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AN IN VITRO INVESTIGATION INTO THE MECHANISM OF ANTI-DIABETIC ACTIVITY OF SELECTED COMMERCIAL POLYHERBAL FORMULATION

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ABSTRACT

Diabetes mellitus is an endocrine disorder, characterized by hyperglycemia with abnormal carbohydrate, protein and lipid metabolism. Currently available treatments have several limitations such as reduced response with prolonged use and toxic side effects. Hence, herbal sources are considered as an alternative for effective management of diabetes mellitus and its associated complications. Commercial antidiabetic polyherbal formulations are available with claimed hypoglycemic activities; yet they lack systematic studies on mechanism of action. In the present study, hydro-alcoholic and aqueous extracts of commercially available antidiabetic polyherbal formulation, Sugar Nil, were assessed for their effects on glucose adsorption, diffusion, amylolysis kinetics and transport across the membrane of yeast cells using *in-vitro* techniques. Our results revealed that the adsorption of glucose increased dose dependently (5mM -100mM), highest glucose binding capacity was observed (64.8±0.54 mM and 60.74±0.74mM) at 100mM concentration for hydro-alcoholic and aqueous extracts respectively. Rate of glucose uptake into yeast cells was linear (5mM - 25 mM) for both the extracts, whereas glucose diffusion was directly proportional to the time (30 - 180 min). GRDI was calculated to be 17.00%and 9.68% at 30 min which reduced to 7.87% and 3.54% at 120 min for hydro-alcoholic and aqueous extracts respectively. Thus, the observed mode of actions might produce a concerted role of Sugar Nil for decrease in the rate of glucose absorption, thereby confirming its hypoglycemic activity.

Keywords: Diabetes mellitus, Polyherbal formulations, Glucose diffusion, Amylolysis kinetics, Yeast

INTRODUCTION

Diabetes mellitus (DM) is an endocrine metabolic disorder, characterised by high blood glucose levels. Insufficiency and unresponsive insulin leads to hyperglycemic condition, with abnormal protein, lipid and carbohydrate metabolism **[1]**. Uncontrolled or mismanaged diabetic condition leads to various macro- and micro-vascular disorders; therefore, it was observed that managed glycaemic state can help in reducing prevalence of secondary complications related to DM **[2, 3, 4]**. In present scenario diabetes is labelled as global pandemic with 415 million affected worldwide **[5, 6]**. Current therapies include oral hypoglycemic agents, prolonged use of which is related to adverse side effects **[7, 8, 19]**. Therefore, herbal formulations with less/no side effects are considered as an alternative treatment for holistic management of DM **[9]**. Plants exhibiting anti-hyperglycemic effects might often be due to their ability to reduce the intestinal absorption of glucose **[10, 11]**.

In recent years there were several reports on traditional medicine for their hypoglycemic claims which lacks in explaining their mode of actions **[12, 13]**. Various commercial antidiabetic polyherbal formulations, with hypoglycemic claims, are available. However, fewer studies were reported on the mechanism of action of these formulations in the management of glucose homeostasis **[14, 15]**. Thus, systematic studies to explore the mechanism of action for these commercial antidiabetic polyherbal formulations are need of the hour.

With the above background, the present study was designed to assess the hypoglycemic potential of hydro-alcoholic (HAE) and aqueous (AQE) extracts of commercially available antidiabetic polyherbal formulation, Sugar Nil, for their effects on glucose adsorption, diffusion, amylolysis kinetics and transport across the membrane of yeast cells using *in-vitro* techniques.

MATERIALS AND METHODS

Chemicals and reagents

Glucose oxidase peroxidase reagent was procured from Agappe Diagnostic Ltd**,** India. Dialysis bags (12,000 MW cut-off) were used from Himedia laboratories, India. Antidiabetic polyherbal formulation, Sugar Nil was obtained from Local market, India. All the other chemicals used in the study were of analytical grade**.**

Preparation of extract

Sugar Nil (5 g) was extracted with 150ml of 70% methanol and distilled water by cold maceration for 24 h. The hydro-alcoholic

(HAE) and aqueous (AQE) extracts were filtered using Whatman filter paper #1.The filtrate was concentrated with rotary evaporator and lyophilizer respectively and stored at 4°C forfurther analysis **[17]**.

