10.5005/jp-journals-10059-0001

RESEARCH ARTICLE

In vitro Studies on Antidiabetic Potential of New Dosage Forms of AYUSH 82

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ABSTRACT

AYUSH 82 powder is an Ayurvedic antidiabetic formulation developed by the Central Council for Research in Ayurvedic Sciences (CCRAS), Ministry of AYUSH, Government of India. It comprises ingredients traditionally used for their beneficial effect in diabetes (*Prameha/Madhumeha*). The hypoglycemic effects of AYUSH 82 powder have been reported in diabetic subjects. In the current study, the antidiabetic potential of AYUSH 82 powder along with its two new dosage forms – AYUSH 82 mixture extract and AYUSH 82 compound extract- was investigated *in vitro* for elucidating mechanism of their action by possible α-amylase inhibitory property, insulin-dependent glucose uptake in skeletal muscle cell line (C2C12 myotubes), and effect on peroxisome proliferator-activated receptor gamma (PPAR-γ) activity.

All the three dosage forms of AYUSH 82 – powder, mixture extract, and compound extract – exhibited inhibition of α-amylase activity. AYUSH 82 mixture extract, however, demonstrated highest extent of inhibition in both methanolic (87.4%) and aqueous (48.2%) format. All the three dosage forms of AYUSH 82 also demonstrated an increase in insulin-dependent glucose uptake in C2C12 myotubes as compared with control. However, none of the test items (TIs) exhibited activation of PPAR-γ expression in tested ranges, indicating that antidiabetic potential of TIs may not be mediated via PPAR-γ activation. Results indicated that the new dosage forms of AYUSH 82 (mixture extract and compound extract) may be useful for making new dosage forms of AYUSH 82 as tablets/capsule, etc.

Keywords: α-Amylase, Antidiabetic activity, AYUSH 82 powder, Compound extract, Glucose uptake assay, Mixture extract, Peroxisome proliferator-activated receptor gamma

How to cite this article: Setia M, Meena K, Madaan A, Srikanth N, Dhiman KS, Sastry JLN. *In vitro* Studies on Antidiabetic Potential of New Dosage Forms of AYUSH 82. J Drug Res Ayurvedic Sci 2017;2(1):1-9.

Source of support: Nil

Conflict of interest: None

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INTRODUCTION

The prevalence of diabetes mellitus in India is predicted to double from 171 million in 2000 to 366 million in 2030, posing a significant health care burden.[1] The major concern regarding this observation relates to the development of chronic complications associated with the condition, either microvascular – retinopathy, nephropathy, and neuropathy – or macrovascular – cardiovascular disease, cerebrovascular accidents, and peripheral vascular disease.[2] It is well recognized that these complications cause considerable morbidity and mortality worldwide and, as such, negatively an increase in disability and death.[3] The costs of caring for diabetes and its related complications are also staggering. The estimated financial burden of diabetes was US$174 billion in 2007 and it is expected to be US$330 billion by 2020 due to the expected increase in new cases in the United States alone. In India, diabetes care can cost low-income households about one-third of their incomes.[4,5]

Providers caring for patients with diabetes recognize that the patients are very interested in alternative supplementation and may choose to supplement their pharmacological regimen with supplementation popularly from natural products, i.e., herbal or botanical sources.[6] From the patient perspective, it is considered very acceptable to include herbal or botanical extracts as part of the medical intervention based on the recognition that the herbal intervention is considered to be natural and that the practice may have been part of the culture for many generations. However, currently, there is a paucity of consistent and reproducible efficacy data in humans to suggest any recommendations for most botanical or bioactive supplements as adjunct treatments for risk factors related to type II diabetes. Firm recommendations for general use would also require an understanding of the mechanism of action, which is not known for most botanicals.[4]

AYUSH 82 is an Ayurvedic hypoglycemic formulation developed by the Central Council for Research in Ayurvedic Sciences (CCRAS), Ministry of AYUSH, Government of India. It comprises ingredients traditionally used for their beneficial effect in diabetes (Prameha/Madhumeha).[7] Glucose-lowering effects of AYUSH 82 powder have been reported in patients of Madhumeha

[noninsulin-dependent diabetes mellitus (NIDDM)] where AYUSH 82 powder (5 gm thrice a day with 500 mg of pure Shilajita twice a day) was found to significantly reduce fasting and postprandial blood sugar levels along with clinical improvement in diabetic subjects after 24 weeks of treatment.[8] In another clinical study, AYUSH 82 administered at similar doses to patients of NIDDM was found to produce significant reduction ($p > 0.001$) in both fasting and postprandial blood sugar and marked decrement in symptoms of diabetes like polyuria, polydipsia, polyphagia, weakness, and exhaustion after a treatment period of 6 weeks.[9]

In the current study, we investigated the antidiabetic potential of AYUSH 82 powder along with two new dosage forms – the mixture extract and the compound extract – through their mechanism of action. All the three dosage forms were investigated for possible α-amylase inhibitory property, insulin-dependent glucose uptake in skeletal muscle cell line (C2C12 myotubes), and effect on peroxisome proliferator-activated receptor gamma (PPAR-γ) activity through *in vitro* assays.

