**EVALUATION OF ALPHA-AMYLASE AND ALPHA-GLUCOSIDASE ACTIVITIES OF BRAHMI NEI-AN IN-VITRO STUDY**

**Abstract**

α- -amylase and α-glucosidase enzymes break down carbohydrates, leading to spikes in postprandial glucose levels in diabetic patients. Suppression of these enzyme activities helps regulate blood glucose levels and lower the risk of diabetes complications. Brahmi together with Ghee helps to regulate postprandial hyperglycemia in diabetic patients. In this study, Brahmi nei was evaluated for their ability to inhibit α-amylase and α-glucosidase activities in in-vitro. The ability of BN to inhibit α-amylase and α-glucosidase activities was measured by the Spectrophotometric method, which involves measuring the changes in light absorption to quantify the enzyme inhibition. BN exhibited α-amylase and α-glucosidase inhibitory potential, with a maximum inhibition of about 53.56±0.55 and 49.80±0.84 and corresponding IC 50 values are 421.67 and 482.87μg/mL. This study shows that the Siddha medicine Brahmi Nei (BN) can inhibit α-amylase and α-glucosidase activities in in-vitro, suggesting its potential anti-diabetic properties.

**INTRODUCTION**

The therapeutic application of medicinal plants for healing is as old as humankind. There is significant proof from much evidence, such as manuscripts, preserved monuments, and even indigenous plant remedies, suggesting that man and his quest for pharmaceuticals within nature have an extended past [1]. Individuals with diabetes who endure peripheral nerve damage can eventually get diabetic neuropathy (DN) [2]. Among DN, diabetic distal symmetric polyneuropathy (DSDN) is the most relevant type. Consequently, the massive expenses of DSDN and its complications will likely rise. Approximately 50% of diabetic patients endure DSDN, and about 20% develop neuropathic pain (painful DSDN).[3] Diabetes mellitus is a chronic metabolic disorder marked by high blood sugar, lipids, and amino acids that causes severe consequences. Around 415 million individuals globally developed DM in 2015, with projected growth of 642 million. Although there are therapies for diabetes, a complete cure is still elusive[4]. Postprandial Hyperglycemia (PPHG) occurs when blood glucose levels remain elevated after eating.PPHG poses a critical hazard in diabetes management and its related complications, such as diabetic neuropathy, nephropathy, and cardiovascular diseases[5].

α-amylase and α-glucosidase enzymes break down carbohydrates into glucose by raising blood glucose levels[6]. Inhibiting these enzymes can decline glucose release, helping to regulate post-meal blood sugar spikes and control PPHG [7]. Currently, Acarbose, Miglito, and voglibose are used to regulate PPHG by inhibiting carbohydrate digesting enzymes. While Acarbose inhibits α-amylase and α-glucosidase, Miglito and voglibose only inhibit α-glucosidase.Although effective in controlling PPGH, these drugs are limited by gastrointestinal effects, making them less suitable for long-term treatment [8][9].

*Bacopa monnieri* (Brahmi) is an ancient medicinal herb that has been extensively utilized for many years for optimizing intellectual and memory functioning. Several bioactive substances, notably flavonoids, alkaloids, and saponins, are abundant in them and can be beneficial for treating neurological conditions and diseases linked to memory.Bacosides,key components of *Bacopa monnieri,*are crucial for neuronal health[10]. Deepak et al,identified and characterized 12 known analogs (bacopasides I-XII), with a distinctive structure incorporating sugar and sterol components[11]. Research shows that bacosides protect neuronal damage, reduce cytotoxicity and even repair damaged neurons potentially alleviating neurological diseases by increasing kinase activity and neuronal synthesis [12]. It reduces oxidative stress caused by diabetes in diabetic models [13]. Many research studies has extensively validated the traditional pharmacological claims of Brahmi, confirming its nootropic,antibacterial,antioxidant, analgesic virtues[14].Cow ghee also exhibits antihyperglycemic properties. Although ghee is an essential food in both traditional culture and commercial activity, it is still a contentious portion among modern nutrition experts [15], and the traditional medical Siddha system possesses distinct views on it. Numerous studies are being carried out on various aspects of ghee, such as conjugated linoleic acid, short-chain fatty acids, and omega-3 fatty acids, indicating that their consumption could potentially benefit brain function[16].According to Siddha literature, Brahmi Nei (BN) is prescribed in the management of DN. Researchers can acquire an enhanced knowledge of Brahmi nei’s mechanism of action to ameliorate DN together with its potential to act as an adjunct or complementary therapy for diabetic management by evaluating its antihyperglycemic activity.Ghee coupled with brahmi is prescribed in traditional medicine for alleviating problems with the neurological system.Therefore,this study evaluated the BN’s ability to inhibit α-amylase and α-glucosidase comparing their effects to the reference drug Acarbose.

