Effect of Areca Nut Extracts on some Digestive Enzymes *in vitro*

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The Areca nut extracts (*n*-hexane and methanol) were used on three important digestive enzymes (α-amylase, α-chymotrypsin and lipase) *in vitro*. The result showed significant reduction of α-amylase activity (between 47-64% inhibition). Whereas, both extracts of *Areca* produced significant increase in lipase activity (54% and 27%, respectively). Significant increase in α-chymotrypsin activity (78%) with *n*-hexane extract and a reduction of (18%) with methanol extract were seen.

Key words: Areca nut, Hexane, Methanol, α-Amylase, α-Chymotrypsin, Lipase.

Introduction

Areca nuts are the seeds of *Areca catechu* L. (Family: Palmae) a feather-palm, 15-17 meter high, which is cultivated in Sri Lanka, the Malay States, South China, the East of Indies, the Philippine Islands and parts of East Africa which are including Zanzibar and Tanzania (Trease and Evans 1978).

The areca nut is about 2.5 cm in length and rounded conical in shape. The seed is very hard, has a faint odor when broken, and has an astringent and somewhat acrid taste. The different types of areca nuts described from different countries are mostly based on the size of the fruits (Raghavan and Baruach 1958).

The areca nuts consist of two distinct parts, the husk and the endosperm. Husk contains pectin, protopectic, hemicellulose, cellulose and lignin (Erfan and Khundkar 1953) while the endosperm is a rich source of alkaloids, tannins, fats, carbohydrates, protein, non-protein nitrogen and minerals (Trease 1952).

The major alkaloids are reduced pyridine derivatives, arecoline (methyl ester of arecain), arecain or arecaidine (*N*-methyl derivatives of guvacine). Two other alkaloids like guavacine (1: 2: 5: 6-tetrahydropyridine-3-carboxylic acid) and guvacoline (methyl ester of guvacine) are also present (Mencke and Holmes 1950).

The areca nuts tannins are predominantly catechol tannins containing tannic acid, catechol, gallic acid and phlobatannin. The endosperm of the areca nuts is also reported to contain fats; varying from 1.3 to 17.0%. The chief components of the fatty acids are lauric (19.5%), myristic (46.2%) and palmitic (12.7%) (Khan and Chughtai 1956).

In addition to the alkaloids, tannins and fats, which have been comparatively more investigated, the endosperm is also stated to contain carbohydrate, protein and non-protein, gums, saponins and vitamin A (Raghavan and Baruach 1958).

Areca nut extract is toxic to cultured fibroblasts and inhibits their proliferation in a concentration-dependent manner (Van Wyk *et al* 1994). Betel quid contains not only genotoxic and cytotoxic agents, but also compounds that stimulate cell proliferation. These compounds may act synergistically in the pathogenesis of oral cancer in the chewers (Jeng *et al* 1994).

Some other studies clearly demonstrate that the HO radical is formed in the human oral cavity during betel quid chewing and is probably implicated in the genetic damage that has been observed in oral epithelial cells of chewers (Nair *et al* 1995).

Concerning the effect on hepatic detoxifying enzymes, the modulator potential of areca nut was assessed on the black mustard that induced changes in hepatic detoxification system in mice. The modulatory effect was assessed on glutathione S-transferase (GST), cytochrome β5 (Cyt. B5) and cytochrome P-450 (Cyt. P-450) and acid-soluble sulfhydryl (-SH) content (Singh and Rao 1993).

The cardiovascular effect of areca nut, were studied in 47 healthy male subjects. Cardiovascular responses were measured, which included heart rate and blood pressure changes following betel chewing. These results indicate that betel chewing can affect cardiovascular function, and that tolerance to the pressure response may occur in habitual users (Chu 1993).

A study (Inokuchi *et al* 1986) had shown that areca II-5-C, a fraction isolated from seeds of *Areca catechu* showed a potent angiotensin-converting enzyme (ACE) inhibitory activity *in vitro*, this means that *Areca* has antihypertensive properties and can be used in therapeutic formulations.

Recent works showed oxidative damage to DNA induced by areca nut extract (Liu *et al* 1996), Cytotoxic and DNA damaging effects in oral mucosal fibroblasts by areca nut extract (Fang *et al* 1997), extract also alters the barrier properties of the epithelium, a factor which may play a role in the deleterious effects on oral mucosa (Van Der Bijl and Thomspon 1998) and effects on cytotoxicity, total and unscheduled DNA synthesis in cultured gingival keratinocytes (Jeng *et al* 1999).