MATERIALS

AYUSH 82 powder was developed by the CCRAS, Ministry of AYUSH, Government of India, for the management of type II diabetes. For the current studies, AYUSH 82 powder and its extracts were procured from M/s Dabur India Ltd., Ghaziabad, Uttar Pradesh, India under technical knowhow from the CCRAS. The composition details of AYUSH 82 are given in Table 1.

Preparation of AYUSH 82 Mixture Extract and Compound Extract

Hydroalcoholic extraction of herbs (*Karvellaka, Jamun, Amra, Gurmar*) was done. The extract was concentrated and dried to fine powder, which was checked for compliance with specification parameters. For AYUSH 82 mixture extract, equal quantities of herbs were extracted individually and then mixed. For AYUSH 82 compound extract, the herbs were mixed together in equal proportions and then extracted.

AYUSH 82 powder (DRDC/2016/006), AYUSH 82 mixture extract (DRDC/2016/008), AYUSH 82 compound extract (DRDC/2016/010) – Batch No. 84/54 (HP), 84/54 (HAE-Dry Pow.) & 84/54 (HAE-Dab.) with Date of manufacturing: April 15, 2016 and expiry 2 years from date of manufacturing were used in the study.

Table 1: Composition details of AYUSH 82 (each 100 gm)

Sanskrit name	*Botanical name*	*Part used*	*Qty (gm)*
Karavallaka	*Momordica charantia* L.	Dried fruit	25
Jambu	*Syzygium cuminii* (L.) Skeels	Seed	25
Amra	*Mangifera indica* L.	Seed	25
Gurmar	*Gymnema sylvestre* R. Br.	Leaves	25

Chemical/Reagent/Media

For α-Amylase Activity

α-Amylase (Sigma), Acarbose (Sigma), Quercetin (Sigma), Dimethyl sulfoxide (DMSO) (Merck), Na_2HPO_4 (Merck), HCl (Qualigens), Iodine (Rankem), Potassium iodide (Fisher Scientific), Methanol (Rankem), and Starch (Fisher Scientific).

For Glucose Uptake in Skeletal Muscles (C1C2 Myotubes)

Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) (Gibco), MTT (Acros Organics), Penicillin/Streptomycin (Himedia), Trypsin (Sigma), 2-NBD Glucose (Life Technologies), Skeletal Muscle Cell Line (C2C12 Myotubes).

For PPAR-γ Activity

The DMSO (Merck), Rosiglitazone (Sigma), Substrate Loading Solution consisted: Solution A (10 mM LiveBLAzer™-FRET B/G Substrate), Solution B, and Solution C (Life Technologies).

METHODS

Inhibition of α-Amylase Activity

The inhibitory potential of the three test items (TIs) on activity of α-amylase was evaluated using *in vitro* cell-free enzyme-based assay in comparison to positive controls – acarbose and quercetin.[10,11] Amylase activity was determined by starch-iodine method using starch as a substrate and measuring the dextrinizing activity of amylase in terms of decrease in the color of iodine.[12,13] Absorbance of samples was read at 620 nm.

Preparation of TIs

Aqueous Formulation

Test items were weighed and dissolved in MilliQ water to prepare stock solution of 250 mg/mL. This stock solution was diluted in 20 mM phosphate buffer to prepare final concentrations ranging from 10 µg/mL to 20 mg/mL.

Methanolic Formulation

Test items were weighed and dissolved in methanol to prepare stock solution of 250 mg/mL. This stock solution was diluted in methanol to prepare final concentrations ranging from 50 µg/mL to 25 mg/mL.

Phosphate Buffer

To make 0.2 M monobasic stock, 2.4 gm sodium phosphate monobasic (NaH_2PO_4) was dissolved in 100 mL of MilliQ water. To make 0.2 M dibasic stock, 2.84 gm sodium phosphate dibasic anhydrous (Na_2HPO_4) was dissolved in 100 mL of MilliQ water. To prepare 0.1 M phosphate buffer (pH 6.9), 45 mL of monobasic stock and 55 mL of dibasic stock were mixed and final volume was adjusted to 200 mL with MilliQ water. This 0.1 M phosphate buffer was diluted to prepare 20 mM phosphate buffer (pH 6.9).

Starch Solution (1%)

Starch 200 mg was weighed and dissolved in 20 mL of 20 mM phosphate buffer. This solution was heated at 40 to 50°C with constant stirring till a clear solution was obtained.

