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Website: www.ayujournal.org
DOI: 10.4103/0974-8520.169000
Quick Response Code:

Pharmacological Study

Experimental studies on glycolytic enzyme inhibitory and antiglycation potential of *Triphala*

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Abstract

Introduction: Imbalance in cellular metabolism of carbohydrates and lipids is observed in diabetes mellitus. Pancreatic α-amylase and α-glucosidases are responsible for the conversion of polysaccharides into glucose that enters in the blood stream. *Triphala* has shown antidiabetic effects (type 2) in human subjects. However, its effects on glycolytic enzymes and protein glycation have not been studied. **Aim:** To evaluate glycolytic enzyme inhibitory and antiglycation potential of *Triphala*. **Materials and Methods:** *Triphala Churna* was extracted with cold water and subjected to phytochemical analysis. Studies on α amylase and α glucosidase inhibition were performed, and its antiglycation potential was determined. **Results:** *Triphala* extract showed prominent α-amylase inhibitory potential (48.66% at concentration 250 μg/ml). Percent α-glucosidase inhibition increased with increasing concentration of the extract (6.32–40.64%). Extract showed remarkable results for antiglycation potential. *Triphala* extract showed glycation inhibition by inhibiting fructosamine; fructosamine inhibition was found to be 37.74%, protein carbonyls were inhibited up to 15.23% whereas protein thiols were inhibited up to 84.81%. **Conclusion:** *Triphala* showed glycolytic enzyme inhibitory and antiglycation potential. Hence, it can be effectively used in the diabetes management.

Key words: α-Amylase, α-glucosidase, antiglycation, diabetes, *Triphala*

Introduction

Triphala, a rejuvenating drug mentioned in Ayurveda, is one of the most common and economical medicinal preparations accessible in India. It is regarded as a therapeutic agent having balancing and revivifying effects on three humors as per Ayurveda viz. *Vata*, *Pitta* and *Kapha*. *Triphala* consists of equal portions of three medicinal herbs as, Indian Gooseberry (*Emblica officinalis* Gaertn.), Chebulic Myrobalan (*Terminalia chebula* Retz.), and Beleric Myrobalan (*Terminalia bellerica* Gaertn.).[1,2]

In the modern era, a number of researches have been performed on *Triphala* that has established antioxidant and revitalizer[3,4] anti-diarrhoeal[5] and antiobesity[6] effects of *Triphala*.

Imbalance in cellular metabolism of carbohydrates and lipids is observed in noninsulin dependent diabetes mellitus. Such a condition predisposes to increase in postprandial blood glucose levels.[7,8] During type 2 diabetes mellitus, a sudden rise in blood glucose levels is observed that is contributed due to starch hydrolysis by pancreatic α-amylase and glucose uptake by α-glucosidase.[9]

Enzyme pancreatic α-amylase is the main enzyme, which plays a key role in starch hydrolysis and converts them into small oligosaccharides. These are then further catalyzed by α-glucosidase that convert them into glucose that after absorption enters into the blood stream. This process ensues in haste that ultimately predisposes the condition of postprandial hyperglycemia. Thus, impeding the process of digestion of starch could play a vital role in the management of diabetes.[10] Drugs such as acarbose, voglibose, and miglitol are known to inhibit glycolytic enzymes. However,

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How to cite this article: Ganeshpurkar A, Jain S, Agarwal S. Experimental studies on glycolytic enzyme inhibitory and antiglycation potential of *Triphala*. Ayu 2015;36:96-100.

their nonspecific response and adverse effects like abdominal discomfort and diarrhea[11] restrict their frequent use. Use of herbal remedies seems to be a promising approach in the treatment of diabetes in terms of no or less side effects and economical.[12]

A number of plant derived polyphenols are known to demonstrate antiobesity and antidiabetic effects by virtue of the inhibiting activity of enzymes such as lipase and α-glucosidase.[13-15] *Triphala* has also shown antidiabetic effects (type 2) in human subjects.[16] However, its effects on glycolytic enzymes and protein glycation have not been studied. Hence, the aim of the present work was to evaluate glycolytic enzyme inhibitory and antiglycation potential of *Triphala*.

