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In Vitro Antidiabetic Activity of Nisamalaki Churna

(Aktiviti Antidiabetik *In Vitro* Nisamalaki Churna)

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ABSTRACT

Nisamalaki Churna is an Ayurveda *formulation used for diabetes, which consists of amalaki and haridra*. In vitro *antidiabetic screening of Nisamalaki Churna by α-amylase inhibition was carried out by starch iodine method, dinitrosalicylic acid method (DNSA) and effect on normal rats blood sugar level was studied. Nisamalaki Churna shows potent α-amylase inhibition IC_{50} 89.44 µg/mL by starch iodine method and IC_{50}100.0 µg/mL by DNS method. Nisamalaki Churna showed potent decrease in blood glucose level in normal rats.*

Keywords: α-amylase inhibition; antidiabetic screening; Nisamalaki Churna

ABSTRAK

Nisamalaki Churna adalah formula Ayurveda *untuk diabetis yang mengandungi amalaki dan haridra. Penyaringan antidiabetis* in vitro *Nisamalaki Churna telah dijalankan. Kesan Nisamalaki Churna terhadap perencatan α-amilase telah dikaji menggunakan kaedah iodin kanji. Dengan menggunakan kaedah asid dinitrosalisilik (DNSA) kesan terhadap aras gula dalam darah tikus biasa telah dijalankan. Nisamalaki Churna menunjukkan kesan perencatan α-amilase yang kuat, IC_{50} 89.44 µg/mL melalui kaedah iodin kanji dan IC_{50}100.0 µg/mL DNS. Nisamalaki Churna menunjukkan penurunan aras glukos yang kuat dalam tikus biasa.*

Kata kunci: Nisamalaki Churna; penyaringan antidiabetis; perencatan α-amilase

INTRODUCTION

India has a rich heritage of traditional medicine constituting with its different components like *Ayurveda, Siddha* and *Unani*. Botanicals constitute a major part of these traditional medicines. Nisamalaki Churna is an *Ayurveda* formulation which is used for antidiabetic, consists of fine powders of *Turmeric* (haridra) – *Curcuma longa* – 1 part and *Emblica* (amalaki) – *Emblica officinalis* – 1 part (Anon. 2000).

Curcuma longa (Zingeberaceae) has been used as spice and coloring agent. Sushruta recommended it for epilepsy and bleeding disorders. Charaka recommends it for skin diseases, to purify the body mind and to help the lungs expel Kapha. In *Ayurveda*, the traditional Indian system of medicine, it has been used in several ways namely as an ingredient in the preparation of medicinal oils, ointment and poultice, in diabetes and leprosy, for stomachache, carminative, tonic, laxative, antirheumatic, blood purifier, vermicide, antiseptic and cure for liver ailments. The raw juice is used to relief gall bladder complaints, dental-troubles, sore throat, common cold, parasitic skin diseases and pile cure. Turmeric helps regulate the female reproductive system and purifies the uterus and breast milk and in men it purifies and builds semen (Chandrashekhara & Srinivasan 1999; Gururaj et al. 2002).

Emblica officinalis (Euphorbiaceae) known as Amla and Indian Gooseberry' is the richest known source of vitamin C. Amalaki is native to India and Southeast Asia and is one of the most revered rejuvenative tonics of ayurvedic medicine. Amalaki is full of powerful antioxidants that protect cells against free-radical damage, acting as an 'anti-aging' tonic super food. It is nicknamed dhatri, or 'the nurse' for its renowned ability to nurture us back to vibrant health. According to *Ayurveda*, amalaki is cooling in energy, predominantly sour in taste and has a sweet 'post-digestive effect' (acts as an overall tonic). It is especially recognized as a detoxifier and rejuvenative tonic for people suffering from excess heat conditions such as hyperacidity, heartburn, infections, inflammations, bleeding disorders, liver problems, skin disorders and biliousness. In addition, it is often recommended for colds, to improve eyesight, to strengthen the bones and for all signs of premature aging (Dey 1980; Naveen 1993). The present investigation was to confirm the antidiabetic activity of Nisamalaki Churna.

