Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 12(3): 1607 - 1614, 2022
Journal of the Institute of Science and Technology, 12(3): 1607 - 1614, 2022

ISSN: 2146-0574, eISSN: 2536-4618

Chemistry **DOI: 10.21597/jist.1109149**

Research Article

Received: 26.04.2022 **Accepted: 09.06.2022**

To Cite: Baş Z, 2022. Investigation of inhibition effect of folic acid (vitamin B9) on angiotensin-converting enzyme activity purified from human plasma. Journal of the Institute of Science and Technology, 12(3): 1607- 1614.

Investigation of inhibition effect of folic acid (vitamin B_9) on angiotensin-converting enzyme activity purified from human plasma

Zehra BAŞ

ABSTRACT: Inhibition of the angiotensin-converting enzyme (ACE, EC 3.4.15.1) is one of the most important hypertension treatments. Here, ACE was purified from human plasma with affinity chromatography. The purity and molecule weight of ACE were identified utilizing the SDS-PAGE and viewed in two bands at around 60 kDa and 70 kDa on the gel. K_M and V_{max} constants from the Lineweaver-Burk graphic were computed to be 0.6 mM and 175.44 (µmol/min).mL^{-1}, respectively. The effects of folic acid (vitamin B_9) on purified ACE were studied. Folic acid on purified ACE demonstrated an inhibitory efficacy. The IC_{50} value for folic acid was calculated to be 127.94 µM. Kind of inhibitory and K_i constant for folic acid were defined. The kind of inhibitory for folic acid was found as non-competitive inhibitory. K_i constant was computed to be 226.59 µM for folic acid. In this study, it was concluded that folic acid, which shows an inhibitor efficacy on ACE, may have both therapeutic and protective impacts against hypertension.

Keywords: Angiotensin-converting enzyme, folic acid (vitamin B_9), inhibition, purification.

Zehra BAŞ (**Orcid ID**: 0000-0002-4071-9744), Van Yüzüncü Yıl University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Van, Turkey.

***Corresponding Author*:** Zehra BAŞ, e-mail: zehrabas@yyu.edu.tr

INTRODUCTION

Hypertension is a common chronic disease that threatens the health of middle-aged and elderly people. It causes serious diseases such as atherosclerosis, heart failure, coronary artery disease, and heart attack (Yu et al., 2020). One of the most important targets for the control of hypertension is the inhibition of the angiotensin-converting enzyme (ACE). The ACE in the renin-angiotensin system is a key enzyme that regulates blood pressure by providing transformation of angiotensin I to angiotensin II and inhibiting the bradykinin component. Therefore, reducing the concentration of angiotensin II by inactivating the ACE in the body is of great importance in patients with hypertension (Tu et al., 2018). However, synthetical ACE inhibitors like benazepril, captopril, and enalapril, which are utilized in the cure of hypertension, have side impacts like allergic reactions, skin rashes, and kidney failure (Liu et al., 2018). In recent experimental and clinical studies, the inhibitory effects of peptides, antioxidant components, herbs, and vitamins, which are determined to have few adverse impacts, on ACE are examined. For example, PNVA and PNLG peptides from sea cucumber-modified hydrolysates showed an inhibitory efficacy on ACE activity. IC_{50} values of these peptides were found as 8.18 ± 0.24 and 13.16 ± 0.39 μM, respectively (Li et al., 2018). The extracts of the *Matricaria chamomilla* L. and *Juniperus excelsa* Bieb. demonstrated an inhibitory impact on ACE from human plasma (Bas et al., 2022; Basi et al., 2019). In our previous work, reduced glutathione (GSH) inhibited ACE purified from human plasma. K_i and IC_{50} constants for GSH were computed as 11.7 μM and 16.2 μM, respectively (Basi and Turkoglu, 2019).

