In vitro Investigation of Deoxyribonucleic Acid Interaction and Anti-Acetylcholinesterase Activity of Turnip (*Brassica Rapa Subsp. Rapa)*

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Abstract

Turnip (*Brassica rapa subsp. Rapa*) is a herbaceous and seasonal plant found in the cruciferous family. It is possible to grow in many regions of Europe and West Asia. Although many studies have been carried out to show that turnip juice obtained from turnip root has high biological activity, there has been no previous study on the interaction of turnip root with DNA or its anti-acetylcholinesterase activity. In this study, the interaction and anti-acetylcholinesterase activity of turnip, which obtained three different extracts by applying Soxhlet extraction, were tried to be determined. The DNA binding properties, DNA protective effects, DNA restorative effects and anti-acetylcholinesterase activity of the obtained water, ethanol and ethyl acetate extracts were calculated and the results obtained were compared with other plants of the same species in the past studies.

Keywords: Turnip, DNA, DNA interaction, acetylcholinesterase, Soxhlet extraction, natural product

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INTRODUCTION

DNA is a nucleic acid that contains the genetic instructions required for the vital activities and biological development of all existing organisms and some viruses as well as these organisms (Nelson et. al., 2013). The coding of genetic information in living things takes place thanks to the sequence of nitrogenous organic bases that run along the backbone of the DNA double helix. The encoded genetic information is read with the help of the genetic code during protein synthesis, and thanks to this reading, the amino acid sequences of the synthesized proteins are determined (Butler, 2001). Damages that occur on the DNA double chain and can occur due to endogenous or exogenous reasons are most frequently encountered by free radicals (Lodish et. al., 2004). These damages are repaired either by destroying the damaged cells or by removing the DNA with their own repair systems (Larsen et. al., 2005).

The non-covalent interactions of DNA with molecules smaller than itself are generally grouped in three groups: Intercalation, electrostatic interactions and binding to grooves (Yıldız et. al. 2015). Intercalation, which can be defined as an intervention in short, is the type of bonding realized by the molecules entering between the double chains in a reversible way (Hasanzadeh and Shadjou, 2016).

In addition to electrostatic interactions that express positive molecules that bind to the negatively charged phosphate-sugar backbone of the DNA chain, molecules can also bind to the grooves of the DNA chain by van der Waals interactions and hydrogen bonds (Rajendiran et. al., 2012).

Acetylcholinesterase, also known as AChE or acetylhydrolase, is an enzyme that is cholinesterase. It is an enzyme that catalyzes the breakdown of acetylcholine and some other choline esters that act as neurotransmitters. AChE is mainly found in cholinergic type chemical synapses and is responsible for terminating synaptic transmission. It belongs to the carboxylesterase enzyme family (Katzung, 2001). Alzheimer's disease is the most common neurodegenerative disease. It can be summarized as the accumulation of extracellular amyloid plaques, shortly acetylcholine. The signal of the acetylcholine molecule is terminated by acetylcholinesterase. Drugs that limit acetylcholine degradation or mimic acetylcholine activity, briefly have anti-acetylcholinesterase activity, are used to increase cholinergic signal transmission in patients with Alzhemier (Nwidu et al., 2017).

Turnip (*Brassica rapa subsp. Rapa*) is a plant in the Brassica genus of the Brassicaceae family (cruciferous or cruciferous) and has a wide cultivation area. The turnip, which loves warm and cool seasons, is resistant to cold despite its structure. Due to the flexible about tolerance and cold temperatures, West Asia and Europe are grown in many regions of Turkey in the overall Adana, Osmaniye, Mersin is grown around. However, it is also possible to grow in colder regions (Padilla et al., 2005).

As stated by the authors, there is no study on the interaction of the turnip root with DNA and its anti-acetylcholinesterase activity. Studies on the biological activity of natural resources have gained importance in recent years, and in this study, the binding, protective and restorative effects and anti-acetylcholinesterase activity of the extracts obtained from turnip by Soxhlet extraction were investigated. The results obtained were compared and interpreted with other plants of the same species.