Iodine Solution (5 mM)

Potassium iodide (KI) 42 mg was dissolved in 50.602 mL of MilliQ water; 5 mM iodine solution: 64 mg iodine was dissolved in 50.394 mL of 5 mM KI solution. This solution was used as iodine solution for termination of the reaction. α-Amylase weighing 11 mg was weighed and dissolved in 14.667 mL 20 mM phosphate buffer to obtain a stock of 0.75 mg/mL.

Positive Control-1 (Acarbose)

Acarbose 4 mg was weighed and dissolved in MilliQ water to prepare 1 gm/mL stock solution. This stock solution was diluted using phosphate buffer to achieve final concentrations of 20, 60, 80, and 100 mg/mL.

Positive Control-2 (Quercetin)

Quercetin 4 mg was weighed and dissolved in DMSO to prepare 1 gm/mL stock solution. This stock solution was diluted using phosphate buffer to achieve final concentration of 10 mM.

Amylase Enzyme Inhibition Assay

Test items of 75 µL at final concentrations (10 µg/mL to 20 mg/mL for aqueous formulation and 50 µg/mL to 25 mg/mL for methanolic formulation respectively) were preincubated with 50 µL of α-amylase solution (0.75 mg/mL) prepared in phosphate buffer in a total volume of 500 µL for 15 minutes at 25°C. Test items at different concentrations in the absence of enzyme were taken as blanks in a total volume of 500 µL. The 50 µL of enzyme alone treated with buffer in a total volume of 500 µL was taken as control for aqueous formulations; 50 µL of enzyme alone treated with 75 µL of methanol in a total volume of 500 µL was taken as control sample for methanolic formulations.

The reaction was initiated by adding 250 µL of substrate (1% starch solution). The reaction mixture was incubated for 30 minutes at 25°C. The reaction was terminated by addition of 0.2 mL of 1 M HCl and 1 mL of iodine solution. The activity of amylase was determined by measuring the absorbance at 620 nm and is inversely proportional to color of the reaction. The inhibitory effect of TIs on α-amylase activity was determined as percent decrease in the enzyme activity with respect to control sample. Percent enzymatic activity of α-amylase was calculated as follows:

$$\left[\frac{\{(\text{OD of Starch} + \text{Iodine}) - (\text{OD of Starch} + \text{Iodine} + \alpha\text{-Amylase})\}}{(\text{OD of Starch} + \text{Iodine})}\right] \times 100$$

Percent inhibition of α-amylase activity was calculated as:

[% Enzymatic activity in Control (buffer treated / Methonal treated) sample − % Enzymatic activity in Test Item treated sample]

Evaluation of Antidiabetic Potential of Various Herbal Formulations using Mouse Skeletal Muscle Cell Line (C2C12 Myotubes) *in vitro*

The study was conducted to determine the effects of different dosage forms of AYUSH 82 on skeletal muscle glucose metabolism utilizing insulin-dependent glucose uptake in mouse skeletal muscle cell line (C2C12 Myotubes).[14]

Noncytotoxic concentration of DMEM + 10% FBS was used as positive control for validation of the assay. It was dissolved in 10% serum-free medium to obtain 10% working concentration range. Glucose uptake was studied utilizing insulin (200 nM). Stock solution of 100 mg/mL of insulin was prepared utilizing 50% methanol as solvent to obtain working concentration range of 1 µg/mL to 1 mg/mL (noncytotoxic concentrations) and 10 µg/mL to 1 mg/mL (for glucose uptake).[15,16]

Differentiation of Skeletal Muscle C2C12 Myoblasts to Myotubes

The skeletal muscle cell line, C2C12 myoblasts, was maintained in DMEM supplemented with 10% FBS at 37°C at 5% CO_2. After reaching confluency, cells were induced to differentiate from myoblasts to myotubes. To induce differentiation, growth medium was replaced with DMEM containing 1% FBS. Myoblasts were differentiated into myotubes after 10 days of daily medium change (DMEM + 0.1% FBS medium). Experiments were performed on differentiated C2C12 myotubes.[17]

Determination of Noncytotoxic Concentrations of TIs on C2C12 Myotubes

The cells were trypsinized and a single-cell suspension was obtained. The cell suspension was counted on a hemocytometer. The cells were seeded at a density of 10×10^3 cells/well in 96-well plate. The seeded plate was incubated overnight at 37°C in a 5% CO_2 incubator. Subsequent to incubation, skeletal muscle C2C12 myoblasts were differentiated to myotubes and treated with different concentrations of TIs ranging from 1 µg/mL to 1 mg/mL in triplicates.[18]

Subsequently, treated cells were incubated for a time period of 24 hours at 37°C in a 5% CO_2 incubator. The untreated cells were used as controls. After treatment of C2C12 myotubes with TIs at concentrations ranging from 1 µg/mL to 1 mg/mL for a time period of 24 hours at 37°C in a 5% CO_2 incubator, the viability of the cells were determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT)-based assay. A total of 20 µL of 5 mg/mL of MTT was added to all the wells and incubated for 3 hours at 37°C in a 5% CO_2 incubator. Subsequent to incubations, the supernatant was aspirated without disturbing the formazan complex and 150 µL of DMSO was added to all wells to dissolve the formazan crystals.

