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Research Article
Coleus forskohlii Root Extract (Forcslim™) as a Prospective Antidiabetic Agent: *In vitro* Glucose Uptake Stimulation and α-Amylase Inhibitory Effects

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Abstract

Background and Objective: One of the major reasons of diabetes and diseases associated with diabetes is hyperglycemia. Few previous reports are available on the *in vitro* antidiabetic activity of *Coleus forskohlii*. This study aims to explore the antidiabetic activity of the ethanolic extract of *Coleus forskohlii* (Forcslim™), an innovative, patented product that contains 10% forskolin. **Materials and Methods:** Forcslim™ was tested for antidiabetic activity in 3T3-L1 cell lines using glucose uptake and Glucose Transporter4 translocation against a standard drug, metformin. The antidiabetic property of Forcslim™ inhibiting the activity of the α-amylase enzyme was compared to the standard drug acarbose using a colorimetric assay. The cytotoxic effect of Forcslim™ was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay. **Results:** After 24 hrs of treatment, *Coleus forskohlii* (Forcslim™) had no cytotoxic effect on the 3T3-L1 cell line. Forcslim™ enhanced glucose uptake in 62.43% of the cells. Glucose Transporter4 expression was 76.86% and standard metformin at 99.54%, respectively. Forcslim™ showed 177.47 μg mL^{-1} of inhibition and the control drug acarbose exhibited 117 μg mL^{-1} of inhibition in α amylase activity. **Conclusion:** These results exposed the potential of Forcslim™ and it could be used as an alternative therapeutic formulation for the treatment of diabetes and the molecular mechanism behind the activities.

Key words: Forcslim™, antidiabetic, GLUT4, 3T3-L1 cells, alpha-amylase, alpha-amylase inhibition, glucose uptake

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes mellitus (DM), an endocrine metabolic disorder, exhibits the signs of hyperglycemia, hyperlipidemia, hyperaminoacidemia and hypoinsulinemia (decreased insulin level)[1]. It is a long-lasting metabolic condition that causes problems with the metabolism of carbohydrates, fats and proteins, as well as postprandial and fasting hyperglycemia. Diabetes-induced hyperglycemia is brought on by either a complete lack of insulin secretion (type 1 DM), insulin action (type 2 DM) or both. The eyes, nerves, kidneys and heart are just a few organs that might suffer harm, dysfunction, or failure as a result of persistent hyperglycemia[2,3]. Insulin resistance is a hallmark of type 2 diabetes. The most common form of diabetes mellitus and it can cause various complications, such as cardiovascular risk factors like hypertension, dyslipidemia and prothrombotic factors[4-6].

One of the most serious metabolic diseases, diabetes mellitus (DM), shows high mortality rates worldwide[7]. It is well recognized that insulin is essential for maintaining glucose homeostasis because it promotes blood glucose transport throughout skeletal muscle[8]. The main characteristics of type 2 diabetes, however, are insulin resistance in target tissues and a deficiency in pancreatic beta-cell generation. A decrease in peripheral glucose uptake by muscle, adipose, or liver cells as well as an increase in endogenous glucose release, which results in a rise in blood glucose levels, are further characteristics of type 2 diabetes[9–11]. Biguanides, such as metformin, are used to manage post-prandial hyperglycemia in people with Non-Insulin-Dependent Diabetes Mellitus (NIDDM). These drugs increase the uptake of glucose by peripheral cells, which would increase the utilization of glucose. As a result, medications that increase glucose absorption in these tissues can treat diabetes and improve insulin resistance[12] and inhibiting α-amylase should lessen the adverse high postprandial blood glucose peak in diabetics. Therefore, finding novel antidiabetic drugs derived from natural sources that promote glucose uptake by peripheral tissues like adipose tissue or muscle cells is highly desirable[13]. Many synthetic antidiabetic medications are on the market, including acarbose, sulfonylurea, miglitol, metformin and thiazolidinedione. Despite the fact that their effectiveness is limited by excessively expensive and negative side effects[14,15], this encourages the development of potent natural antidiabetic medicines with fewer negative side effects.

A significant medicinal plant with Indian origins, *C. forskohlii* has been utilized for thousands of years in Ayurvedic medicine to treat conditions affecting the circulatory, pulmonary, hypoglycemic, gastrointestinal and neurological systems[16]. It contains the significant chemical component "forskolin", a diterpene that is widely known to elevate c-AMP concentration by activating adenylate cyclase and has a variety of therapeutic effects[17,18]. In order to confirm the use of ethanolic extract of *C. forskohlii* roots (Forcslim™) in the treatment of diabetes, the *in vitro* antidiabetic activity on 3T3-L1 cell lines was examined in this study.

