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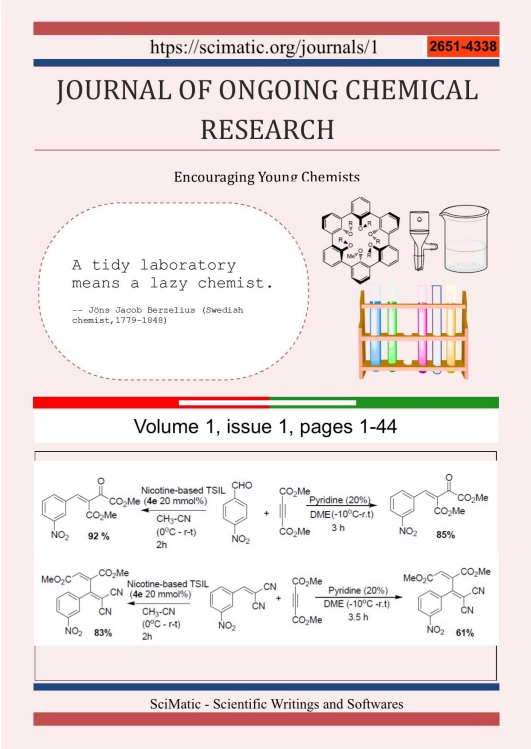

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Research Article

Can Sunflower Honey Have a Protective Effect Against Alzheimer's Disease?

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Abstract

Alzheimer disease is neurodegenerative disease which has no exact cure. In this study the anticholinesterase activity of sunflower honey samples produced in four regions of Adana city and various wholesalers through Turkey was screened. The anticholinesterase activity was performed according to the Elman's method against acetylcholinesterase and butyrylcholinesterase which are the chief enzymes against Alzheimer disease. All sunflower honey samples exhibited anticholinesterase activity. The honey samples produced under control dose dependently inhibited acetylcholinesterase and butyrylcholinesterase in the range of 28.33-34.86% and 27.57-39.75% at 10 mg/mL, respectively. The sunflower honey samples from various wholesalers, however, served 14.77-21.36 and 14.18-23.64% at the same concentration, respectively. The anticholinesterase activity of processed sunflower honey samples exhibited less or no activity when compared to those of produced under control ones. It was concluded that the filtering process and honey sample's shelf life might affect the lesser enzyme inhibitory activity. Moreover, the possibilities such as storage and processing of honey are among the factors that affect the composition of honey. Consuming or using honey freshly, therefore, advised to be benefited from the anticholinesterase property of honey.

Keywords: Anticholinesterase, Alzheimer, Butyrylcholinesterase, Enzyme Inhibitory, Sunflower Honey

INTRODUCTION

Alzheimer's disease is one of the most common diseases today. Alzheimer's is a type of dementia that causes memory, thinking, and behavior problems. Alzheimer's disease (AD) is the most common disease of dementia. According to Alzheimer's Association Report (2019), AD constitutes 60-80% of the cases by the reports received. The pathophysiology of Alzheimer's disease is related to the depletion of the neurotransmitter acetylcholine which is hydrolyzed by the acetylcholinesterase enzyme. Therefore, the inhibition of acetylcholinesterase enzyme is also the only known hypothesis in the pathology of Alzheimers disease (Howes et al., 2003). Thus, acetylcholinesterase inhibitory drugs have been development in the treatment of the AD. For this purpose, synthetic and natural cholinesterase inhibitors have been developed in Alzheimer's treatment such as galantamine physostigmine (Melzer, 1998). However, most of the drugs used against AD damages liver and causes some side effects such as bradycardia. For this purpose, the scientist have been searching new acetylcholinesterase and butyrylcholinesterase inhibitory drugs which have no side effects.

Honey contains more than 200 compounds and is considered as one of the most complete nourishments for humans. This food is an important natural product that mainly contains supersaturated sugar solution of glucose and fructose (do Nascimento et al., 2018; Guzelmeric et al., 2020). The minor components are amino acids, enzymes, vitamins, proteins, organic acids, flavonoids and other phenolic compounds (Guzelmeric et al., 2020). Phenols and flavonoids are found in pollen collected by honeybees, and these medicinal compounds are mixed with bees when honey is being produced by bees. In recent years, honey is reported that it contains antioxidant compounds (Gul and Pehlivan, 2018; Sarhan and Azzazy, 2015; Bonta et al, 2020; Maric et al, 2021). According to the literature, the compounds indicating antioxidant activity may exhibit anticholinesterase activity (Atta-ur Rahman and Choudhary, 2001). Regarding to having antioxidant activity, it is aimed to study the anticholinesterase activity of the sunflower honey. Herein, the acetylcholinesterase and butyrylcholinesterase inhibitory activities of sunflower honey samples produced in four regions of Adana city and various wholesalers through Turkey were studies. The goal of the study is to compare the freshly produces sunflower honey under control with those of obtained from honey wholesalers.