The optical density of each well was read at 540 nm on a multiplate Biotek reader. Noncytotoxic concentrations of TIs were determined by calculating percent viability of C2C12 myotubes as follows:

$$\%\ \text{Viability} = \frac{100 - R - X \times 100}{R}$$

where X = OD of cells treated with TI(s); R = OD of untreated control cells.

Glucose Uptake

C2C12 myoblasts were plated in 48-well plate at a density of 30×10^3 cells per well and incubated overnight at 37°C in 5% CO_2 incubator after which they were differentiated with predetermined noncytotoxic concentrations of TIs ranging from 10 µg/mL to 1 mg/mL and insulin (200 nM). The treated cells were incubated for 24 hours at 37°C in 5% CO_2 incubator. Following the incubation period, these were washed twice with phosphate-buffered saline (PBS; pH 7.4) and were incubated for 30 minutes at 37°C in PBS (pH 7.4).

Subsequent to incubation, C2C12 myotubes were washed twice with PBS (pH 7.4) at 37°C.

A total of 450 µM of 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino]-2-deoxy-D-glucose (2-NBDG) prepared in PBS (pH 7.4) was added to all the wells and incubated for 30 minutes at 37°C. Cells were lysed by freeze thaw in PBS (pH 7.4). Fluorescence of 2-NBDG were measured in cell lysates using multiplate Biotek reader at an emission wavelength of 528 nm and excitation wavelength of 485 nm.[17-19]

Percentage increase in the glucose uptake by the TI(s)-treated cells as compared with control untreated cells was calculated as follows:

$$\%\ \text{Increase in Glucose Uptake} = \frac{X - R}{R} \times 100$$

where X = Fluorescence of cells treated with TI(s); R = Fluorescence of untreated control cells.

Determination of Noncytotoxic Concentrations of TIs on C2C12 Myotubes

Criterion for evaluation of noncytotoxic concentrations was cell viability ≥70% as compared with control cells. All the TIs were found to be noncytotoxic at concentrations ranging from 1 µg/mL to 1 mg/mL for 24 hours on C2C12 myotubes and safe to use for further evaluation of antidiabetic potential of TIs.

Effect on PPAR-γ Activity using *in vitro* Assay

The study was conducted to evaluate the effect of TIs on PPAR-γ expression, for their antidiabetic potential *in vitro* utilizing cell-based assays. UAS-bla HEK 293H cell line containing the ligand-binding domain of the human PPAR-γ was treated with TIs for 24 hours at different concentration ranges. The activation of PPAR-γ expression was monitored by fluorescence-based assay.[20]

Preparation of Stock Solution

The DMSO stocks of TI were prepared (AYUSH 82 powder and mixture – extract 50 mg/mL; AYUSH 82 compound extract – 100 mg/mL), which were treated as 1,000× stock solution. These were serially diluted (10 point ½-log increments) in 100% DMSO to obtain final tested concentrations of 0.0015 µg/mL to 50 µg/mL for TI 1 and 2 and 0.003 µg/mL to 100 µg/mL for TI 3. A total of 40 nL of 1,000× test compound or known activator [4 µL of a 10× serial dilution of rosiglitazone (positive control agonist starting concentration, 316 nM)] plus 4 µL of assay media was added to 384-well poly-D-lysine assay plate.[21,22]

The PPAR-γ-UAS-bla HEK 293H cells were thawed and resuspended in assay media (DMEM phenol red free, 2% CD-treated FBS, 0.1 mM NEAA, 1 mM sodium pyruvate, 100 U/mL/100 µg/mL Pen/Strep) to a concentration of 937,500 cells/mL. A total of 32 µL of cell suspension (30,000 cells) was added to each well; 4 µL of assay media was added to all wells to bring the final assay volume to 40 µL. The plate was incubated for 24 hours at 37°C/5% CO_2 in

a humidified incubator; 8 µL of 1 µM substrate loading solution was added to each well and the plate was incubated for 2 hours at room temperature. The assay plate was read on a fluorescence plate reader (Tecan Safire2) and the data were analyzed for percent activation. Vehicle (DMSO)-treated cells were used as control.[23]

RESULTS

Effect on α-Amylase Activity

Aqueous formulations of AYUSH 82 mixture extract and AYUSH 82 compound extract and the methanolic formulations of all the three dosage forms of AYUSH 82 demonstrated inhibitory effect on α-amylase activity in a dose-dependent manner, indicating the antidiabetic potential. AYUSH 82 mixture extract demonstrated highest extent of inhibition of α-amylase activity in both aqueous (48.2%) and methanolic (87.4%) formulations at concentration of 20 and 25 mg/mL respectively (Tables 2 and 3; Graphs 1 and 2).