Folate, also known as vitamin B_9, is a water-soluble vitamin. Folate is an essential vitamin naturally obtained from foods, especially dark green leafy vegetables. Folic acid is the manufactured form of vitamin B_9 commonly found in supplements and foods enriched with vitamins. For folic acid to become metabolically active in humans, it must first be converted to dihydrofolate (DHF) and then to tetrahydrofolate (THF) by the enzyme DHF reductase (DHFR). THF participates in single carbon transfer reactions necessary for the synthesis of nitrogenous organic bases during DNA and RNA replication during cell division. Folic acid plays a major role in the maturation of red blood cells (Botez, 1976; Weinstein et al., 2003; Liew et al., 2016). In folate vitamin deficiency, many negative diseases such as megaloblastic anemia, hyperhomocysteinemia, cardiovascular diseases, neurological and cognitive impairment, cancer onset, and neural tube defects have been observed. At the same time, in many experimental studies, it has been designated that folic acid supplementation is protective and curative against many diseases like hypertension, cancer, coronary artery disease, and stroke (Liew et al., 2016).

Here, the in vitro inhibitory efficacy of folic acid (vitamin B_9) on ACE purified from human plasma was explored. Folic acid displayed a substantial inhibitory efficacy on purified ACE. The efficacy of folic acid on ACE in human plasma has not been explored to date.

MATERIALS AND METHODS

Materials

Folic acid, lisinopril, N-[3-(2-Furyl)acryloyl]-L-phenylalanyl-glycyl-glycine (FAPGG), $Na_2B_4O_7.10H_2O$, HepesNa, and lisinopril from Sigma-Aldrich were purchased. NHS-activated Sepharose 4 Fast Flow was purchased from GE Healthcare Life Sciences.

Obtain the Human Plasma

Blood instances were got from Van Red Crescent Blood Center of Turkey. The blood instances were gathered in tubes including anticoagulant, afterwards, the tubes were centrifuged (1500 ×*g*,

20 min) and the plasma was attentively split from the blood. The plasma was anew centrifuged for 1 h (8500 ×*g*, 4 °C) to separate the intact cells and ghosts. A clean plasma was gained. The acquired plasma was utilized for tests.

Purification with Affinity Chromatography Procedure

NHS-activated Sepharose 4 Fast Flow (gel) was added onto the colon (1cm×10 cm) by a equilibration tampon (pH 8.0, 20 mM Tris, 0.3 M NaCl). The flux rates were adjusted to 40 mL/h with a peristaltic pump. Human plasma added to the affinity column was equilibrated with an equilibration buffer. Cleaning of the gel was continued until the absorbance measurement at 280 nm was 0.1. Next, the elution buffer $Na_2B_4O_7.10H_2O$ (pH 9.0, 50 mM) was passed through the column with a peristaltic pump. The elution with $Na_2B_4O_7.10H_2O$ was acquired to be 1.5 mL fragments. The ACE in the fragments was gauged at 345 nm. The purified ACE was sliced into fragments and stored in a deep freeze (Pantoliano et al., 1984; Sabeur et al., 2001; Basi and Turkoglu, 2018).

ACE Activity Specification

The ACE activity was described at 35 ^oC utilizing the Holmquist process at 345 nm. The measuring cuvette, whose absorbance was measured, contained human plasma ACE, 50 mM HepesNa tampon (pH 7.5, 10 µM $ZnCl_2$, 0.3 M NaCl), and 1 mM FAPGG (Holmquist et al., 1979; Andújar-Sánchez et al., 2003).

Protein Specification

Protein concentrations of the purified fractions and human plasma hemolysate were specified by the Bradford method (Bradford, 1976).

Molecule Weight Specification by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The purity and molecule weight of ACE were defined utilizing the SDS-PAGE procedure (Laemmli, 1970).

Preparation of Folic acid (vitamin B_9) Solution

5 mg of folic acid (vitamin B_9) was resolved in a little pure water and it was complemented to 5 mL utilizing pure water.

