Journal Of Harmonized Research (JOHR)

Journal Of Harmonized Research in Pharmacy
8(3), 2019, 37-43

ISSN 2321 – 0958

Original Research Article

ANTIDIABETIC AND ANTIOXIDANT ACTIVITY OF AQUEOUS ROOT EXTRACT OF *CHLOROPHYTUM BORIVILIUANUM* (SAFED MUSLI)

Hemlata Rathore[1], Bhushan Hatwar[1] & Shishupal Bodhankar[2]

[1]School of Pharmacy, Chouksey Engineering College, Bilaspur (C.G.)
[2]Bajiraoji Karanjekar College of Pharmacy, Sakoli (M.S.)

Abstract: Objective: To investigate the antidiabetic effect of aqueous extract of *Chlorophytum borivilianum* (safed musli) root. **Methods:** The collected roots were washed; shade dried and was pulverized with a mechanical pulverizer for the size reduction. It was then passed through mesh of size # 60 and the fine powder was collected and was used for the experiment for powder microscopy and preparation of extract. The root powder (1000 g) was subjected to cold maceration in 2 L of sterile distilled water for 48 hours at room temperature, filtered into a clean round bottom flask. The α-Glucosidase inhibitory activity was conducted where 100µl of a sample of different concentrations was incubated with 50µl α-glucosidase (1.0 U/ml) (from Saccharomyces cerevisiae) in phosphate buffer (0.1 M, pH 6.8) for 10 min at 37°C. The reaction was initiated by addition of 50 µl of substrate: 5 mM, p-nitrophenyl-α-D glucopyranoside in a 0.1 M phosphate buffer at pH 6.8. P-nitrophenol's release kinetics. The α -Amylase Inhibition where 250µL of the extract (5mg/mL) was preincubated with 250µL of -amylase solution for 10min at 25°C in one set of tubes. In another set of tubes -amylase was preincubated with 250µL of phosphate buffer (pH 6.9). 250µL of starch solution at increasing concentrations (0.30–5.0mg/mL) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10min at 25°C and then boiled for 5min after the addition of 500µL of DNS to stop the reaction. **Results:** In this study acarbose was also used as a standard drug for α-glucosidase inhibitor. Acarbose at a concentration of (100-1000 µg/ml) showed α-glucosidase inhibitory activity from 30. 31±0.1084to 84.05±0.2075% with an IC value 411.436567µg dry extract, & acarbose was also used as a standard drug for α amylase inhibitor. Acarbose at a concentration of (100-1000 µg/ml) showed α-glucosidase inhibitory activity from 23.33±0.1415 to 62.05±0.2725% with an IC value 236.774194µg dry extract.
Conclusions: As a result, we found that the extract of C. boriviliamum have free radical scavenging activity and inhibitory activity against α-amylase and α-glucosidase and this therapeutic potentiality could be exploited in the management of post prandial hyperglycemia in the treatment of type 2 diabetes mellitus.

Key words- *Chlorophytum boriviliuanum,* Antidiabetic & Antioxidant

Introduction: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia [1]. As a consequence, diabetics have high risk of developing metabolic related complications such as ketoacidosis, hyperosmolar non-ketotic coma (HONK) [2] , atherosclerosis [3], coronary artery disease [4] and pancreatitis [5]. These increased risks were due to co-existence of hyperlipidemia, which was characterized by hypertriglyceridemia, reduced high-density lipoprotein (HDL) and increased low density lipoprotein (LDL) [6] cholesterol levels.

Oxidative stress plays important role in the pathogenesis of diabetes and its complications [7]. Oxidative stress is caused by increased free radical formation, which were generated through several mechanisms such as glucose auto-oxidation, protein glycosylation and accumulation of advanced glycation end products (AGEs) [8]. Oxidative stress could affect pancreatic β-cells [9] which could be destroyed through a direct insult by the free radicals [10]. Abnormally high levels of reactive oxygen species (ROS) in the pancreas and low levels of antioxidant defense mechanisms could lead to increased oxidative damage which causes reduced insulin secretion [11]. *Chlorophytum borivilianum*, commonly known as Safed Musli, is a highly valued medicinal plant in India and Southeast Asia. This plant has been shown to possess anticancer, antimicrobial, antifungal, antiulcer, antipyretic, antiarthritic and immunomodulatory activities [12]. Preliminary findings by Panda *et al*, [13] and Mujeeb *et al*, [14] reported that C. borivilianum could induce acute reduction of blood glucose levels in diabetic rats. Despite of these reports, the effect of long-term administration of *C. borivilianum* root on blood glucose, lipid profiles and insulin levels are currently unknown, Kenjale *et al*, [15] reported that *C. borivilianum* root aqueous extract can cause significant reduction in blood glucose, TG and cholesterol levels in rats exposed to cold stress as well as displays in-vitro anti-oxidant activity which could help to protect against oxidative stress.