Table 2: Percent α-amylase activity of AYUSH 82 (aqueous formulations)

Sample	*Conc.*	*% α-Amylase activity*	*% Inhibition of α-Amylase activity (wrt Control)*
	Control	100	0.0
AYUSH 82 Powder (DRDC/2016/006)	10 µg/ml	99.9	0.1
	100 µg/ml	99.8	0.2
	1 mg/ml	99.6	0.4
	2.5 mg/ml	98.8	1.2
	5 mg/ml	98.4	1.6
	10 mg/ml	97.9	2.1
	20 mg/ml	95.5	4.5
AYUSH 82 Mixture Extract (DRDC/2016/008)	10 µg/ml	100.0	0.0
	100 µg/ml	99.7	0.3
	1 mg/ml	97.3	2.7
	2.5 mg/ml	94.3	5.7
	5 mg/ml	88.2	11.8
	10 mg/ml	76.2	23.8
	20 mg/ml	51.8	48.2
AYUSH 82 Compound Extract (DRDC/2016/010)	10 µg/ml	100.6	-0.6
	100 µg/ml	98.6	1.4
	1 mg/ml	98.2	1.8
	2.5 mg/ml	95.2	4.8
	5 mg/ml	93.8	6.2
	10 mg/ml	88.8	11.2
	20 mg/ml	81.0	19.0
Acarbose (PC-1)	20 mg/ml	92.9	7.1
	60 mg/ml	83.2	16.8
	80 mg/ml	80.2	19.8
	100 mg/ml	76.7	23.3
Quercetin (PC-2)	10 mM	39.9	60.1

Table 3: Percent α-amylase activity of AYUSH 82 (methanolic formulations)

TI/dosage form	*Conc.*	*Percent α-amylase activity*	*Percent inhibition α-amylase activity (wrt control)*
	Control	100	0
AYUSH 82 powder	50 µg/mL	99.2	0.8
	500 µg/mL	97.6	2.4
	2.5 mg/mL	97.2	2.8
	5 mg/mL	89.0	11.0
	12.5 mg/mL	78.8	21.2
	25 mg/mL	54.7	45.3
AYUSH 82 mixture extract	50 µg/mL	100.0	0.0
	500 µg/mL	99.4	0.6
	2.5 mg/mL	96.1	3.9
	5 mg/mL	89.8	10.2
	12.5 mg/mL	62.5	37.5
	25 mg/mL	12.6	87.4
AYUSH 82 compound extract	50 µg/mL	98.9	1.1
	500 µg/mL	97.9	2.1
	2.5 mg/mL	90.9	9.1
	5 mg/mL	81.5	18.5
	12.5 mg/mL	73.0	27.0
	25 mg/mL	25.5	74.5
Acarbose (PC-1)	20 mg/mL	92.9	7.1
	60 mg/mL	83.2	16.8
	80 mg/mL	80.2	19.8
	100 mg/mL	76.7	23.3
Quercetin (PC-2)	10 mM	39.9	60.1

Glucose Uptake in Skeletal Muscle Cell Line (C2C12 Myotubes)

