The effect of ginseng plant on the activity of monoamino oxidase and peroxidase

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Abstract

**Background:** Ginseng is a medicinal herb that has been used in many laboratory experiments because of its pharmacological activities of its some constituents including ginsenosides, phytosterols, sesquiterpenes, flavonoids, polyacetylenes, alkaloids, and phenolic compounds. This herb has been used in many European and Middle Eastern countries as a traditional remedy for several diseases due to its antithrombotic, antioxidative, anti-inflammatory, and anticancer effects. So we designed this study to detect the effect of various concentrations of ginseng herb on the catalytic activity of monoaminoxidase (MAO) and peroxidase through in vivo and in vitro study. **Methods:** The in vivo study included the effect of Ginseng extract on the catalytic activity of MAO. Eighteen female mice were collected and distributed to three groups. The first group consist of six mice was not treated with ginseng extract (control group). Each of second and third groups consisted of six mice that were orally injected with 250 mg/kg and 450 mg/kg of body weight of ginseng extract respectively during fourteen consecutive days. The activity of MAO was calorimetrically measured at 272nm in the isolated brain mitochondrial fractions. The in vitro study of ginseng extract was calorimetrically tested in human serum of both MAO and peroxidase enzymes at 272nm, 510 nm respectively through two experiments; the first experiment includes measuring the activity of the enzymes using different concentrations of ginseng extract. The second experiment consisted of measuring the activity of the enzyme using different concentrations of substrate and constant concentration of ginseng extract. **Results:** Through in vivo and in vitro study, the outcomes manifested that ginseng extract is good inhibitor towards MAO and peroxidase enzymes. The results also revealed that the inhibition capacity of ginseng extract increased with growing its concentration. Where higher percentage of inhibition at highest concentration of ginseng extract (0.1 mg/mL) was 83.26% and 64.6% for MAO and peroxidase respectively. The inhibition Kinetic characteristics of ginseng extract were \( V_{\text{max}} = 20 \mu\text{mol}\text{min}\text{L}^{-1}, K_i = 0.03 \) for MAO and \( V_{\text{amx}} = 83.3 \mu\text{mol}\text{minL}^{-1} \), \( K_i = 0.003 \) (mol L) for peroxidase, this results refer that ginseng extract is competitive inhibitor with MAO while is un competitive inhibitor with peroxidase. **Conclusion:** The results of this study revealed that the inhibitory capacity of ginseng extract towards both MAO and peroxidase. The inhibitory of properties of ginseng extract opens up new horizons towards the medicinal uses of this herb.

Keywords: - ginseng, MAO, peroxidase, inhibition, competitive inhibitor, un competitive inhibitor

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1-Introduction

Ginseng is a herbal product belongs Araliaceae family that has several uses. It was produced only in China, Korea, Canada, and the USA. It is currently distributed in 35 countries around the world. The Korean market is the biggest market worldwide its value calculable to $1,140 million. It has widely been applied for a long time as a traditional drug in European and eastern Asia nations due to its antithrombotic, antioxidative, anti-inflammatory, and anticancer effects. It was also found that this herb has a strong influence on some functions of the body, including learning, memory and behavior, cardiovascular, neuroendocrine, and immune. As well as it has been observed that Ginseng...
effectiveness on the metabolism of carbohydrate and lipid. There are other uses of this herb, including employ it to overcome fatigue, enhance the immune system, improve physical stamina, and stimulate the appetite [3,4].

Many previous studies that carried on pharmacological mechanisms of Panax ginseng (P. ginseng) reached that ginsenosides represent the most important active bio constituents, besides ginsenosides there are many bioactive components including phytosterols, sesquiterpenes, flavonoids, polyacetylenes, alkaloids, and phenols [4,5]. Some findings reported that ginsenosides alone do not work they act with the help of bio active ingredients in order to perform a beneficial effect [6].

Previous studies discovered that the age factor can effect on the concentration of triterpene saponins or ginsenosides. Where the increase in the age of plant increases the concentration of ginsenosides with maximum concentration at the age of five [7,8]. Recent investigation revealed that extract of ginseng roots has pharmacological effects against diabetic and obesity diseases [8,9]. In vivo study of ginseng extract detected impact of ginseng extract on the levels of both antioxidant and lipid. Furthermore, orally administered ginseng extracts significantly improved the lipid profile and increased the antioxidant activity in rats [7].

