

The inhibitory effects of lycopene and thymoquinone on angiotensin converting enzyme from human plasma (An *in vitro* study)

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Abstract: Angiotensin converting enzyme (ACE, EC 3.4.15.1) is an important enzyme responsible for regulating blood pressure. Inhibition of this enzyme is an important treatment approach in the treatment of hypertension, and natural or synthetic ACE inhibitors are often used for this purpose. In this study, the preventive effect of two important antioxidant compounds, lycopene (LYC) and thymoquinone (TQ) on ACE activity in human plasma was investigated. Human plasma was used as ACE source. ACE activity was calculated absorbance at 345 nm after incubation for 30 minutes at 35°C. TQ and LYC showed inhibitory effect on ACE activity. IC₅₀ values for TQ and LYC were determined as 314 μM and 182 μM, respectively. Type of inhibition for TQ and lycopene from plot Line weaver-Burk was designated as non-competitive inhibition. The K_i constants of TQ and LYC were determined to be 707 μM and 167 μM, respectively. It was concluded that TQ and LYC may have significant potential as ACE inhibitors.

Keywords: Angiotensin converting enzyme, *in vitro*, inhibition, lycopene, thymoquinone.

INTRODUCTION

Angiotensin converting enzyme controls blood pressure by converting, angiotensin I (a decapeptide protein), to angiotensin II (an octapeptide protein). ACE inhibitors are one of the drug compounds often used in the treatment of hypertension by lowering high blood pressure. It has been determined that many new angiotensin-I-converting enzyme inhibitory compounds significant inhibitory effect on hypertension. Therefore, in the some studies have investigated ACE inhibitors obtained from various sources such as *Nigella sativa* (*N. sativa*) (Yur *et al*, 2013, Ahmad *et al*, 2017, Basi and Turkoglu, 2018, Enayatfard *et al*, 2018, Tu *et al*, 2018).

Hypertension is a very important health problem worldwide and can lead to more dangerous diseases such as cardiovascular diseases if left untreated. Also, it is thought that oxidative stress plays an important role in the development of hypertension. In many studies, it has been observed that reactive oxygen species (ROS) cause both oxidative stress and increase hypertension (Sinha and Dabla, 2015, Guzik and Touyz, 2017).

Therefore, it has been determined that antioxidant compounds can be effective in oxidative stress and hypertension treatment (Ahmad *et al*, 2017, Massaro *et al*, 2019).

Lycopene is a carotenoid and found naturally in fruits and vegetables (tomatoes and tomato products). Lycopene, one of the strongest antioxidants and free radicals scavenging, plays an important role in reducing oxidative

stress. Also, it was observed that foods containing lycopene and lycopene had antihypertensive effect. For example, it was reported that lycopene supplement (>12 mg/day) might effectively reduce systolic blood pressure (SBP). Also, lycopene has shown this antihypertensive effect by inhibiting the angiotensin converting enzyme (Ozmutlu *et al*, 2012, Li and Xu, 2013, Belovic *et al*, 2016, Khan *et al*, 2016, Han and Liu, 2017, Karahan *et al*, 2018).

Thymoquinone has been exist in many medical plants like different type of the Lamiaceae family and the Cupressaceae family. The main bioactive component in *Nigella sativa* essential oil, thymoquinone (TQ), is a compound that has antioxidant, antihypertensive, antimutagenic effects, antibacterial, antidiabetic, anti-inflammatory and anticancer effects (Darakhshan *et al*, 2015, Cobourne-Duval *et al*, 2016, Farkhondeh *et al*, 2018).

This study was planned to determine the inhibitory effects and types of TQ and LYS, the well-known antioxidants, to be used as candidate antihypertensive agent in future studies.

MATERIALS AND METHODS

Preparation of plasma pattern

Human plasma was used as ACE source. Blood samples required obtaining plasma; Turkey was obtained from Van Crescent Blood Center. Blood samples were placed in heparinized tubes. Tubes with heparin were centrifuged to obtain plasma (20 minutes, 1500 x g). The plasma collected above was carefully removed. The collected

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plasma was then centrifuged (1hour, 4°C, 8500Xg). Thus, cell ghosts, intact cells and clear plasma were obtained.

Preparation of thymoquinone and lycopene solutions

TQ and LYC solutions were prepared to determine ACE inhibitor activities. TQ (Sigma-Aldrich, Darmstadt, Germany) was dissolved in DMSO (10 mg / 50 µL). This solution was made up to 10 mL with bidistilled water. LYC (25 mg) (DMS, Basel, Switzerland) was dissolved in bidistilled water and its volume was completed to 10 mL.