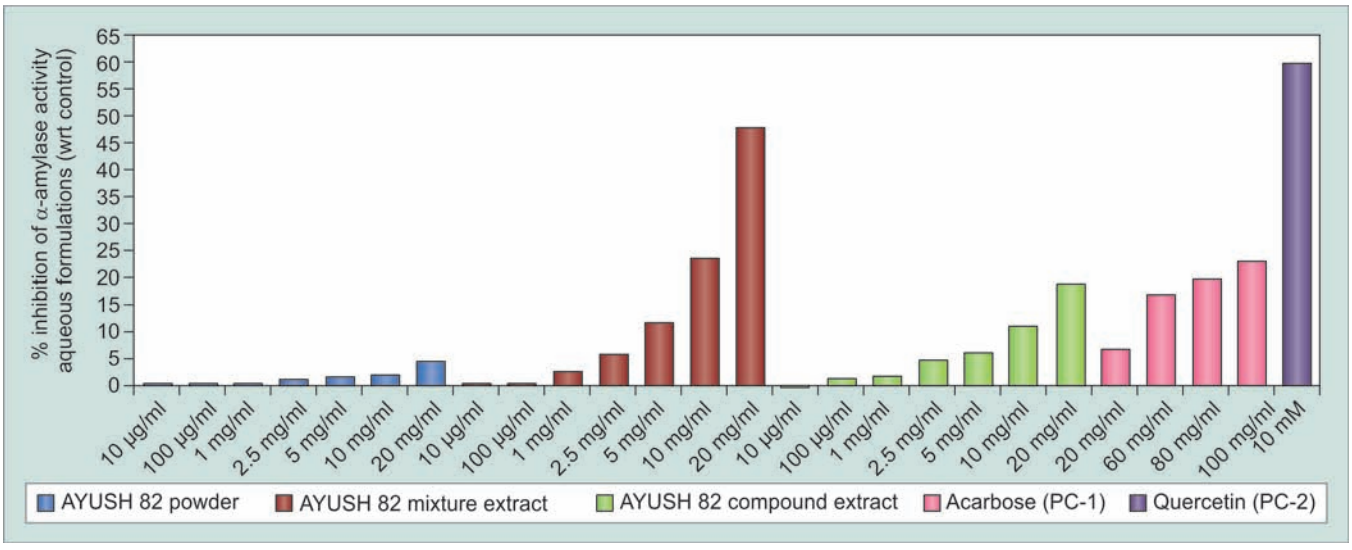
All the TIs at tested concentrations (10 µg/mL, 50 µg/mL, 100 µg/mL, 500 µg/mL, and 1 mg/mL) showed increase in glucose uptake by the skeleton muscle cell line (C2C12 myotubes) as compared with control. Insulin (positive control) at a concentration of 200 nM demonstrated 137% increase in glucose uptake as compared with control, indicating potential antidiabetic activity (Table 4; Graph 3).

Effect on PPAR-γ Activity

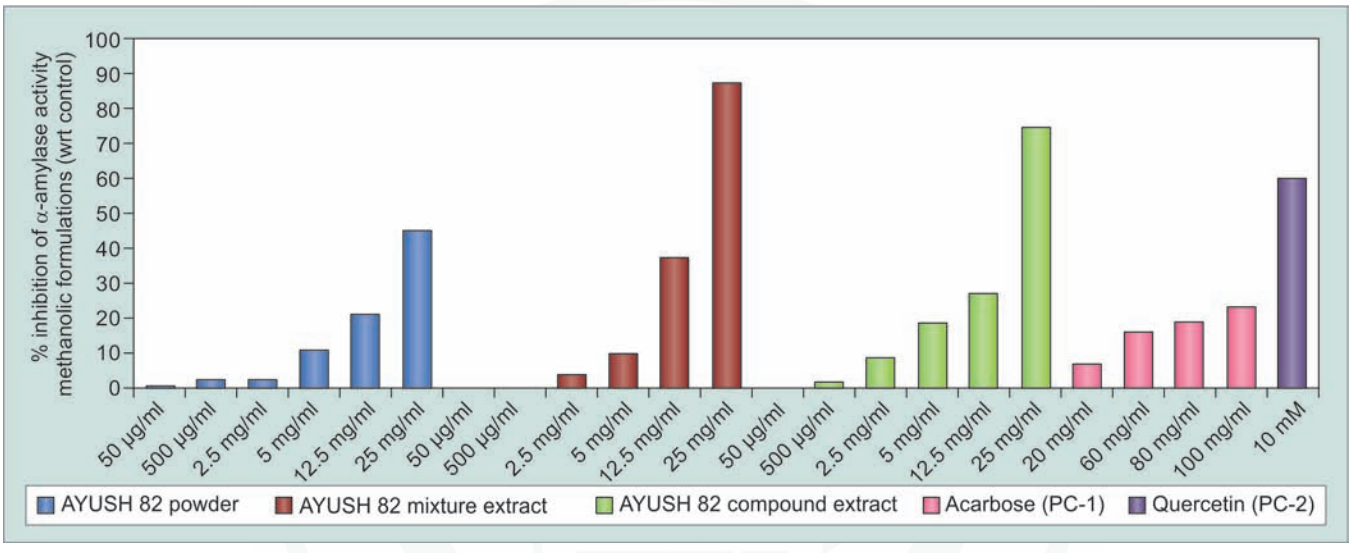
There was no activation of PPAR-γ as compared with vehicle control (DMSO) by any of the TIs in the tested concentration range. Positive control (rosiglitazone) demonstrated strong activation of PPAR-γ with an IC50 of 3.15 nM.

