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ALPHA AMYLASE AND ALPHA GLUCOSIDASE INHIBITORY ACTIVITY OF AMALAKI RASAYANA-AN AYURVEDIC FORMULATION

Dr. Prashant B. Bedarkar*

Assistant Prof., Dept. of Rasashstra and Bhaishajya Kalpana, IPGT & RA, Gujarat Ayurved University, Jamnagar, Gujarat, India.

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***Corresponding Author**
Dr. Prashant B. Bedarkar
Assistant Prof., Dept. of Rasashstra and Bhaishajya Kalpana, IPGT & RA, Gujarat Ayurved University, Jamnagar, Gujarat, India.

ABSTRACT

Introduction- Powder of fruit pulp of Indian gooseberry (*Amalaki*) levigated multiple times with its juice i.e. *Amalaki swarasa bhavita Amalaki churna* (ASBAC) is commonly regarded as *Amalaki Rasayana* (AR) is recommended in Ayurvedic classics and practiced in the management (treatment, prevention of complications) of *Madhumeha* (Diabetes). Alpha amylase and alpha glucosidase activities of *Amalaki Rasayana* has not been evaluated till the date. Hence, its in-vitro study was conducted. **Aims and objectives-**To evaluate α- amylase and α- glucosidase activities of *Amalaki Rasayana*. **Materials and methods-** *Amalaki Rasayana* was prepared with 16 times levigation (*Bhavana*) of fine powder of fruit pulp of Indian gooseberry (*Amalaki, Emblica officinalis Gaertn.*) with its fresh fruit pulp juice equal in quantity for each levigation leading to 36.33% of powder and 63.66% of total solid content derived from 581.39% of fresh fruit pulp juice. Methanolic extract of *Amalaki Rasayana* was tested for Porcine pancreatic α- amylase inhibition and intestinal α- glucosidase activities at 4 concentrations (40, 80, 120 and 160 µg/ml) through starch as base and chromogenic DNSA (3, 5-dinitrosalicylic acid) as colouring agent, in 2 different solutions by recording absorbance with UV VIS Spectrophotometer. **Results-***Amalaki Rasayana* exhibited comparatively better α- amylase inhibition and α-glucosidase inhibition by acetic acid buffer and inhibition was dose dependent at all concentration. **Conclusion-** *Amalaki Rasayana* exhibited inhibition of Pancreatic Alpha amylase and intestinal alpha glucosidase in dose dependent manner in the concentration of 40, 80, 120 and 160 µg/ml thus have potential to reduce post prandial hyperglycemia.

KEYWORDS: Alpha amylase, Alpha glucosidase, *Emblica officinalis*, *Amalaki Rasayana.*

INTRODUCTION

Formulation prepared by soaking method of *Bhavana* (Levigation) from Powder and juice of fruit pulp of *Emblica officinalis* Gaertn., known as "*Amalaki Rasayana*"[1] is mentioned in ancient most Ayurvedic text of medicine "Charaka samhita", which is vitalizer and possess wide preventive and therapeutic arena.[2,3,4,5,6,7,8] Powder of fruit pulp of Indian gooseberry (*Amalaki*) levigated multiple times with its juice i.e. *Amalaki swarasa bhavita Amalaki churna* (ASBAC) is commonly regarded as *Amalaki Rasayana* (AR) and is widely practiced formulation for rejuvenation as general tonic.[9] Indian gooseberry (especially juice) is recommended in Ayurvedic classics, proven efficacious and widely practiced in the management (treatment, prevention of complications) of Diabetes.[10,11] *Amalaki Rasayana* assures perennial availability of drug, improves palatability, shelf life and potentiate the classical dosage forms of *Amalaki* i.e. *Churna* and *Swarasa*. Although *Amalaki* has been proven to posses Alpha amylase and alpha glucosidase inhibitory properties through in vitro studies, formulation *Amalaki Rasayana* has not been evaluated for these activities till the date, hence, its in-vitro studies i.e. Porcine pancreatic α-amylase and intestinal α- glucosidase inhibitory was conducted. Though the disease *Prameha* as per Ayurvedic concepts consists of constellation of signs and symptoms– it is equated in limited sense to the diabetes as described in modern medicine. The major pathological event is hyperglycemia which leads to series of associated complications. Enzymes, pancreatic α- amylase and intestinal α-glucosidase affect glucose degradation and thus absorption. Rapid degradation of dietary starch by α-amylase leads to elevated postprandial hyperglycemia (PPHG). It has been shown that activity of HPA (human pancreatic α-amylase) in the small intestine correlates to an increase in post-prandial glucose levels, the control of which is therefore an important aspect in treatment of type 2 diabetes.[12] Inhibitors of pancreatic α-amylase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the post-prandial serum glucose levels.[13,14,15]