MATERIAL and METHOD

Source Plant and Extraction Process

To be used in the experiments, the turnip plant was used for this study, purchased from a local market in Küçükçekmece district of Istanbul province. The plant itself can be consumed both in the form of leaves and in the form of a root, and the interaction with DNA and anti-acetylcholinesterase activity were investigated, by the extracts provided by Soxhlet extraction. In order to obtain the extracts, Soxhlet extraction with 100 mL water, ethanol and ethyl acetate solvents for 4 hours each was applied to the 50 gram samples taken from the turnip root. Then, the solvents were removed from the extracts using a rotary evaporator, oven and fume hood respectively. Both the plant and the extracts obtained were stored at +4 °C throughout the study.

Enzyme Activity Assay

Anti-acetylcholinesterase Activity Assay

While studying anti-acetylcholinesterase activity, Ingkaninan et al., (2003)'s method was followed (Ingkaninan et. al., 2003). At the first step, 325 µL Tris-HCl buffer (at 50 mM and pH 8) was added to each 100 µL of extract samples prepared at a concentration of 1 mg/mL. 25 µL of enzyme solution (0.28 U/mL) was added on each of the samples to which buffer was added, and the samples were incubated for 15 minutes at room temperature.

After 15 minutes, 75 µL of acetylcholine iodide (15 mM) and then 475 µL of DTNB (3 mM) solution were added to each sample, respectively. After the addition of DTNB, the absorbance values at 405 nm of the samples, which were incubated for another 30 minutes at room temperature, were read. During the measurements, Tris-HCl buffer was used as a blank solution, and samples containing each sample's own solvent were used as the control solution. While calculating the % anti-acetylcholinesterase activity, the equation where A_0 corresponds to the absorbance of the control solution and A_1 to the absorbance of the samples was applied.

$$\text{Anti-acetylcholinesterase Activity (\%)} = [1 - (A_1 / A_0)] \times 100 \qquad (1)$$

Interaction with DNA

DNA For studies involving DNA binding effect, DNA protective effect and DNA restorative effect assays, a working solution of 3×10^{-2} mM from CT-DNA was prepared using Tris-HCl buffer solution. This prepared working solution and the main stock DNA solution were stored at +4 °C throughout the studies. In all studies on DNA, water extract, ethanol extract and ethyl acetate extract obtained from the turnip root were prepared at a concentration of 1 mg/mL and using Tris-HCl buffer solution.

DNA Binding Effect

Before observing the change in the absorbance values of the samples added to the DNA solution, in the wavelength range of 200-400 nm, absorbance values of 3×10^{-2} mM DNA working solution and water, ethanol and ethyl acetate extracts at a concentration of 1 mg/mL against the Tris-HCl buffer used as a blank solution were measured and recorded separately. Subsequently, the changes in absorbance values were observed by adding 10 µL of each extract up to 100 µL with the addition of 10 µL onto 1 mL and 3×10^{-2} mM DNA working solution. All measurements were repeated at the 24th hour with samples stored at +4 °C in order to observe the time-dependent changes in the DNA binding capacity of water, ethanol and ethyl acetate extracts.

DNA Protective Effect

In studies for DNA protective effect, changes in absorbance values were observed with the help of extracts added to DNA working solutions that were denatured with ethanol and UV light, separately. During the study of denaturation by the addition of ethanol, an equal volume of DNA working solution and ethanol were mixed first and the its absorbance between 200-400 nm against Tris-HCl buffer was measured.

In order to observe the DNA protective effect of the extracts, first, 250 µL of ethanol and then 500 µL of all three extracts each were added to the 250 µL DNA working solution, respectively, and the measurements were taken in the range of 200-400 nm. In order to compare the protective effect more accurately, the absorbance value of pure ethanol was also read in the same wavelength range.

All measurements were repeated at 24th hour with samples stored at +4 °C to observe the time-dependent change of the protective effect of the extracts. However, it was observed that repeated measurements at 24th hour did not give a different result from the first measurements.

During the investigation of denaturation with UV light, the wavelengths of 254 nm and 365 nm were studied, respectively. 500 µL of each extract was added onto 500 µL of DNA working solution and the solutions were exposed to UV light for 30 minutes, separately.

