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Alpha-glucosidase inhibitory and hypoglycemic activities of *Areca catechu* extract.

Senthil Amudhan M.and Hazeena Begum V.

Department of Siddha Medicine, Faculty of Science, Tamil University, Vakaiyur, Thanjavur 613 005, Tamilnadu, India.

ABSTRACT

α-glycosidase *in-vitro* inhibitory activity and hypoglycemic effect by oral administration in rats of arecanut ethanol extracts have been investigated. Arecanut extract showed *in-vitro* inhibitory activity of intestinal α -glucosiadse enzymes maltase and sucrase and IC_{50} values of maltase and sucrase activity was found to be 12µg/ml and 30 µg/ml of arecanut extract respectively. The purpose of study is to know that arecanut extract alpha-glycosidase inhibition could reduce intestinal absorption of monosaccharides by inhibiting disaccharide hydrolysis. The post prandial elevation in blood glucose level at 30 and 60 min after administration of maltose with ethanol arecanut extracts (250 mg/kg and 500 mg/kg doses) showed significant suppression compared to control group rats. These results suggest that the arecanut extract has potent α-glucosidase inhibitors and would be effective in suppression of elevation in blood glucose after oral administration of maltose to rats.

KEY WORDS: Areca nut- hypoglycemic- Alpha -glycosidase inhibition

INTRODUCTION

Intestinal glycosidase enzymes play an important role in carbohydrate digestion and absorption. Therefore an inhibitor of intestinal glucosidase could be expected to retard carbohydrate digestion and absorption. A reasonable way to control these carbohydrate dependent diseases would be to limit intestinal carbohydrate digestion. It has been recognized that alpha glucosidase inhibitors can be used to prevent some disorders such as diabetes, obesity, hyperlipidaemia and hyperlipoproteinaemia(1) and also show anti-HIV activity (2).

It is essential for hyperglycemic conditions that the intestinal absorption of dietary carbohydrates be suppressed by inhibiting intestinal glycosidase, which delay the digestion of oligosaccharides and disaccharides to monosaccharide and reduce the rate of glucose absorption, rise in blood glucose levels and insulin response. Research has recently been conducted on glucosidase inhibitors obtained from plant sources which show reduction in postprandial blood glucose concentrations, e.g., onion (3), clove (4), tea (5). A high postprandial blood glucose response is associated with micro- and macro-vascular complications in diabetes, and is more strongly associated with the risk for cardiovascular disease than are fasting glucose levels (6). Potent glucosidase inhibitors such as acarbose and voglibose have already been clinically used for diabetic and obese patients.

Arecanut (*Areca catechu*) belongs to *Palmaceae* family, cultivated in South Asian countries. Vagbhata (4th century AD) described its medicinal properties and its effective use against leucoderma, leprosy, cough, fits, worms, anemia and obesity. The powered nuts are used in diarrhea and urinary disorders Arecanut is considered useful as an external application on ulcer and in skin disorders. (7).

Arecanut showed the most potent angiotensin-converting enzyme (ACE) inhibitory activity (8) and relaxed aortic ring preparations of isolated rat aorta (9) MAO-A inhibition (10). Arecanut extract showed potent anti-oxidative activity (11) and inhibition of free radicals and reactive oxygen species (12). The arecoline in betel nut showed hypoglycemic activity (13). The arecanut seed or nut contains polyphenol, alkaloids, polysaccharide, fat, protein (14). Main polyphenol subgroups in arecanut are proanthocyanidins, catechin, epicatechin. Polyphenolic compounds in plants recognized to inhibit the activities of digestive enzymes due to their ability to bind with enzyme protein (15). Hence, we have conducted this present study to know arecanut extract *in vitro* inhibitory activity on α-glucosidase enzymes such as maltase and sucrase and subsequent *in vivo* reduction of glucose absorption by inhibiting disaccharide digestion.

MATERIAL AND METHODS

Preparation of test material- Arecanut has been

obtained from central plantation crops research institute (CPCRI),Vittal Karnataka. Dried arecanut 25g were powdered and it was extracted with 70% ethanol in soxhlet apparatus for 24 hrs. After filtration and evaporation of ethanol the residue was 8.4% and it was used for experiment.

Male Wistar rats (150-200 g) were fed with a standard diet and water *ad libitum*. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2°c and 12 hours light / dark cycle) throughout the experimental period. Animal experiments were carried out following the guidelines of the animal ethics committee of the institute.

In vitro a- glucosidase inhibitory activity

Arecanut ethanol extract was used to investigate the *in-vitro* inhibitory effect of α –glucosiadse enzymes. After fasting, rat small intestine between duodenum and above cecum was cut, rinsed with ice-cold saline and homogenated with maleate buffer (pH 6). We have used small intestine homogenate as an enzyme source.