For Correspondence:
bhushanpharmacology@gmail.com.
Received on: May 2019
Accepted after revision: June 2019
DOI: 10.30876/JOHR.8.3.2019.37-43

We hypothesized that *C. borivilianum* root was able to prevent impairment of blood glucose, lipid profile and insulin levels and damage to the pancreas due to increased oxidative stress in diabetes. This study therefore aimed to investigate *C. borivilianum* root extract effect on alpha - glucosidase, alpha amyles and antioxidant activity and glucose homeostasis indices as well as oxidative stress parameters (lipid peroxidation product-LPO and activity levels of antioxidant enzymes) in the pancreas of diabetic rats. In addition, the histopathological changes of the pancreas in diabetic rats following treatment with the root extract will also investigated.

Chlorophytum borivilianum is a herb with lanceolate leaves, from tropical wet forests in peninsular India. The Hindi name is safed musli (also commonly known as musli).Binomial Name(s): *Chlorophytum borivilianum* Family: Liliaceae Parts Used: roots. (21)

Materials and Methods: **Plant Collection and Preparation of Plant Extract**

Plant Material: The plant material such as root of *Chlorophytum borivillianum*, commonly known as safed musli were collected from Champa region in Chhattisgarh in 2016. The collected roots were washed; shade dried and was pulverized with a mechanical pulverizer for the size reduction. It was then passed through mesh of size # 60 and the fine powder was collected and was used for the experiment for powder microscopy and preparation of extract. The root powder (1000g) was subjected to cold maceration in 2L of sterile distilled water for 48 hours at room temperature, filtered into a clean round bottom flask.

Plant authentication: On 19-May-2017, the Raw Material Herbarium and Museum, Delhi (RHMD) authenticate, that dried roots sample of Chlorophytum borivillianum, safed musli has been found correct as dried roots of Chlorophytum borivillianum Sant. & Fernandez which is commonly known as safed musli. The identification has been done on the basis of macroscopic studies of the sample followed by detailed scrutiny of literature and matching the sample with authentic sample deposited in the (RHMD).

Fig: 1 Chlorophytum borivillianum,

Chemicals: α-Glucosidase from Saccharomyces cerevisiae EC.3.2.1.20, 4-Nitrophenyl α-D-glucopyranoside, Acarbose, L-Ascorbic acid, 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ); 2,2′-Azobis(2-methyl-propionamidine) dihydrochloride; Fluorescein Sodium Salt, Folin-Denis' reagent, 2,2-diphenyl-1-picrylhydrazyl, Gallic acid, Quercetin, Vanillin, (+)-Catechin hydrate, Iron(II) sulfate heptahydrate, Iron (III) chloride hexahydrate and Sodium carbonate . Phosphate buffered saline and anhydrous Aluminum chloride were purchased from Hi Media Laboratories, Mumbai, India. All other chemicals, reagents, kits and solvents used in this study were of analytical grade.

Phytochemical screening: A qualitative and quantitative phytochemical evaluation was performed on the aqueous root extract to determine the presence of carbohydrates (Barfoed's test), flavonoids (test of Shinoda), phytosterols (Libermann Buchard test), phenols (ferric chloride test), alkaloids (Dragendorff test), proteins (Biuret test) and saponins (Saponification test)(22.)

Biological effects:

α- Glucosidase inhibitory activity: The slightly modified method of Rao *et al.*[17] was adopted to determine the α-glucosidase's inhibitory activity. Briefly, in a 96-well microplate 100 µl of a sample of different concentrations was incubated with 50 µl α-glucosidase (1.0 U/ml) (from Saccharomyces cerevisiae) in phosphate buffer (0.1 M, pH 6.8) for 10 min at 37°C. The reaction was initiated by addition of 50 µl of substrate: 5 mM, p-nitrophenyl-α-D glucopyranoside in a 0.1 M phosphate buffer at pH 6.8. P-nitrophenol's release kinetics was measured spectrophotometrically with a microplate spectrophotometric reader Multiskan MS (Labsystems, Minneapolis, USA) for 5 min with intervals of 30 seconds at 405 nm. Acarbose was used as reference. The IC50 (concentration required to decrease the reaction rate to 50%) was then determined from the concentration-dependence curve.