MATERIALS AND METHODS

PREPARATION OF NISAMALAKI CHURNA

Curcuma longa (10 g) and *Embolic officinalis* (10 g) are finely powdered and passed through 100 No. sieve and mixed. Nisamalaki Churna was extracts with distilled water and the extract is dried.

IN VITRO ALPHA AMYLASE INHIBITION ASSAY

3, 5-Dinitrosalicylic acid method (DNSA) The inhibition assay was performed according to Miller (1959) using DNS method. Aqueous extract of Nisamalki churna of varied concentrations in 500 µL were added to 500 µL of 0.02 M sodium phosphate buffer (pH6.9 containing 6 mM sodium chloride) containing 0.04 units of α-amylase solution and were incubated at 37°C for 10 min, followed by addition of 500 µL of a 1% starch solution in 0.02 M sodium phosphate buffer (pH6.9) all the test tubes. The reaction was stopped with 1.0 mL of 3, 5 DNSA reagent. The test tubes were then incubated in a boiling bath water for 5 min and cooled to room temperature. The reaction mixture was then diluted after adding 10 mL distilled water and absorbance was measured at 540 nm. The control samples were also prepared accordingly without any plant extracts and were compared with the test samples containing various concentrations of the plant extracts prepared with different solvents. The results were expressed as % inhibition calculated using the formula:

$$\% \text{ Inhibition activity} = \frac{\text{Abs (Control)-Abs (Extract)}}{\text{Abs (Control)}} \times 100.$$

Starch-iodine Colour Assay Screening of Nisamalaki Churna for α-amylase inhibitors was carried out according to Xiao et al. (2006) with slight modification based on the starch-iodine test. Aqueous extract of Nisamalki churna of varied concentrations in 500 µL were added to 500 µL of 0.02 M sodium phosphate buffer (pH6.9 containing 6 mM sodium chloride) containing 0.04 units of α-amylase solution and were incubated at 37°C for 10 min. Then 500 µL soluble starch (1%, w/v) was added to each reaction well and incubated at 37°C for 15 min. 1 M HCl (20 µL) was added to stop the enzymatic reaction, followed by the addition of 100 µL of iodine reagent (5 mM I_2 and 5 mM KI). The colour change was noted and the absorbance was read at 620 nm on a microplate reader. The control reaction representing 100% enzyme activity did not contain any plant extract. To eliminate the absorbance produced by plant extract, appropriate extract controls without the enzyme were also included. Inhibition of enzyme activity was calculated as:

$$\text{Inhibition of enzyme activity (\%)} = (C\text{-}S) / C \times 100,$$

where S is the absorbance of the sample and C is the absorbance of blank (no extract).

ANIMALS

Male Wistar rats weighing 150-250 g were acclimatized to the experimental room at temperature 23 ± 2°C, controlled humidity conditions (50-55%) and 12 h light and 12 h dark cycle. They were caged with a maximum of two animals in polypropylene cage and were fed with standard food pellets (Kamadenu Enterprises, Bangalore) and water *ad libitum*. All the studies conducted were approved by the institutional animal ethical committee of Sri K.V. College of Pharmacy, Chickballapur, Karnataka, according to prescribed guidelines of CPCSEA, Government of India (Reg. No. 117/2000/CPCSEA).

ANTIDIABETIC SCREENING IN NON-FASTED RATS

Prior to the study, blood samples were collected from all the animals to check random glucose levels. The animals which had a glucose level >130 gm/dL were excluded from the study. Five groups of non-fasted rats, six in each were grouped as follows:

Group I – Control (0.5% w/v CMC) administered;
Group II – Glucose control ;
Group III – Standard drug treatment (metformin-250 mg/kg, p.o.);
Group IV – Nisamalaki Churna (90 mg/kgp.o.)
Group V – Nisamalaki Churna (180 mg/kgp.o.)

The test and standard substances were suspended in 0.5% w/v carboxy methyl cellulose (CMC). Group II to group V animals were given 5 g/kg glucose additionally after 30 min of standard drug/ test substance administration. Blood samples were collected from retrooribatal puncture for glucose estimation at various time points like pre dose, 1.0, 2.0, 3.0 and 4.0 h after test substance/ standard drug treatment. Glucose was estimated by using Lab-India auto-analyzer with the help of Lab kit enzymatic kit (Nyunai 2006; Vogel 2002).

