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

INTRODUCTION:

*Borago officinalis* (or *Borage*) is an herbaceous annual plant belonging to the family Boraginaceae. The high levels of gamma- linolenic 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 like respiratory, cardiovascular and gastrointestinal disorders (Wannes et al., 2009).

Monoamine oxidases (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 norepinephrine (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 40°C, then dried in oven at 37°C and refrigeration until used (Nagappan, 2012).

Chemical detection of active compounds in Borage officinalis:
Detection of Alkaloides: Used the method in Pandey & Tripathi (2014).
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
*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*.

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>+ve</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+ve</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+ve</td>
</tr>
<tr>
<td>Phenols</td>
<td>+ve</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Alcohol extract of <em>Borage officinalis</em> (mg/ml)</th>
<th>MAO activity (μmol/ml/min)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>39.87</td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td>38.16</td>
<td>4.3</td>
</tr>
<tr>
<td>0.01</td>
<td>30.22</td>
<td>24.20</td>
</tr>
<tr>
<td>0.05</td>
<td>20.14</td>
<td>49.50</td>
</tr>
<tr>
<td>0.1</td>
<td>10.09</td>
<td>74.70</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Alcohol extract of <em>Borage officinalis</em> (mg/ml)</th>
<th>AChE activity (μmol/ml/min)</th>
<th>(% ) inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>6.70</td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td>5.68</td>
<td>15.20</td>
</tr>
<tr>
<td>0.01</td>
<td>4.09</td>
<td>35.95</td>
</tr>
<tr>
<td>0.05</td>
<td>3.14</td>
<td>53.13</td>
</tr>
<tr>
<td>0.1</td>
<td>1.06</td>
<td>84.18</td>
</tr>
</tbody>
</table>
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 officinalis* 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*.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Vmax</th>
<th>Km</th>
<th>Type of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAO</td>
<td>0.2</td>
<td>20</td>
<td>competitive</td>
</tr>
<tr>
<td>AChE</td>
<td>0.09</td>
<td>5</td>
<td>uncompetitive</td>
</tr>
</tbody>
</table>

**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 officinalis* 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 officinalis* 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 Borago officinalis flowers include tannins, saponins, alkaloids, flavanoids and phenols, which is responsible for inhibitory effects on MAO enzyme and AChE enzyme.

REFERENCES