α -Amylase Inhibition: The mode of inhibition of α -amylase by the root extract was conducted using the extract with the lowest IC50 according to the modified method described by Ali *et al.* [19]. Briefly, 250µL of the extract (5mg/mL) was preincubated with 250µL of -amylase solution for 10min at 25°C in one set of tubes. In another set of tubes -amylase was preincubated with 250µL of phosphate buffer (pH 6.9). 250µL of starch solution at increasing concentrations (0.30–5.0mg/mL) was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 10min at 25°C and then boiled for 5min after the addition of 500µL of DNS to stop the reaction. The amount of reducing sugars released was determined spectrophotometrically using a maltose standard curve and converted to reaction velocities. A double reciprocal plot (1/

versus 1/()) where is reaction velocity and () is substrate concentration was plotted. The type (mode) of inhibition of the crude extract on -amylase activity was determined by analysis of the double reciprocal (Line weaver-Burk) plot using Michaelis-Menten kinetics [20].

Antioxidant activity: 2, 2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging assay Free radical scavenging activity was determined using 2,2-diphenyl-1-picrylhydrazil (DPPH), as described by Povichit *et al.* with slight modifications [18]. Briefly, in a 96 well microplate, 180 µl of DPPH solution (6.10-5 M in Methanol) and 20 µl of the plant extract at different concentrations were added. The reaction mixture was shaken and incubated in the dark for 30 min at 37°C. The absorbance was read at 540 nm against blank using the microplate spectrophotometric reader. Ascorbic acid was used as positive control. DPPH radical scavenging activity was calculated according to the formula:

[(Abs. of control - Abs. of sample)/Abs. of control]100

The IC50 value for each sample was calculated from the non-linear regression curve.

Statistical analysis:

All experiments were performed at least in triplicate, and results are expressed as mean ± SEM. Statistical analysis was performed using the statistical software XLSTAT (version 2012. 1.01, Addinsoft, Paris, France). The results are analyzed by the univariate ANOVA test followed by Dunnett/Tukey test for multiple comparisons and determination of significance level. Group means were considered to be significantly different at $P < 0.05$.

Result:

Table 1: Phytochemical Screening of *Chlorophytum borivilianum* of root extract

Phytochemical	**Quantity**
Carbohydrates	40 %
Protein	10%
Saponins	17%
Alkolaids	15-25%
Fibers	20-30%

Table 2: Physico-chemical screening of *Chlorophytum borivilianum* of root extract

Quantitative estimation	**Value (mg/g)**
Protein	3.61031
Reducing sugar	0.98648
Non-reducing sugar	1.98731
Starch	2.82716
Saponins	1.1552

Table 3: α glucosidase inhibition by C.boriviliamum root aqueous extract

Sample	**Concentration (µg/ml)**	**% inhibition**	**IC_{50}ug/ml**
CD	100	30.31±0.1084	411.436567
	200	39.15±0.1084	
	400	55.16±0.1294	
	800	65.08±0.1355	
	1000	84.05±0.2075	
ACARBOSE (standard)	100	40.55±0.1275	236.774194
	200	48.87±0.5424	
	400	63.38±0.4169	
	800	71.15±0.5003	
	1000	91.21±0.7036	

CD=411.436567; N=5

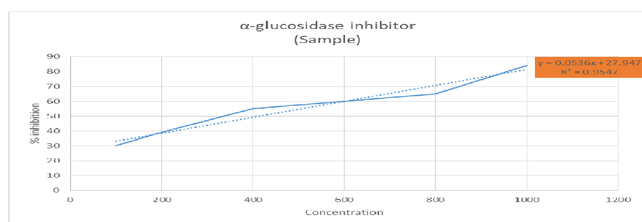

Fig 1: α glycosidase inhibition (Sample)

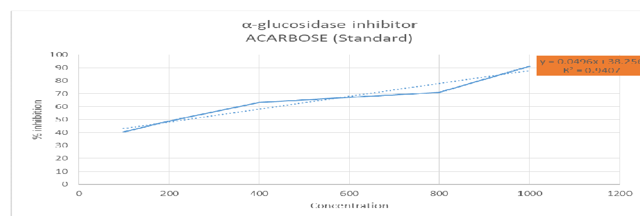

Fig 2: α glycosidase inhibition (ACARBOSE)

Table1: α amylase inhibition by *C.boriviliamum* root aqueous extract

Sample	Concentration (µg/ml)	% inhibition	IC_{50}ug/ml
CD	100	23.33±0.1415	**672.524038**
	200	32.15±0.1084	
	400	40.16±0.1294	
	800	56.43±0.1316	
	1000	62.05±0.2725	
ACARBOSE (standard)	100	32.55±0.2457	**336.514423**
	200	48.41±0.3992	
	400	58.47±0.3618	
	800	69.67±0.1719	
	1000	74.89±0.3636	

