

## Ace (Angiotensin Converting Enzyme) Inhibitory Activity of Apple Extract

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### ABSTRACT:

Hypertension is causative risk issue for disorder. RAAS (Renin-Angiotensin-Aldosterone-System) is one among the components that contribute in maintenance of circulatory pressure and electrolytes balance. ACE (Angiotensin changing Enzyme) is main player of RAAS system. Purpose of the study was to try to find ACE repressive activity of apple extract. varied dosages of the extract were used and ACE activity was calculable. therefore, on discover the causative issue phytochemical examination of apple extract was done. Finding of this study shows that significantly ( $p < 0.001$ ) ACE repressive activity of apple extract in a dose dependent manner. Highest ability to inhibit ACE activity was seen in 500  $\mu$ l extract of apple.

**Keywords:** ACE Inhibitory Activity, Apple, Natural ACE Inhibitor

### 1- INTRODUCTION:

Hypertension is one in all the greatest causative factor of inability, mortality on the people and contribute within the improvement of a few cardiovascular pathology including atherosclerosis, coronary conduit ailments, myocardial infarction, cardiovascular breakdown, renal inadequacy, stroke [1] it's notable that among a number of categories of antihypertensive medicine ACE (Angiotensin changing Enzyme) inhibitors are one in all the influential medicine for the treatment of cardiovascular disease, they in addition suggested for treating patients with Secondary disease [2]. ACE is a conjugated protein, peptidyl dipeptidase hydrolyase, whose basic capability is to divide dipeptide (Histidin-leusine) from Angiotensin-I (Ang-I) to Angiotensin-II (Ang-II) one of the effector peptide of RAAS (Renin-Angiotensin-Aldosterone-System) which expect a critical participation in fluid and electrolyte homeostasis and has been involved as a causative factor for hypertension [3]. Synthetic ACE inhibitors, for instance, Captopril, enalapril, lisinopril and rampril are generally utilized for the treatment of cardiovascular disease, nonetheless these medications exhibit some unfavorable impacts, for example, postural hypotension, cough, kidney failure and angioedema [4]. Therefore, recently attention has been targeted towards natural and mineral preparation that is traditionally used as potential therapeutic agents in interference and management of cardiovascular diseases. *In vitro* screening of medicinal plants is a fast method often used to search new ACE inhibitory compounds. a number of plants, for instance, *Hibiscus sabdariffa* [5], *Vitex doniana* [6] and cell reinforcement peptides (decreased glutathione and carsine-related peptides) are reported for their ACE repressing activity.

However, Apples are a widely consumed, rich source of phytochemicals, and epidemiological studies have linked the consumption of apples with reduced risk of some cancers, cardiovascular disease, asthma, and diabetes. In the laboratory, apples have been found to have very strong antioxidant activity, inhibit cancer cell proliferation, decrease lipid oxidation, and lower cholesterol. [7]. Purpose of the study was to search out ACE repressing activity of apple extract.

### 2- MATERIAL AND METHODS:

**2.1- Materials:** Hippuryl-1-histidyl-1-leucine (HHL) was bought from letter Aldrich; St. Louis, MO. Cyanuric chloride was bought from Alfa Aesar, Lancaster. HEPES (N-(2-hydroxy ethyl)- piperazine-N'- 2-ethan sulphonic acid) support was bought from loba synthetics.

**2.2- Extraction of Fruit:** The fruit was bought from neighborhood market. liquid extract of apple was ready by mixing one gram of fruit pulp and also the five volumes of water; this was shaken for one day. Following

day mix was re-extracted with water and concentrates were pooled to 50ml with water and from this 100µl - 500µl aliquots were ready.

**2.3- Enzyme Source:** ACE protein was extracted from lungs of freshly killed male goat, that was dropped at centre from the slaughter house, placed at Rajkot underneath frozen condition. One gram of tissue was homogenate with 10ml of 100mM salt buffer, containing 50mM KCl; pH eight.3. The homogenate was centrifuged at twenty,000rpm for twenty min. at 4°C. To avoid the interference of molecules having low relative molecular mass in protein activity supernatant was dialyzed against 20-volume of same buffer and hold on at -20°C throughout experiment.

**2.4- Ace Assay:** ACE protein was studied utilizing method depicted by MallikarjunaRao[8]. In short, 50µl of enzyme source and 0.2ml of 5mM HHL, organized in 200mM salt buffer, containing 1000mM KCL, PH 8.3, was incubated at 37°C for 30 min. Volume of the mixture was pooled up to 1ml with distilled water. The reaction was end by addition 2ml of 100mM HEPES (N-(2-hydroxy ethyl)- piperazine-N'- 2-ethan sulphonic corrosive) buffer, containing 2.5mM EDTA, PH-9.0. To this 1ml 136mM Cyanuric chloride, prepared in 1, 4-dioxane was add and mix well. The hippuric acid concentration of reaction mixture was measured at 405nm. For ACE repressive activity of apple extract 100µl, 200µl, 300µl, 400µl, 500µl doses were prepare in distilled water and add to reaction mixture and analyze with utilizing same technique. The hippuric acid concentration within the lung homogenates were examine from the standard curve, prepared from numerous concentration of hippuric acid (250-2500µM). One unit of ACE was process because the quantity of enzyme catalyzing release of hippuric acid over incubation time.