Recently Yang et al., 2019 [11] found through in vivo and in vitro study that Panax ginseng inhibits the metabolism of diester alkaloids which have toxic effects on cardiac and neurons through decreasing the activity of CYP3A4 enzyme by the Pregnane X Receptor (PXR) that act as a regulator of PXR.

Because of the high cost of most drugs used. Most researchers are interested in the use of natural products to treat many conditions because of its rich antioxidant content and cheap prices compared to the prices of medicines. So we design this study to evaluate the impact of ginseng extract through in vitro and in vivo experiments on the activities of MAO and peroxidase enzymes.

MAO-A and MAO-B belong to the FAD-dependent enzyme family, which has several actions including the metabolism of neurotransmitters such as adrenaline, dopamine, serotonin, and noradrenaline. In addition to its role in deactivation of exogenous arylalkyl amines. They are linked to the external mitochondrial membrane and stimulate oxidative deamination of its substrates [12].

Because of ginseng’s role in the metabolism of neurotransmitters, Researchers are now turning to use it as a treatment for neurological diseases and depression [12,13].

Specifically, MAOs seem to be shape the main row of protection from monoamines such as tyramine and 3-phenylethanolamine, which might else output an indirect sympathomimetic reply ensuing inside the acute altitude in blood pressure [14]. Identification of inhibitors of MAO has a remarkable role in drug discovery [15]. New researches for MAO-A and MAO-B have focused on using specialist inhibitors towards these enzymes. Where selective inhibitors of MAO-A were used to treat depression [16], and for the treatment of several diseases including depression, Alzheimer’s disease and Parkinson’s disease, MAO-B inhibitors were used [17,18]. Evaluation of enzyme inhibitor components has an important role in softening neurological troubles and their possible interactions with drugs and monoamine-rich food [19]. Natural merchandises were proposed as remarkable supply for MAOs inhibitors. The conventional employment of these natural products has helped to remedy several diseases such as despair, Parkinson’s sickness and different neuropsychiatric in addition to neurological issues [20]. In particular, the nutritional complements, as well as herbaceous extracts with β-carboline harmala alkaloids, reveal notable inhibition towards MAO-A. It is recommended to use it because of its effect on psychological characteristics [12]. Other enzyme we studied in this study are Peroxidases. Theses enzymes belong to oxidoreductases class which has different roles in some metabolic pathways of aerobic organisms. They exist in animal and plant tissues as well as in microorganisms, Peroxidases catalyze the oxidation - reduction reaction that occurs between H₂O₂ and various reductants [21,22].

Hydrogen peroxide is (H₂O₂) a signaling molecule that has an important role in the regulation of many biological processes [23,24].

Previous finding reported that Inositol phosphoglycan-like compounds decrease the activity of the two enzymes including peroxidases and catalase, with increasing the concentration of cellular H₂O₂. Although high concentrations of H₂O₂ consider toxic, but slightly increase in H₂O₂ level is associated to several metabolic pathways [22].

2- Material and methods:-

2.1 Chemicals:

Di-sodium hydrogen phosphate (Na₂HPO₄), sodium di-hydrogen orthophosphate(NaH₂PO₄), glutathione (GSH), 4-amino antipyrine) and phenol were obtained from BDH ,United Kingdom.
Sucrose was purchased from Thomas Baker Pvt. Ltd., Mumbai, India. Tris (hydroxymethyl) amino methan was provided from SD Fine -Chem ltd., Mumbai, India. EDTA di-sodium salt, Dipotassium hydrogen phosphate (K$_2$HPO$_4$) and potassium dihydrogen phosphate (KH$_2$PO$_4$) were obtained from Fluka, Germany. Hydrogen Peroxide was bought from Sigma -aldrich, USA.

2.2 Preparation of ginseng extract:

Ginseng powder was purchased from local market, and then dissolved in appropriate volume of water in order to prepare different concentrations of Ginseng extract. This extract is stored in the refrigerator until it is used.

2.3 Studying and treatment of animals

Eighteen females' mice, weighing around 22-24 g, were obtained from the animal house in Department of Biology - college of science/ Mustansiriyah University - Iraq. The animals were kept separately in three cages under suitable laboratory conditions where the temperature was between 22-25 °C.

