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Neerizhivu kudineer (a traditional siddha polyherbal antidiabetic medicine) inhibits α- amylase enzyme and α-glucosidase enzyme

Jayaraman Raamachandran[1], Arul Amuthan[2,3]

1 *Retired Professor, Indian Institute of Technology (IIT), Madras and Siddha medical practitioner, Chennai, India*
2 *Assistant Professor, Department of Pharmacology, Melaka Manipal Medical College, Bukit Baru, Melaka, Malaysia*
3 *Team Leader, Division of Siddha, Center for Integrative Medicine and Research, Manipal Academy of Higher Education, Manipal, Karnataka, India*

ABSTRACT

Objective: To investigate the α-amylase and α-glucosidases inhibitory activity of *Neerizhivu kudineer* (NK). **Methods:** The polyherbal formulation NK was prepared as per traditional Siddha medical literature and aqueous extract was taken. In-vitro α-amylase and α-glucosidases inhibitory activity of NK was evaluated using various concentrations of NK. Acarbose was used as standard drug. The percent inhibition values were determined and the dose verses percent inhibition was plotted in MS excel. Using the linear trend line, the concentration required for 50% inhibition (IC_{50} value) were calculated. Results: NK exhibited dose dependent inhibition on α- amylase and α-glucosidase with the IC_{50} value of 6.90 µg/ml and 8.51 µg/ml respectively, whereas the standard drug acarbose exhibited IC_{50} at 5.04 µg/ml and 5.50 µg/ml respectively. **Conclusion:** *Neerizhivu kudineer* has the inhibitory action on α-amylase and α-glucosidases enzyme.

Keywords: Ayurveda, Traditional and complementary medicine, Diabetes, Glucose absorption.

INTRODUCTION

The Traditional Tamil Medicine (TTM), now popularly known as Siddha medicinal system is the oldest medicinal system of India. If it is old it is equally scientific in its approach be it diagnosing the disease or prescribing the medicine or preparing the same. In everything there is a scientific temper that as good as or even better than modern approach to medicines. The Siddha medicinal system is based on three humors vaLi (wind), azhal (bile) and aiyam (phlegm); five elements viz, fire, water, earth, ether and wind and six tastes like sweet, bitter, sour, astringent, salt and spicy. The combinations of these are related to one's health or lack of it. Siddha system has enormous and elaborate amount of literatures that deal with the diabetes [1]. The derangement of the three humors (wind, bile and phlegm) are responsible for many diseases. As for as diabetes known as "*neerizhivu*" in Tamil is concerned, there are twenty different types; some of which are designed not curable. Elaborate urine tests are prescribed as to which category the patient's urine falls and medicines are prescribed accordingly [2].

Neerizhivu kudineer (NK) is a polyherbal Siddha drug successfully used for treating diabetes mellitus without any side effect. The ingredients include Strychnos *potatorum (thaeEtraan*), *Terminalia chebula* (*kadukkaay*), *Cassia auriculata (aavaarai)* and *Limonia acidissima (vilaa maram),* and is to be consumed along with buttermilk. Siddhar Theraiyar described this drug as follows; [3]

தேற்றான் விரைகடுக்காய் செப்பரிய வாவாரை
யேற்ற விளாம்பிசினோ டித்தனையுங்-கோற்றொடியாய்
பங்கா யறுகாற் பசுவின் மோரிற்பருகப்
பொங்கி வரு நீரிழிவு போம்

- தேரையர் குடிநீர் 76/100

thEtraanvirai kadukkaay cheppariya aavaarai
Aetra viLaampisinodu iththanaiyung – kOtrodiyaay
Pangaay aRukaaR pasuvin mOrilparukap
Pongivaru neerozhivi pOm

- Thaeraiyar kudineer 76/100

***Corresponding author:**
Dr. Arul Amuthan
Assistant Professor, Department of Pharmacology, Melaka Manipal Medical College, Bukit Baru, Melaka, Malaysia
Email: arul.amuthan[[at]manipal.edu
dramuthanmd[at]gmail.com

Strychnos potatorum seed is stomachic, demulcent, emetic and is useful in diarhoea, diabetes and gonorrhea. *Terminalia chebula* is a laxative, stomachic, tonic and alterative. It is said that the seed nourishes a person like a mother nourishes her children [4]. *Cassia auriculata* herb was very much praised by the Siddhars for its curative properties. There is a saying in Tamil "can you see a person dying when *Cassia auriculata* blossoming?", which means it can save patients life. Clinically, 2-4 gm should be taken along with 100-150 ml of cow's butter-milk every two hours in the case of very severe or chronic diabetes to reduce the elevated blood glucose level drastically.