DISCUSSION

Natural α-amylase inhibitors of herbal origin are an attractive therapeutic approach to control postprandial hyperglycemia via reducing the glucose release from starch and delaying carbohydrate absorption. These



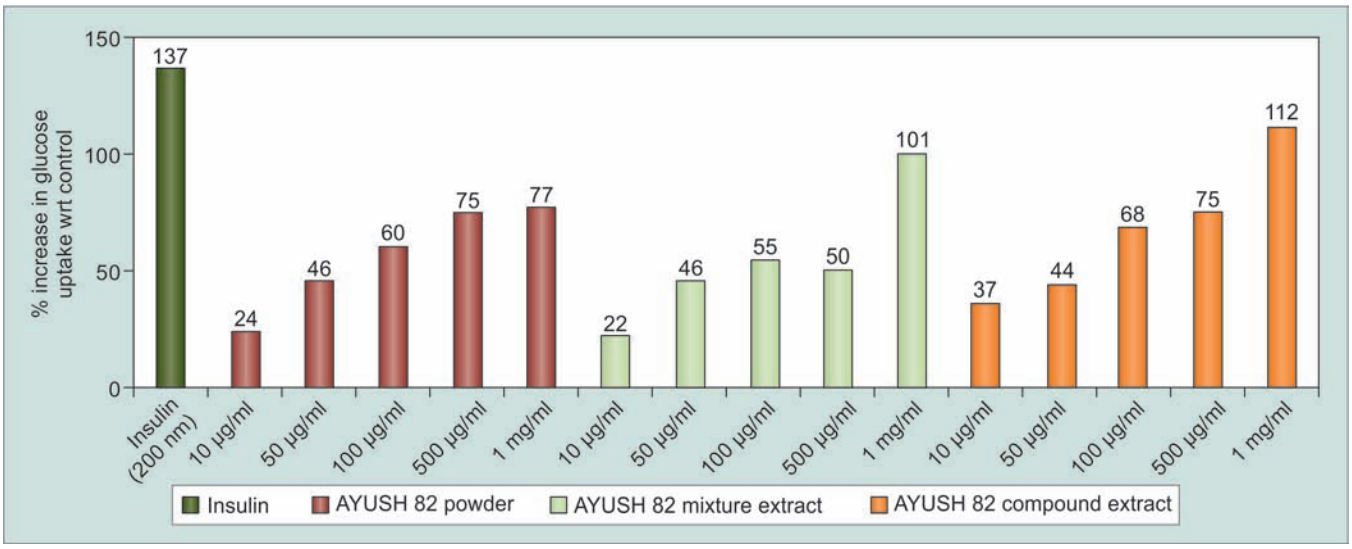
Graph 1: Percent inhibition of α-amylase activity by AYUSH 82 (aqueous formulation)



Graph 2: Percent inhibition of α-amylase activity by AYUSH 82 (methanolic formulation)

Table 4: Glucose uptake analysis in C1C2 myotubules of AYUSH 82

TI/dosage form	*Conc.*	*F1*	*F2*	*Mean (fluorescence unit)*	*Percent increase in glucose uptake wrt control*
Control	Untreated cells	167	159	163	0
Insulin	Insulin (200 nM)	383	388	386	137
AYUSH 82 powder	10 µg/mL	142	263	203	24
	50 µg/mL	245	232	239	46
	100 µg/mL	254	269	262	60
	500 µg/mL	283	287	285	75
	1 mg/mL	294	284	289	77
AYUSH 82 mixture extract	10 µg/mL	166	232	199	22
	50 µg/mL	219	257	238	46
	100 µg/mL	248	256	252	55
	500 µg/mL	248	242	245	50
	1 mg/mL	325	330	328	101
AYUSH 82 compound extract	10 µg/mL	202	243	223	37
	100 µg/mL	252	219	236	44
	200 µg/mL	277	272	275	68
	500 µg/mL	358	213	286	75
	1 mg/mL	313	377	345	112



Graph 3: Percent increase in glucose uptake with different dosage forms of AYUSH 82

compounds are able to inhibit the activity of the carbohydrate hydrolyzing enzymes in the small intestine and are potentially useful in control of diabetes.[24] Results of the current study indicate that methanolic formulations of all the three dosage forms of AYUSH 82 – powder, the mixture extract, and the compound extract – could inhibit α-amylase activity in a dose-dependent manner. AYUSH 82 mixture extract, however, demonstrated highest extent of inhibition in both aqueous (48.2%) and methanolic (87.4%) formulation.

Skeletal muscle is one of the major insulin-target tissues responsible for the maintenance of whole body glucose homeostasis and accounts for the bulk of insulin-stimulated glucose disposal (70–80%) after a meal.[21] Insulin and exercise play central roles in the regulation of glucose transport and its metabolism in the muscles. The maintenance of glucose homeostasis is critical for normal physiology, and any alteration of blood glucose levels directly affects a variety of insulin actions. For example, in chronic hyperglycemia, the predominant metabolic state of diabetes can exacerbate defective glucose disposal by interfering with insulin action in insulin-target tissues, including skeletal muscle.[21] In the current study, all the three dosage forms of AYUSH 82 at tested concentrations (10 µg/mL, 50 µg/mL, 100 µg/mL, 500 µg/mL, and 1 mg/mL) showed an increase in glucose uptake by the skeleton muscle cell line (C2C12 myotubes) as compared with control. AYUSH 82 compound extract, however, showed maximum increase in glucose uptake in a dose-dependent manner (112% increase at concentration of 1 mg/mL).

