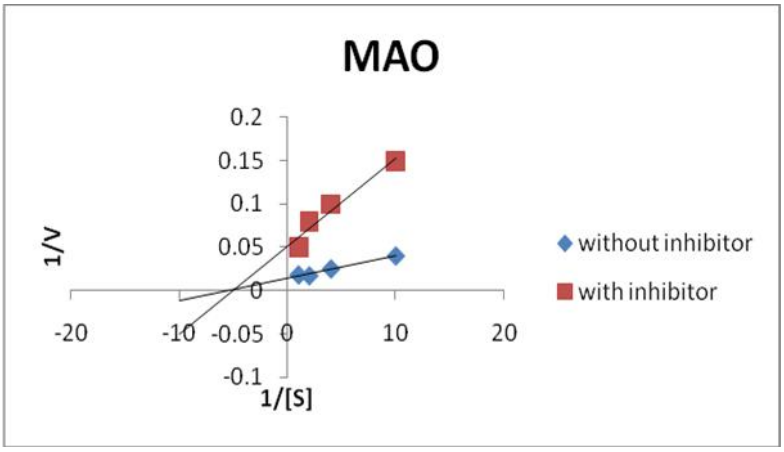

Figure (2): A- Line Weaver- Burk plots for alcoholic extract of *Borage officinalis* on MAO enzyme.

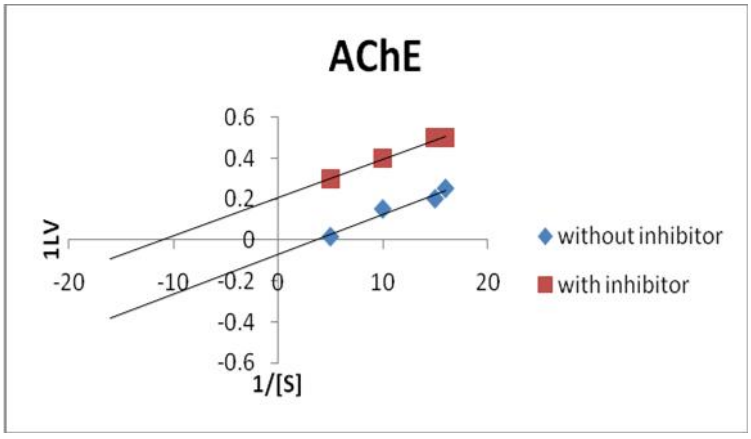

Figure (2): B- Line Weaver-Burk plots for alcoholic extract of *Borage officinalis* on AChE enzyme.

The alcoholic extract of *Borage officinalies* exhibited inhibition influence on MAO enzyme because it is consist of highly bioactive compounds such as alkaloids, phenols, saponins, tannins and flavonoids ,which is responsible for inhibitory effects on MAO enzyme **(Giri *et al.*, 2012)**.

The results from the study showed that alcoholic extract of *Borage officinalies* exhibited inhibition effects on AChE enzyme as a results of the highly content of phenolic compound, which is able to inhibit the enzyme because the hydroxyl group of the amino acid

series residue attacks the inhibitor and forms inhibitor – enzyme complex instead of attacking the carbonyl group of acetyl choline. **(Abdulsada, 2016)**.

CONCLUSION

The primary chemical constituents of *Borage officinalis* flowers include tannins, saponins, alkaloids, flavanoids and phenols, which is responsible for inhibitory effects on MAO enzyme and AChE enzyme.

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