Enzyme inhibition analysis

Previously prepared stock inhibitor solutions (thymoquinone and lycopene) were prepared by dilution. The inhibitory effect of inhibitor solutions diluted in different doses on enzyme activity was determined spectrophotometrically and a decrease in enzyme activity was observed. The reduction of activity was determined periodically. Inhibitor types, Ki values and IC50 (inhibitor concentrations causing 50% inhibition) of TQ and LYC were estimated according to classical methods (Holmquist *et al*, 1979, Andújar-Sánchez *et al*, 2003).

ACE activity assay

The previously prepared human plasma was used as an ACE source. ACE enzyme, a divalent dipeptidyl carboxyl metalloproteptidase, exists in a soluble form in blood and multiple body fluids. Substrates of enzymes are specific to them. Each enzyme is unique to its own substrate. FAPGG is a substrate specific for the ACE enzyme. It specifically binds to this enzyme.

The glass tubes were taken as blank and samples. The prepared human plasma was used as an ACE source. 100 µL of human plasma was added to each tube. 900µL HEPES buffer (50mM HEPES, 0.3M NaCl, 10µM ZnCl2 pH 7.5) was added to the blank tube and mixed. The spectrophotometer was reset with an empty tube. 50 mM HEPES buffer (pH 7.5), inhibitors (separately for each dilution for TQ and LYC) and 1mM substrate (FAPGG: furanacryloyl-L-phenylalanyl-glylyl-glycine-ACE-specific substrate to be completed to sample tubes to 1000µL in total volume) was added and mixed. The samples were incubated for 30 minutes at 35°C. After 30 minutes, the absorbance of the tubes was measured spectrophotometrically (345 nm) and the amount of decrease in absorbance was determined according to classical methods (Holmquist *et al*, 1979; Andújar-Sánchez *et al*, 2003).

The calibration curve was used to determine the ACE inhibitory activity (% inhibition). The following equation was obtained:

$$\% \text{ ACE inhibition} = \frac{\text{Uninhibited activity} - \text{Inhibited activity}}{\text{Uninhibited activity}} \times 100$$

In vitro effects of LYC and TQ on ACE activity

To determine the concentration range and ACE activities in this study, LYC and TQ sediments were added to the

measurement tubs (50 mM Hepes Na, 0.3 M NaCl, 10µM ZnCl2, 1mM FAPGG, 100µL enzyme solution). The activity plot was plotted against inhibitor concentration. LYC and TQ IC50 (inhibitory concentration inhibiting 50% enzyme activity) values were calculated from the inhibition plot equations. Line weaver-Burk charts were drawn using five different FAPGG concentrations (in the range of 0.15 to 0.25mM) and three different concentrations. The Ki value and inhibition types of LYC and TQ concentrations were determined from these plots according to well knowing classical procedure (Lineweaver and Burk, 1934).

RESULTS

In this study, the effect of lycopene and thymoquinone compounds on ACE activity in human plasma was studied, separately. Lycopene and thymoquinone showed inhibition effect on human plasma ACE activity. IC50 values for LYC and TQ were determined to be 182 µM and 314µM, respectively (figs. 1 and 2).