**Materials and Methods:**

1. *Chemicals and reagents*

The α-amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method by Miller 1959 & Akbar et al 2023 [17]. The α-glucosidase inhibition assay was determined using a method described by Kim et al 2004 & Jeddi et al 2023[18].

1. *Collection of plant materials*

Whole parts of *Bacopa* *monnieri* were procured from Jalagamparai near Yelagiri, Tirupattur district, Tamilnadu,India and identified by Pharmacology department, Government Siddha Medical College,Chennai.Purified Ghee were procured from Aavin diary product. A voucher number Botanical name of the plant was verified from published literature and database (The Plant List 2015).

c)Purification and Preparation of the medicine

Fresh Brahmi plant and Cow’s ghee were purified as mentioned in Sikitcha Rathna Deepam Ennum Vaidhya Nool. Two parts of fresh juice of Brahmi plant taken with one part of Cow’s ghee and the mixture were allowed to boil till it reaches ghee consistency.

**Anti-diabetic activity**

**Sample preparation**

One mL of BN siddha formulation was dissolved in 1mL of DMSO and mixed vigorously in a water bath at 40℃ for 5 minutes to achieve uniform mixing of sample and used as a stock solution. From these further dilutions were made for further assay.

**In vitro α-amylase inhibitory studies**

α -Amylase randomly cleaves α - (1-4) glycosidic linkages of amylase to yield dextrin, maltose, or maltotriose. Whereas α -glucosidase hydrolyzes the terminal non-reducing 1-4 linked a-glucose to release glucose molecules. Incubation of the a-amylase with the substrate leads to the generation of maltose; however, the addition of the test sample significantly inhibited the liberation of maltose in a dose-dependent manner, compared with acarbose.

**In-vitro α-amylase inhibiton study**

This study was done by Spectrophotometric assay method.The assay mixture consisted of 500 mL of 0.02M sodium phosphate buffer [containing 6mM sodium chloride (NaCl), pH 6.9], 0.05 units of α-amylase, and BN siddha formulation at a concentration of 10 to 500µg/mL (w/v). The assay mixture was pre-incubated at 37°C for 20 minutes. After incubation, 250 mL of 0.5% (v/v) starch solution in the above-mentioned buffer was added to the tubes and incubated for 15 minutes at 37°C. The reaction was terminated by adding 1 mL of dinitrosalicylic acid reagent and then incubated in a boiling water bath for 10 minutes.The tubes were cooled and the absorbance was measured at 540 nm using ELISA reader. A tube with α-amylase but without test sample the control with 100% enzyme activity. Acarbose (10 mg/ml) as stock an amylase inhibitor, was the positive control.

The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the equation given below: The % α-amylase inhibition was plotted against the extract

concentration and the IC50values were obtained from the graph.

% α amylase inhibition = 100 × Abs 100% control-Abs Sample

Abs100% Control

*ii) α-glucosidase inhibiton*

The α-glucosidase inhibition assay was determined using a method described by Kim et al 2004 & Jeddi et al 2023[18].The assay mixture consisted of 150 mL of 0.1M sodium phosphate buffer (containing 6mM NaCl, pH 6.9), 0.1 unit of α-glucosidase, and BN siddha formulation at a concentration of 10 to 500 µg/mL (w/v). The assay mixture was pre-incubated at 37°C for 10 minutes. After incubation, 50 mL of 2mM para-nitrophenyl a-D-glucopyranoside in 0.1M sodium phosphate buffer was added to the mixture and incubated at 37°C for 25 minutes.The reaction was terminated by adding 50 mL of 0.1M sodium carbonate (Na2CO3). The yellow color that developed was measured at 405 nm using colorimeter. The tube with α-glucosidase but without test sample served as the control with 100% enzyme activity, and acarbose served as the positive control.