**2.5- Phytochemical Analyses of Fruit Extract:**Quantitative examinations of fruit extract were completed for Total phenols, flavonoids, tannins, saponins, vitamin C and FRAP (ferric decreasing cell reinforcement potential). Total phenols, flavonoids were evaluated by Folin-ciocalteu's chemical agent as mention by Ebrahimzadeh and Niknam [9] and Malick and Singh [10]. Tannins were evaluated with Folin Denis technique given by Schanderi [11], saponins content was calculable utilizing vanillin-sulphuric acid chemical agent as describe by Thimmaiah [12]. Vitamin C and FRAP were assessed utilizing technique given by Schaffert and Kingsley [13] and Benzie and Strain [14]

**2.6-Statistical Analysis:**All the analyses were wiped out triplet. Result were expressed mean  $\pm$  S.E.M. variations were calculated by one-way analysis of variance (ANOVA) check completed by Tukey's Multiple Comparison check with utilizing prism Graphpad. The differences were significant at  $p < 0.001$ .

### 3- RESULT:

Treatment of extract to lung homogenate reveled that ACE activity ranged from 32.86 units to as low as 21.43 units. There was seen decline in ACE activity because the dose of apple extract was increase as compare to control. 100µl dose of apple extract showing most ACE activity i.e. 32.86 units. Whereas moderate inhibition of ACE activity was seen by 300µl dose of apple extract i.e. 26.33 units and most inhibition of ACE activity was seen by 500µl dose of apple extract i.e. 21.43 units. therefore, ACE inhibition by apple extract was in dose depended manner. for example, percentage inhibition of ACE activity was 58.16 to 37.93; at 100µl to 500µl doses compare to control. (Table.1)

Apple extract was analyzed for total phenols, flavonoids, tannins, saponins, vitamin C and FRAP. Among mention phytochemical vitamin C content was showing in apple extract i.e.0.0612mg/ml. whereas Phenol content and flavonoids content of extract were showing, 1.89mg/ml, and 1.513mg/ml respectively. Antioxidative activity was examined as FRAP price i.e. 4.81mg/ml. but saponins and tannins content of 2.04mg/ml and 2.15mg/ml respectively.(figure-2)

### 4-DISCUSSION:

ACE could be a key regulator of RAAS, it increases blood pressure by cleaving dipeptide from Ang-I and convert it into Ang-II, an effector vasoconstrictor peptide of RAAS and inactivate bradykinin, a potent dilator. Thus, ACE inhibitors are valuable drug for treatment of cardiovascular disease and connected diseases, however these synthetic ACE inhibitors are responsible for adverse effects. In stand of ACE inhibitors several plant derived compounds area beneficial like peptides [15], fatty acids [16], triterpenes [17]. Purpose of the study was to try to find ACE repressive activity of apple extract. varied dosages of the extract were used. apparently apple extract exerts significantly ( $p < 0.001$ ) reduction in ACE activity in a remarkably dose dependent manner, however degree of ACE inhibition varies among all doses. 100µl dose of extracts exhibit weaker ACE inhibition compare with all alternative doses. but most ACE inhibition of ACE enzyme was found with treatment of 500µl doses. (Figure-1) Previous study on bamboo powder

demonstrate methanol and ethyl alcohol soluble fraction of exhibited stronger ACE inhibition in dose dependent manner than water soluble fraction and quardary soluble fraction [18].

However, ACE is Zn containing metallopeptidase, that have three parts in the active site, a carboxylate binding functionality like guanidium group of Arg, a pocket that accommodates a hydrophobic facet chain of C- terminal aminoalkanoic acid residue and a Zn atom, an energetic center of Zn dependent metallopeptidase. Some author suggested that the activity flavonoids and other polyphenols are due to the formation of chelate complex with zinc atom within the active sites of zinc dependent metallopeptidase [19]. Possibly it also results from the formation of hydrogen bound between the inhibitor and amino acid near the active site [20]. Reducing power of extract assayed often used to evaluate the ability of natural antioxidant to donate an electron or hydrogen, which may be related to presence of phenolic compounds due to substitution in aromatic ring [21]. FRAP value of apple extract mention in figure-2.

Secondary metabolites of plant like phenols, flavonols [19], tannins [22], have potential to inhibit ACE. so as to estimate quantity of those molecules in apple extract phytochemical assayed was disbursed results of that shows variation in quantity of total phenol, flavonols, saponins, vitamin C, tannins. A highest quantity of tannins was found (figure-2). Additionally, to plant derived antioxidants like water-soluble vitamin, vitamin E, P-carateone and Co accelerator letter were recently shown to possess hypotensive properties, which can be secondary to increasing convenience of dilator gas, which may have an effect on ACE activity [23].