STATISTICAL ANALYSIS

The values were expressed as mean ± SEM (n=5) for each group. The significant difference between groups was determined using one-way ANOVA followed by Dunett's test. P value less than 0.05 was considered significant.

RESULTS AND DISCUSSION

IN VITRO α-AMYLASE INHIBITION ASSAY OF NISAMALAKI CHURNA

Drugs that inhibit carbohydrate hydrolyzing enzymes have been demonstrated to decrease postprandial hyperglycemia and improve impaired glucose metabolism without promoting insulin secretion of NIDDM patients. The results of *in vitro* studies showed that Nisamalaki Churna inhibits α-amylase activity. Natural health products of vegetable origin were clearly indicated as a promising avenue for the prevention of chronic diseases (Punitha & Manoharan 2006). Figures 1 and 2 show that Nisamalaki Churna significant inhibition of α-amylase enzyme. IC_{50} values of Nisamalaki Churna by DNSA and Starch Iodine method were 89.44 and 100.0 µg/mL, respectively.

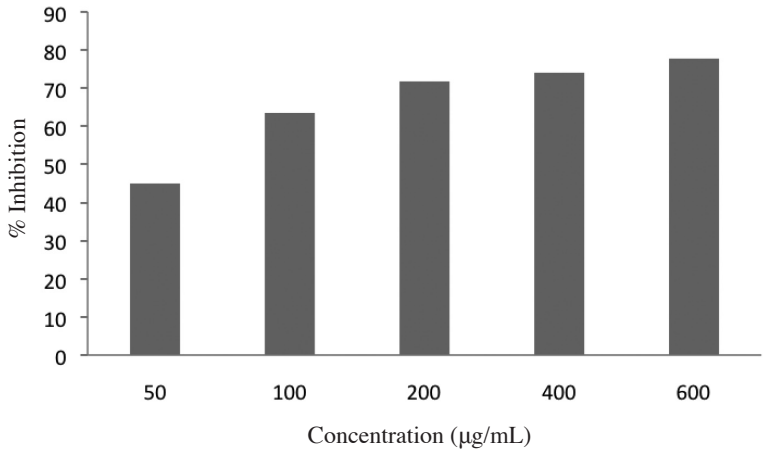

FIGURE 1. Effect of Nisamalaki Churna on α-amylase inhibition activity by Starch Iodine method

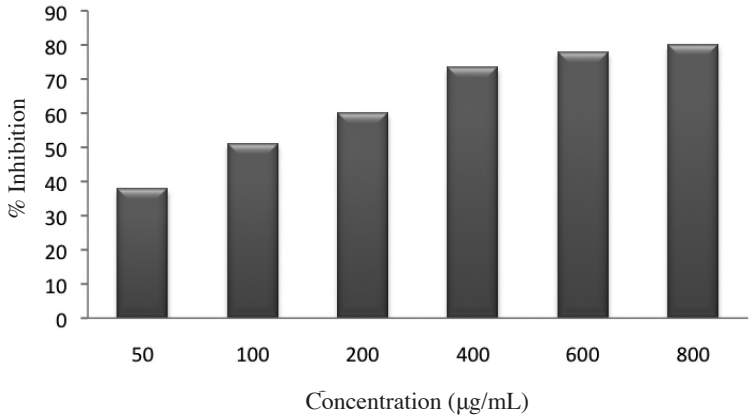

FIGURE 2. Effect of Nisamalaki Churna on α-amylase inhibition activity by DNS method