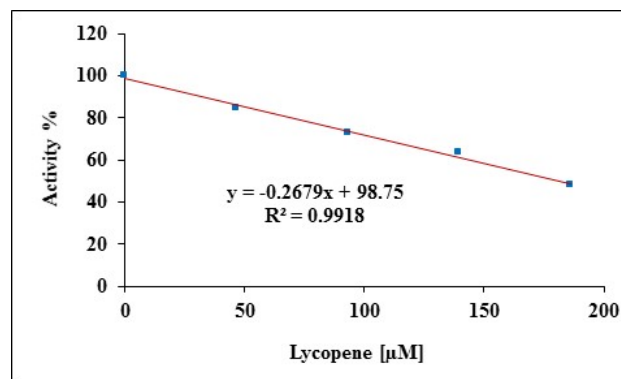


Fig. 1: The inhibition effect of LYC on the ACE activity from human plasma

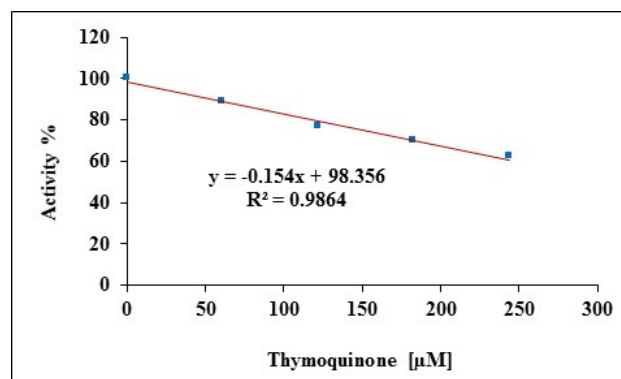


Fig. 2: The inhibition effect of TQ on the ACE activity from human plasma

Also, the inhibition type of lycopene and thymoquinone was determined to be reversible non-competitive type, according to Line weaver-Burk plot calculation (figs. 3 and 4).

DISCUSSION

There are the studies about inductive effects of oxidative stress on increasing hypertension and in the pathogenesis of hypertension. It is reported that there is a strong relationship between blood pressure and oxidative stress related parameters. Therefore, antioxidant compounds have been observed to reduce high blood pressure while treating oxidative stress. One class of antihypertensive drugs is ACE inhibitors (Hou *et al*, 2003, Baradaran *et al*, 2014, Ahmad *et al*, 2017, Mozos *et al*, 2018, Borghi and Omboni, 2020, Chiu *et al*, 2021, Martinelli *et al*, 2021).

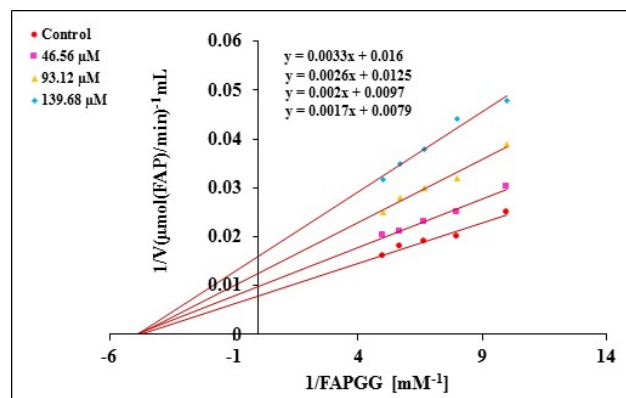


Fig. 3: Line weaver-Burk plot with five different substrate concentrations (FAPGG) and three different LYC concentrations used for the determination of inhibition type

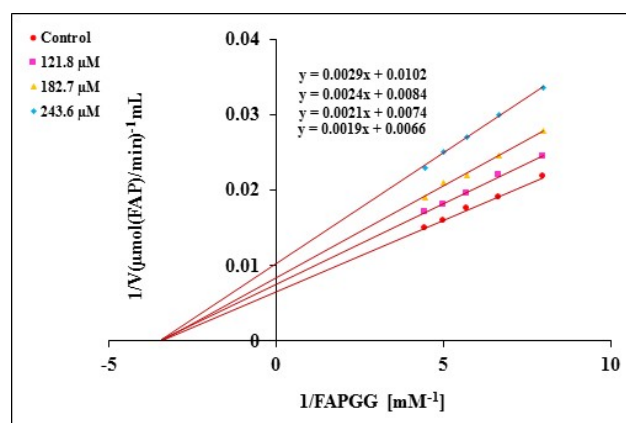


Fig. 4: Line weaver-Burk plot with five different substrate concentrations (FAPGG) and three different TQ concentrations used for the determination of inhibition type

In recent studies, antihypertensive and inhibitory effect on ACE activity of antioxidant compounds has been investigated. In a study, the effect of one of important antioxidant compound glutathione (both forms: GSSG, GSH) on ACE activity was investigated and it was reported that GSSG peptide has activator effect and GSH peptide has inhibitor effect on ACE activity. IC_{50} values for GSH peptide (16.2 μ M) and lisinopril (0.781 nM)

were calculated (Basi and Turkoglu, 2019). Hou *et al* (2003), reported that GSH (IC_{50} : 32.4 μ M), carnosine (IC_{50} : 5.216 mM), homocarnosine (IC_{50} : 6.147 mM), and anserine (IC_{50} : 6.967 mM) showed inhibitory effects on ACE activity. K_i values for GSH and carnosine were calculated as 49.7 μ M and 3.899 mM.