Materials and Methods

Chemicals

The various chemicals used in this study includes porcine pancreatic amylase (PPA), gallic acid (SRL, Mumbai), acarbose (Bayer Pharmaceutical), trichloro acetic acid, ascorbic acid (Merck, Mumbai), ammonium molybdate (Qualigens, Mumbai), 3,5-dinitrosalicylic acid (Loba Chemie, Mumbai). *Triphala Churna* was purchased from local market.

Extraction

A total of 50 g of *Triphala* powder was extracted with 100 ml distilled water (cold perfusion) for 6 h. The extract was then filtered and concentrated. The extract was subjected to phytochemical analysis as per reported method.[17]

Glycolytic enzyme inhibition studies

α-*Amylase inhibitory assay*

Porcine pancreatic amylase inhibitory assay was performed by following standard method.[18] About 2 mg of starch was suspended in each of the tubes containing 0.2 ml of 0.5 M Tris-HCl buffer (pH 6.9) and 0.01 M $CaCl_2$. The tubes containing the substrate solution were boiled for 5 min and were then incubated at 37°C for 5 min. About 0.2 ml of extract was taken in each tube containing different concentrations (10, 20, 40, 60, 80, and 100 μg/ml) of dimethyl sulfoxide (DMSO). PPA was dissolved in Tris-HCl buffer to form a concentration of 2 units/ml and 0.1 ml of this enzyme solution were added to each of the above mentioned tubes. The reaction was carried out at 37°C for 10 min and was stopped by adding 0.5 ml of 50% acetic acid in each tube. The reaction mixture was centrifuged at 3000 rpm for 5 min at 4°C. The absorbance of the resulting supernatant was measured at 595 nm using a spectrophotometer (Shimadzu 1700 spectrometer). The α-amylase inhibitory activity was calculated as follows:

$$= [(Ac+) - (Ac-)] - [(As - Ab)]/[(Ac+) - (Ac-)] \times 100,$$

where Ac+, Ac−-, As and Ab are defined as the absorbance of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme activity), a test sample (with enzyme), and a blank (a test sample without enzyme), respectively.

α-*Glucosidase inhibitory activity*

The α-glucosidase inhibitory activity was determined using the standard method.[19] 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 (BSA). It was diluted further to 1:10 with phosphate buffer just before use. Sample solutions were prepared by dissolving 4 mg sample extract in 400 μl DMSO. Five concentrations; 50, 100, 150, 200, and 250 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 the sample against sample blank (containing DMSO with no sample) at 400 nm using UV–visible spectrophotometer.

Antiglycation potential

Glycation of bovine serum albumin

Albumin glycation was performed with certain modifications.[20] Glycated BSA samples were prepared with BSA (10 mg/ml) fructose (250 mM) in potassium phosphate buffer (200 mM, pH 7.4 containing 0.02% sodium azide) with extract. These were incubated in the dark for 4 days at 37°C in sealed tubes. Positive control (BSA + fructose) was also maintained in similar conditions. All the incubates were made in triplicates. The unbound form of fructose in the solution was obtained by dialysis against the phosphate buffer (200 mM, pH 7.4) and was stored at 4°C. The resultant obtained was used for determination of antiglycation activity of the extract by estimation of fructosamines adducts, protein carbonyls, protein thiols, and Congo red absorbance.

Estimation of fructosamine

Nitrobluetetrazolium assay was used for the determination of fructosamine.[21] About 0.8 ml of nitrobluetetrazolium (0.75 mM) in sodium carbonate buffer (100 mM, pH 10.35) was added to the aliquots of glycated samples and positive control (40 ml) and these were incubated at 37°C for 30 min. After incubation, the absorbance was taken at 530 nm and percent inhibition of fructosamines by extract was calculated by –

$$\text{Inhibitory activity } (\%) = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance value of the positive control and A_1 is the absorbance of the glycated albumin samples co-incubated with extract.

Carbonyl group estimation

For protein carbonyls, absorbance was taken at 365 nm and the concentration was calculated by molar extinction coefficient.[22] The results obtained were given as percentage inhibition and was calculated by the formula used in the estimation of fructosamine.

Protein thiol estimation

The estimation of thiol groups of glycated albumin samples and positive control was performed by dithionitrobenzoic acid (DTNB).[23] In this assay, 250 μl samples and control were incubated with three volumes of 0.5 mM-DTNB (750 μl) for about 15 min and then the absorbance was taken at 410 nm. The free thiol concentration in the solution was taken by the

standard curve of various BSA concentrations (0·8–4 mg/ml) as nM thiols/mg protein. The percentage protection was calculated by the formula used in the estimation of fructosamine.