The assay mixture consisted of 100 mM maleate buffer, maltose (2%) and sample arecanut extract (10-200 ug/ml). It was pre-incubated after 5 minutes at 37^0C and reaction was initiated by adding crude glycosidase solution, followed by incubation the glucose released in the reaction mixture was determined. Glucose concentration was measured by glucose kit. In case of maltase inhibitory test, maltose was used as a substrate. But in the sucrase inhibitory test sucrose was used a substrate.

Evaluation of hypoglycemic activity in arecanut fed rat using maltose tolerance test

Normal Wistar rats were randomly divided in to 3 groups. (6 rats/group). Animals in-group I treated with saline as control and the experimental rats are groups II animals treated with arecanut extract 250 mg/kg and group III animals treated with arecanut 500 mg/kg.

After overnight fasting (18 hr) the rats were orally administrated with a maltose (2g/kg body weight) alone in control animals in group I. But in case of experimental rats both group II and group III were orally administrated maltose (2g/kg body weight) along with arecanut ethanol extract 250 mg/kg, 500 mg\kg body weight respectively and blood samples were taken from the lateral tail vein at various times during 0-120 minutes. The blood glucose concentration was measured by the glucose oxidase method using glucose kit.

Statistical analysis- All values were expressed as mean ± S.E.M. The differences were compared using one way analysis of variance (ANOVA) followed by Tukey's test. P values< 0.05 were considered as significant.

RESULTS

In vitro effect of a- glucosidase inhibitory activity

Figure1 shows the effect of arecanut ethanol extract inhibitory activities on maltase and sucrase *in vitro* (experiment 1). Arecanut ethanol extract inhibited sucrase activity in a dose-dependent manner and 12 µg/ml of arecanut extract resulted in 50% maltase enzymatic inhibitory activity. Arecanut ethanol extract inhibited, another carbohydrate metabolizing enzyme, maltase activity in dose dependent manner, which 30 µg/ml of arecanut caused 50% maltase inhibitory activity.

Evaluation of hypoglycemic activity in arecanut fed rat using maltose tolerance test

Figure 2 shows the changes in the levels of blood glucose in group I control and experimental arecanut fed groups group II and group III after oral administration of maltose (2g/ kg). Arecanut treated both rat groups, 250 mg/kg and 500 mg/kg doses fed rats, showed suppression of blood glucose elevation at 30 min and 60 min significantly ($p< 0.05$) compared to control (maltose) rat group. In this study, arecanut extract significantly ($p< 0.05$) suppressed the post-prandial elevation in blood glucose compared with control group during 30min to 60 min period after maltose loading. The blood glucose level of the arecanut extract administered rats was identical to the level in control group during period from 60 and 120 min. These results showed that arecanut extract had a suppressive effect on the post-prandial elevation in blood glucose after maltose oral administration in rats.

DISCUSSION

This present study results show that ethanol arecanut extract had inhibitory activities against both sucrase and maltase that both present in small intestinal mucosa with IC_{50} value 12 µg/ml and 30 µg/ml respectively. This is accordance with recent research conducted on glucosidase inhibitor obtained from plant source, on assumption that could suppress the post-prandial blood glucose level. The methanol extracts of *S. reticulata* and *S. oblonga* strongly inhibited rat intestinal maltase and sucrase *in vitro* IC_{50} values were 42 and 66 µg/ml, respectively, for maltase, and 32 and 24 µg/ml, respectively, for sucrase and maltase inhibitory activity of arecanut were equivalent to the effect of *S. reticulata* and *S.* oblonga (16).

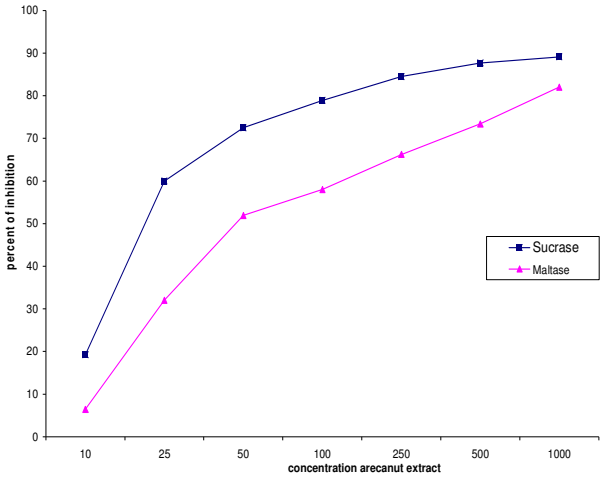

Figure 1. Rat intestinal maltase and sucrase inhibitory activities of arecanut extract at various concentrations Maltase activity of arecanut extract (square), sucrase activity of arecanut extract (triangle). IC_{50} values of maltase and sucrase activity were 12 µg/ml and 30 µg/ml of arecanut extract