CD= 672.524038; N=5

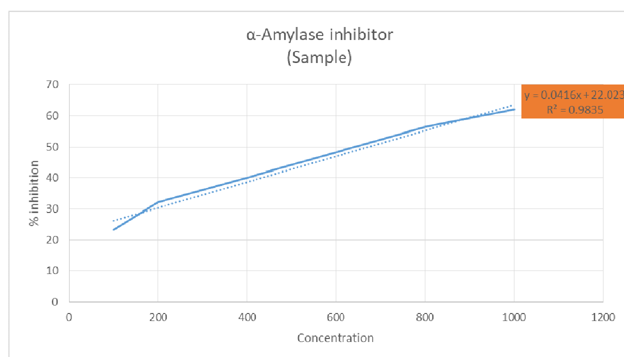

Fig 1: α amylase inhibition (Sample)

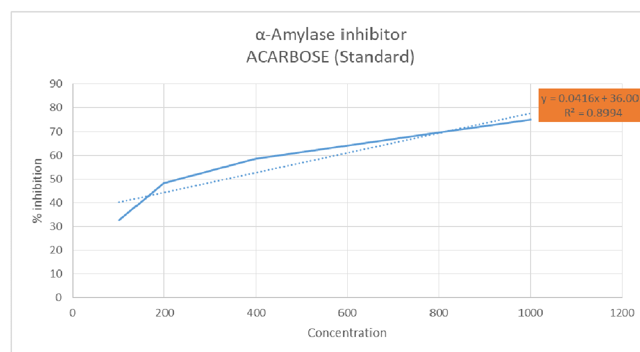

Fig 2: α amylase inhibition (ACARBOSE)

Table 4. Antioxidant activity of *Chlorophytum borivilianum* root aqueous extract using DPPH-scavenging model

Concentration (µg/ml)	% Antioxidant activity	
	Ascorbic acid	Aqueous extract
10	9	8.9
20	20.2	10
40	20.2	13.1
60	56.9	15.2
80	77.2	18.1
100	93.3	20.1

Discussion: In this study acarbose was also used as a standard drug for α-glucosidase inhibitor. Acarbose at a concentration of (100-1000 µg/ml) showedα-glucosidase inhibitory activity from 30. 31±0.1084to 84.05±0.2075% with an IC value 411.436567 µg dry extract, & acarbose was also used as a standard drug for α amylase inhibitor. Acarbose at a concentration of (100-1000 µg/ml) showed α-glucosidase inhibitory activity from 23.33±0.1415 to 62.05±0.2725% with an IC value 236.774194 µg dry extract. This indicates that the aqueous extract of C. boriviliamum is a potent α-amylase and α-glucosidase Inhibitor in comparison with acarbose. Thus the inhibition of the activity of alpha glucosidase by *Chlorophytum borivilianum* would delay the degradation of Carbohydrate , which in turn cause a decrease in the absorption of glucose as a result the reduced of postprandial blood glucose level elevation This could be justified that the nature of some extract constituents (phenols, flavonoids saponins, steroids, alkaloids, terpenoids) present in the extract could be responsible as being effective inhibitors of α-amylase and α-glucosidase.

In DPPH-scavenging model, the aqueous extract of *Chlorophytum borivilianum* showed the

highest 20.1% (100µg/ml) scavenging of DPPH minimum by 8.9% (10µg/ml).

Conclusion: To investigate the biological activities of C. boriviliamum root, the antioxidant and antidiabetic activities of the aqueous extract of the plant had been analysed. As a result, we found that the extract of C. boriviliamum have free radical scavenging activity and inhibitory activity against α-amylase and α-glucosidase and this therapeutic potentiality could be exploited in the management of post prandial hyperglycemia in the treatment of type 2 diabetes mellitus. Although the effects of C. boriviliamum extract have been established in vitro, these results indicate that C. boriviliamum has potential as a crude drug and a dietary health supplement. The plant showed significant enzyme inhibitory activity, so the compound isolation, purification and characterization which are responsible for inhibiting activity, have to be done for the usage of antidiabetic agent. Further studies are also required to elucidate whether the plant have antidiabetic potential by in vivo for corroborating the traditional claim of the plant. It has been reported that Flavonoid, coumarin, lignan, flavone, Saponin, and Tannin are responsible for antioxidant activity in many plants and it was detected that both plants consist of flavonoid, Saponin which could be responsible for antioxidant activity.