Binding of Congo red

Congo red binding was measured by taking the absorbance at 530 nm.[24] For this assay, the samples (500 µl) were incubated with 100 µl of Congo red (100 µM) in phosphate-buffered saline with 10% (v/v) ethanol for 20 min at room temperature. The absorbance was recorded for both Congo red incubated samples as well as for Congo red background. The results were expressed as percentage inhibition calculated by the same formula used in estimation of fructosamine.

Statistical analysis

The results are expressed as mean ± standard error of mean. Experiments were always performed in triplicates. Statistical comparison was performed using analysis of variance followed by Bonferroni's test ($P < 0.001$).

Results

Triphala extract was found to be rich in tannins, polyphenols, alkaloids, and glycosides. The percentage yield was 5.4%. The present work was focused to establish the inhibitory activity of *Triphala* against α-amylase and α-glucosidase. The percentage inhibition displayed by each extract [Figure 1], which justifies that *Triphala* extract showed prominent α-amylase inhibitory potential (48.66% at concentration 250 µg/ml). This percentage of inhibition ranged from 8.23 to 48.66. The α-glucosidase inhibitory activity of *Triphala* extract is shown in Figure 2. For all tested concentrations, percent α-glucosidase inhibition increased with increasing concentration of the extract. Inhibition in enzyme activity ranged from 6.32% to 40.64%.

In glycation inhibitory activity, extract showed remarkable results for antiglycation potential. For inhibition of glycation potential inhibition, the various parameters were calculated like fructosamine inhibition was found to be 37.74%, protein carbonyls were inhibited up to 15.23% whereas protein thiols were inhibited up to 84.81% [Table 1].

Discussion

According to the International Diabetes Federation, type-2 diabetes presently has an effect on 246 million people globally and is anticipated to augment to 380 million by 2025. Type-2 diabetes is observed as impairment in glucose tolerance, elevated cholesterol and blood pressure predisposing to cardiovascular risk.[25] Thus, it becomes necessary to search some safe drugs against such condition. As per World Health Organization, >80% of the world's population depends on traditional medicine and folklore for healthcare. Use of herbal remedies in Asian subcontinent depicts a long history of use of medicinal plants for maintenance of good health. The use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic, as well as infectious diseases.[26]

Administration of *Triphala* for a period of 45 days, produces a significant reduction in blood glucose levels.[16] Current study aimed to determine the effect of *Triphala* on activity of glycolytic enzymes as α-amylases and α-glucosidase along with protein glycation studies. In this work, *Triphala* extract significantly inhibited ($P < 0.01$) activity of amylase and glucosidase. It was observed that nonenzymatic glycation in between proteins and reducing sugars leads to the formation of advanced glycation products. Such products are believed to progress pathogenesis of diabetic and aging complications.[27] Hence, inhibition of such glycation end products may demonstrate a key role in inhibiting diabetic complications. Some previous reports pertaining to

Table 1: Percentage inhibition of different glycated proteins at 33 mg/ml concentration by *Triphala* extract

Parameters	Percentage inhibition
Fructosamine	37.74
Protein carbonyls	15.23
Protein thiols	84.81
Protein amyloids	Not found

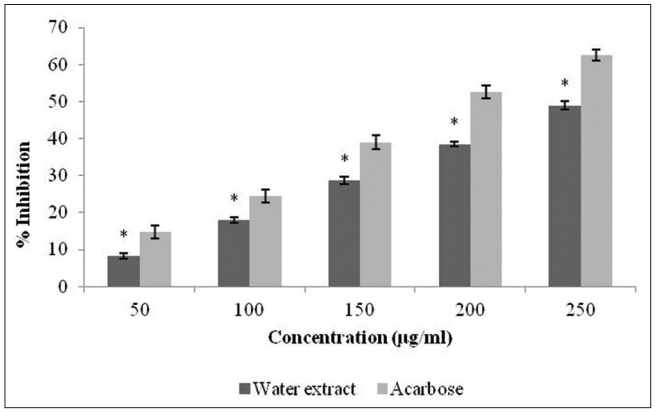

Figure 1: Inhibitory activity of *Triphala* extract against α-amylase. The results are expressed as mean ± standard error of mean. Statistical comparison was performed using analysis of variance followed by Bonferroni's test (*$P < 0.001$)