Then, absorbances of the samples were read against the Tris-HCl buffer solution used as a blank solution in the wavelength range of 200-400 nm. For a better interpretation of the results, pure water extract, ethanol extract, ethyl acetate extract and DNA working solution were kept under UV light for 30 minutes and absorbance values were measured in the same wavelength range. In the protective effect tests performed after a second 30-minute UV light denaturation, it was determined that the second period had no effect.

DNA Restorative Effect

In order to determine whether the turnip extracts have a restorative effect, the DNA working solution was first kept under UV light for 30 minutes at 254 and 365 nm, respectively. Then, the absorbance between 200-400 nm was scanned against the Tris-HCl buffer solution. Afterwards, absorbances of the solutions formed by mixing the UV-damaged DNA working solution and the extracts in an equal volume were studied to see if the extracts had a restorative effect. In the experiments carried out with another 30-minute UV light denaturation, it was determined that the second 30-minute period had no effect.

RESULTS and DISCUSSION

Enzyme Activity Assay

Anti-acetylcholinesterase Activity Assay

The inhibition percentages for the anti-acetylcholinesterase activities of the samples obtained from the turnip root were found to be 0.76% for water extract, 1.38% for ethanol extract and 3.70% for ethyl acetate extract.

Interaction with DNA

In order to determine whether the extracts obtained from turnip root have an antimutagenic effect on CT-DNA, the values reached by the measurements should be interpreted through the wavelength changes divided into two as hypsochromic effect and batochromic effect, and absorbance values, which are divided into two as hyperchromic effect and hypochromic effect. In studies on DNA, a purity control should be calculated by proportioning the absorbance of DNA at 260 nm to 280 nm absorbance and A_{260}/A_{280} ratio should be 1.8 or more than value of 1.8. For the CT-DNA used in the study, the value of absorbance ratio calculated as A_{260}/A_{280} = 2,4209/1,3126 = 1,8444.

DNA Binding Effect

Initially, the maximum absorbance of the pure DNA sample was obtained at 258 nm and 2,4378. After the addition of 100 µL water extract, the maximum absorbance value of 2.0194 was obtained at the same wavelength, 258 nm. By adding 100 µL ethanol extract to the pure DNA sample, the maximum absorbance was again at 258 nm, but this time with a value of 2.0014. After the addition of 100 µL of ethyl acetate extract to the pure DNA sample, the maximum absorbance value of the DNA sample was again measured at 258 nm and this time as 1.9735.

Although the wavelength at which the DNA solution gives maximum absorbance does not change with the addition of all three extracts, the absorbance at this wavelength has changed and this decrease is expressed as a hypochromic effect. Based on the numerical data, it can be interpreted that the binding that occurs between all three extracts and DNA is intercalated, separately. Studies based on the binding effect between DNA solution and water, ethanol and ethyl acetate extracts were repeated after 24 hours, and no significant changes were observed in the second measurements.

Maximum wavelength of 100 µL water extract, ethanol extract and ethyl acetate extract interacting with DNA, maximum wavelength change caused by these extracts, the hypochromic effect percentages of the extracts and the binding constants of their binding with DNA are given in Table 1. Equation 2 was used for hypochromic effect values calculated in percentage, and Equation 3 was used for binding constants calculated by absorbance values and DNA concentration. In these equations, A_0 indicates the absorbance value that the DNA sample in free state reaches at the maximum wavelength, while A_1 indicates the absorbance value reached by the DNA sample to which the extract is attached at the maximum wavelength, and C_{DNA} indicates the concentration of the DNA working solution.

$$H\ (\%) = 100 \times [(A_0 - A_1) / A_0] \quad (2)$$

$$K_b = (A_0 - A_1) / (A_1 \times C_{DNA}) \quad (3)$$

DNA Protective Effect

Denaturation with Ethanol

During the examination of the protective effects of the extracts on DNA denatured with ethanol, firstly, the maximum wavelength of DNA and ethanol mixed in equal volume was determined as 259 nm, while the absorbance value at this wavelength was found to be 2.4032. When water extract was added to DNA and ethanol, the maximum wavelength did not change, while the absorbance value was observed as 0.7727. While the maximum absorbance value of the ethanol extract added DNA and ethanol solution was read at 260 nm, the absorbance value at this wavelength was read as 0.9958. The addition of ethyl acetate extract decreased the wavelength at which the maximum absorbance was read to 256 nm, while the absorbance value was measured as 0.8637. The decreasing absorbance value in all three extracts indicates the hypochromic effect. In terms of wavelength change, it can be said that there is a batchromic effect, which means the π-π^* transition in the ethanol extract, and a hypsochromic effect in the ethyl acetate extract, except for the water extract which did not perform any change. In the light of these results, it can be interpreted that all three extracts obtained from the turnip root are intercalated to DNA.