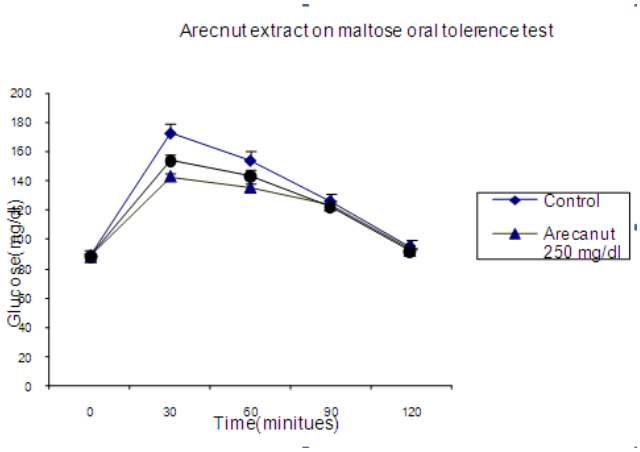

Figure 2 Postprandial serum glucose response after maltose ingestion in Wistar Rats. The arecanut extract (250 mg/kg and 500 mg/kg) was administered to wistar rats with maltose (2 g/kg).Serum samples at each fixed time (0, 30, 60, and 120 min) were subjected to the glucose assay. Data are expressed as mean (mg/dlof serum)_S.E.M. Significant differences between control and test groups at each time were examined with Tukey's -test (n-6, p <0.05). Arecanut extract at dose 250 mg/ kg and 500 mg/kg produced a significant decrease in blood glucose levels compared with control (P<0.05) at 30 and 60 min period.

It had been reported that digestive enzymes such as lipase, α-amylase, and α-glucosidase, were inhibited by proanthocyanidins and tannins in young chicks, which decreased the digestibility of protein, starch and lipid (17, 18). The mechanism of inhibition on both sucrase and maltase intestinal enzymes by ethanol arecanut extract could be, polyphenol content, which constitute 15% to 20%. For example, comparing the inhibitory effect on a-amylase IC_{50} value of tea extract e.g., 20 µg/ml mainly including tannins were on the same level (19). Similarly, Arecanut extract showed inhibition of elastase and hyaluronidase on skin tissues, which purified by each fraction of solvents and was identified as a phenolic substance that showed competitive inhibition with the substrate (20) and in other study tea polyphenol such as catechin have been found to inhibit glucosidase activity and glucose transport (21).

It has been reported that arecanut polyphenols, mostly flavonols, include about 10 per cent of (+) catechin, 2.5 per cent epicatechin, 12 per cent of (+) leucocyanidin, the remaining portion being complex

flavonoids in varying degrees of polymerization (22). A series of dimeric, trimeric, and tetramertic procyanidins has been isolated from seeds of *Areca cateheu* (23).

Tannins (polyphenol) have specific property such as the ability to precipitate some proteins. This precipitation is presumed to occur by the formation of hydrogen bonds between the hydroxy groups of the tannins and the peptide linkages of the proteins (24). In our study arecanut tannins were present in sufficiently high concentrations might have significantly precipitated the enzymes such as maltase and sucrase.

The physiological effects of arecanut had been demonstrated in animal experiments, include inhibitory effect on pancreatic cholesterol esterase (pCEase) *in vitro* and decrease in absorption of cholesteryl oleate (25), intestinal free cholesterol and also lowering activity of small intestinal pCEase significantly (26) .

In this present study, an extent of arecanut extract was examined for its *in vitro* inhibition of rat intestinal α-glucosidase and its *in vivo* effect on suppression of elevating blood glucose level. Arecanut extract treated rat in both group's (250mg/kg and 500 mg/kg doses) showed significant suppression ($p<0.05$) in blood glucose elevation at 30 min and 60 min significantly ($p< 0.05$) compared to maltose loading control rat group. These results suggest that arecanut extract had a suppressive effect on post prandial elevation in blood glucose after oral administration of maltlose to rats. This study in accordance with earlier report stated that anthocyanins inhibited α-glucosidase activity and reduced blood glucose levels after starch-rich meals (27).

The results strongly suggest that arecanut extract inhibited blood glucose elevation by inhibiting glucosidase activity, however, it is able to take part any other mechanism. It is necessary to investigate the action mechanism of arecanut extract on glucose transport and insulin secretion. α-glucosidase inhibitors are used worldwide for the treatment of diabetes and α-glucosidase inhibit reversibly the enzymatic cleavage of complex carbohydrates to simple absorbable sugars and hence slow the absorption of carbohydrate from the small intestine, thereby lowering postprandial hyperglycemia.

In conclusion, our findings show that ethanol arecanut extract inhibition on maltase and sucrase may be due to several polyphenolic compounds present within the extract. More studies and in vivo experiments in diabetic conditions are required to ascertain the compounds and its mechanism of action, may provide a natural hyperglycemic control treatment, improve plasma glucose level and thus decrease risk for diabetes, cardiovascular diseases and to further define the physiologic extent of these effects. This arecanut polyphenol may be attracted a great deal of attention as a new food material, which had high added values and new characteristics with useful functional ingredients. However, further studies are needed before arecanut polyphenol can be used safely as food additives and supplements.

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