Each cage contains six mice. The first group represent untreated control group, the second group administrated with a 250 mg/kg dose of ginseng extract, and the third group administrated with a 450 mg/kg dose of ginseng extract). The dosage was for fourteen consecutive days. The experiments of animals were conducted in line with World Animal Protection Guidelines Low.

2.4 In vivo determination of ginseng effect on monoaminoxidase activity:

After 14 followed days of administrated with ginseng extract, mice were killed, the mouse brain was isolated and brain mitochondria fraction of mouse were prepared according to the methods described in [25, 26]. MAO activity was measured calorimetrically in collected mitochondrial brain fractions of mouse for the three groups (untreated control group, group administrated with a 250 mg/kg dose of ginseng extract, a group administrated with a 450 mg/kg dose of ginseng extract) at wave length 242nm in methodology delineated by Mcwen and Cohen method [27]. This procedure can be summarized, by mixing the buffer washed brain sample with nine volumes of cold 0.25M buffer (pH 7.4) contain Sucrose-0.1M Tris-0.02M EDTA. The last mixture is centrifuged for two times using cooling centrifuge at 4°C at 800 g for 10 min. the pellet has been removed but the supernatant is recentrifuged using the same centrifuge at 12000 g for 20 min. 100 ml of sucrose-tris- EDTA buffer was used to wash the precipitate for two times. The precipitate suspended in nine volumes of 10 mM cold sodium phosphate buffer (pH 7.4) containing 320 mM sucrose and mixed well at 4°C for 20 min. after that the mixture was separated at 15000 g for 30 min under 0°C. Finally In the cold sodium phosphate buffer, the pellets were resuspended. For estimating of MAO. 600 µl of mitochondrial brain buffer , 750 µl of sodium phosphate buffer (0.2 M), pH 7.2) and 150 µl of 0.008 M benzylamine were mixed and placed in water bath for 3 hrs. at 37°C, and then 150 µl of perichloric acid and 1.5 ml of cyclohexane were added. The mixture of reaction was centrifuged for 10 min and the absorbance of supernatant was read at 242nm by double beam spectrophotometer (Shimadzu, Japan).

2.5 In vitro estimation of ginseng effect on monoaminoxidase activity:

MAO was assayed in serum by Mcwen and Cohen methods [27] that described above. Different concentrations of ginseng (0.1, 0.05, 0.01, 0.001)(mg/ml) were used. Ginseng extract should be added with buffer solution as 500 µl buffer mix with 250 µl extract of ginseng. To determine the type of inhibition, we used constant concentration of inhibitor (ginseng extract) and various concentrations of substrate that were made of 0.008 mol/L benzylamine after that MAO activity was measured in the presence and absence of inhibitor. The equation of Lineweaver–Burk was used by drawing 1/v versus 1/s to determine values such as ki, Apparent Vmax , and Apperent km.

2.6 In vitro determination of ginseng effect on peroxidase activity

The Peroxidase activity was estimated colorimetrically according to [28] using various hydrogen donors. In this study 4- aminoantipyrine were used as hydrogen donor. Briefly the mixture of reaction consist of 1.4ml that contain (0.17 mol/L phenol mix with 0.0025 mol/L 4aminoa ntipyrine solution), and 1.5ml of 0.0017M hydrogen peroxide in 0.2M potassium phosphate buffer (pH 7.0) as substrate. The activity
is estimated by gauge the change of absorbance through 5 min at = 510 nm which resulting from the decomposition of H\textsubscript{2}O\textsubscript{2} per time of incubation (ΔA/min). The in vitro inhibition of peroxidase was also estimated by using the same method with adding 20 μl of different inhibitor concentrations(0.001,0.01,0.05,0.1) mg/ml to serum. The type of inhibition was determined depending on Lineweaver–Burk equation and using constant concentration of inhibitor and various concentration of hydrogen peroxide (0.0011,0.0013,0.0015,0.0017)mol/L.

2.7 -Statistical analysis

This study analyzed their data by a statistical programme SPSS version10. The results were expressed as mean ±SD. Statistically differences are regarded significant with p value of less than 0.05.