References:

1. Packard C, Olsson A. Management of hypercholesterolaemia in the patient with diabetes. Int J Clin Pract Suppl. 2002:27–32. [PubMed]

2. Gouni-Berthold I, Krone W. Diabetic ketoacidosis and hyperosmolar hyperglycemic state. Medizinische Klinik (Munich, Germany: 1983) 2006; 101:100–5. [PubMed]

3. Lambrinoudaki I, Tsouvalas E, Vakaki M, Kaparos G, Stamatelopoulos K, Augoulea A. *et al*. Osteoprotegerin, Soluble Receptor Activator of Nuclear Factor- κ B Ligand, and Subclinical Atherosclerosis in Children and Adolescents with Type 1 Diabetes Mellitus. Int J Endocrinol. 2013; 2013:8. [PMC free article] [PubMed]

4. Rajmohan L, Deepa R, Mohan A, Mohan V. Association between isolated hypercholesterolemia, isolated hypertriglyceridemia and coronary artessry disease in south Indian type 2 diabetic patients. Indian Heart J. 2000; 52:400–6. [PubMed]

5. Khan AS, Latif SU, Eloubeidi MA. Controversies in the etiologies of acute pancreatitis. JOP. 2010; 11:545–52. [PubMed]

6. Saravanan G, Ponmurugan P. Ameliorative potential of S-allylcysteine: effect on lipid profile and changes in tissue fatty acid composition in experimental diabetes. Exp Toxicol Pathol. 2012; 64:639–44. [PubMed]

7. Ramachandran B, Ravi K, Narayanan V, Kandaswamy M, Subramanian S. Protective effect of macrocyclic binuclear oxovanadium complex on oxidative stress in pancreas of streptozotocin induced diabetic rats. Chem Biol Interact. 2004; 149:9–21. [PubMed]
8. Elosta A, Ghous T, Ahmed N. Natural products as anti-glycation agents: possible therapeutic potential for diabetic complications. Curr Diabetes Rev. 2012; 8:92–108. [PubMed]
9. Rossini AA, Like AA, Chick WL, Appel MC, Cahill GF. Studies of streptozotocin-induced insulitis and diabetes. PNAS. 1977; 74:2485–9. [PMC free article] [PubMed]
10. Bandyopadhyay U, Das D, Banerjee RK. Reactive oxygen species: oxidative damage and pathogenesis. Current Science. 1999; 77:658–66.
11. Coskun O, Kanter M, Korkmaz A, Oter S. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β-cell damage in rat pancreas. Pharmacol Res. 2005; 51:117–23. [PubMed]
12. Khanam Z, Singh O, Singh R, Bhat IUH. Safed musli (*Chlorophytum borivilianum*): A review of its botany, ethnopharmacology and phytochemistry. J Ethnopharmacol. 2013; 150:421–41. [PubMed]
13. anda SK, Si SC, Bhatnagar SP. Studies on Hypoglycaemic and Analgesic Activities of *Chlorophytum borivilianum* Sant & Ferz. J Natu Remed. 2007; 7:31–36.
14. Mujeeb M, Khan SA, Ali M, Mal A, Ahmad A. Antidiabetic activity of the aqueous extract of *Chlorophytum borivilianum* L. in Streptozotocin induced- hyperglycemic rats-a preliminary study. J Pharm Res. 2009; 2:51–53.
15. Kenjale R, Shah RK, Sathaye SS. Anti-stress and anti-oxidant effects of roots of *Chlorophytum borivilianum* (Santa Pau & Fernandes) Indian J Exp Biol. 2007; 45:974–9
16. Harborne AJ. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis 3, Revised: Springer; 1998.
17.Rao RR, Tiwari AK, Reddy PP, Babu KS, Ali AZ, Madhusudana K, Rao JM. New furanoflavonoids, intestinal α-glucosidase inhibitory and free radical [DPPH) scavenging, activity from antihyperglycemic root extract of Derris indica (Lam) Bioorg Med Chem. 2009
18.Povichit N, Ampai P, Maitree S, Chaiyavat C, PimpornLee l. Phenolic content and in vitro inhibitory effects on oxidation and protein glycation of some Thai medicinal plants. Pak J Pharm Sci. 2010; 23:403–408.
19.H. Ali, P. J. Houghton, and A. Soumyanath, "α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to Phyllanthus amarus," Journal of Ethnopharmacology, vol. 107, no. 3, pp. 449–455, 2006.
20. D. L. Nelson and M. M. Cox, Lehninger Principles of Biochemistry, W. H. Freeman, New York, NY, USA, 5th edition, 2008.
21. Dr. Kokate C.K; Pharmacognosy; Nirali Prakashan; First Edition 2008.
22. Dr. Khandelwal K. R., "Pharmacognosy" practical book, Nirali Prakashan, edition 13th