Experimental Protocols

MATERIALS AND METHODS

The test drug: *Amalaki Rasayana* (AR) or *.Amalaki swarasa bhavita Nisha Amalaki Churna* (ASBAC)] was prepared in the Department of R.S. & B.K, IPGT & RA, Gujarat Ayurved University, Jamnagar, India. It was prepared with 16 times levigation (*Bhavana*)[16] of fine

powder of fruit pulp of Indian gooseberry (*Amalaki, Emblica officinalis Gaertn.*) with its fresh fruit pulp juice equal in quantity for each levigation. Thus prepared formulation *Amalaki swarasa bhavita Nisha Amalaki Churna* (ASBAC) was composed of 36.33% of powder and 63.66% of total solid content derived from 581.39% of fresh fruit pulp juice of *Emblica officinalis*.

α -Amylase Inhibitory Activity

The following procedure was followed for present study:

- Chemicals and Reagents: Phosphate buffer, Acetic acid buffer, PPA (Porcine pancreatic α-amylase) and Intestinal alpha glucosidase were analytical grade and acquired from Himedia and Difco.
- Test drug "*Amalaki Rasayana*"ASBAC (as Inhibitor of alpha amylase enzyme): Four concentrations of Methanolic extract of ASBAC prepared by method of Alcohol soluble extractive of Ayurvedic pharmacopoeia further solidified and dehydrated at bellow 40^0C with 4 different concentrations (4, 8,12 and 16%) were taken for analysis.

Alpha amylase inhibitory activity

The analysis of ASBAC for PPA inhibition was initially performed qualitatively by starch-iodine colour assay. The lead extracts were further quantified with respect to PPA inhibition using the chromogenic DNSA (3, 5-dinitrosalicylic acid) method.

The α-amylase inhibitory activity was determined according to the method described by Miller.[17] Briefly, different solutions were prepared and different concentrations of inhibitor (Methanolic extract of *Amalaki Rasayana*) were incorporated in 4 same concentrations ranging from 4% to 16% (40, 80, 120 and 160 µg/ml) and were incubated at room temperature for 15 min and followed by addition of 1% starch in all test tubes. The reaction was determined the addition of 400 µl of 3,5 di nitro salycylic acid (DNSA) color reagent, placed in boiling water for 5 min, cooling to room temperature and diluted with 15 ml of distilled water. The absorbance measured at 540 nm (Schimadzu UV-VIS spectrophotometer) in triplicate and average values were taken for calculations. 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 2 different solvents. The results were expressed as % inhibition calculated using the formula:

$$\text{Inhibitory activity of alpha amylase enzyme} = \frac{\text{Abs (control) - Abs (extract)} \times 100}{\text{Abs of (control)}}$$

α - Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity was determined using the standard method.[18] The enzyme solution was prepared by dissolving 0.5 mg α-glucosidase in 10 ml phosphate buffer (pH 7.0) containing 20 mg bovine serum albumin. It was diluted further to 1:10 with phosphate buffer just before use. Sample solutions were prepared by dissolving 4 mg sample extract (Aqueous extract of *Amalaki Rasayana*) in 400 µl DMSO. Four concentrations: 40, 80, 120 and 160 µg/ml were prepared and 5 µl each of the sample solutions or DMSO (sample blank) was then added to 250 µl of 20 mM p-nitrophenyl-α-D-glucopyranoside and 495 µl of 100 mM phosphate buffer (pH 7.0). It was pre-incubated at 37°C for 5 min and the reaction started by addition of 250 µl of the enzyme solution, after which it was incubated at 37°C for exactly 15 min. 250 µl of phosphate buffer was added instead of enzyme for blank. The reaction was then stopped by addition of 1000 µl of 200 mM Na_2CO_3 solution and the amount of p-nitrophenol released was measured by reading the absorbance of sample against a sample blank (containing DMSO with no sample) at 400 nm using UV visible spectrophotometer in triplicate.

