**INTRODUCTION**

*Nishamalaki* (NA), various combination formulations of Turmeric (*Haridra, Curcuma longa* Linn.) and Indian gooseberry (*Amalaki, Emblica officinalis* Gaertn.); is recommended in Ayurvedic classics,[1-3] proven efficacious and widely practiced in the management (treatment, prevention of complications) of *Madhumeha* (Diabetes). Alpha amylase and alpha glucosidase activities of *Nishamalaki*, prepared by levigation method has not been evaluated till the date, hence, its in-vitro study was conducted. *Nishamalaki* yoga (NA) was prepared with 16 times levigation (*Bhavana*) of combination of fine Turmeric powder [*Haridra churna* (HC)] and fine powder of fruit pulp of goose berry (*Amalaki churna*) in equal quantity with equal quantity of fresh fruit pulp juice of *Amalaki* (*Swarasa*) as that of combined powder for each levigation followed by drying. Prepared *Nishamalaki* consists of 53.3% of combined powder and 46.69% of total solid content of *Amalaki* *Swarasa*. Methanolic extract of *Nishamalaki* 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. Results and Conclusion: *Nishamalaki* exhibited comparatively better α-amylase inhibition and α-glucosidase inhibition by acetic acid buffer in the concentration of 40, 80, 120 and 160 μg/ml and inhibition was dose dependent at all concentration thus have potential to reduce post prandial hyperglycemia.

EXPERIMENTAL PROTOCOLS
MATERIALS AND METHODS
The test drug: Nishamalaki (NA) or Amalaki swarasa bhavita Nisha Amalaki Churna (ASBNAC) was prepared with 16 times levigation (Bhavanid) of equal quantity of fine powder of Turmeric and fine powder of fruit pulp of Indian gooseberry (Amalaki, Emblica officinalis Gaertn.) with fresh fruit pulp juice of Amalaki equal in quantity as that of combined powders for each levigation. This was followed by drying of mixture after spreading it in the form of thin sheets in oven at bellow 60°C and micronization by grinding in pestle and mortar and laboratorial mixer juicer (blender) to fine powder and stored in air tight containers. Thus prepared formulation Nishamalaki consists of 53.3% of combined powder and 46.69% of total solid content derived from 426.43% of fresh fruit pulp juice.

(I) 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 “Nishamalaki” ASBNAC (as Inhibitor of alpha amylase enzyme): Four concentrations of Methanolic extract of ASBAC prepared by method of Alcohol soluble extractive of Ayurvedic pharmacopeia further solidified and dehydrated at bellow 40°C 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 chromogenic DNA (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 Nishamalaki) 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 salicylic 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 (Shimadzu 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.

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

RESULTS AND DISCUSSION
(I) α-AMYLASE INHIBITORY ACTIVITY OF NISHAMALAKI (ASBNAC)
Table No. 1: α-Amylase inhibitory activity on the basis of concentration of inhibitor and solvent variation.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>% Inhibitions with respects to different solutions(Average)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phosphate buffer</td>
</tr>
<tr>
<td>16%</td>
<td>60.23</td>
</tr>
<tr>
<td>12%</td>
<td>57.25</td>
</tr>
<tr>
<td>8%</td>
<td>48.44</td>
</tr>
<tr>
<td>4%</td>
<td>38.25</td>
</tr>
</tbody>
</table>

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

(II) 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 Nishamalaki) 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 Na₂CO₃ 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.
Figure 1: α-Amylase inhibitory activity of Nishamalaki at different concentrations with different solvents.

(II) α-GLUCOSIDASE INHIBITORY ACTIVITY OF NISHAMALAKI (ASBNAC)

Table No. 2: α-Glucosidase inhibitory activity on the basis of concentration of inhibitor and solvent variation.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Inhibitions with respect to different solutions (Average)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phosphate buffer %</td>
</tr>
<tr>
<td>16%</td>
<td>50.54</td>
</tr>
<tr>
<td>12%</td>
<td>48.56</td>
</tr>
<tr>
<td>8%</td>
<td>40.25</td>
</tr>
<tr>
<td>4%</td>
<td>33.66</td>
</tr>
</tbody>
</table>

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 Nishamalaki at different concentrations with different solvents.

Results of both the study depicts that, Amalaki Swarasa Bhavita Nishamalaki 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. Swarasas; 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.[19] Researches on significance of Amalaki Swarasa as that of dried form present 20 times and 18 times more Ascorbic acid content than that of Amalaki Kwatha and Amalaki Kwatha bhavita Amalaki Churna.[20]

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 and Turmeric, “Nishamalaki” i.e. “Amalaki swarasa bhavita Nishamalaki Churna, (ASBNAC)” from their powder and juice dosage forms was formulated and tested.

Although Nishamalaki and Amalaki rasayana (Amalaki Swarasa bhavita Amalaki churna) Bhavita Amalaki Churna had 20 times and 18 times more Ascorbic acid content than that of Amalaki Kwatha and Amalaki Kwatha bhavita Amalaki Churna.[20] still the results may not be equivocal and
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 multidimensional pharmaceutical and therapeutic implications. Bhavana has 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] Hence there is need to evaluate status of α- amylase and α-glucosidase properties of levigated product prepared from powder of both the drugs possessing wide range of therapeutics.

Various groups of phytochemicals present in Emblica officinalis and Curcuma longa are known to possess 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 Acorbose on the enzymes 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 α-amylase inhibitory effect.[26] 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.[27] Previous studies on Nishamalaki also suggest that there is possibility of additive or synergistic effect among 2 drugs Turmeric and Amalaki when formulated into a compound formulation.[28,29] Studies also suggest synergistic antioxidant effect among these 2 drugs.[30,31]

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

CONCLUSION
Methanolic extract of Amalaki Swarasa Bhavita Nishamalaki churna exhibited inhibition of Pancreatic Alpha amylase and intestinal Alpha glucosidase in Phosphat buffer as well as in Acetic acid buffer reaction mixture, in dose dependent manner in the concentration of 4.8,12 and 16%, thus have potential to reduce Post Prandial hyperglycemia.

AKNOWLEDGEMENT
Author is thankful to SMR services, Rajkot, Gujarat, India, for supporting for analytical facilities and Dr VJ Shukla, Head, Pharmaceutical chemistry laboratory, IPGT & RA for guidance, Head, Dept of Rasashastra & BK, IPGT & RA and Director, IPGT & RA, for technical support.

Conflict of Interest: None declared.

REFERENCES


31. Department of Pharmaceutical Science, Faculty of Medicine, Maharaj Hospital, Chiang Mai University, Chiang Mai, Thailand.