MATERIALS AND METHODS

Study area: This *in vitro* study was performed at Stellixir Biotech Pvt. Ltd., Bangalore, Karnataka in November, 2022.

Chemicals and reagents: Dulbecco's Modified Eagle's Medium (DMEM) without glucose (#AL186, Himedia), fetal bovine serum (#RM10432, Himedia), DMEM high glucose (#AL219A, Himedia), Dulbecco's Phosphate Buffered Saline (DPBS) (#TL1006, Himedia), Acarbose (#A8980, Sigma), metformin (#PHR 1084, Sigma), 2 (N (7 nitrobenz 2 oxa 1,3 diazol 4 yl) amino)-2 deoxyglucose (2 NBDG) (Invitrogen: Cat No. 13195), mouse anti GLUT4–fluorescein isothiocyanate (FITC) antibody (#NBP1 49533F, Novus Biologicals), dimethyl sulfoxide (DMSO) (#PHR1309, Sigma), 3 (4,5 dimethythiazol2 yl)-2,5 diphenyltetrazolium bromide (MTT) Reagent (#4060 Himedia), FACS Calibur (BD Biosciences, USA), Microplate reader (#ELx800™, BioTek Instruments Inc., Vermont, USA), HPLC (Make-SHIMADZU, Model: LC-2030C Plus, Kyoto, Japan), Forskolin standard (Lot #BCBW0503, Make-Sigma-Aldrich), Acetonitrile (HPLC Gradient Grade, B. No: Q4400SP, Make-Qualigens), Milli-Q Water (Make-Sartorius-Arium).

Preparation of *Coleus forskohlii* extract (Forcslim™): Forcslim™ is mass-produced and registered by Star Hi Herbs Pvt. Ltd., Jigani, Bangalore, Karnataka, India. High-Performance Liquid Chromatography (HPLC) was performed to analyze the presence of forskolin in the ethanolic root extract of *Coleus forskohlii*. The forskolin content from the extract is determined by the in-house validated method.

The 3T3-L1 cell line was procured from the NCCS (National Centre for Cell Science), Pune, Maharashtra, India and cultured in high glucose DMEM supplemented with 10% FBS, 10,000 units of penicillin G, 10,000 µg mL^{-1} streptomycin sulfate and 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid) at 37°C humidified incubator (Heal Force, China) with humidified atmosphere of 5% CO_2.

Cytotoxicity assay: The MTT assay was used to examine *Coleus forskohlii* (Forcslim™)'s potential cytotoxicity. An MTT assay is a colorimetric assay that quantifies the amount of mitochondrial succinate dehydrogenase that reduces

yellow-colored MTT to an insoluble, dark purple formazan product[19,20]. As a result, the decrease in MTT indicates the quantity of viable cells in the culture. The 3T3-L1 cells were initially seeded in 96-well plates at a density of 20×10^4 cells per well. Overnight, the cells were given time to adhere. Following that, cells were examined with Forcslim™ in a range of doses (25-400 g mL^{-1}) for 24 hrs. As 0.5 mg mL^{-1} of MTT reagent was added to the cells for post-treatment and the cells were then incubated at 37°C for 2 hrs. After the MTT reagent was taken out, 20 L of DMSO was added to dissolve the formazan crystals. The absorbance at 570 nm was measured using a microplate reader (#ELx800™, BioTek Instruments Inc., Vermont, USA) and the formula for calculating the percentage of viable cells was applied[21]:

$$\text{Viability (\%)} = \frac{\text{Mean OD of test sample at 570 nm}}{\text{Mean OD of untreated cells at 570 nm}} \times 100$$

Glucose uptake assay: A six-well plate was seeded with 3T3-L1 cell lines at a density of 2×10^5 cells per well. Following a D-PBS wash, the cells from an overnight culture were treated for 2 hrs with 100 g mL^{-1} of *C. forskohlii* (Forcslim™), with 100 mM of the reference medication metformin serving as the control. The cells were then treated with 2 NBDG for an additional 2 hrs. The cells were used after trypsinization, PBS washing and resuspension in 0.5 mL of D-PBS. Using Flow Cytometry (FACSCalibur, BD Biosciences), the cellular absorption of 2-NBDG was assessed and the data were examined using Cell Quest Pro Software, Version 6.0.