The percentage inhibition was calculated using the following equation:

% of Inhibition = (Absorbance of control – Absorb ance of test) x 100

(Absorbance of control)

Table 1-Percentage of inhibition in α-Glucosidase assay

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Concentration**  **µg/mL** | **% of inhibition in α-Glucosidase assay** | **IC 50** | **% of inhibition in α-amylase assayµ** | **IC 50** |
| (T) 10 | 11.69 ±1.54 | 421.67 | 27.89±2.72 | 482.87 |
| (T) 50 | 20.72± 0.96 | 32.95±0.19 |
| (T) 100 | 31.63±0.34 | 41.14±0.94 |
| (T) 250 | 39.34±0.49 | 40.68±0.96 |
| (T) 500 | 53.56±0.55 | 49.80±0.84 |
| (S) 10 | 41.03±0.24 | 12.25 | 35.05±2.0 | 100.98 |
| (S) 50 | 56.17±1.03 | 43.06±0.51 |
| (S) 100 | 62.84±0.68 | 56.78±1.60 |
| (S) 250 | 73.16±0.61 | 69.80±0.84 |
| (S) 500 | 88.15±1.49 | 81.91±1.57 |

**S)- Standard- acarbose (10 mg/ml); (T) - BN Test- (1000 µg/ml)**

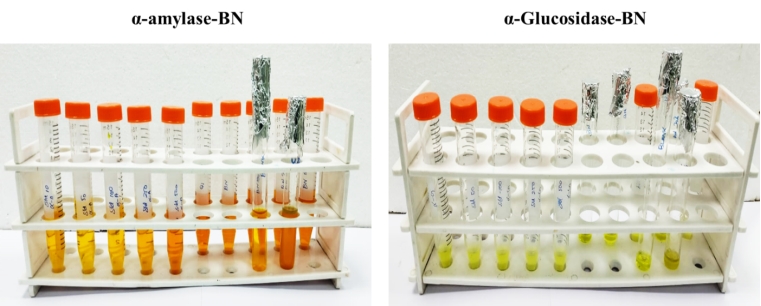


Fig 1- Sample analysis

A graph of different sizes and colors

Description automatically generated with medium confidence

Fig 2- Variation in alpha amylase and alpha glucosidase with different concentration



Fig 3- Laboratory analysiss

**RESULTS AND DISCUSSION**

This study inspected the α-amylase inhibitory effects of BN as a possible ameliorative approach for type 2 diabetes. The results showed that BN had a notable ability to inhibit α- amylase activity with minimum inhibition of 49.80±0.84% and corresponding IC50 is 482.87 µg/mL and α-glucosidase with minimum inhibition of 53.56±0.55% and corresponding IC50 is 421.67 µg/mL. Inhibiting the carbohydrates-breaking enzymes is a crucial strategy in the management of Diabetes mellitus. Generally, *Bacopa monnieri* was traditionally used to treat a variety of ailments that exhibit damage to oxidative stress. In addition, it could be utilized to alleviate the oxidative stress brought on by elevated glucose levels, which is the key component of diabetic complications [19]. Among two ingredients of BN, B*acopa monnieri* contains Bacosine, triterpene is responsible for the neuroprotective and anti-diabetic effects. Rajagopal et, al have reported a strong inhibitory effect on the enzymes α- amylase and α-glucosidase by reducing carbohydrate digestion and glucose absorption [20]. In a study conducted by Ghosh et.al, Bacosine significantly decreased the blood glucose levels in diabetic rats, showing a substantial reduction compared to untreated diabetic controls after single and multiple administrations [21]. Research studies on *Bacopa* *monnieri* have shown promising results, with its ethanolic extract exhibiting antioxidant and antihyperglycemic activity in mice. Additionally, Bacosine, a triterpene molecule has been shown to have a significant impact as compared to the control drug α-tocopherol with an IC50 value of 7.44µg/mL[22].AA Thant et,al conducted the study isolated and identified apigenin and luteolin from aerial parts of B. *monnieri* and screening for in vitro antidiabetic activity.The isolated compounds apigenin and luteolin possessed antidiabetic activity due to their α-amylase inhibitory effects and α-glucosidase inhibitory effects comparable with the standard drug metformin . The higher percent enzyme inhibition with the lowest IC50 value, the percent inhibition of ethanol extract and luteolin was nearly the same as that of standard metformin [23].

Recent studies have shown the potential beneficial effects of dairy fats dispelling previous concerns about them [24]. According to the Siddha Literature, Ghee has extremely penetrating features, allowing it to deeply nourish all seven bodily constituents. It enhances the absorption of herbal and metallic preparations by acting as an excellent carrier. Kumar et,al conducted the study, suggested that certain components in Cow’s Ghee helps to prevent and manage diabetes,and its complication.The beneficial compounds include carotenoids,Vit A,D and E,Mg and Ca which have antidiabetic properties[25].According to Dwivedi et al.,Animal studies have confirmed many beneficial effects of ghee,inclusive of dose-dependent decreases in serum total cholesterol, low density lipoprotein, very low density lipoprotein, and triglycerides; reduction in liver total cholesterol, triglycerides, and cholesterol esters; and a lower level of non-enzymatic induced lipid per oxidation in liver homogenate[26].