Inhibition Effects of Folic Acid (vitamin B_9) on ACE

The efficacy of folic acid (vitamin B_9) on ACE from plasma was explored. Several concentrations of folic acid were attached to the test tube containing 100 µL ACE, 50 mM HepesNa tampon, and 1 mM FAPGG for the description of the ACE activities and the concentration range. With the obtained concentration values, the Activity% versus inhibitor graphic was formed. The IC_{50} constant of folic acid was computed from the equality of the inhibitory graphic. The Lineweaver-Burk graphic was formed with 5 diverse FAPGG values and 3 diverse values of folic acid. The inhibitory type and K_i value of folic acid from this graphic were defined (Lineweaver and Burk, 1934).

RESULTS AND DISCUSSION

ACE plays a substantial role in the arrangement of blood pressure. Herein, ACE in the human plasma was purified by the affinity chromatography procedure. NHS-activated Sepharose 4 Fast Flow to be matrix and lisinopril was utilized as a ligand. NHS activated Sepharose 4 has the high flux and steadiness features of Fast Flow column packing. Therefore, in this study, purification with very high

purity and in one step was successfully carried out. Also, the in vitro inhibitor efficacy of folic acid (vitamin B_9) on the purified ACE was explored.

The ACE enzyme, which is a membrane protein located on the lumen surface of the cell membrane, has two forms, namely the somatic form and the germinal form (Bernstein et al., 2018). In a work by Strittmatter et al, the molecule weight with SDS-PAGE of ACE from rat lung and brain corpus striatum was determined to be 175 kDa and 165 kDa, respectively (Strittmatter et al., 1985). In our before works, the molecule weight of the ACE from sheep lungs and sheep kidneys was defined to be 70 and 60 kDa with SDS-PAGE (Aydin et al., 2021; Kiylik et al., 2022). In this study, the molecule weight and purity of the ACE from human plasma were described using SDS-PAGE, and 70 kDa and 60 kDa bands were analyzed on the gel (Figure 1).

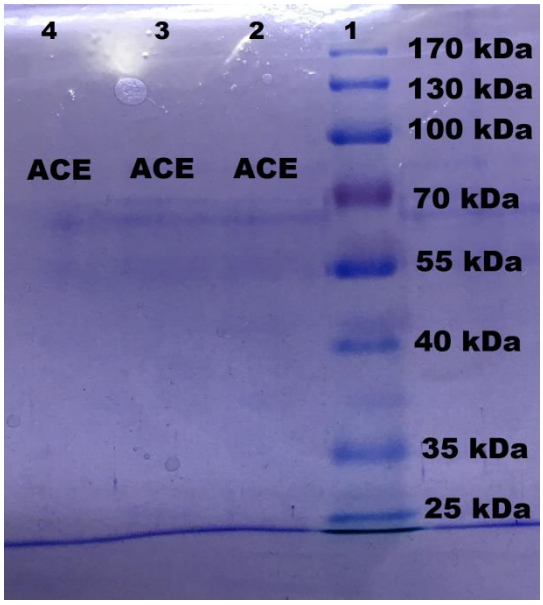

Figure 1. SDS-PAGE gel electrophoresis of ACE purified. Lane 1: Standard proteins (Fermentas unstained protein ladder SM0671). Lanes 2, 3, and 4: purified ACE from human plasma.