In addition, as a result of repeated measurements of the DNA protective effect at the 24th hour, no significant difference was observed from the values obtained in the first measurements. The results for the effect of preventing the denaturation in DNA with ethanol, studied for water extract, ethanol extract and ethyl acetate extract, were calculated using Equation 1 and Equation 2 and shown in Table 2.

Denaturation with UV Light

During the investigation of the reparative effect of water, ethanol and ethyl acetate extracts against denaturation with UV light on DNA, the maximum wavelength of the DNA sample exposed to UV light and the change in the absorbance value at this wavelength were considered.

When water extract was added to the DNA sample denatured by UV light, the maximum wavelength did not change at both 254 nm and 365 nm, while the maximum absorbance value decreased. Changes of 0.9497 and 0.9735, respectively, can be expressed as a hypochromic effect. In the measurements performed with ethanol extract, the maximum absorbance value decreased while the maximum wavelength increased at both 254 nm and 365 nm. While the wavelength increasing from 258 nm to 260 nm expresses the batchromic effect, the changes in absorbance values of 0.8425 and 0.8636, respectively, can be expressed as hypochromic effect. During the examination of the restorative effect of the ethyl acetate extract, both at 254 nm and 365 nm, the maximum wavelength and maximum absorbance value decreased as a result of the addition of ethyl acetate extract. While the wavelength from 258 nm to 257 nm is described as hypsochromic effect, the changes in absorbance values of 0.9549 and 0.9788, respectively, can be expressed as hypochromic effect. In the second measurements repeated 30 minutes after the first measurements, no serious difference was observed for all three extracts. The values calculated for the DNA protective effect at both 254 nm and 365 nm using Equation 1 and Equation 2 are shown in Table 3 and Table 4, respectively, for two wavelengths.

DNA Restorative Effect

When the effects of the extracts obtained from turnip root on DNA to restore the denaturation caused by UV light are examined, it is seen that the maximum absorbance values decrease with the addition of all extracts. Moreover, the maximum wavelength unchanged with the addition of water extract increased after the addition of ethanol extract and decreased after the addition of ethyl acetate extract. As a result, it can be interpreted that all extracts cause hypochromic effect, additionally there is a batchromic effect in ethanol extract and a hypsochromic effect in ethyl acetate extract. The results obtained in the second measurements performed after a period of 30 minutes do not differ significantly from the first measurement results. The values calculated using Equation 1 and Equation 2 at 254 nm and 365 nm, which are two wavelengths where the DNA restorative effect is studied, are given in Table 5 and Table 6, respectively.

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Table 1. λmax, Δλ, H% and K_b values of water extract, ethanol extract and ethyl acetate extract

Sample	**$\lambda_{maximum}$ (nm)**	**Δλ**	**H%**	**K_b (x 10^5 M^{-1})**
Distilled water extract with DNA	258	0.4184	17.1630	0.0691
Ethanol extract with DNA	258	0.4364	17.9014	0.0727
Ethyl acetate extract with DNA	258	0.4643	19.0459	0.0784

Table 2. λmax, Δλ, H% and K_b values of DNA denaturation of water extract, ethanol extract and ethyl acetate extract with ethanol

Sample	**$\lambda_{maximum}$ (nm)**	**Δλ**	**H%**	**K_b (x 10^5 M^{-1})**
Distilled water extract with DNA and ethanol	259	0.7727	67.8470	0.7034
Ethanol extract with DNA and ethanol	260	0.9958	58.5636	0.4711
Ethyl acetate extract with DNA and ethanol	256	0.8637	64.0604	0.5941