In one of the studies obtained as a result of the enzyme activity studies of honey, it was observed that the enzyme activities decreased after 1-2 years from the harvest date (Uzun, 2011).

RESULTS AND DISCUSSION

There are some methods to determine anticholinesterase activity. Among the Elman method is the easy to screen and gives accurate results. Therefore, the Elman method was used to determine the enzyme inhibitory activity potential of sunflower honey samples. In this assay, The potent inhibitor was incubated for 15 minutes at room temperature. As soon as the substrate acetylcholine was added to the mixture, the remaining enzyme hydrolises the acetylcholine to acetyl and colin. The latter was reacted with the Elmann reactive which resulted with the yellow color absorbs at 412 nm.

Table 1 shows the acetylcholinesterase inhibitory activity of the sunflower honey samples. In the acetylcholinesterase assay, the sunflower honey samples produced in Adana under control showed higher activity than the processed wholesalers' samples (Table 1). Produced fresh sunflower honey samples exhibited in the range of 28.33 and 34.86% at 10 mg/mL concentration. Since none of the sunflower displayed more than 50%, the activity of lower concentrations did not study. This percentage range belongs to the freshly produced honey. Among them, **the sample produced in Yumurtalık (South) displayed the highest activity (**34.86 ± 0.48%**), followed by Yumurtalık (East), Kozan (North), and Kozan (East) with the percentage inhibitions of** 29.45 ± 0.39, 29.44 ± 0.65%, and 28.33 ± 0.51, respectively. It can be said that all samples demonstrated close activity to each other. There was no statistical difference found between the sunflower honey samples. At the same conditions, the galantamine displayed 84.97 ± 1.01% at 100 µg/mL.

These results indicate that the sunflower honey has more or less anticholinesterase capacity the samples provided from wholesalers possessed percentage inhibition in the range of 14.77 ± 1.03 and 21.36 ± 0.88% at the same concentrations. The activity of the processed honey samples was lesser than the honey samples produced under control.

Table 1 also shows the butyrylcholinesterase inhibitory activity of the sunflower honey samples. In this assay, the sunflower honey samples produced in Adana under control showed higher activity than the processed wholesalers'samples (Table 1). Produced fresh sunflower honey samples exhibited in the range of 27.57 and 39.75% at 10 mg/mL concentration. Since none of the sunflower displayed more than 50%, the activity of lower concentrations did not study.

Table 1. Anticholinesterase inhibition activity results of Sunflower honey

	Collection place		Anticholinesterase Inhibition Activity (Inhibition %)	
	Region	Samples	AChE	BChE
The honey get produced freshly	ADANA	Kozan (North)	29.44±0.65	27.57±1.18
		Kozan (East)	28.33±0.51	39.75±1.54
		Yumurtalık (South)	34.86±0.48	32.57±0.77
		Yumurtalık (East)	29.45±0.39	28.28±1.42
The honey wholesalers	KONYA	Honey 1	15.41±1.05	17.46±1.31
		Honey 2	18.28±0.57	20.58±0.32
	İZMİR	Honey 3	14.88±0.75	19.73±1.27
		Honey 4	19.22±0.56	16.81±1.17
	ANKARA	Honey 5	14.77±1.03	14.18±1.73
		Honey 6	15.49±0.74	23.64±1.01
		Honey 7	15.98±1.05	14.18±1.73
	ADANA	Honey 8	16.01±0.43	15.91±0.59
		Honey 9	21.36±0.88	14.64±0.96
		Honey 10	15.47±0.46	22.56±0.75
	Standards Anticholinesterase	Galantamina	84,97±1.01	79.97±1.01

a: The results presented herein are mean ± Standard error meaning (S.E.M.) of three parallel measurements ($p>0.05$

This percentage range belongs to the freshly produced honey. Among them, **the sample produced in Kozan (East) displayed the highest activity (**39.75 ± 1.54%**), followed by Yumurtalık (South), Yumurtalık (East), and Kozan (North) with the percentage inhibitions of** 32.57 ± 0.77, 28.28 ± 1.42%, and 27.57 ± 1.18, respectively. It can be said that all samples demonstrated close activity to each other. There was no statistical difference found between the sunflower honey samples. At the same conditions, the galantamine displayed 79.97 ± 1.01% at 100 µg/mL.