One of the most important causes of hypertension is the high activity of the ACE enzyme. Therefore, synthetical ACE inhibitors are generally utilized in the cure of this disease. However, these drugs accumulate in the body and cause many side effects. For this reason, many works have investigated the efficacy of native ingredients like herbs, peptides, and vitamins on ACE. For example, in a work, two ACE inhibitory peptides Glu–Ala–Leu–Val–Ser–Gln–Leu–Thr–Arg and Ala–Asn–Ser–Glu–Val–Ala–Gln–Trp–Arg were obtained from *Trichiurus lepturus* myosin. IC_{50} values of these peptides were computed to be 91.48 µM and 89.58 µM, respectively (Fu et al., 2019). In our previous work, NADH showed inhibitory effects on ACE in sheep kidneys. K_i and IC_{50} constants of NADH were designated as 175.08 µM and 244.33 µM, respectively (Kiylik et al., 2022). In another work, the IC_{50} constants for fosinopril, captopril, and lisinopril, which indicate an inhibitory efficacy on ACE purified from bovine lung, were designated as 1.159 µM, 0.835 nM, and 4.085 nM, respectively. GSH peptide and nicotinamide (vitamin B_3) indicated inhibitory effects on ACE in sheep kidneys. IC_{50} constants for these components were designated as 7.3 µM and 14.3 µM and K_i constants were 6.7 µM and 15.4 µM. The inhibitory type of these components was designated as non-competitive (Bas, 2021). Extracts of *Thymbra sintenisii*, *Coriandrum sativum*, and *Chaerophyllum macropodum* plants demonstrated inhibitory effects on ACE in human plasma (Gür et al., 2020; Çelikezen et al., 2021).

Herein, the inhibition efficacy of folic acid (vitamin B_9) on pure ACE was examined. Folic acid displayed a major inhibitory efficacy with IC_{50} values of 127.94 µM (Figure 2). K_i values and

inhibition type were determined by Lineveawer-Burk charts. The type of inhibitory for folic acid was described to be reversible non-competitive inhibitory (Table 1). K_i value of folic acid was calculated as 226.59 µM (Figure 3).

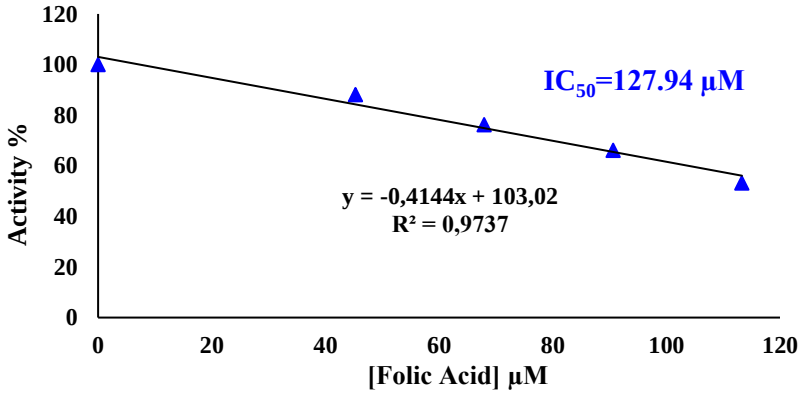

Figure 2. The inhibitory efficacy of folic acid (vitamin B_9) on ACE from human plasma. Four diverse folic acid (from 45.32 to 113.3 µM) values on ACE were examined.