#### **5-CONCUSION:**

ACE is a key regulator of RAAS, it increases blood pressure. Thus, ACE inhibitors are more beneficial drug for treatment of hypertension and related diseases. Therefore, recently attention has been focused towards natural ACE inhibitor. Present study shows ACE inhibitory activity of apple extract in a dose dependent manner. Thus the inhibitory activity of secondary metabolites likes phenols flavonoids, tannins and ascorbic acid may be responsible for the ACE inhibitory effect of apple extract.

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#### **7-CONFICT OF INTEREST:**

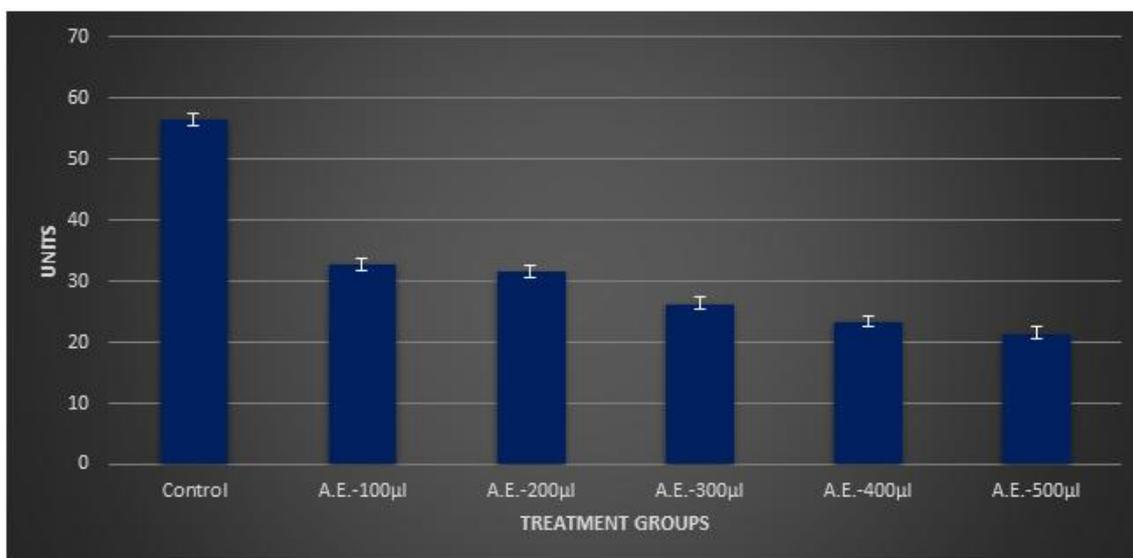
There was no conflict.

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## 9. FIGURE:



**Figure No. 1- ACE Inhibitory Activity of Apple Extract**

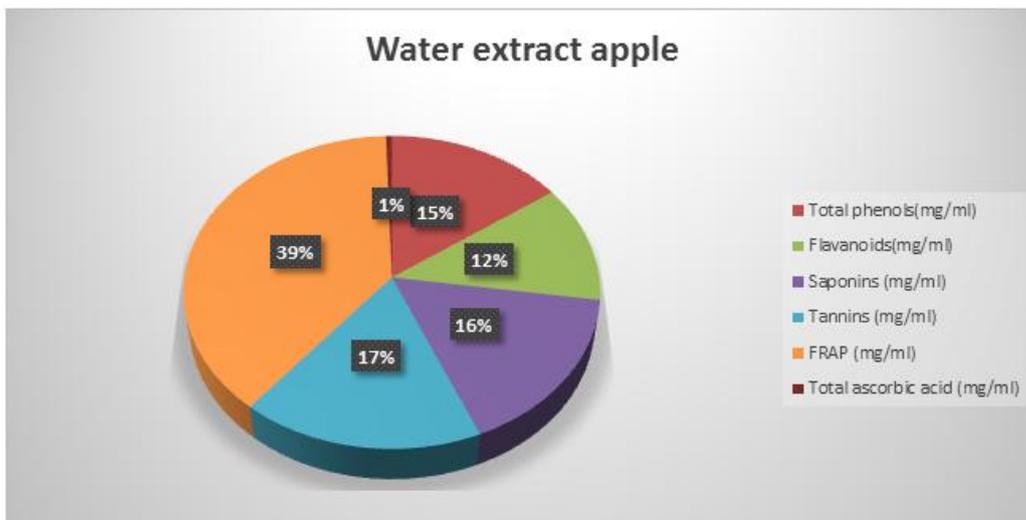


Figure no. 2-Phytochemical Analysis of Apple Extract

10-TABLE:

Table.1 ACE Activity of Apple

Control	Dose of Apple	ACE activity
56.49 ± 0.51	100µl	32.86 ± 0.059* (- 58.16%)
	200µl	31.55 ± 0.74* (-55.85%)
	300µl	26.33 ± 0.19* (-46.61%)
	400µl	23.43 ± 0.38* (-41.47%)
	500µl	21.43 ± 0.56* (-37.93%)

Values are express as mean ±SEM P<0.001 each comparisons were done between control and treatment groups.