RESULTS AND DISCUSSION

α -Amylase Inhibitory Activity of Amalaki Rasayana (Asbac)

Table 1: α-Amylase Inhibitory Activity On The Basis Of Concentration Of Inhibitor And Solvent Variation.

Concentration	% Inhibitions with respects to different solutions(Average)	
	Phosphate buffer	Acetic acid buffer
16%	69.25	71.22
12%	62.33	63.33
8%	54.55	55
4%	48.36	49.2

It is evident from Table 1 that, comparatively more percent inhibition of α-amylase enzyme was demonstrated by sample ASBAC in Acetic buffer than that of Phosphate buffer at all tested concentrations.

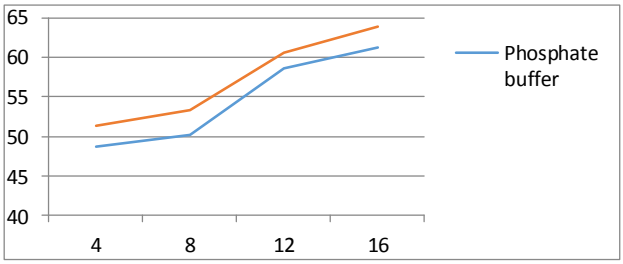

Figure 1: α-Amylase inhibitory activity activity of *Amalaki Rasayana* at different concentrations with different solvents.

α -Glucosidase Inhibitory Activity of Amalaki Rasayana

Table 2: α-Glucosidase inhibitory activity on the basis of concentration of inhibitor and solvent variation.

Concentration	Inhibitions with respect to different solutions (Average)	
	Phosphate buffer %	Acetic acid buffer %
16%	61.25	63.88
12%	58.66	60.54
8%	50.22	53.33
4%	48.66	51.25

It is evident from Table 2 that, comparatively more inhibition of α-amylase enzyme was demonstrated by sample ASBAC in Acetic buffer than that of Phosphate buffer at all tested concentrations.

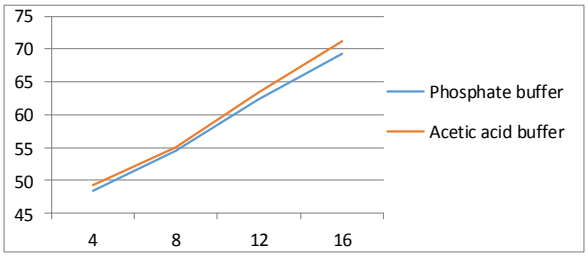

Fig. 2: α-Glucosidase inhibitory activity of *Amalaki Rasayana* at different concentrations with different solvents.

Results of both the study depicts that, *Amalaki Swarasa Bhavita Amalaki churna* exhibited inhibition of Pancreatic Alpha amylase and intestinal alpha glucosidase in Phosphate buffer as well as in Acetic acid buffer reaction mixture, in dose dependent manner in the concentration of 4,8,12 and 16%.

Fresh juice (*Swarasa*) is most frequently preferred dosage form of *Amalaki* in Ayurvedic classics, apart from its powder form (*churna*) for the management of Diabetes. *Swarasa*; a crude galanical is most potent dosage form among 5 basic dosage forms of Ayurveda

(*Pachavidha kashaya*), due to assurance of all chemical ingredients and thus therapeutic attributes. Researches on significance of *Amalaki Swarasa* as that of dried form suggest that, Ascorbic acid content of fresh fruits (329mg/100gm) was more than that of *Amalaki Churna* (39.5mg/100gm). *Amalaki swarasa* and *Amalaki swarasa Bhavita Amalaki Churna* had 20 times and 18times more Ascorbic acid content than that of *Amalaki Kwatha* and *Amalaki Kwatha bhavita Amalaki Churna.*[19]

As *Amalaki swarasa* is not available throughout the year, therefore it is need of time to modify the formulation. In the present study, comparatively more durable, probably more potent and palatable dosage form of *Amalaki*, "*Amalaki Rasayana"* i.e. "*Amalaki swarasa bhavita Amalaki Churna,* (ASBAC)*"* from its powder and juice dosage forms was formulated ad tested.