These results indicate that the sunflower honey has more or less butyrylcholinesterase inhibitory capacity. The samples provided from wholesalers possessed percentage inhibition in the range of 14.18 ± 1.73 and 23.64 ± 1.01% at the same concentrations. The activity of the processed honey samples was also lesser than the honey samples produced under control.

Among the sunflower honey samples, the honey produced in Adana at four regions exhibited the higher anticholinesterase activity in both assays than the

sunflower honey samples purchased from wholesalers. Different factors may be affected this result. One of these is that the wholesalers filter the honey with less than 0.2 mm sieve to avoid honey crystallization. During this process, the pollens of honey are removed from the honey. Therefore, most of the compounds having inhibitory potentials may also be removed from the honey. Another reason is the holding period after filtration. In this case, the shelf life of the honey samples becomes essential. It is seen from the study that the freshly produced sunflower honey samples, therefore, contain more potent compounds.

EXPERIMENTAL

2.1. Chemicals and reagents

Butyrylcholinesterase (BChE) from horse serum (EC 3.1.1.8, 11.4 U/mg, Sigma, St. Louis, MO), acetylcholinesterase (AChE) from electric eel (Type VI S, EC 3.1.1.7, 425.84 U/mg, Sigma, St. Louis, MO), 5,5′ dithiobis (2 nitrobenzoic acid) (DTNB), galantamine, butyryl-thiocholine chloride, acetylthiocholine iodide were purchased from Sigma Chemical Co. (Sigma Aldrich GmbH, Steinheim, Germany). Optical densities for bioassays were read on a 96 well microplate reader, SpectraMax340 PC^{384} (Molecular Devices, Silicon Valley, CA). Solvents and chemicals were of analytical grade.

2.2. Collection of Honey Samples and preparation study solution.

The Sunflower honey samples were get produced by the beekeepers in Adana in four locations in June 2020. The locations were in **Kozan (North), Kozan (East), Yumurtalık (East), and Yumurtalık (South) districts.** The produced honey samples were used for the comparison. The processed sunflower honey samples were purchased from different wholesalers in Konya, Ankara, İzmir, and Adana. Ten sunflower honey samples were purchased from wholesalers.

Each Honey sample (2 g) was diluted with 10 mL distilled water. The stock solution was used to analyze the anticholinesterase activity.

2.3. Anticholinesterase activity

The inhibition activity of acetylcholinesterase (AChE) and butyryl-cholinesterase (BChE) were measured by Elman's method with slight modification (Ozturk et al., 2014), using 96-well microplate reader (SpectraMax PC340, Molecular Devices, USA). The substrates of the reaction of both enzymes were acetylthiocholine iodide (0.71 mM) and butyrylthiocholine chloride (0.2 mM) respectively. In a 96 well plate, 10 µL of sample in ethanol at different concentrations were mixed with 150 µL sodium phosphate buffer (100 mM, pH = 8) and 20 µL of enzymes [AChE (5.32×10^{-3U}) or BChE (6.85×10^{-3U})]. After 15 min incubation at 25 °C, 10 µL of Ellman's Reagent (DTNB 0.5 mM) and 10 µL of substrates were added to make 200 µL final volume. The absorbance of each sample was measured at 412 nm for 10 minutes. Each experiment was carried out in triplicate and galantamine was used as the standard. Percentage inhibitory activity against AChE or BChE were determined by comparison of reaction rates of samples relative to control using the formula:

$$\text{Inhibition\%} = (E - S)/E \times 100$$

Where: E: the activity of the enzyme with control. S: the activity of the enzyme with the sample.

2.4. Statistical analysis

All data on bioactivity tests were averages of triplicate analyses. Data were recorded as mean ± SEM (standard error of the mean). p values <0.05 were regarded as significant.

CONCLUSION

The results indicate that the sunflower honey has enzyme inhibitory capacity. The honey can more or less inhibit the acetylcholinesterase and butyrylcholinesterase which are the chief enzymes of Alzheimers disease. The results suggested that ten grams consume of sunflower honey a day protects the human being against Alzheimers disease. But the honey should be kept raw and placed in markets without any process. The crystallization, which could be formed naturally, does not show the honey's poor quality. Therefore, as mentioned in the Turkish honey codex (No. 2020/7), the crystallization of honey is natural. The study results indicate that the sunflower honey, which could crystalize easily, should be placed in markets. To overcome the prejudices of people, it is necessary to reveal all the properties of sunflower honey and share these inhibitory activity properties with people. Moreover, the sunflower honey should not be filtered by the wholesalers to protect its nutrition and bioactivity.

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