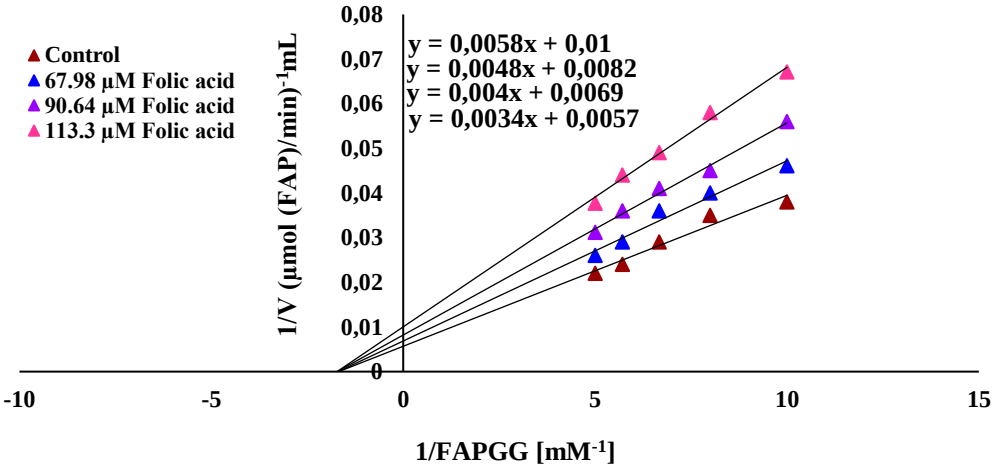

Figure 3. Lineweaver–Burk graph with five diverse substrate concentrations (FAPGG) and three diverse folic acid (vitamin B_9) concentrations used for the determination of the inhibitory type and K_i.

Table 1. Comparison table of IC_{50}, K_i values, and inhibitory types acquired from regression analysis graphs for ACE in the presence of diverse inhibitors and peptides concentrations.

ACE Inhibitor	IC_{50}	K_i	Inhibition type	References
Folic acid (Vitamin B_9)	127.94 µM	226.59 µM	Non-competitive	This work
Reduced glutathione (GSH)	16.2 µM	11.7 µM	Non-competitive	(Basi and Turkoglu, 2019).
PNVA peptide	8.18±0.24 µM	-	-	(Li et al., 2018)
PNLG peptide	13.16±0.39 µM	-	-	(Li et al., 2018)
NADH	244.233 µM	175.08 µM	Non-competitive	(Kiylik et al., 2022).
Nicotinamide (Vitamin B_3)	14.3 µM	15.4 µM	Non-competitive	(Bas, 2021).
Reduced glutathione (GSH)	7.3 µM	6.7 µM	Non-competitive	(Bas, 2021).

Biochemical features were designated to further characterize the ACE purified from human plasma. Evaluations were performed for the ACE at 5 diverse FAPGG values. Then, the Lineweaver-Burk graphic was formed with these concentrations (Figure 3). With this graphic, K_M and V_{max} constants were found to be 0.6 mM and 175.44 (µmol/min).mL^{-1}, respectively.

Folic acid or folate, called vitamin B_9, is one of the B vitamins. Folic acid, which is transformed into folate by the body, is used as a nutritional supplement and in food supplements. Folate is necessary for metabolizing amino acids required for cell division and the production of DNA and RNA (Choi et al., 2014; West et al., 2020). In many in vivo works, it has been observed that folic acid has preventive and therapeutical impacts against many diseases such as cardiovascular diseases, hypertension, coronary artery disease, and cancer. For example, in twelve randomized controlled trials, a folic acid supplement of at least 5000 mg/day for 2 to 16 weeks was administered to hypertensive subjects. The pooled estimate of the efficacy on systolic blood pressure (SBP) and diastolic blood pressure (DBP) after folic acid supplementation was -2.03 mm Hg. Folic acid supplementation reduced SBP (McRae, 2009). A study by Qin et al. observed that folic acid addition substantially decreased the advance of carotid intima-media thickness, especially in subjects with elevated cardiovascular disease or chronic kidney disease (Qin et al., 2012). A meta-analysis based on randomized studies found that folic acid supplementation decreased the stroke risk by 8% (Hou et al., 2012).

CONCLUSION

Hypertension, a serious health problem, is a significant risk factor responsible for cardiovascular diseases. One of the causes of hypertension is high ACE activity. Therefore, one of the most used drugs in hypertension patients is ACE inhibitors. However, synthetic drugs like fosinopril, captopril, and lisinopril have been found to have many adverse impacts. Therefore, in recent works, the inhibitory efficacy of native components on ACE has been explored. Folic acid (vitamin B_9) displayed a significant inhibitor efficacy with IC_{50} values of 127.94 μM on ACE activity. Vitamins are essential organic compounds taken from natural foods for the growth and development of living things. Also, many in vivo works have indicated that folic acid supplementation has a treatment efficacy on many illnesses like hypertension, stroke, cardiovascular diseases, cancer, kidney disease, and hyperhomocysteinemia. In this in vitro study, it was concluded that folic acid may be protective against hypertension by showing an inhibitory effect on ACE.