This study was undertaken to provide some scientific grounds to understand some of the biochemical and pharmacological properties of areca nut. To achieve this goal, this study was conducted by extraction of the active ingredients by using two solvents of different polarities and testing the various extracts on three enzymatic systems (*in vitro*) that have important roles in the digestion of carbohydrates, proteins and fats. α-Amylase (α-1, 4-glucan-4-glucanhydrolase, EC 3.2.1.1) is present in the salivary glands and the pancreas, which is primarily responsible for the digestion of starch to maltose as a major end product. α-Chymotrypsin (a proteolytic enzyme, EC 3.4.21.1) is present in the pancreas as one of several proteases that preferentially hydrolyzes peptide bonds involving carboxyl groups of aromatic amino acid residues (Calbreath 1992). Lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) is present mainly in the pancreas, and is responsible for the hydrolysis of triglycerides .

Materials and Methods

Material. Samples of areca nut (*Areca catechu*) was bought from a local market and used in this study.

Extraction of samples. Dried weights of 43 g and 50 g of areca nut (whole) was soaked for 20 days in dark bottle containing 0.5 liter of *n*-hexane and 0.3 liter of methanol, respectively. By filtering through a filter paper, the residues were discarded and the crude *n*-hexane or methanol extracts were refiltered with Whatman filter paper (42 ashless, Whatman Ltd., UK). The solvents were removed by evaporation, at 80°C for *n*-hexane extract, and 90°C for methanol extract for maximum extraction, using model R110 Buchi Rotary Evaporator (Abo-Khatwa and Kubo 1987). The weights of the *n*-hexane extract and methanol extract were recorded and each sample was dissolved in 10 ml acetone each.

Chemicals. All chemicals and solvents used in this study were of purest analytical grade. α-amylase (bacterial source), α-chymotrypsin (from bovine pancreas), starch (soluble), succinyl-Ala-Ala-Pro-Phe-p-nitroanilide (a substrate for α-chymotrypsin), tris (hydroxymethyl) aminomethane, olive oil (substrate for serum lipase), sodium azide and thymolphthalein were purchased from Sigma Chemical Co. Calcium chloride and sodium chloride were purchased from Merck Co. Maltose was purchased from Emscope Laboratories Ltd. Potassium hydroxide, sodium hydroxide, 3, 5-dinitrosalicylic acid and acetone were purchased from BDH chemicals Ltd. Ethanol, *n*-hexane, potassium sodium tartarate and nitric acid were purchased from Fluka AG.

Determination of α-amylase activity. Fischer and Stein method (1961) was employed, where they used a saccharogenic assay to measure α-amylase activity. α-Amylase catalyses the hydrolysis of α-1-4 links of starch with the production of reducing sugars. Measuring the increase in concentration of reducing sugars using 3, 5-dinitrosalicylic acid reagents were followed in this reaction. When an alkaline solution of 3, 5-dinitrosalicylic acid was reduced to 3-amino-5-nitrosalycilic acid, the absorbance at 540 nm was measured. The enzyme activity unit was micromoles maltose liberated per mg enzyme in reaction (Units/mg).

Determination of α-chymotrypsin activity. α-chymotrypsin activity was determined by the method of Delmer *et al* 1979. α-chymotrypsin catalyses the hydrolysis of the substrate (succinyl-Ala-Ala-Pro-Phe-P-nitroaniline) into amino acids and p-nitroaniline which has maximum absorbance at 380 nm. The enzyme activity unit is micromoles P-nitroaniline liberated/mg enzyme in reaction (Units/mg).

Determination of lipase activity. Serum lipase activity was determined by the method of Tietz and Fierech (1966). The amount of fatty acids formed by the action of enzyme was determined by titration with sodium hydroxide solution as a direct measure of lipase activity.

Statistical analysis. The data collected were entered into personal computer and analysis of data was performed using SPSS statistical package. T-test was used for comparing means (Norusis 1989) and X2-test (chi s-test) was used for comparing the frequency of occurrence of variable in two or more occasions. P-value was considered to be statistically significant if <0.05.

Results and Discussion

Table 1 shows the dry weight of areca nut used in this study and its *n*-hexane and methanol extracts. From dried areca nut, *n*-hexane extract yielded 7.20 %, whereas methanol extract yielded 7.28 %.

The effect of *n*-hexane and methanol extracts of areca nut on α-amylase activity *in vitro* is presented in Table 2. *n*-hexane extract at 78 ppm showed a significant reduction of α-amylase activity (about 53% inhibition), and methanol extract at

Table 1
The percentage yield of Areca nut

Samples	Dry weight of sample (g)	Dry weight of extract (g)	%
Areca (whole) extract in *n*-hexane	43.30	3.12	7.20
Areca (whole) extract in methanol	49.30	3.59	7.28

90 ppm also showed a significant reduction of α-amylase activity (about 36% inhibition), respectively.

The effect of *n*-hexane and methanol extracts of areca nut on α-chymotrypsin activity *in vitro* is presented in Table 3. *n*-Hexane extract at 100 ppm showed a significant increase of α-chymotrypsin activity (about 78% increase), whereas, methanol extract at 123 ppm showed a reduction of chymotrypsin activity (about 18% inhibition), respectively.