Cow’s Ghee is a rich source of Conjugated Linoleic Acid (CLA), and has been shown in animal studies to have anti-diabetic effects by influencing gene expression, lowering body fat, increasing insulin sensitivity, and improving muscle function[27]. Sharma H et al, indicate that 10% of cow’s ghee in the dietary habits, has a beneficial effect on serum lipid profiles.CLA mitigates the synthesis of pro-inflammatory mediators such as interleukins, prostaglandins, and leukotrienes. Furthermore, ghee’s potential to reduce the release of leukotrienes and arachidonic acid metabolites in preventing diabetes-related complications, such as cardiovascular disease and atherosclerosis [28]. In Wister rats, Kathirvelan et al. investigated the effects of CLA on preventing atherosclerosis and enhancing antioxidant enzymes [29].

Research suggested that two ingredients (Brahmi and Cow’s ghee) in Brahmi Nei,a traditional Siddha formulation possess anti-diabetic properties.This study evaluated that the extract of Brahmi nei significantly inhibited α- amylase and α-glucosidase activities,indicating its potential anti-diabetic agent.

**CONCLUSION**

The inhibitory potential of Brahmi Nei (BN) on α- amylase and α-glucosidase enzymes presents a compelling prospect in the quest for effective anti-diabetic agents.In B.monnieri, its unique triterpenoids saponins known as bacosides are a promising modulator of various biological processes.In Ghee, Conjugated Linoleic Acid (CLA) notably have anti-diabetic effects and may help mitigate diabetes risk factors and improve glucose metabolism. The in-vitro experiments conducted in this study have demonstrated that the Siddha formulation BN exerts a substantial inhibitory effect on both α- amylase and α-glucosidase enzymes.While laboratory results are promising,more researchis needed to confirm the safety and efficacy of BN for anti-diabetic activity.In-vivo studies are necessary to determine optimal dosage and validate the potential benefits.

**CONSENT**

This study did not involve human subjects and therefore, does not require informed consent.

**ETHICAL** **APPROVAL**

Ethical approval was not required for this study as it did not involve human participants.

**REFERENCE**

1. Petrovska BB. Historical review of medicinal plants' usage. Pharmacogn Rev. 2012 Jan;6(11):1-5. doi: 10.4103/0973-7847.95849. PMID: 22654398; PMCID: PMC3358962.

2.Jain PK, Das D, Jain P, Jain P, Jain PK. The pharmacognostic and pharmacological aspect of Bacopa monnieri - a review. Innovare J Ayurvedic Sci. (2016) 4, 7 ˝ U-11.

3. Schreiber AK, Nones CF, Reis RC, Chichorro JG, Cunha JM. Diabetic neuropathic pain: Physiopathology and treatment. World J Diabetes. 2015 Apr 15;6(3):432-44. doi: 10.4239/wjd.v6.i3.432. PMID: 25897354; PMCID: PMC4398900.

4. Atlas D. International Diabetes Federation. IDF Diabetes Atlas. 7th ed.Brussels: International Diabetes Federation (2015).

5.Gin H, Rigalleau V. Post-prandial hyperglycemia. Post-prandial hyperglycemia and diabetes. Diabetes Metab. 2000;4:265–72.

6. Lordan S, Smyth TJ, Soler-Vila A, Stanton C, Ross RP. The α-amylase and α-glucosidase inhibitory effects of Irish seaweed extracts. Food Chem.2013;141:2170–6.

7. Lebovitz HE. α-glucosidase inhibitors. Endocrinol Metab Clin North Am. 1997;26:539–51.

8. Van de Laar FA. α-glucosidase inhibitors in the early treatment of type 2 diabetes. Vasc. Health Risk Manag. 2008;4:1189–95.

9. Etxeberria U, de la Garza AL, Capion J, Martnez JA, Milagro FI. Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic α amylase. Expert Opin Ther Targets.2012;16:269–97.

10. Fatima U, Roy S, Ahmad S, Ali S, Elkady WM, Khan I, Alsaffar RM, Adnan M, Islam A, Hassan MI. Pharmacological attributes of Bacopa monnieri extract: Current updates and clinical manifestation. Front Nutr. 2022 Aug 18;9:972379. doi: 10.3389/fnut.2022.972379. PMID: 36061899; PMCID: PMC9436272.