Effect of extracts on glucose adsorption capacity

HAE and AQE (250 mg) were separately added to 25 mL of glucose solution of increasing concentrations (5, 10, 20, 50 and 100 mM). The reaction mixture was agitated and incubated in a shaker incubator at 37°C for 6 h, centrifuged at 4,000 x g for 20 min. Glucose content in the supernatant solution was determined by Glucose oxidase-peroxidase method, Absorbance was read at 520nm, Acarbose was taken as positive control. Glucose adsorption capacity was determined according to the following formula **[16, 18]**.

$$\text{Glucose Bound} = \frac{(\text{Glucose}_1 - \text{Glucose}_6) \times \text{Vol. solution}}{\text{Weight of the extracts}}$$

$Glucose_1$: Concentration of glucose original solution.

$Glucose_{6.}$ Concentration of glucose after 6 hrs.

Effect of extracts on *in-vitro* glucose diffusion

Twenty mM glucose solution (25 ml) and 0.25 g of Sugar Nil extracts and Acarbose were dialyzed against 200ml of distilled water at 37°C [8]. Further, glucose concentration in the dialysate was determined at time intervals i.e., 30, 60, 120 and 180 min using glucose oxidase peroxidase kit. Control test without addition of the extract was also performed. Glucose dialysis retardation index (GDRI) was calculated according to the following formula.

$$\text{GDRI (\%)} = \frac{(100 - \text{Glucose content + extract})}{\text{Glucose content of the control}} \times 100$$

Effect of extracts on *in-vitro* amylolysis kinetics

Twenty five millilitres of 4% starch solution with 0.4% of α-amylase and 1% of Sugar Nil extracts were dialysed against 200 ml of distilled water at 37°C (pH-7). Concentration of glucose in the dialysate was determined at various time intervals 30, 60, 120 and 180 min and a control test without addition of the extract was also performed **[16, 18]**.

Effect of extracts on glucose uptake by yeast cells

Commercial baker's yeast (EasyGrow Baker's) was washed in distilled water with repeated centrifugation (3,000 x g; 5 min) till a clear supernatant was obtained; further 10% (v/v) suspension was prepared with the same. Different concentrations of both the extracts (1—5 mg) were added to 1 mL of glucose solution (5—25 mM), mixture was incubated for 10 min at 37°C. Reaction was initiated by adding 100µL of yeast suspension, vortexed and incubated at 37°C. After 60 min, tubes were centrifuged (2500 x g, 5 min) and glucose was estimated in the

supernatant. Precent increase in glucose uptake by yeast cells was calculated using the following formula **[16, 20]**.

$$\% \text{ Increase glucose uptake} = \frac{\text{Abs. C} - \text{Abs. S} \times 100}{\text{Absorbance control}}$$

Abs= Absorbance; C= control; S= Sample

Statistical analysis

All the experimental works were carried out in triplicates and the obtained data were analysed by ANOVA. Values were considered at $P \leq 0.05$. Graphs were plotted using Graph Pad Prism 8 software.

RESULTS AND DISCUSSION

Effect of extracts on glucose adsorption capacity

HAE and AQE extracts of Sugar Nil were assessed for their glucose adsorption capacity. It was observed that with an increase in glucose concentration there was an increase in bound glucose **(Figure 1)**. Adsorption capacity of the extracts is directly proportional to the molar concentrations of glucose. Higher adsorption capacity was observed with HAE as compared to AQE in all five concentrations of glucose. Standard drug, Acarbose was considered as control. Maximum bound glucose content was observed for HAE and AQEat 100mM concentration respectively (64.8±0.54 mM and 60.74±0.74mM). In the present study, HAE and AQE had the capacity to bind glucose at lower concentration i.e., 5mM. Therefore it will ensure decreasing glucose availability for transport across the intestinal lumen which might be able to manage hyperglycemia **[21, 22, 34]**. Adsorption capacity of extracts might be due to presence of soluble fibres, insoluble fibres and bioactive constituents. Similar interpretations were reported for insoluble fiber-rich fractions isolated from *Averrhoa carambola* **[23]**.