The PPAR-γ has been the focus of intense research during the past decade because ligands for this receptor have emerged as potent insulin sensitizers used in the treatment of type II diabetes. Agonists of the nuclear receptor PPAR-γ (thiazolidinediones) are therapeutically used to combat hyperglycemia associated with the metabolic syndrome and type II diabetes.[25] The effect of all the three dosage forms of AYUSH 82 was also studied on PPAR-γ expression as many natural products are reported to have PPAR-γ activating potential. Results of the current study, however, demonstrated that none of the TIs exhibited activation of PPAR-γ expression in tested ranges, indicating that antidiabetic potential of tested AYUSH 82 may not be mediated *via* PPAR-γ activation but by other mechanisms.

CONCLUSION

All the tested dosage forms of AYUSH 82 – powder, mixture extract, and the compound extracts – showed potential antidiabetic activity mediated either through inhibition of α-amylase or regulation of insulin-dependent glucose uptake in mouse skeletal muscle cell line. However, none of the three dosage forms of AYUSH 82 exhibited activation of PPAR-γ expression in tested ranges, indicating that antidiabetic potential of tested AYUSH 82 may not be mediated *via* PPAR-γ activation.

In conclusion, all the three formulations (AYUSH 82 powder, mixture extract, and the compound extract) have shown antidiabetic activity though inhibition of α-amylase activity or regulation of insulin-dependent glucose uptake in mouse skeletal muscle cell line. Out of the three, the AYUSH 82 mixture extract demonstrated highest extent of inhibition of α-amylase activity while the compound extract showed highest insulin-dependent glucose uptake in muscles. These new formulations (mixture extract and compound extract) may be useful for making new dosage forms of AYUSH 82 as tablets/capsule.

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हिन्दी सारांश

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आयुष 82 पाउडर सीसीआरएएस (केन्द्रीय आयुर्वेदीय विज्ञान अनुसंधान परिषद्, आयुष मंत्रालय, भारत सरकार) द्वारा विकसित एक आयुर्वेदिक मधुमेहरोधी यौगिक है। इसमें मधुमेह (प्रमेहा/मधुमेह) में उनके लाभकारी प्रभाव के लिए उपयोग की जाने वाली पारंपरिक सामग्री शामिल है। आयुष 82 के हाइपोग्लेस्मिक प्रभावों को मधुमेह विषयों में प्रतिवेदित किया गया। वर्तमान अध्ययन में आयुष 82 पाउडर की मधुमेहरोधी संभावना की दो नए खुराक के प्रकार यथा- आयुष 82 मिश्रण सत्त एवं मिश्रित सत्व का संभव α–एमाइलेज निरोधात्मक गुण, कंकाल की मांसपेशीय कोशिका रेखा (सी2 सी12 मायोट्यूब) में इंसुलिन निर्धारित ग्लुकोज़ अपटेक एवं पेरोक्सिसोम प्रोलिफिरेटर–एक्टिवेटेड रिसेप्टर गामा (पीपीएआर-γ) गतिविधि पर प्रभाव के कारण कार्यवाही की व्यवस्था के माध्यम से इन विट्रो जांच की गई।

आयुष 82 की सभी तीन प्रकार की खुराक – पाउडर, मिश्रण सत्त एवं मिश्रित सत्त ने α–एमाइलेज गतिविधि की अंतर्बाधा का प्रदर्शन किया। आयुष 82 मिश्रण सत्त ने तथापि दोनों जलकृत (48.2%) एवं मैथोनोलिक फॉर्मुलेशन (87.4%) में उच्चतम सीमा तक अंतर्बाधा का प्रदर्शन किया। आयुष 82 की सभी तीन प्रकार की खुराक ने नियंत्रण की तुलना में सी2 सी12 मायोट्यूब में इंसुलिन निर्धारित गुलुकोस अपटेक में भी वृद्धि का प्रदर्शन किया। जबकि परीक्षण सीमा में पीपीएआर-γ एक्सरप्रेशन की सक्रियण का किसी भी जांच वस्तु ने प्रदर्शन नहीं किया जिससे यह इंगित होता है कि पीपीएआर-γ सक्रियण के माध्यसम से प्रशिक्षित जांच वस्तुओ की मधुमेहरोधी क्षमता को मध्यस्थित नहीं किया जा सकता। परिणाम से यह निष्कर्ष निकलता है कि आयुष 82 (मिश्रण सत्त एवं योगिक सत्त) के नए योगो को टेबलेट/कैप्सूल आदि के रूप में आयुष 82 के नए प्रकार की खुराक के निर्माण हेतु उपयोग किया जा सकता है।