Glucose Transporter 4 (GLUT4) translocation studies: Using flow cytometry, the GLUT4 expression levels were examined. The 3T3-L1 cells were cultivated overnight in a six-well plate after being seeded at a density of $2x10^5$ cells. Then, they were treated with 100 µg mL^{-1} of *C. forskohlii* (Forcslim™) and 100 µM of the positive control, metformin, after the used media was sucked. After 24 hrs, the cells were trypsinized, washed with D-PBS and then resuspended in 0.5 mL of D-PBS. After that, the cells were treated for 30 min in the dark with a mouse anti-GLUT4-FITC antibody (#NBP1 49533F, Novus Biologicals). Using a BD FACSCalibur flow cytometer and the FL1 channel, the cells were examined for the expression of GLUT4 after the unbound antibody had been removed with DPBS. Software called Cell Quest Pro (Version 6.0) was used to scrutinize the data.

α-Amylase inhibition assay: An approach that has been previously reported was used to estimate the amylase inhibitory activity[22]. Briefly, different concentrations of *Coleus forskohlii* (Forcslim™), ranging from 100 to 500 g mL^{-1} was prepared in DMSO. Forcslim™ and amylase were combined and they were then incubated in microtubes for 10 min at 37°C. Each microtube received 100 mL of 1% soluble starch dissolved in buffer and the mixture was then incubated at 37°C for 30 min. The reaction was then stopped by adding 200 µL of dinitrosalicylic acid color reagent and the microtubes were then incubated at 100°C for 5 min. A 96-well microplate was filled with 50 µL of the reaction mixture once the Forcslim™ was cooled to room temperature. A microplate reader (#ELx800™, BioTek Instruments Inc., Vermont, USA) was used to measure the absorbance at 540 nm after the sample had been diluted with 150 µL of DW (distilled water). The following formula was used to compute the percentage of inhibition and the findings were related to the inhibitory effect of the control medication, acarbose[23].

$$\text{Inhibition (\%)} = \frac{\text{Mean OD of untreated control-Mean OD of test samples}}{\text{Mean OD of untreated control}} \times 100$$

Statistical analysis: A linear regression graph (concentration vs. percentage enzyme inhibition) was used to determine the IC_{50} values in enzyme inhibition experiments. The findings of each experiment were performed in triplicate and the mean percentage inhibition ± the standard deviation (n = 3) is how they were calculated. One-way analysis of variance and a Bonferroni *post hoc* test for multiple comparisons were used to establish the statistical significance, with *p<0.05 being regarded as statistically significant. Software from Graph Pad Prism (San Diego, California), version 3.1, was used for all statistical analyses and IC_{50} value estimation.

RESULTS

Cytotoxic effect of *Coleus forskohlii* (Forcslim™) on the 3T3-L1 cell line: The cytotoxic effects of *Coleus forskohlii* (Forcslim™) were tested for 3T3-L1 cell lines at concentrations of 25-400 µg mL^{-1}. Metformin, a standard antidiabetic drug, has been used to conduct *in vitro* cell-based cytotoxicity against 3T3-L1 cells and it showed 91.46% viability at 100 µM concentration. The difference in the percentage of survival and cytotoxic effect of the 3T3-L1 cell lines treated with different concentrations of Forcslim™ and metformin was presented in Fig. 1-3.

Effect of glucose uptake on the 3T3-L1 cell line: The fluorescent deoxyglucose analogue, 2-NBDG, was used as a probe for the cellular uptake of glucose in 3T3-L1 cells. The results showed that the percentage of cells taking up 2-NBDG was higher in the population of cells treated with lower and higher concentrations of *Coleus forskohlii* (Forcslim™) when compared to the untreated cells. The Forcslim™ showed