The α-amylase is a pancreatic enzyme that involves the initial step in hydrolysis of starch to a smaller oligosaccharides such as maltotriose, maltose, and a number of other oligoglucans. These smaller oligosaccharides are further acted on by another enzyme α-glucosidases, which is a membrane bound enzyme at the epithelium of small intestine. α-glucosidases further degrades the smaller oligosaccharides to glucose in the small intestine, which eventually gets absorbed into systemic circulation. Post-prandial hyperglycemia occurs if the dietary starch gets rapid degradation by these two enzymes. There is a direct relationship between the activity of pancreatic α-amylase as well as α-glucosidases in the small intestine and the post-prandial glucose levels; the control of which is therefore delays the absorption of ingested carbohydrates, reducing the postprandial glucose. This is an important aspect in the treatment of type 2 diabetes. Hence, pancreatic α-amylase inhibitors as well as α-glucosidases inhibiting drugs or herbals could be the effective strategy to control post-prandial hyperglycemia.

Since no previous study reports were available on this polyhedral formulation, this study was aimed to evaluate the antidiabetic activity of NK through α-amylase and α-glucosidases inhibitory action.

MATERIALS AND METHODS

Preparation of neerizhivu kudineer [3]

Before using the herbals (raw materials) for medicinal purposes, a process known as "*suththi seythal*" to be done that might remove toxic things from them. This purification process involved in the herbals used in the NK were as follows; *Strychnos potatorum* seed was soaked in cow milk for half an hour. Then washed with water and allowed to be dried. Then seed was soaked in *Amaranthus tricolor* (*chiru keerai* in Tamil) juice (4 times the seed weight) and boiled till the volume is reduced to one-eighth of its original volume. Then washed with water and dried. This was used to prepare NK. *Terminalia chebula* dried fruit was soaked in rice-washed water over night for three days by changing the water every day. On third day, filter and peel the skin and throw the seed kernel inside. Dry the peeled skin thoroughly which was used for medicine.

About 100 gm of each *Strychnos potatorum* seed, rind of *Terminalia chebula*, flower of *Cassia auriculata* and the gum of *Limonia acidissima* were fine powdered. (Figure 1).

Extraction and phytochemical screening

Aqueous extract from the NK was taken for the study as follows; 10 mg of NK was soaked into 10 ml of distilled water, vortexed for 15 minutes and kept for 24 hrs in room temperature. Then filtered and collected. The phytochemical screening of the extract was done by following standard protocol [5].

Figure 1: Ingredients of *neerizhivu kudineer*. A. *Strychnos potatorum* seeds. B. *Cassia auriculata* flower C: *Terminalia chebula*, soaked in rice washed water D. *Limonia acidissima* gum

α- Amylase inhibitory activity

The α amylase inhibitory activity was determined by measuring the presence of starch in the assay medium. A dark-blue, yellow and brownish colour formation indicates the presence of starch, absence of starch and partially degraded starch respectively in the reaction mixture.

The α- amylase inhibitory activity was determined by the previously described method [6,7]. The total assay mixture composed of 40 µl 0.02 M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride), 0.02 units of Porcine Pancreatic α-amylase (PPA) solution and NK aqueous extract at concentration from 0.1-1.5 mg/ml (w/v) were incubated at 37°C for 10 min. Then soluble starch (1%, w/v) was added to each reaction well and incubated at 37°C for 15 min. Solvent control DMSO, Positive control (Acarbose- known antidiabetic drug), 9 concentrations of test sample (0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 5, 10 µg/ml) were added and triplicates (n=3) were also maintained. To stop the enzymatic reaction, 1 M HCl (20 µl), followed by 100 µl of iodine reagent (5 mM I2 and 5 mM KI) was added. The absorbance in control or experimental groups was read at 620 nm on a microplate reader. The percent inhibition values were determined using the formula; [control - experimental / control] X 100. The plots of percent inhibition versus concentration was plotted in MS excel and the linear trend line with the formula (Y = mX + c) was drawn, in which Y is the % inhibition, X is the dose, m is the slop and c is the intercept. The IC_{50} value of NK and well as acarbose were calculated using the formula; x = (50-c)/m.

