ORIGINAL ARTICLE

In-vitro α amylase and glycosidase inhibitory effect of ethanolic extract of antiasthmatic drug — *Shirishadi*

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ABSTRACT

Asthma and diabetes have strong relationship; both are cause and effect of each other. Oxidative stress due to bronchial asthma may cause insulin resistance whereas lack of proper insulin can cause defective smooth muscle relaxant. There is no single medicine available that can manage both diseases, rather the mainstay treatment of bronchial asthma causes hyperglycemia. Keeping this problem in focus, in this study the hypoglycemic effect of an indigenous antiasthmatic Ayurvedic drug *Shirishadi* was evaluated. Pancreatic alpha amylase and glucosidase inhibitors offer an effective strategy to lower the level of post prandial hyperglycemia via control of starch breakdown. For evaluation of hypoglycemic activity of drug, *in-vitro* alpha amylase and alpha glucosidase enzyme inhibition was calculated. Ethanolic extract of compound showed 76.40% + 0.88% reduction in alpha amylase activity and 63.85% + 0.36% in alpha glucosidase activity with IC_{50} 0.68 mg/ml and 2.89 mg/ml, respectively. This study suggests that the ethanolic extract of *Shirishadi* polyherbal compound effectively acts as alpha amylase and glucosidase inhibitor leading to a reduction in starch hydrolysis and hence acts as antiasthmatic as well as hypoglycemic drug.

Key words: Alpha amylase, alpha glucosidase, diabetes, *Shirishadi* polyherbal compound

INTRODUCTION

Shirishadi is a self-experienced polyherbal drug use for the management of allergic diseases (mainly allergic asthma) in Ayurvedic system of medicine. It consist of three herbs namely *Shirisha* (*Albizia lebbeck* L. Benth.), *Nagarmotha* (*Cyprus rotundus* L.), and *Kantakari* (*S. xanthocarpum* L.). Asthma and diabetes have strong relationship, having asthma enhances the possibility of acquiring type-2 diabetes as most of the known life stressors that lead to diabetes. Presently the mainstay medication for the management of asthma includes steroid-based formulations and inhalers and steroids are known to raise blood sugar as part of their mechanism of action. In this study, we evaluate the hypoglycemic effect of *Shirishadi* compound to exclude the side effects of mainstay contemporary medicines. Diabetes mellitus is a chronic disease characterized by elevated blood glucose levels, disturbance in carbohydrate, lipid, and protein metabolism. Absorption of glucose can be delayed by reducing the rate of digestion of starch.[1] Pancreatic α-amylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to a mixture of smaller oligosaccharides consisting of maltose, maltotriose, and a number of α-(l-6) and α-(1-4) oligoglucans. These are then acted on by alpha glucosidases and further degraded to glucose that on absorption enters the blood stream. Degradation of this dietary starch proceeds rapidly and leads to elevated PPHG (postprandial hyperglycemia). It has been shown that activity of HPA (human pancreatic α-amylase) in the small intestine correlates to an increase in postprandial glucose levels, the control of which is therefore an important aspect in treatment of type-2

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diabetes.[2,3] Inhibitors of pancreatic α-amylase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the postprandial serum glucose levels. Glucosidase inhibitors are widely studied and isolated from different sources such as plants and microbes. In 1970s, it was realized that inhibition of all or some of the intestinal disaccharidases and pancreatic α-amylase by inhibitors could regulate the absorption of carbohydrate and these inhibitors could be used therapeutically in the oral treatment of the noninsulin-dependent diabetes mellitus (type-2 diabetes).[4] More advancement in the pharmaceutical research showed that new era antiasthmatics are those that inhibit aldose reductase (AR) which is an important link between asthma and diabetes. Recent studies have indicated that AR inhibition prevents the NF-κB-dependent generation of pro-inflammatory cytokines and chemokines in mouse models of allergic airway inflammation indicating the potential use of AR inhibition as a novel tool to control allergic responses.[5] Therefore, it was decided to assess the hypoglycemic effect of above mentioned antiasthmatic drug by assessing its *in-vitro* activity on alpha amylase and alpha glucosidase and to postulate indirect evidence of its action on AR inhibition.