Effect of extracts on *in-vitro* glucose diffusion

In-vitro glucose dialysis retardation index (GDRI) is a convenient method to analyse the effect of extracts on interruption of glucose adsorption in gastrointestinal tract **[24, 30]**. Both HAE and AQE extracts exhibited significant inhibitory effects on movement of glucose into external solution when compared to control. The rate of glucose diffusion across the dialysis membrane was found to be directly proportional with time. The GRDI reduced over time for both the extracts with highest values observed at 180min, **(Table 1)**. HAE exhibited similar GRDI when compared with standard Acarbose. Our results corroborate with the studies on different samples where GRDI reduced over time **[8, 22]**.

Previous studies has proposed several possible mechanism for lowering of glucose which might be due to adsorption or

inclusion of the smaller glucose molecules into the structure of particles present in the extracts. Another possible explanation for glucose diffusion ability might be the capability of polysaccharides forming gelatinous compounds in aqueous solution, which decreases glucose availability in small intestine, resulting in reducing postprandial glucose levels **[24, 26, 27]**.

Effect of extracts on *in-vitro* amylolysis kinetics

Effect of tested extracts on starch digestibility and glucose dialysis retardation index were presented **(Table 2)**. The rate of glucose diffusion were analysed at every 30 min interval, diffusion rate was nil at 30min i.e., 100%. Further from 60 min the rate of glucose diffusion reduced significantly ($p \leq 0.05$), highest GDRI was observed at 180min. Present analysis revealed that GRDI value reduced gradually as time increases which is in comparable with previous reports **[16, 22, 32]**. Inhibition of α-amylase can be considered as a limiting step for the release of glucose from starch thus retarding glucose diffusion. α-amylase inhibition might be due to various factors such as fibre content, inhibitors on fibres, encapsulation of starch, thereby decreasing availability of starch to the enzyme **[18, 21, 25]**.

Effect of extracts on glucose uptake by yeast cells

Rate of glucose uptake across the yeast cell can be measured by the remaining glucose content in the medium after defined time interval. Glucose transport across cell membrane in yeast cells system is depicted **(Figures 2 and 3)**. A linear uptake of glucose was observed for both the extracts, percent increase in glucose uptake by the yeast cells was found to be inversely proportional to glucose concentration. Glucose uptake decreased with higher molar concentration of the glucose solution. Similar studies were reported with various traditional hyoglycemic plants on glucose uptake by yeast cells **[8, 16, 19]**.

Previous studies on movement of various sugars and glycosides have suggested its transport across the cell membrane which might be due to presence of stereospecific membrane carriers or mediators further enhancing facilitated diffusion process **[28, 29, 16]**. Our results are in accordance with previous reports on medicinal plants **[31, 33]**.

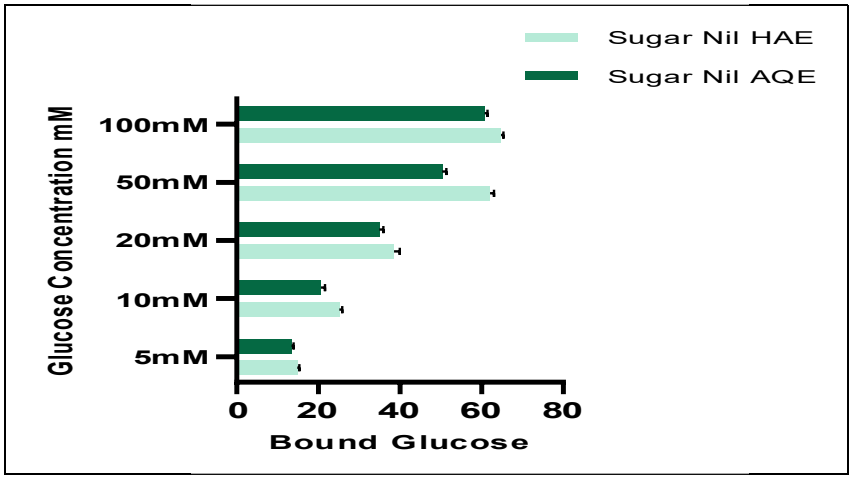

Figure 1: Glucose binding capacity of HAE and AQE at different glucose concentrations
***Values are mean ± SE of triplicate determinations**

