Research Paper

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Evaluation of In Vitro Angiotensin-Converting Enzyme Inhibitory Activity of Psidium Guajava Linn. Leaf Extract and A Study on Enzyme Kinetics

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KEYWORDS : Angiotensin Converting Enzyme; Enzyme Kinetics; Hypertension

Jomon Sebastian — Department of Biotechnology and Biochemical Engineering, Sree Buddha College of Engineering, Pattoor P.O., Alappuzha, Kerala-690 529,

Jency Blesson — Department of Biotechnology and Biochemical Engineering, Sree Buddha College of Engineering, Pattoor P.O., Alappuzha, Kerala-690 529

ABSTRACT

Psidium guajava Linn. leaf is used traditionally in Indian medicine to control hypertension in human. The aim of this work was to study the hypotensive effect of Psidium guajava leaf extract in human. Hypertension is due to the activity of the Angiotensin-Converting Enzyme that catalyzes the conversion of the inactive Angiotensin I to the active Angiotensin II. Angiotensin II functions as a potent vasoconstrictor causing hypertension. Hence, ACE inhibitors are considered to be the first choice treatment of hypertension. The ACE inhibitory activity of Psidium guajava leaf extract in human was studied by its in vitro inhibitory effect on human serum-huACE using the substrate FA-PGG (N-(3-(2-Furyl) acryloyl)-L-phenylalanyl-glycyl-glycine). The enzyme kinetics of ACE inhibition was investigated by Michaelis–Menten kinetics and Lineweaver–Burk graph and Vmax and Km values were calculated. The results show that there is a decrease in both Vmax and Km values in the presence of Psidium guajava leaf extract. The decreased Vmax and Km seen with Psidium guajava indicates that Psidium guajava binds to other alternative sites rather than the active site of the ACE and hence the inhibition is uncompetitive. The decreased Vmax/Km value indicates decreased catalytic affinity of enzyme-substrate due to the presence of the inhibitor Psidium guajava leaf extract. From these it is concluded that Psidium guajava leaf extract inhibit human serum-ACE uncompetitively and it has hypotensive effects in human.

INTRODUCTION

Hypertension is one of the most common worldwide diseases afflicting humans. It is mainly due to our food habits and life style changes. It is a major risk factor for the occurrence of cardiovascular diseases and kidney failure. Hypertension is often called a "Silent killer" because persons with hypertension are often asymptomatic for years. Hypertension is a condition in which the blood pressure in the arteries is elevated by the vasoconstriction of arteries. Angiotensin Convertin Enzyme (ACE) is a zinc carboxypeptidase (Skeggs et al., 1956), synthesized by the endothelium. It is present on the luminal surface of the endothelial cell membrane and in plasma (Baudin et al., 1997). It catalyzes the conversion of inactive angiotensin-I to active angiotensin-II, a potent vasoconstrictor. The enzyme also catalyzes the hydrolysis of bradykinin, a strong vasodilator. Formation of angiotensin-II and degradation of bradykinin by the activity of ACE causes vasoconstriction and elevation of blood pressure. Hence ACE inhibitors are considered to be the first choice treatment of hypertension.

The function of an enzyme is to catalyze and accelerate a reaction by lowering the activation energy. According to Michaelis–Menten kinetics, the rate of the reaction increases with increasing substrate concentration [S], asymptotically approaching its maximum rate (Vmax). The rate of enzymatic reactions in terms of Michaelis–Menten kinetics is given by;

$$V = \frac{V_{max}\,[S]}{K_m + [S]}$$

The enzyme inhibitors bind to the enzyme and bring down the rate of reaction. The enzyme inhibitors can be competitive, non-competitive or uncompetitive depending on their binding site on the enzyme such as active site, allosteric site or site on Enzyme-Substrate complex respectively. The enzyme inhibition kinetics can be studied with the help of Lineweaver-Burk Plot which is given by;

$$\frac{1}{V} = \frac{K_m + [S]}{V_{max}\,[S]} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$$