MATERIALS AND METHODS

Collection of Plant Material and Sample Extraction

The plants *A. lebbeck* (L.) Benth, *C. rotundus* L., and *S. xanthocarpum* L. were collected from local market of Varanasi, India. The identification of the plants was done by Prof. A. K. Singh, Department of Dravyaguna, S.S.U., Varanasi. The *Shirishadi* group contains *Shirisha* (*A. lebbeck* L. Benth), *Nagarmotha* (*Cyperus rotandus* L.), and *Kantakari* (*S. xanthocarpum* L.) Hydroalcoholic Extraction (Distilled water: Ethanol = 2:1) of drug were done separately by hot percolation method through soxhlet apparatus. Thereafter, extract was dried using rotary evaporator and dried extract was put to the process of standardization.[6,7]

In-vitro alpha Glucosidase Inhibition Study

The tissue homogenate prepared from small intestine of rats was used as enzyme source. A small piece of small intestine was taken out in precooled phosphate buffer saline (PBS), thoroughly cleaned, dried on blotting paper, weighed, and then homogenized in glass Teflon homogenizer. The homogenate was centrifuged at 5,000 g for 30 min and its supernatant was used as enzyme source. Final volume of supernatant was maintained to 20% (w/v).

Content of *Shirishadi* ayurvedic nebulizer

Name of the drug	Botanical name	Part used	Approx. quantity in 100 ml of extract
Shirisha	*Albezia lebbeck* (L.)	*Twaka* (Bark)	20 mg
Nagarmotha	*Cyperus rotundus* L.	*Kanda* (Rhizome)	20 mg
Kantkari	*Solanum xanthocarpum* L.	*Panchanga* (whole plant)	20 mg

The spectrophotometric assay method was used with slight modification.[8] Here, 40 µl tissue homogenate was mixed with 80 µl of test, standard, drug vector and incubated for 15 min at 37°C. Thereafter, 280 µl maltose (37 mM) was added and further incubated for 30 min. Finally, the reaction was stopped by putting the tubes in boiling water for 10 min. The tubes were centrifuged and glucose concentration was assessed in the supernatant by glucose oxidase/presence of peroxidase (GOD/POD) method based kit. The change in activity was expressed as:

% inhibition (Absorbance Control – Absorbance Test/ Absorbance Control) ×100

In-vitro Alpha Amylase Inhibition Study

The spectrophotometric assay method was used with slight modification.[9] The 90 µl of homogenate-supernatant was mixed with 180 µl of 40 mM phosphate buffer (pH 6.9), test sample, and positive control of various concentrations and incubated at 37°C for 15 min. To this reaction mixture, 360 µl of substrate, 2-Chloro-4-Nitrophenyl-α-Maltotrioside ($CNPG_3$ 0.5 mg/ml) was added, mixed and for incubated 37°C for 10 min. Finally, absorbance was measured at 405 nm against blank in spectrophotometer. A control reaction was carried out without the test sample.

RESULT AND DISCUSSION

Shirishadi polyherbal compound is an indigenous Ayurvedic drug mainly used clinically for the management of allergic bronchial asthma, allergic rhinitis, and other respiratory conditions associated with dyspnea. Experimental and clinical studies showed that it has potent bronchodilator,[10] anti-inflammatory,[11] and anti-anaphylactic[12] effects. It significantly improves the peak expiratory flow rate (PEFR) and forced vital capacity (FVC) in the patients of bronchial asthma. All the three herbs of *Shirishadi* polyherbal compound are reported for having potent alpha amylase or alpha glucosidase inhibitory activities. *A. lebbeck* (*Shirisha*) is reported for having alpha amylase inhibitory activity; a research study by Faiyaz *et al*. showed the presence of a novel isoform of proteinaceous a-amylase inhibitor (a-AI) from seed extract of *A. lebbeck*.[13] The alpha amylase inhibitory activity is shown by some of the phenolic compound isolated from *C. rotundus* (L.).[14] In addition to this *C.*

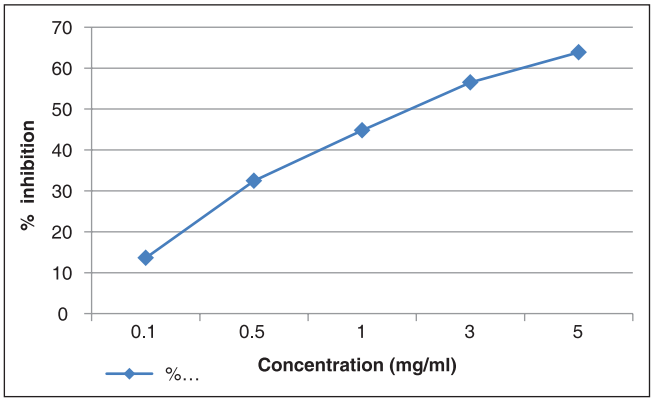

Figure 1: The percentage inhibition of α-glucosidase activity by ethanolic extract of *Shirishadi* compound