Table 1 Effect of Sugar Nil extracts on glucose diffusion and GDRI				
Sample	Glucose content in dialysate (mM)			
	30 min	60 min	120 min	180min
Control	0.929±0.081	1.31±0.043	1.503±0.04	1.744±0.13
AQE	0.84±0.04 (9.68)	1.26±0.03(3.54)	1.45±0.00(3.55)	1.54±0.008(11.75)
HAE	0.77±0.02 (17)	1.19±0.03(9.46)	1.38±0.06 (7.87)	1.53±0.02(11.83)
Values in parenthesis indicate GDRI. Values are represented as Mean±SE (n = 3) (P ≤ 0.05).				

Table 2: Effect of Sugar Nil extracts on starch digestibility and GDRI				
	Glucose content in dialysate (mM)			
	30 min	60 min	120 min	180min
Control	0.0	0.28±0.005	0.354±0.003	0.446±0.009
AQE	0.0 (100)	0.141±0.03(49.64%)	0.273±0.05 (22.74%)	0.37±0.03 (17.01%)
HAE	0.0 (100)	0.137±0.04 (51.07%)	0.195±0.03 (43.76%)	0.317±0.048(28.89%)
Values in parenthesis indicate GDRI. Values are represented as Mean±SE (n = 3) (P ≤ 0.05).				

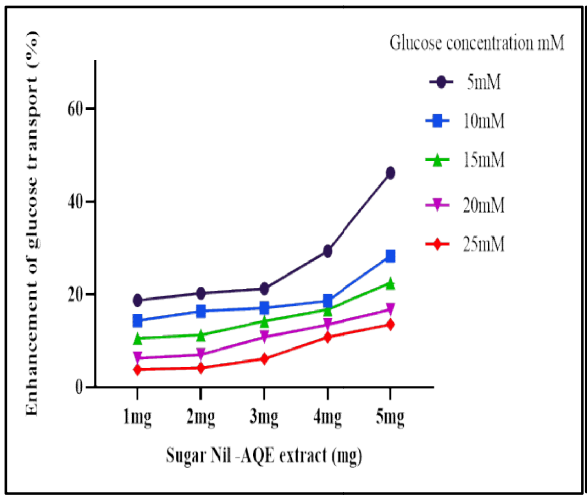

Figure 2: Effect of AQE extract on the uptake of glucose by yeast cells. Values are mean of triplicate determinations

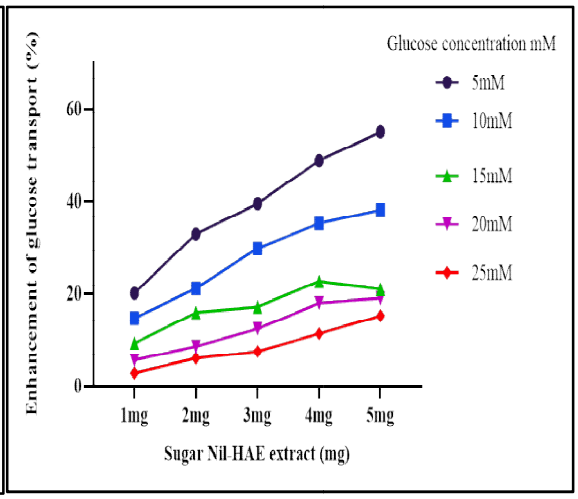

Figure 3: Effect of HAE extract on the uptake of glucose by yeast cells. Values are mean of triplicate determinations

CONCLUSION

Our study primarily focuses on revealing the various mechanisms for the hypoglycemic activity by which an antidiabetic polyherbal formulation, Sugar Nil might be managing blood glucose levels, thus authenticating claims for the same. The observed results validate the antidiabetic activities of HAE and AQE of Sugar Nil by several *in-vitro* methods viz. glucose adsorption, glucose diffusion and glucose uptake at cellular levels by *in-vitro* yeast cells model.