11.Deepak M, Amit A. The need for establishing identities of’bacoside A and B’, the putative major bioactive saponins of Indian medicinal plant Bacopa monnieri.Phytomedicine. (2004) 11:264. doi: 10.1078/0944-7113-00351

12. Kishore K, Singh M. Effect of bacosides, alcoholic extract of Bacopa monniera Linn. (brahmi), on experimental amnesia in mice. Indian J Exp Biol. (2005) 43:640–5

13. Kapoor R, Srivastava S, Kakkar P. Bacopa monnieri modulates antioxidant responses in brain and kidney of diabetic rats. Environ Toxicol Pharmacol. (2009)27:62–9. doi: 10.1016/j.etap.2008.08.007

14. Abdul Manap AS, Vijayabalan S, Madhavan P, Chia YY, Arya A, Wong EH, et al. Bacopa monnieri, a neuroprotective lead in Alzheimer Disease: a review on its properties, mechanisms of action, and preclinical and clinical studies. Drug Target Insights. (2019) 13:1177392819866412. doi: 10.1177/1177392819866412

15.Deepshikha Kataria, Gurmeet Singh,Health benefits of ghee: Review of Ayurveda and modern science perspectives,Journal of Ayurveda and Integrative Medicine,Volume 15, Issue 1,2024,100819,ISSN 0975-9476,https://doi.org/10.1016/j.jaim.2023.100819.

16.Wenzel TJ, Gates EJ, Ranger AL, Klegeris A. Short-chain fatty acids (SCFAs) alone or in combination regulate select immune functions of microglia-like cells. Mol Cell Neurosci 2020;105. <https://doi.org/10.1016/J.MCN.2020.103493>.

17.Kim YM, Wang MH, Rhee HI. A novel alpha-glucosidase inhibitor from pine bark. Carbohydr Res 2004;339:715–7.

18. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 1959;31:426–8.

19. Rao MU, Sreenivasulu M, Chengaiah B, Reddy KJ, Chetty CM. Herbal medicines for diabetes mellitus: a review. Int J PharmTech Res. (2010) 2:1883–92.

20. Rajagopal        Kanagalatha and    Venugopal    Tamilselvi,  2024. Inhibitory   Potential  of A Amylase and  A  Glucosidase  Enzymes  by Bioactive    Fraction    of    Bacopa Monnieri.  In  Vitro  Study  for Anti‐Diabetic Activity.  Res. J. Med. Sci.,  18:  101‐108,  doi:  10.59218/ makrjms.2024.5.101.108

21.Ghosh T, Maity TK, Singh J. Antihyperglycemic activity of bacosine, a triterpene from Bacopa monnieri, in alloxan-induced diabetic rats. Planta Med.(2011) 77:804–8. doi: 10.1055/s-0030-1250600

22.Ghosh T, Sengupta P, Dash D, Bose A. Antidiabetic and in vivo antioxidant activity of ethanolic extract of Bacopa monnieri Linn. aerial parts: possible mechanism of action. Iran J Pharm Res (IJPR). (2008) 7, 61–8.

23. Thant, A. A., Win, Y. Y., Khine, M. M., & Than, n. N. Identification of apigenin and luteolin isolated from the aerial parts of bacopa monnieri (l.) Wettst in vitro evaluation of α-amylase and α-glucosidase inhibition activity.

24.Kataria D, Singh G. Health benefits of ghee: Review of Ayurveda and modern science perspectives. J Ayurveda Integr Med. 2024 Jan-Feb;15(1):100819. doi: 10.1016/j.jaim.2023.100819. Epub 2024 Jan 5. PMID: 38181707; PMCID: PMC10789628.

25.Ravi K. Critical review of cow ghee intake and its relation with prameha or diabetes. World J Pharm. 2018; 7:459–66.

26. Dwivedi C, Crosser AE, Mistry VV, Sharma HM. Effects of dietary ghee (clarified butter) on serum lipids in rats). J Appl Nutr. 2002; 52:65–8.

27. Ryder JW, Portocarrero CP, Song XM, Cui L, Yu M, Combatsiaris T,et al. Isomer-Specific Antidiabetic properties of Conjugated Linoleic Acid Improved Glucose Tolerance, Skeletal Muscle Insulin Action, and UCP-2 Gene Expression). Diabetes. 2001;50:1149–57.

28.Sharma H, Zhang X, Dwivedi C. The effect of ghee (clarified butter) on serum lipid levels and microsomal lipid peroxidation. AYU (An Int Quarterly J Res Ayurveda). 2010;31:134–40.

29.Kathirvelan C, Tyagi A, Krishnamurthi P. Influence of conjugated Linoleic acid ghee feeding on cancer incidences and histopathologicalchanges in 7, 12 dimethlybenz (a) anthrazene induced mammary gland carcinogenesis in rats. . Veterinary Archiv. 2008;78:51120.