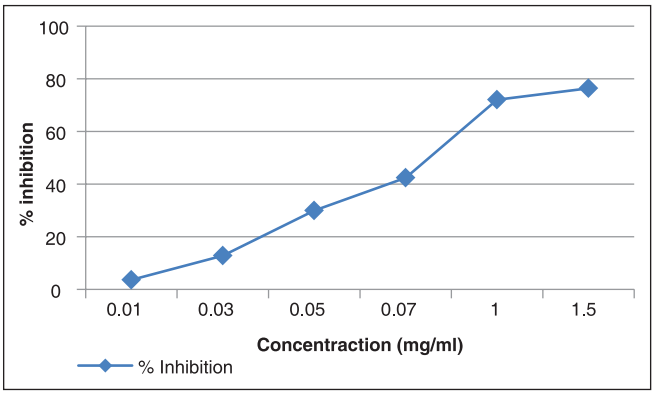

Figure 2: The percentage inhibition of α-amylase activity by ethanolic extract of *Shirishadi* compound

rotundus is also reported for having the antidiabetic activity.[15] An experimental study showed that the extracts of *C. rotundus* had displayed 100% inhibition of alpha glucosidase at the concentration of 100 µg/ml.[16] *S. xanthocarpum* is also reported for having alpha amylase and alpha glucosidase inhibitory activities.[17] Evidences showed that insulin produces a shift toward a hypercontractile state suggests that poorly controlled diabetics may, as a result of reduced plasma insulin levels, display reduced airway smooth muscle contractile activity.[18] Moreover, oxidative stress due to bronchial asthma increase susceptibility for diabetes mellitus; this situation becomes nastiest, as the mainstay contemporary medicine for the treatment of bronchial asthma causes hyperglycemia. Thus, both these two disease are cause and effect of each other. And it is very essential to consider that drug chosen for the management of bronchial asthma should not increase the blood glucose level. Natural α-amylase and α-glycosidase inhibitors from food-grade plant sources offer an attractive therapeutic approach for the treatment of postprandial hyperglycemia by decreasing glucose release from starch and delaying carbohydrate absorption by inhibiting the activity of the carbohydrate hydrolyzing enzymes in the small intestine and may have potential for use in the treatment of diabetes mellitus and obesity.[19,20] On the basis of the prevalence, the delay or inhibition of carbohydrate digestion would contribute to optimize a postprandial blood glucose level. There are many natural resources with the α-glucosidase inhibitory activity and some of them are more specific for sucrose inhibition rather than maltase inhibition. This research suggest that the presence of polyphenolic compounds of *Shirishadi* compound may have a potentially important role in managing diabetes via the inhibition of α-amylase and α-glucosidase enzyme activities. The α-glucosidase and α-amylase inhibitory activity of *Shirishadi* ethanolic extract, 63% and 76% was confirmed in this study.

Nonetheless, it is important to mention here that α-amylase breaks down starch into disaccharides that are acted upon by isomaltase, especially α-glucosidase to release glucose. The presence of potent α-glucosidase inhibitory activity, therefore, appears more important in controlling the release of glucose from disaccharides in the gut than α-amylase inhibition. However, moderate α-amylase inhibition with potent α-glucosidase inhibitory activity may offer better therapeutic strategy that could slowdown the availability of dietary carbohydrate substrate for glucose production in gut. Food-grade phenolic α-amylase inhibitors from dietary plant extracts are potentially safer and may, therefore, be a preferred alternative for modulation of carbohydrate digestion and control of glycemic index of food products. Furthermore, the present results demonstrate that the ethanolic extract of *Shirishadi* compound contained potent α-glucosidase, α-amylase inhibitors and were effective for suppressing postprandial hyperglycemia.

CONCLUSION

In conclusion, ethanolic extract of *Shirishadi* polyherbal compound demonstrates good α-glucosidase and α-amylase inhibitory activity [Figures 1 and 2]. From previous studies, it has been found that extract contains compounds like phenylpropanoyl esters of catechol glycosides that are reported for having hypoglycemic activity. *Shirishadi* extracts have the dual advantage of having α-glucosidase and pancreatic α-amylase inhibitor action; hence, it may prove to be best drug for the management of bronchial asthma associated with diabetes mellitus. As the drug was reported for having anti-inflammatory and anti-allergic activities, it can be recommended that its action on aldose reductase should also be evaluated.

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