Lineweaver-Burk Plot is used to evaluate Michaelis–Menten's parameters Km (substrate concentration at which reaction rate is half of Vmax) and Vmax. In competitive inhibition, in order to achieve the same Vmax, the Km is to be raised. In non-competitive inhibition the Km value is unchanged while Vmax is lowered and in uncompetitive inhibition both Vmax and Km are lowered. The commonly used ACE inhibitors are Captopril, Enalapril and Lisinopril. These are synthetic drugs and cause adverse side effects such as dry cough, headache, dizziness, fatigue, nausea and renal impairment (Fitzgerald and Meisel, 2000). Therefore, it is desirable to find safe and natural ACE inhibitors for the prevention and remedy of hypertension. The leaf of Psidium guajava Linn. (Myrtaceae) is used traditionally in Indian medicine to manage, control and treat a number of human ailments including hypertension. The aim of this study was to investigate the enzyme kinetics of human serum-Angiotensin-Converting Enzyme inhibition by Psidium guajava leaf extract and to study the hypotensive effects of Psidium guajava leaf extract in human.

MATERIALS AND METHODS

Extraction of Leaves. The fresh leaves of Psidium guajava were collected from Cherthala, in Alappuzha district of Kerala, India during the month of May. The leaves were dried in hot air oven at 70oC for 24 hours. The leaves were powdered (coarse) and subjected to Soxhlet extraction using ethanol as solvent. The extracts obtained were evaporated in rotary evaporator to get a powdery mass. One gram of powder was infused in freshly boiled 20mL sterile phosphate-buffered saline (PBS) for 10min. The infusion was then filtered twice, first through a Whatman No.1 filter (11µm) and then through a Millipore filter (0.2 µm). The concentrations of the filtrates obtained were determined as 1:20 and they were frozen in aliquots at -20oC until use.

Serum Collection. Human blood was collected from healthy volunteers. Vacutainer tubes without anticoagulants were used to collect blood for serum-ACE. Approximately 5ml of blood was collected from each individual. After 2 hours the tubes were centrifuged at 10,000rpm for 20min, at 4oC. Serum was transferred to plastic tubes and ACE activity was analyzed on the same day.

The study was approved by the ethical review board at Sree Buddha College of Engineering, Alappuzha, Kerala, India.

ACE Inhibition Kinetics. N-[3-(2-furyl) acryloyl] – L-phenylalanylglycylglycine (FAPGG), synthetic tripeptide substrate of ACE, is hydrolyzed to furylacryloylphenylalanine (FAP) and glycylglycine. Hydrolysis of FAPGG results in a decrease in absorbance at 340nm. The ACE activity in the sample is determined directly as the rate of decrease in the absorbance at 340nm.

The assay was performed after modification according to Shalaby et al. (2006). The substrate used was 0.1, 0.2 and 0.4mM N-(3-(2-Furyl) acryloyl)-L-phenylalanyl-glycyl-glycine (FAPGG) in 50mM tris-HCl containing 0.3M NaCl, pH 7.5. Human serum diluted in PBS to the ACE concentration 0.25units/L was freshly

prepared each day. 50µL of serum and 5.6µL of Psidium guajava leaf extract or Control taken in three different ependorf, were preincubated for 10min at 37oC. Then 500µL of substrate solution (0.1, 0.2 and 0.4mM) was added to each ependorf during less than 1min and the reaction started. The absorbance at 340nm was measured in spectrophotometer for every 1min for a running time of 10min. The concentration of Psidium guajava leaf extract used in this study was 1:80. The experiment repeated for three times. Enzyme activity was calculated as the rate of decrease in the absorbance at 340nm.

Chemicals.N-(3-(2-furyl) acryloyl)-L-phenylalanyl-glycyl-glycine (FAPGG) was obtained from Sigma-Aldrich Chemicals Co. (St Louis, MO, USA) and Tris-HCl was bought from Merck. All the other reagents used were of analytical grade and locally purchased.

Calculations. Statistical calculations were performed with GraphPad Prism 5.0.