The effect of *n*-hexane and methanol extracts of areca nut on lipase activity *in vitro* is presented in Table 4. *n*-Hexane extract

Table 2
Effect of Areca nuts extracts on α-amylase activity *in vitro*

Sample	Extract concentration (ppm)	Mean activity U/mg of sample extract (±SD)	Significance	% of Relative activity	% of Activation/ (Inhibition)
Control *	00.00	41.00 (±0.1)		100.00	----
Areca (whole) extract in *n*-hexane	78.00	19.25 (±3.8)	+	46.95	53.00
Areca (whole) extract in methanol	90.00	26.10 (±0.03)	+	63.66	36.00

Results represent the average of 3 independent experiments.
*Acetone alone was used as control; += P<0.05 level; n.s= non-significant P>0.05.

Table 3
Effect of Areca nuts extracts on α-chymotrypsin activity *in vitro*

Sample	Extract concentration (ppm)	Mean activity U/mg of sample extract (±SD)	Significance	% of Relative activity	% of Activation/ (Inhibition)
Control *	00.00	1.150 (± 0.02)		100.00	----
Areca (whole) extract in *n*-hexane	100.00	2.050 (± 0.04)	+	178.26	78.00
Areca (whole) extract in methanol	123.00	0.942 (± 0.04)	n.s	81.91	18.00

Results represent the average of 3 independent experiments.
*Acetone alone was used as control; += P<0.05 level; n.s= non-significant P>0.05.

Table 4
Effect of Areca nuts extracts on serum lipase activity *in vitro*

Sample	Extract concentration (ppm)	Mean activity U/mg of sample extract (±SD)	Significance	% of Relative activity	% of Activation/ (Inhibition)
Control *	00.00	506.8 (±4.4)		100.00	----
Areca (whole) extract in *n*-hexane	90.00	781.6 (±11.4)	+	154.22	54.00
Areca (whole) extract in methanol	100.00	642.1 (±17.2)	+	126.69	27.00

Results represent the average of 3 independent experiments.
*Acetone alone was used as control; += P<0.05 level.

at 90 ppm showed a significant activation of serum lipase activity (about 54% increase), and methanol extract at 90 ppm showed lower activation of lipase activity (about 27% increase), respectively.

The extraction of the areca nut with *n*-hexane yielded variable amounts of lipid soluble fraction. The residual fraction 7.2% based on dry weight was obtained in this solvent. Areca nut is rich in fats, and its distribution in the inner and outer seed had been analyzed (Kartha 1971). In general, the lipid fraction of areca contains a major alkaloid (arecoline) and several other apolar substances, in addition to triglycerides (fats), fatty acids, vitamin A and carotenes (Raghavan and Baruach 1958).

The use of a more polar solvent system such as methanol to extract the active principles from areca nuts gave yield of 7.28 %. Methanolic fraction from *Areca* contains carbohydrates, proteins, saponins, gums and mineral salts (Raghavan and Baruach 1958).

In this study, both *Areca* extracts (*n*-hexane and methanol) at concentration of 78 and 90 ppm caused significant reduction of α-amylase activity (between 47-64% inhibition). This finding is in full agreement with the work of Reddy *et al* (1980) who found that α-amylase activity in the saliva of betel (*Areca*) chewers was decreased significantly as compared to non-chewers. However, chewers tend to secrete more saliva, and that the excessive salvation could be due to the presence of arecoline (Reddy *et al* 1980). It should be noted that α-amylase activity in human serum is used clinically to monitor acute and chronic inflammation, viral infection (such as hepatitis), liver diseases and cancer (Calbreath 1992). It is also known that several chemical substances influence α-amylase activity such as codeine, morphine, glucosteroids and oral contraceptive (Calbreath 1992).

Various extracts of areca nut produced in general a small increase and decrease in chymotrypsin activity, respectively.

In a sharp contrast with the previous findings, both extracts of areca (*n*-hexane and methanol) produced significant increase in lipase activity. Thus, at concentration of 90 and 100 ppm lipase activity increased by 26 and 54%, respectively. It is clinically established that serum lipase activity increases sharply in case of acute pancreatitis (especially two weeks after the attack). Chronic pancreatitis on the other hand, caused loss of pancreatic tissue, and therefore decreases lipase activity (Calbreath 1992).

It is also known that various species such as ginger, cumin, fenugreek and mustard seeds cause significant increase in increase in intestinal lipase, sucrase and mutase activities (Platel and Srinivasan 1996). Similar results were obtained when two varieties of betel leaves were administered orally to rat (Prabhu *et al* 1995). While the betel leaves did not influence bile secretion and composition, they had a significant stimulatory effect on pancreatic lipase activity. Therefore, areca nut as well as other spices (such as ginger, cumin and fenugreek) could be useful as digestive aids particularly in case of patients suffering from gallbladder disorders, which render them unable to digest fatty meals properly.

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