Although *Amalaki* has been proven to posses α- amylase and α- glucosidase inhibitory properties through in vitro studies,[20,21] still the results may not be equivocal and comparable due to various reasons like different standard of drug, different manufacturing process, different study protocol, different source of standard enzyme etc which have been prove have their impact of variation in study outcomes. Control materials with α-amylase of nonhuman origin were not commutable with the enzyme in human sera and should not be used for inter - method calibration.[22]

Bhavana (unique Ayurvedic pharmaceutical process)*;* besides wet trituration process is also a size reduction technology, frequently used in Ayurvedic pharmaceutics is an example of drug combination. It has multi-dimensional pharmaceutical and therapeutic implications. *Bhavana* have its utility in almost all pharmaceutical processing; affecting the physicochemical and biological properties of dosage form. Process of *Bhavana* to drug in powder form with liquid extract of same drug increases its potency.[23] Modified process of levigation i.e. Soaking method especially mentioned for *Amalaki* by ancient most text of Ayurvedic Medicine, Charaka Samhita is specifically mentioned for preparation of *Amalaki Rasayana,* which was used in preparation of formulation "*Amalaki Rasayana*" in present study. Hence there is need to evaluate status of α- amylase ad α- glucosidase properties of fresh *Amalaki* fruit pulp in its popular dosage form "*Amalaki Rasayana*", possessing wide range of therapeutics. In view of multiple pharmaceutical and practical analytical experimental variants in in-vitro study of Pancreatic α- amylase and intestinal α-glucosidase, with potential to affecting test results, it

is advisable to plan comparative study of evaluation of effect of *Amalaki Swarasa, Churna* and *Amalaki swarasa bhavit Amalaki churna* (ASBAC) in single experiment.

Various groups of phytochemicals present in *Emblica officinalis* are known to posses inhibitory effect on Pancreatic Alpha Amylase and Intestinal Alpha Glucosidase in in vitro studies. Effect of combination of Gallic acid (GA) on inhibitory effect of Acarbose on the ezymes showed that, mixtures of the samples (50% acarbose & 50% GA; 75% acarbose &25% GA; and 25% acarbose & 75% GA) were prepared. The results revealed that the combination of 50% acarbose and 50% GA showed the highest α-glucosidase inhibitory effect, while 75% acarbose & 25% GA showed the highest a-amylase inhibitory effect.[24] Phenolic compounds such as phenolic acids and flavonoids bind covalently to alpha amylase and change its activity due to the ability to form quinones or lactones that react with nucleophilic groups on the enzyme molecule.[25]

In view of potent α- amylase and intestinal α- glucosidase activity of formulation "*Amalaki Rasayana*", and synergistic inhibitory effect of Gallic Acid with Acarbose, it could best combination to prevent side effects of α- Amylase and α- glucosidae inhibitors i.e. bloating, belching, fullness of abdomen and diabetic gastropathy as it probably will show synergistic activity o these 2 enzymes and posses mild laxative effect, thus further may reduce gastric emptying time favouring to reduce post prandial hyperglycemia. Hence studies of *Amalaki Rasayana* on Drug-drug interaction with Alpha Amylase and Alpha glucosidae inhibitors is potent area of research, are recommendable, which could started from retrograde clinical survey studies.

CONCLUSION

Methanolic extract of *Amalaki Swarasa Bhavita Amalaki churna* exhibited inhibition of Pancreatic Alpha amylase and intestinal Alpha glucosidase in Phosphate buffer as well as in Acetic acid buffer reaction mixture, in dose dependent manner in the concentration of 4,8,12 ad16%, thus have potential to reduce Post Prandial hyperglycemia.

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Conflict of Interest-None declared.

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