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REFERENCES

[1] Srivastava AK A, Maurya R, Antihyperglycemic activity of compounds isolated from Indian medicinal plants, Indian Journal of experimental biology, 48(3), 2010, 294-298.

[2] Carene M N P, Subratty H A, Mahomoodally F M, Inhibitory Potential of Five Traditionally Used Native Antidiabetic Medicinal Plants on α-Amylase, α-Glucosidase, Glucose Entrapment, and Amylolysis Kinetics *In Vitro*, Advances in Pharmacological Sciences, 2014, 2014, 1-7.

[3] Pasupathi P, Chandrasekar V, Kumar U S, Evaluation of oxidative stress, enzymatic and non-enzymatic antioxidants and metabolic thyroid hormone status in patients with diabetes mellitus, Diabetes and Metabolic Syndrome: Clinical Research & Reviews, 3(3), 2009, 160-165.

[4] Mayur B, Sabdesh S S, Sung-yum S, Antioxidant and α-glucosidase inhibitory properties of *Carpesium abrotanoides* L, Journal of Medicinal Plants Research, 4, 2010, 1547-1553.

[5] Ogurtsova K, Fernandes JD R DA, Huang Y, Cavan D, Shaw JE, Makaroff LE. IDF Diabetes Atlas: global estimates for the prevalence of diabetes for 2015 and 2040, Diabetes Research in Clinical Practice, 128, 2010, 40–50.

[6] Marín-Peñalver J J, Martín-Timón I, Sevillano-Collantes C, Cañizo-Gómez D J f, Update on the treatment of type 2 diabetes mellitus, World journal of Diabetes, 7(17), 2016, 354-395.

[7] Bandawane D, Juvekar A, Juvekar M, Antidiabetic and antihyperlipidemic

effect of *Alstonia scholaris* Linn bark in streptozotocin induced diabetic rats, Indian Journal of Pharmaceutical Education and Research, 45(2), 2011, 114-120.

[8] Bhutkar M, Bhise S, In vitro hypoglycemic effects of *Albizzia lebbeck* and *Mucuna pruriens*, Asian Pacefic Journal of Tropical Biomedicine, 3(11), 2013a, 866-870.

[9] Basha K S, Kumari S V, In vitro antidiabetic activity of *psidium guajava* leaves extracts, Asian Pacific Journal of Tropical Disease, 2012, 2012, S98-S100.

[10] Handa S S, Mundkinajeddu D, Mangal A K, Indian pharmacopeia, Government of India, ministry of Health and Family Welfare, Delhi: Controller of Publications, 1996.

[11] Randive D S, Sayyad S F, Bhinge S D, Bhutkar M, Preparation of Arjunarishta using microbes isolated from *Woodfordia fruticosa* flowers (Dhayati), Ancient Journal of Life Science, 13(1), 2016a, 42-47.

[12] Randive D S, Bhinge S D, Sayyad S F, Bhutkar M A, Wadkar G H, Comparative standardization of marketed formulations of fermented biomedicine — Arjunaristha, Indonesian Journal of Pharmacy, 27(04), 2016b, 220-225.

[13] Grover J K, Yadav S, Vats V, Medicinal plants of India with antidiabetic potential, Journal of Ethnopharmacology, 81, 2002, 81—100.

[14] Ojo C M, Osunsanmi O F, Zaharare E G, Mosa A R, Cele D N, Oboh O M, OpokuR A, In-vitro Anti-diabetic and Antioxidant Efficacy of Methanolic Extract of *Encephalartos ferox* leaves, Pharmacognosy Journal, 11(3), 2019, 455-460.

[15] Thengya S, Thiantongin P, Sontimuang C , Ovatlarnporn C, Puttarak P, α-glucosidase and α-amylase inhibitory activities of medicinal plants in Thai antidiabetic recipes and bioactive compounds from *Vitex glabrata* R.Br. stem bark, Journal of herbal medicine, 19, 2019, 1-8.

[16] Sayyed F J, Wadkar G H, Studies on in-vitro hypoglycemic effects of root bark of *Caesalpinia bonducella*, Annales Pharmaceutiques Franc-aises, 525, 2017, 1-6.