α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity in this study was determined by measuring the release of 4-nitrophenol from 4-Nitrophenyl–D-Glucopyranoside (4NPGP) using Spectrophotometer at 405 nm absorbance.

The α-glucosidase inhibitory activity was determined by previously described method [8,9]. About 20 mg of NK aqueous extract was dissolved in DMSO to make stock solution and serially diluted to make different concentrations. The solvent DMSO control, positive acarbose drug control, and 9 test drug concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 5, 10 µg/ml) with triplicates (n =3) were used for the study. The concentration of α-glucosidase inhibitor required to inhibit 50% (IC_{50}) of the α-glucosidase enzymatic activity under the assay conditions was calculated (based on the absorbance). The Bio-Rad microplate absorbance reader was used for the assay. The percent inhibition values were determined using the formula; [control - experimental / control] X 100. The plots of percent inhibition versus concentration was plotted in MS excel and the linear trend line with the formula (Y = mX + c) was drawn, in which Y is the % inhibition, X is the dose, m is the slop and c is the intercept. The IC_{50} value of NK and well as acarbose were calculated using the formula; x = (50-c)/m.

RESULTS

Phytochemical screening

The aqueous extract of the *neerizhivu kudineer* showed presence of saponins, steroids, falvanoids, phenols, tannins, carbohydrates and proteins.

α- Amylase and α-Glucosidase inhibitory activity

Neerizhivu kudineer exerted dose dependent α- amylase and α-glucosidase inhibitory activity with the IC_{50} value of 6.90 µg/ml (α-amylase) and 8.51 µg/ml (α-Glucosidase) , whereas the standard drug acarbose exhibited IC_{50} at 5.04 µg/ml (α- amylase) and 5.50 µg/ml (α-Glucosidase). Compare to the acarbose, the *Neerizhivu kudineer* exhibited slightly lesser potential in its inhibitory activity (Table 1-2, figure 2).

Table 1: α Amylase inhibitory activity and α-Glucosidase inhibitory activity of *neerizhivu kudineer* and acarbose

Concentration (µg/ml)	% inhibition on α Amylase		% inhibition on α-Glucosidase	
	Neerizhivu *kudineer*	Acarbose	Neerizhivu *kudineer*	Acarbose
0.1	1	2	1	1
0.2	4	3	3	7
0.4	17	16	8	16
0.6	25	24	19	32
0.8	34	47	26	41
1	40	74	30	55
2	48	88	37	65
5	60	91	45	80
10	71	100	58	98

Table 2: IC_{50} values of *neerizhivu kudineer* and acarbose to inhibit α-Amylase and α-Glucosidase enzyme

Treatment	IC_{50} values (µg/ml)	
	α Amylase	α-Glucosidase
Neerizhivu kudineer	6.90	8.51
Acarbose	5.04	5.50