REFERENCES

Andújar-Sánchez M, Cámara-Artigas A, Jara-Pérez V, 2003. Purification of angiotensin I converting enzyme from pig lung using concanavalin-A sepharose chromatography. Journal of Chromatography B, 783: 247-252.

Aydin F, Turkoglu V, Bas Z, 2021. Purification and characterization of angiotensin-converting enzyme (ACE) from sheep lung, Molecular Biology Reports 48: 4191-4199.

Bas Z, 2021. Inhibition effect of nicotinamide (vitamin B_3) and reduced glutathione (GSH) peptide on angiotensin-converting enzyme activity purified from sheep kidney, International Journal of Biological Macromolecules, 189: 65-71.

Bas Z, Turkoglu V, Goz Y, 2022. Investigation of inhibition effect of butanol and water extracts of *Matricaria chamomilla* L. on angiotensin-converting enzyme purified from human plasma. Biotechnology and Applied Biochemistry, 69: 273–280.

Basi Z, Turkoglu V, 2018. Purification of angiotensin-converting enzyme from human plasma and investigation of the effect of some active ingredients isolated from *Nigella sativa* L.extract on the enzyme activity. Biomedical Chromatography, 32: e4175.

Basi Z, Turkoglu V, 2019. In vitro effect of oxidized and reduced glutathione peptides on angiotensin converting enzyme purified from human plasma. Journal of Chromatography B Analyt. Technol. Biomed. Life Sci., 1104: 190-195.

Basi Z, Turkoglu N, Turkoglu V, Karahan F, 2019. In vitro effect of ethyl acetate, butanol and water extracts of *Juniperus excelsa* Bieb. on angiotensin-converting enzyme purified from human plasma. Chemical Papers, 73: 2525–2533.

Bernstein KE, Khan Z, Giani, JF, Cao DY, Bernstein, EA, Shen XZ, 2018. Angiotensin-converting enzyme in innate and adaptive immunity. Nature reviews. Nephrology,14: 325–336.

Botez MI, 1976. Folate deficiency and neurological disorders in adults. Med Hypotheses. 1976; 2(4):135-40.

Bradford MM, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72: 248-54.

Choi JH, Yates Z, Veysey M, Heo YR, Lucock M, 2014. Contemporary issues surrounding folic Acid fortification initiatives. Preventive Nutrition and Food Science, 19 (4): 247–260.

Çelikezen FÇ, Türkoğlu V, Fırat M, Baş Z, 2021. The Effects of *Coriandrum sativum* L. and *Chaerophyllum macropodum* Boiss. (Apiaceae) on human plasma angiotensin-converting enzyme (ACE) in vitro. BEU Journal of Science, 10 (3): 710-718.

Fu W, Chen C, Zeng H, Lin J, Zhang Y, Hu J, Zheng B, 2019. Novel angiotensin-converting enzyme inhibitory peptides derived from Trichiurus lepturus myosin: Molecular docking and surface plasmon resonance study. LWT, 110: 54-63.

Gür T, Karahan F, Baş Z, Türkoğlu V, 2020. The Determination of Inhibition Effect of Extracts of *Thymbra sintenisii* Bornm. et Aznav. Subsp on Angiotensin Converting Enzyme. Journal of the Institute of Science and Technology, 10 (3): 1848-1856.

Holmquist B, Bünning P, Riordan JF, 1979. A continuous spectrophotometric assay for angiotensin converting enzyme. Analytical Biochemistry, 95 (2): 540-548.

Huo Y, Qin X, Wang J, Sun N, Zeng Q, Xu X, Liu, L Xu, X, Wang X, 2012. Efficacy of folic acid supplementation in stroke prevention: new insight from a meta-analysis. International Journal of Clinical Practice, 66 (6): 544-51.