[17] Telapolu S, Kalachavedu M, Punnoose M A, Bilikere D, MD-1, a

poly herbal formulation indicated in diabetes mellitus ameliorates glucose uptake and inhibits adipogenesis – an *in vitro* study, BMC Complementary and Alternative Medicine, 18(113), 2018, 1-11.

[18] Ou S, Kwok K C, Li Y, Fu L, In vitro study of possible role of dietary fiber in lowering postprandial serum glucose, Journal of Agricultural and Food Chemistry, 49, 2001, 1026-1029.

[19] Bhutkar M A, Bhise S B, Studies on antiglycation potential of some traditional antidiabetic plants, Asian Journal of Plant Science and research, 3(6), 2013b, 60-63.

[20] Cirillo V P, Mechanism of glucose transport across the yeast cell membrane, Journal of Bacteriology, 84, 1962, 485-491.

[21] Ahmed F, Sairam S, Urooj A, In vitro hypoglycemic effects of selected dietary fiber sources, Journal of Food Science and Technology, 48(3), 2011, 285–289.

[22] Chau C F, Huang Y L, Lee M H, In vitro hypoglycemic effect of different insoluble fiber-rich fractions prepared from the peel of *citrus sinensis* L.cv. Liucheng, Journal of Agriculture and Food Chemistry, 51,2003, 6623- 6626.

[23] Chau C F, Chen C H, Lin C Y, Insoluble fiber-rich fractions derived from *Averrhoa carambola*: hypoglycemic effects determined by in vitro methods, Lebensmittel Wissenschaft Technologie, 37, 2004, 331–335.

[24] Lopez G, Ros G, Rincon F, Periago M J, Martinez M C, Ortuno J, Relationship between physical and hydration properties of soluble and insoluble fiber of artichoke, Journal of Agriculture and Food Chemistry, 44,1996, 2773–2778.

[25] Bailey C J. Textbook of diabetes. Blackwell Science, Oxford. pp 1–73, 2003.

[26] Gallagher A M, Flatt P R, Duffy G, Abdel-Wahab Y H A, The effects of traditional antidiabetic plants on in vitro glucose diffusion, Nutrition research, 23, 2003, 413-424.

[27] Wood P J, Beer M U, Butler G, Evaluation of role of concentration and molecular weight of oat beta-glucan in determining effect of viscosity on plasma glucose and insulin following oral glucose load,

British Journal of Nutrition, 84, 2000, 19–23.

[28] Teusink B, Diderich J A, Westerhoff H V, Van Dam K, Walsh M C, Intracellular glucose concentration in derepressed yeast cells consuming glucose is high enough to reduce the glucose transport rate by 50%, Journal of Bacteriology, 180, 1998, 556-562.

[29] Illiano G, Cuatrecasas P, Glucose transport in fat cell membranes, Journal of Biochemistry, 246, 1971, 2472-2479.

[30] Harish M, Ahmed F, Urooj A, In vitro hypoglycemic effects of *Butea monosperma* Lam. leaves and bark M, Journal of Food Science and Technology , 51, 2011, 308-314.

[31] Pitchaipillai R, Ponniah T S, In Vitro Antidiabetic Activity of Ethanolic Leaf Extract of *Bruguiera cylindrica* L. – Glucose Uptake by Yeast Cells Method, International biological and biomedical journal, 2(4), 2016.

[32] Bhutkar M A, Bhinge S D, Randive D S, Wadkar G H, Hypoglycemic effects of *Berberis aristata* and *Tamarindus indica* extracts in vitro, Bulletin of Faculty of Pharmacy, Cairo University, 55, 2017, 91–94.

[33] Bhinge S D, Bhutkar M A, Randive D S, Wadkar G H, Hasabe T, In vitro hypoglycemic effects of unripe and ripe fruits of *Musa sapientum*, Brazilian Journal of Pharmaceutical Sciences, 53(4), 2017, 1-6.

[34] Randive D S, Mangesh ID, Bhutkar A, Somnath D B, Kiran P S, Patil D P, Mane A S, Hypoglycemic effects of *Lagenaria siceraria*, Cynodon dactylon and *Stevia rebaudiana* extracts, Journal of Herbmed Pharmacology, 8(1), 2019, 51-55.