Table 3. λmax, Δλ, H% and K_b values for the restorative effect of extracts against UV light denaturation of 254 nm in DNA

Sample	**$\lambda_{maximum}$ (nm)**	**Δλ**	**H%**	**K_b (x 10^5 M^{-1})**
Distilled water extract with DNA and UV Light	258	0.6971	57.6694	0.4541
Ethanol extract with DNA and UV Light	260	0.8043	51.1234	0.4029
Ethyl acetate extract with DNA and UV Light	257	0.6919	57.9852	0.4566

Table 4. λmax, Δλ, H% and K_b values for the restorative effect of extracts against UV light denaturation of 365 nm in DNA

Sample	**$\lambda_{maximum}$ (nm)**	**Δλ**	**H%**	**K_b (x 10^5 M^{-1})**
Distilled water extract with DNA and UV Light	258	0.7145	57.6718	0.4542
Ethanol extract with DNA and UV Light	260	0.8244	51.1611	0.3492
Ethyl acetate extract with DNA and UV Light	257	0.7092	57.9858	0.4600

Table 5. λmax, Δλ, H% and K_b values for the restorative effect of extracts against UV light denaturation at 254 nm on DNA

Sample	**$\lambda_{maximum}$ (nm)**	**Δλ**	**H%**	**K_b (x 10^5 M^{-1})**
Distilled water extract with DNA and UV Light	258	1.3623	19.1823	0.0736
Ethanol extract with DNA and UV Light	259	1.4368	8.6387	0.0525
Ethyl acetate extract with DNA and UV Light	256	3.4798	8.6387	0.0413

Table 6. λmax, Δλ, H% and K_b values for the restorative effect of extracts against UV light denaturation at 365 nm on DNA

Sample	**$\lambda_{maximum}$ (nm)**	**Δλ**	**H%**	**K_b (x 10^5 M^{-1})**
Distilled water extract with DNA and UV Light	258	1.2770	18.0966	0.0791
Ethanol extract with DNA and UV Light	259	1.4436	13.6175	0.0315
Ethyl acetate extract with DNA and UV Light	256	3.4436	11.0323	0.0315

CONCLUSION

Synthetic antioxidants such as BHA, BHT, Trolox and α-tocopherol have been used for a long time for Today, in daily life, many changes occur in DNA structure due to many endogenous and exogenous factors. These changes, which are important and damaging enough to affect the continuation of life, cause mutations on the DNA molecule and diseases such as cancer that are very difficult to reverse. The fact that some natural sources have antitumor and anticarcinogen effects allows them to have a protective and restorative effect on DNA. In addition, the number of natural resources used for the treatment of many neurodegenerative diseases, especially Alzheimer's, is rapidly increasing.

In the study, turnip (*Brassica Rapa Subsp. Rapa*) was subjected to Soxhlet extraction and three different extracts were obtained with the help of water, ethanol and ethyl acetate solvents which have different polarity. With the help of different parameters, binding, restorative and protective effects and anti-acetylcholinesterase activity on the DNA molecule were investigated.

In addition to many natural sources that have been investigated for enzyme inhibition and interaction with DNA, the fact that turnip has not been included in such a study before has caused some limitations to compare our results with similar studies. Likewise, turnip could not be compared with other plant samples in Brassica, since no study has been conducted on these subjects. Interaction with DNA was not studied in any plant of Brassica species, and only one reference of the same species was found for anti-AChE activity. In a study conducted with red cabbage (Brassica oleracea var. Capitata f. Rubra), the high anti-AChE activity of the plant stands out (Archana et. al., 2018).

In conclusion, the study used a material that had not been evaluated in any similar study before. Comparison of the obtained results with the literature is not easy in this respect. The results obtained in the study show that the interaction of turnip with DNA molecule changes depending on the parameter studied. Similarly, the anti-acetylcholinesterase activity of turnip could not be obtained at high values due to the results compared with other plant in the same family. The importance of the study appears before us in terms of giving an idea for the future studies. In the future, the interaction with DNA and anti-acetylcholinesterase activity of turnip root, the content of which will be completely purified and determined, will be revealed more clearly.

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