Karahan F, Turkoglu V, 2021 Enhanced purification protocol for the angiotensin-converting enzyme from bovine systems and investigation of the in vitro effect of some active substances Chemico-Biological Interactions, 347: 109604.

Kiylik A, Turkoglu V, Bas Z, 2022. Purification of angiotensin-converting enzyme (ACE) from sheep kidney and inhibition effect of reduced nicotinamide adenine dinucleotide (NADH) on purified ACE activity. Cell Biochemistry and Biophysics, 80: 115-122.

Laemmli UK, 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Li J, Liu, Z, Zhao Y, Zhu X, Yu R, Dong S, Wu H, 2018. Novel natural angiotensin converting enzyme (ACE)-inhibitory peptides derived from sea cucumber-modified hydrolysates by adding exogenous proline and a study of their structure–activity relationship. Marine Drugs, 16 (8): 271.

Liew SC, 2016. Folic acid and diseases - Supplement it or not? Revista da Associação Médica Brasileira, 62 (1): 90-100.

Lineweaver H, Burk D, 1934. The determination of enzyme dissociation constants. Journal of the American Chemical Society, 56: 658–660.

Liu CL, Fang L, Min WH, Liu JS, Li HM, 2018. Exploration of the molecular interactions between angiotensin-I-converting enzyme (ACE) and the inhibitory peptides derived from hazelnut (*Corylus heterophylla* Fisch.). Food Chemistry, 245: 471–480.

McRae MP, 2009. High-dose folic acid supplementation effects on endothelial function and blood pressure in hypertensive patients: a meta-analysis of randomized controlled clinical trials. Journal of Chiropractic Medicine, 8 (1): 15-24.

Pantoliano MW, Holmquist B, Riordan JF, 1984. Affinity chromatographic purification of angiotensin converting enzyme. Biochemistry, 23 (5): 1037-1042.

Qin X, Xu M, Zhang, Y, Li J, Xu X, Wang X, Xu X, Huo Y, 2012. Effect of folic acid supplementation on the progression of carotid intima-media thickness: a meta-analysis of randomized controlled trials. Atherosclerosis, 222 (2): 307-313.

Sabeur K, Vo AT, Ball BA, 2001. Characterization of angiotensin-converting enzyme in canine testis. Reproduction, 122 (1): 139-146.

Strittmatter SM, Thiele EA, Kapiloff MS, Snyder SH, 1985. A rat brain isozyme of angiotensin-converting enzyme unique specificity for amidated peptide substrates. The Journal of Biological Chemistry, 260 (17): 9825-9832.

Tu M, Wang C, Chen C, Zhang R, Liu H, Lu W, Jiang L, Du M, 2018. Identification of a novel ACE-inhibitory peptide from casein and evaluation of the inhibitory mechanisms. Food Chemistry, 256: 98-104.

Weinstein SJ, Hartman TJ, Stolzenberg-Solomon R, Pietinen P, Barrett MJ, Taylor PR, Virtamo, J, Albanes D, 2003. Null association between prostate cancer and serum folate, vitamin B(6), vitamin B(12), and homocysteine. Cancer Epidemiology Biomarkers & Prevention, 12: 1271-1272.

West AA, Caudill MA, Bailey LB, 2020. "Folate". In BP Marriott, DF Birt, VA Stallings, AA Yates (eds.). Present Knowledge in Nutrition, Eleventh Edition. London, United Kingdom: Academic Press (Elsevier). Pp: 273–88.

Yu ZP, Guo H, Shiuan D, Xia CS, Zhao WZ, Ding L, Zheng FP, Liu JB, 2020. Interaction mechanism of egg white-derived ACE inhibitory peptide TNGIIR with ACE and its effect on the expression of ACE and AT1 receptor. Food Science and Human Wellness, 9 (1): 52–57.