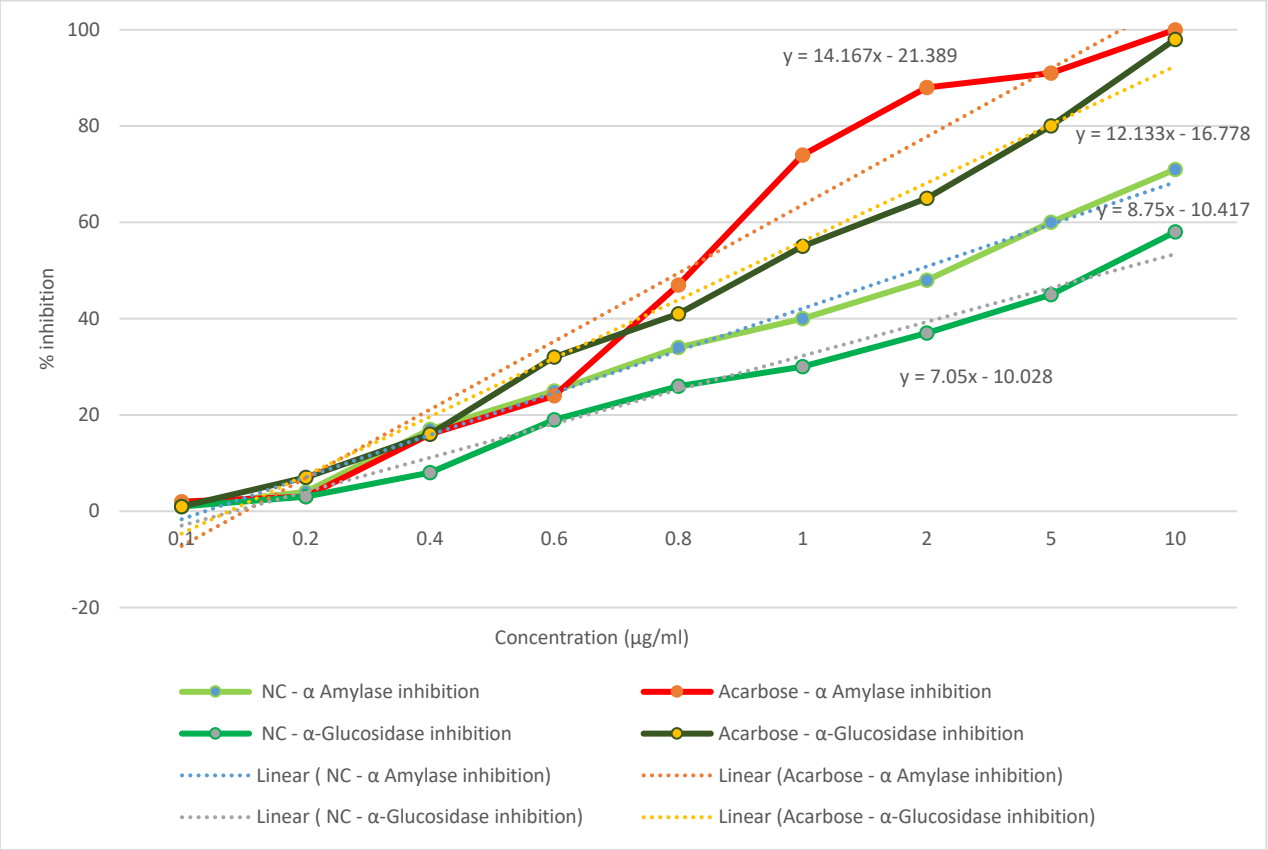

Figure 2: Dose dependent inhibitory activity on α Amylase and α-Glucosidase by neerizhivu kudineer and acarbose

DISCUSSION

Drugs that reduce post-prandial hyperglycemia by suppressing hydrolysis of starch such as α- amylase as well as α-glucosidase inhibitors were proven as the suitable drugs to control post prandial hyprglcemia. Many traditional Indian systems of medicines are currently used for the treatment of diabetes and few of them have been reported for their anti-diabetic activities. *Neerizhivu kudineer* is one such Traditional Siddha polyherbal formulation consists of four herbs, which was evaluated for α-amylase and α-glucosidase enzyme inhibitory activity. It exhibited dose dependent inhibitory activity on both enzymes. The ingredients are *Strychnos potatorum, Terminalia chebula, Cassia auriculata* and *Limonia acidissima*, and is to be consumed along with buttermilk.

Many preclinical studies have demonstrated the antidiabetic activities of *Strychnos potatorum* seed [10,11], *Terminalia chebula* fruit [12,13] and *Cassia auriculata* flower [14-16]. Already the α-amylase and α-glucosidase enzyme inhibitory activity have been studied in *Strychnos potatorum seed* [17], *Terminalia chebula* fruit [18] and *Cassia auriculata* flower [14]. The antidiabetic activity of *Limonia acidissima* gum has not been reported, but the fruit and leaf of the plant have shown antidiabetic activity in animal models [19,20]. Future studies may be directed to find why the gum is preferred than other parts of *Limonia acidissima* in treating diabetes. The NK is advised to consume with buttermilk which contains many beneficial bacilli. There are number of studies to show the imbalance if gut microbiota in diabetes patients and probiotic supplements improved the diabetes [21]. That is the reason, ancient Siddha literature included buttermilk as one of the ingredient of this polyherbal formulation. The multiple mechanisms and their synergistic activities of this polyherbal formulation in managing diabetes and preventing diabetic complications are to be studied.

CONCLUSION

This study demonstrated that n*eerizhivu kudineer* the inhibitory activity of α- amylase inhibitory and α- amylase, which is one of the pharmacological approach to control post prandial hyperglycemia. Hence, n*eerizhivu kudineer* could be used to control post prandial hyperglycemia. However, further detailed studies are to be warranted.

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Source of Support

None.

Conflict of Interest

None declared.

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