3 - Results

Table 1 shows the effect of ginseng extract on MAO activity through in vivo study in mice brain in 3 groups: the first group G1(untreated control group), the second group G2(mice administrated with 250 mg/kg of ginseng extract) and the third group G3(mice administrated with 450 mg/kg of ginseng extract). We observed a significant decrease in MAO activity in G2 (80U/g) and G3 (67 U/g) compared to G1 (109 U/g), (p < 0.05). The table 2 and fig 1 show the concentration of ginseng extracts and its inhibition effect on MAO activity, the inhibition percentage directly proportional with gradual increment of ginseng extract concentration. Where the highest percentage of inhibition of MAO 83.26 % was achieved at the highest concentration of ginseng extracts 0.1 mg/mL.

As well as the table 3 and fig 2 shows the ginseng extracts act as inhibitor for peroxidase activity, as with MAO the inhibition percentage of ginseng extract directly proportional with gradual increment of ginseng extract concentration with highest inhibition ratio of peroxidase activity at 0.1mg/ml of ginseng extracts (64.60%).

The kinetics data of MAO and peroxidase enzymes are presented as double reciprocal Lineweaver-Burk plots (table 4) where ginseng extract causes competitive inhibition for MAO. The calculated Vmax and Kmax were 20 μmol\min\L and 0.03 (mol\L) respectively. While it causes uncompetitive inhibition for peroxidase. The calculated Vmax and Kmax were 83.3 μmol\min\L and 0.003 (mol\L) respectively.

Table1. Impact of ginseng extract on in vivo MAO activity

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>Dose (mg/kg)</th>
<th>MAO activity (mean±SD) (U/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>---</td>
<td>109±17</td>
</tr>
<tr>
<td>G2</td>
<td>250</td>
<td>80±8*</td>
</tr>
<tr>
<td>G3</td>
<td>450</td>
<td>67±5*</td>
</tr>
</tbody>
</table>

G1: control, G2: The second group represented by mice injected with 250 mg/kg, G3: The third group represented by mice injected with 450 mg/kg, *: p≤ 0.05

Table2. The inhibitory effect of various concentrations of ginseng extract on MAO activity in human serum

<table>
<thead>
<tr>
<th>Inhibitor concentration (mg\mL)</th>
<th>MAO activity (U/L)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>nil</td>
<td>39.14</td>
<td>---</td>
</tr>
<tr>
<td>0.001</td>
<td>25.93</td>
<td>33.75</td>
</tr>
<tr>
<td>0.01</td>
<td>17.40</td>
<td>55.54</td>
</tr>
<tr>
<td>0.05</td>
<td>11.62</td>
<td>70.31</td>
</tr>
<tr>
<td>0.1</td>
<td>6.55</td>
<td></td>
</tr>
</tbody>
</table>

%: percentage of inhibition for ginseng extract
Fig. 1 Kinetic properties of human MAO inhibition with ginseng
[s]: represent concentration of substrate, \( v \): velocity of enzymatic reaction
Blue color: represent the kinetic values of enzyme without inhibitor
Red color: represent the kinetic values of enzyme with ginseng inhibitor

Table 3. Effect of various concentrations of ginseng extract on peroxidase enzyme in human serum.

<table>
<thead>
<tr>
<th>Inhibitor concentration (mg/mL)</th>
<th>Peroxidase activity (µmol/min/L)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>nil</td>
<td>148.16</td>
<td>---</td>
</tr>
<tr>
<td>0.001</td>
<td>93.78</td>
<td>36.70</td>
</tr>
<tr>
<td>0.01</td>
<td>87.63</td>
<td>40.85</td>
</tr>
<tr>
<td>0.05</td>
<td>70.31</td>
<td>52.54</td>
</tr>
<tr>
<td>0.1</td>
<td>52.44</td>
<td>64.60</td>
</tr>
</tbody>
</table>

Fig. 2. Lineweaver-Burk equation for inhibition of peroxidase by ginseng
blue color: represent the kinetic values of enzyme without inhibitor
red color: represent the kinetic values of enzyme with ginseng inhibitor
Table 4. Values of kinetic inhibition functions for MAO and peroxidase win the presence of ginseng extract.