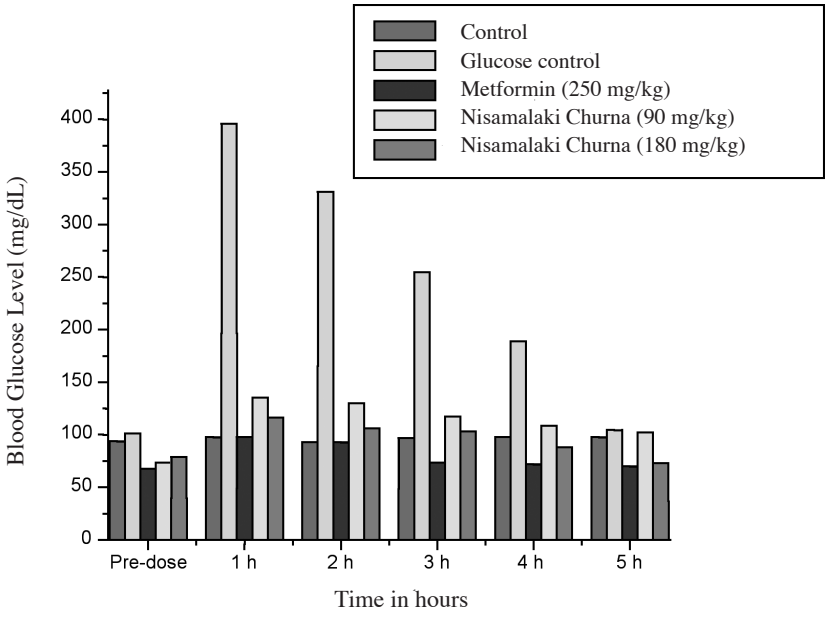

FIGURE 3. Effect of Nisamalaki Churna on blood glucose levels of non fasted rats

ANTIDIABETIC SCREENING IN NON-FASTED RATS

The oral hypoglycaemic effect of Nisamalaki Churna was studied in non-fasted Wistar rats. The Nisamalaki Churna significantly reduced ($p< 0.05 – 0.01$) hyperglycemia at the dose levels of 90 and 180 mg/kg, compared with glucose treated group (group-2). The glucose control group showed significant ($p< 0.01$) increase in glucose levels after 30 min of glucose administration in all the animals when compared with normal group animals. The hypoglycemic effect of Nisamalaki Churna comparably equivalent to the standard metformin (Figure 3). The results of this study indicated that Nisamalaki Churna showed appreciable antidiabetic activity. This study supports the ayurvedic use of Nisamalaki Churna.

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REFERENCES

Anon. 2000. *Vaidya Yoga Ratnavali (Formulary of Ayurvedic Medicines) Impcops* (The Indian Medical Practitioner's Co-Operative Pharmacy & Stores Ltd), Chennai.

Chandrashekhara, N. & Srinivasan, K. 1999. Health beneficial effects of spices. *Trends in Food Science and Technology-Global Perspective*. Mysore, India. Association of Food Scientist and Technologist.

Dey, A.C. 1980. In *Indian Medicinal Plants Used in Ayurvedic Preparations* edited by Bishen Singh, Mahendra Pal Singh & Dehra Dun.

Gururaj, A.E., Belakawadi, M., Venkatesh, D.A., Marme, D. & Salimath, B.P. 2002. Molecular mechanisms of antiangiogenic effect of curcumin. *Biochem. Biophys. Res. Commun.* 297: 934-942.

Miller, G.L. 1959. Use of Dinitrosalicylicacid reagent for determination of reducing sugar. *Analytical Chemistry* 31: 426-428.

Naveen, P. 1993. *The Garden of Life: An Introduction to the Healing Plants of India*. New York: Doubleday.

Nyunai, N., Njikam, N., Mounier, C. & Pastoureau, P. 2006. Blood glucose lowering effect of aqueous leaf extracts of *Ageratum conyzoides* in rats. *African Journal of Traditional, Complementary and Alternative Medicines* 3(3): 76-79.

Punitha, R. & Manoharan, S. 2006. Antihyperglycemic and antilipidperoxidative effects of *Pongamiapinnata* (Linn.) Pierre flowers in alloxan induced diabetic rats. *J. Ethanopharmacol.* 105: 39-46.

Vogel, G.H. 2002. *Drug Discovery and Evaluation Pharmacological Assays*. 2nd ed. Berlin Heidelberg, Germany: Springer-Verlag.

Xiao, Z., Storms, R. & Tsang, A. 2006. A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. *Anal. Biochem.* 351(1): 146-148.

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