In addition to these compounds, there are some studies done by preparing plants extract on ACE activity. Water and butanol extracts of *Juniperus excels* Bieb. plant exhibited an inhibition effect on ACE activity purified from human plasma (Basi and Turkoglu, 2019). In another study, it was determined that Ser-Tyr (SY-a dipeptide purified from jellyfish gonad protein hydrolysates) showed significantly ACE inhibitory antioxidant activity (Zhang *et al*, 2018).

Reducing oxidative stress using antioxidants is important in regulating blood pressure. For this purpose, Diets rich in antioxidant substances are thought to have beneficial effects in protection and treatment against hypertension and cardiovascular risk factors (Hou *et al*, 2003, Baradaran *et al*, 2014, Ahmad *et al*, 2017, Mozos *et al*, 2018, Borghi and Omboni, 2020, Chiu *et al*, 2021, Martinelli *et al*, 2021).

Lycopene is the well knowing antioxidant, it naturally present in fruits and vegetables. There are many experimental and epidemiological to verify the protective role of carotenoids including lycopene in chronic diseases caused by oxidative stress. Known as effective antioxidants, carotenoids (lycopene, beta carotene and vitamin E etc.) are found in abundant products such as tomato extract. Carotenoids are scavenged the peroxy radicals more efficiently as compared to any other ROS (Li and Xu, 2013, Ghaffari and Roshanravan, 2020).

Lycopene has increasing effect of the antioxidant capacity, and has beneficial effects on immune system, antioxidant capacity, DNA damage. Lycopene shows a stronger singlet oxygen extinguishing ability than α -tocopherol. Patients treated with low-dose ACE inhibitors (calcium channel blockers, low-dose diuretics) have been reported to have a clinically significant reduction in blood pressure when given lycopene-rich tomato extract (Zhu *et al*, 2011, Ozmutlu *et al*, 2012, Karahan *et al*, 2018).

Ozmutlu *et al*. (2012) investigated the effect of lycopene on controlling ACE enzyme activity, which plays an important role in the pathogenesis of diabetes complications, and stated that it may be beneficial for the treatment and prognosis of the disease.

In recent years, some important researchers have been conducted to explain the use and effects of *N. sativa* and TQ, which is its main constituent, in various diseases such as inflammation, diabetes, dyslipidemia, hypertension and obesity (Mohtashami *et al*, 2016).

TQ is the most active ingredient of the essential oil of *N. sativa* seeds, which is well known and has been studied, with antioxidative properties and vasodilating effects. There have been some studies showing that *N. sativa* has a beneficial effect on cardio metabolic risk factors, including blood pressure. TQ contributes to improving endothelial function in part aging by preventing oxidative stress and regulation the angiotensin system (Idris-Khodja and Schini-Kerth, 2012, Sahebkar *et al*, 2016, Fatima Shad *et al*, 2021).

N. sativa and TQ supplements increase mean arterial pressure and heart rate. It also reduces systolic and diastolic blood pressure through antioxidant activity (Fallah-Huseini *et al*, 2017).

In a study by Khattab and Nagi (2007), the thymoquinone compound was observed to have therapeutic effect against experimentally induced hypertension and kidney damage in rats.

Basi and Türkoğlu (2018) studied on the inhibition effect of fatty acids isolated from *N. sativa* plant on ACE activity was investigated. The 6 isolated fractions were found to have an inhibition effect based on IC50 values (1.597 mg/mL, 0.053 mg/mL, 0.527 mg/mL, 0.044 mg/mL, 0.136 mg/mL, respectively). In this study, the inhibitory effect of TK, which is one of the important antioxidant substances and the component of *N. sativa*, was determined. Inhibition types of TQ and LYC are non-competitive inhibition.

CONCLUSION

As a result, the inhibitory effect of lycopene and thymoquinone, an important antioxidant compounds, on ACE enzyme activity was investigated *in vitro* and found to be effective by non-competitive inhibition. It was concluded that these data could be used as an important criterion in determining the importance and location of lycopene and thymoquinone among possible ACE inhibitors. This suggests that antioxidant compounds may also have antihypertensive effect, which reduces oxidative stress.

These antioxidant compounds, which reduce oxidative stress, have also been found to have antihypertensive effect. Unlike synthetic ACE inhibitors with side effects, these natural compounds have no side effects. This aspect is also very advantageous. The obtained results can evaluate to prepare new drug and approach related that TQ and LYC for future studies.

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