<table>
<thead>
<tr>
<th>enzymes</th>
<th>Vmax µmol/min/L</th>
<th>Kmax (mol/L)</th>
<th>Inhibition type</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAO</td>
<td>20</td>
<td>0.03</td>
<td>Competitive</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>83.3</td>
<td>0.003</td>
<td>Uncompetitive</td>
</tr>
</tbody>
</table>

4-Discussion:

Over the past few decades, the ginseng extract has shown that it has an effect on many diseases. This effect stems from the possession of this herb for certain properties including antioxidants, anti-tumors and hepatoprotective activities, neuroprotective, and immunomodulatory. The possession of ginseng for many biological and pharmacological properties may be attributed to its contain many components in their structure including ginsenosides, polyacetylenic, phenol, fatty acids, polyacetylenes, peptides, phytosterols and polysaccharides. These ingredients are characterized by having different effects on immune systems, neurodegenerative, blood vessels, CVS and central nervous system (CNS) function, fat metabolism and carbohydrates. Therefore we planned to check the impact of this herb on the activity of MAO and peroxidase enzymes consequent to their role in several metabolic pathways. The results given during this paper demonstrated that ginseng extracted have inhibition properties towards these enzymes. In order to achieve compatibility in the results of MAO activity during this study, the observation of in vivo inhibition of MAO that carried out in the brains of mice receiving ginseng extract for 14 continuous day was supported by an in vitro inhibition results on MAO activity that worked on human serum. Lineweaver-Burk equation results revealed competitive inhibition of ginseng extract towards MAO so the value of Michaelis-Menten constant (Km) was higher than its value without the presence of the extract but the maximum enzyme activity (Vmax) not effected, this result refers to this inhibitor (ginseng extract) compete with substrate for binding to the active site on MAO.

This result confirmed with observations of Sloley et al. who found that HT-1001 , which is a commercially available extract from the root of American ginseng, has significant inhibition towards MAO-A and MAO-B during in vitro study; however, this effect was only observed at very high concentrations of this extract (1 and 10 mg/mL %). The inhibitory effect of ginseng is due to the fact that this herb contains many phenolic compounds that are characterized as a competitive inhibitors towards the MAO enzyme. New and/or processed ginseng characterized by the fact that it contains more than 10 phenolic compounds besides ginsenosides such as caffeic acid, ferulic acid, vanillic acid, p-hydroxybenzoic acid, gentisic acid, and syringic acid. They have various biological functions including their action as anticancer and antioxidant.

Bhattacharya and Mitra found during in vivo study using ginseng herb extract on a sample of rats and mice suffer anxiety that the effect of this herb on mice is close to the effect of diazepam drug. The enzyme peroxides have a large variety of specialized substrates, including guaiacol, o-phenylenediamine, o-dianisidine, pyrogallol and p-aminoantipyrine which oxides during reaction wish H2O2. It was suggested that the electrostatic field arising from charged enzyme residues act on the enzyme's active site's reduction potential. The results of kinetic study of this enzyme revealed ginseng is un competitive inhibitor with peroxidase. According to Lineweaver-Burk plot which shown in fig 2, the calculated Kmax and Vamx were 83.3 µmol/min/L and 0.003 (mol/L) respectively, where both kmax and Vmax drop in presence of un competitive inhibitor. The few Kmax mean high affinity of enzyme towards substrate. As a result of this mechanism, ginseng extract favorably modulates the intracellular concentrations of H2O2, which work in many metabolic pathways function as a signal transducer.

Recently, Altın et al., concluded that peroxidase is highly inhibited by citric acid and Cetyltrimethylammonium bromide (CTAB) where the percentage inhibition was 94% and 95% respectively.

In conclusion, our data suggest that the ginseng extract is good inhibitor towards both MAO and peroxidase enzymes and these finding may be helpful for grasp ginseng’s clinical value and scientific and sensible implementation of ginseng. Where it opens up new horizons towards the medicinal uses of this herb.

5-Acknowledgments
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6-Conflict of Interest Statement

We confirm as authors there is no conflict of interest, including any economic, personal or other relationship with other individuals or organizations that may affect our work

7-References

[14] Finberg JPM ,Gillman K. selective inhibitors of monoamine oxidase type b and the ‘‘cheese effect”’. INT REV NEUROBIOL. 2011; 100.;169-190.