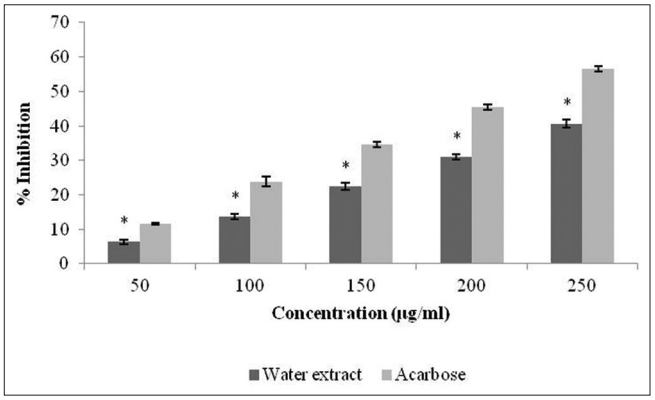

Figure 2: Inhibitory activity of *Triphala* extract against α-glucosidase. The results are expressed as mean ± standard error of mean. Statistical comparison was performed using analysis of variance followed by Bonferroni's test (*$P < 0.001$)

antiglycation effects of polyphenols have been established.[28,29] *Triphala* extract effectively inhibited protein glycation which is contributed due to presence of tannins.

Conclusion

Triphala extract demonstrated α-amylase and α-glucosidase inhibitory potential which may serve as a lead for isolation and identification of compounds responsible for it. However, more systematic studies are needed to confirm these results.

Acknowledgment
Authors are thankful for Rewa Shiksha Samiti for providing grant for research purpose.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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हिन्दी सारांश

त्रिफला का ग्लायकोसाइडिक एन्जाइम निरोधात्मक एवं एन्टिग्लायकेशन क्षमता पर प्रयोगात्मक अध्ययन

आदित्य गणेशपुरकर, शुंभागी जैन, सोनम अग्रवाल

डायाबिटिस मेलाइटस में कार्बोहाइड्रेट्स एवं लिपिड के चयापचय में अस्थिरता आती है। अग्न्याशय से स्त्रावित होने वाले एन्जाइम, अमाइलेज एवं ग्लुकोसाइडेज कार्बोहाइड्रेट्स को ग्लुकोज में परिवर्तित करते हैं, जिसके पश्चात् ग्लुकोज रक्त में प्रवेश करता है। त्रिफला का मानव पर एन्टिडायाबिटिक प्रभाव देखा गया है। किंतु अभी तक त्रिफला का ग्लायकोसाइडिक एन्जाइम एवं प्रोटीन ग्लायकेशन क्षमता पर अध्ययन नहीं किया गया है। वर्तमान कार्य का उद्देश्य त्रिफला का ग्लायकोसाइडिक एन्जाइम निरोधात्मक एवं एन्टिग्लायकेशन क्षमता देखना है। त्रिफला चूर्ण का शीतल जल में सत्व निकाला गया एवं अध्ययन किया गया। अल्फा अमाइलेज, अल्फा ग्लुकोसाइडेज एन्जाइम पर कार्बोहाइड्रेट चयापचय का प्रभाव एवं एन्टिग्लायकेशन क्षमता का अध्ययन किया गया। त्रिफला सत्व ने मात्रा अनुसार अल्फा अमाइलेज एन्जाइम पर निशेधात्मक प्रभाव छोड़ा। सत्व की बढती मात्रा के अनुसार अल्फा ग्लाईकोसाइडेज इन्जाइम की गतिविधि कम हुई। सत्व द्वारा फ्रुक्टोजामाइन (३७.७४%), प्रोटीन कार्बोनाइल (१५.२३%) एवं प्रोटीन थायोल (८४.४%) में कमी पाई गई। इस अध्ययन से त्रिफला के अल्फा अमाइलेज, अल्फा ग्लुकोसाइडेज पर ऋणात्मक प्रभाव हुआ। इस शोध से एन्टिग्लायकेशन प्रभाव भी सिद्ध हुआ। अतः त्रिफला का प्रयोग डायबिटिस के प्रबंधन में किया जा सकता है।