RESULTS AND DISCUSSION

ACE Inhibition Kinetics. The rate of decrease in the absorbance per minute (ΔA/min) were calculated for both Control and Psidium guajava leaf extract inhibition which corresponds to the rate of conversion of substrate into product by enzyme human serum-ACE. The reciprocals of substrate concentration and rate of reaction were calculated and they are given in Table 1. These values were used to plot Lineweaver–Burk graph in order to calculate Vmax and Km values. The graphs are shown in Figure 1a & 1b.

	1/S	1/V
Control	2.5	224.7
	5	526.32
	10	714.28
P. guajava Inhibition	2.5	625
	5	880.95
	10	1294.1

Table 1: Reciprocals of substrate concentration and rate of reaction

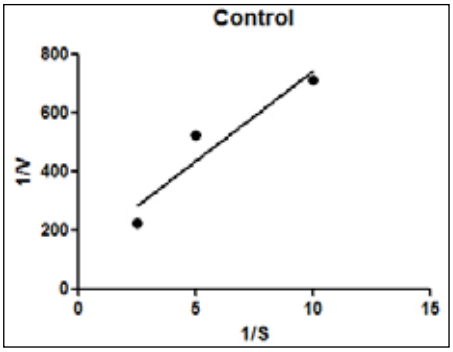

Figure 1a: Lineweaver–Burk graph of Control, r^2=0.899

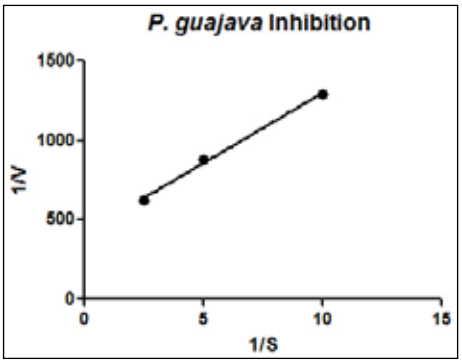

Figure 1b: Lineweaver–Burk graph of Psidium guajava Inhibition, r^2=0.996

The Lineweaver–Burk graph showed Vmax=0.0077µM/min and Km=0.472µM for Control and Vmax=0.0023µM/min and Km=0.203µM for Psidium guajava leaf extract inhibition. The Vmax/Km values were calculated to be 0.016min-1 for Control and 0.011min-1 for Psidium guajava leaf extract inhibition. The values are given in Table 2.

	Control	P. guajava Inhibition
Vmax (µM/min)	0.0077	0.0023
Km (µM)	0.472	0.203
Vmax/Km (min-1)	0.016	0.011

Table 2: V_{max} and K_m values of Control and Psidium guajava Inhibition

Vmax is the rate at which a substrate is converted to a product once bound to the enzyme and Km shows how effectively the enzyme binds to the substrate. These two values show the affinity of enzyme-substrate and give the quantity value of the enzyme inhibition. The decreased Vmax and Km (Vmax=0.0023µM/min; Km=0.203µM) seen with Psidium guajava as compared to Control (Vmax=0.0077µM/min; Km=0.472µM) show that the inhibitor uncompetitively binds to the enzyme.

The decreased Vmax and Km seen with Psidium guajava indicates that Psidium guajava binds to other alternative sites rather than the active site of the enzyme, and this might be due to the presence of flavonoids in Psidium guajava leaf extract. The Vmax/Km value represents the catalytic affinity of enzyme-substrate. The decreased Vmax/Km value of Psidium guajava (0.011min-1) compared to that of Control (0.016min-1) indicates decreased catalytic affinity of enzyme-substrate due to the presence of the inhibitor Psidium guajava leaf extract.

CONCLUSIONS

The ACE inhibitory activity of P. guajava leaf extract in human was studied by its in vitro inhibitory effect on human serum-ACE using the substrate FAPGG. The results show that there is a decrease in both Vmax and Km values in the presence of P. guajava leaf extract. The decreased Vmax/Km value indicates decreased catalytic affinity of enzyme-substrate due to the presence of the inhibitor P. guajava leaf extract. From these results, it is concluded that P. guajava leaf extract has hypotensive effects in human.