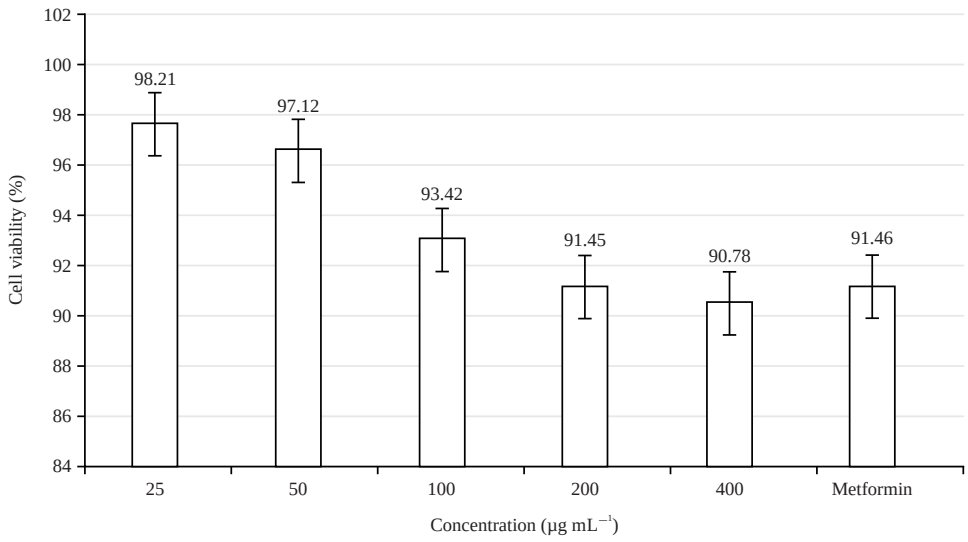


Fig. 1: Effect of Forcslim™ on 3T3-L1 cell line viability
Data were shown as the Mean±Standard deviation of triplicate experiments (*p<0.05)

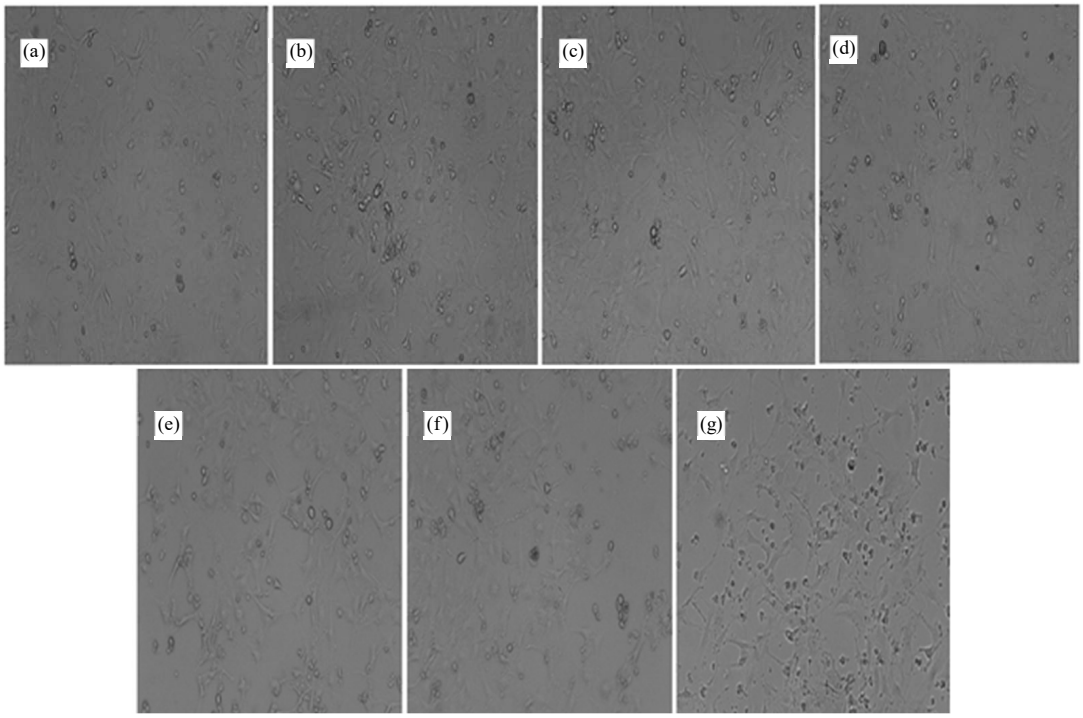


Fig. 2(a-g): Images of 3T3-L1 cells after exposure to Forcslim™, (a) Control, (b) 25 µg mL^{-1}, (c) 50 µg mL^{-1}, (d) 100 µg mL^{-1}, (e) 200 µg mL^{-1}, (f) 400 µg mL^{-1} and (g) Metformin (standard) treated cells for 24 hrs

62.43% of 2-NBDG uptake and the metformin-treated cells showed 99.74% of the highest cellular uptake of 2-NBDG, as shown in Fig. 4.

GLUT4 translocation studies: The GLUT4 expression on *C. forskohlii* (Forcslim™) was analyzed using flow cytometry. Metformin (100 µM) was used as standard. The flow cytometric analysis revealed that the 3T3 L1 cells treated with Forcslim™ expressed 76.86% of GLUT4 expression and metformin treated cells showed 99.74% of GLUT4 expression at 100 µg mL^{-1}, respectively (Fig. 5).

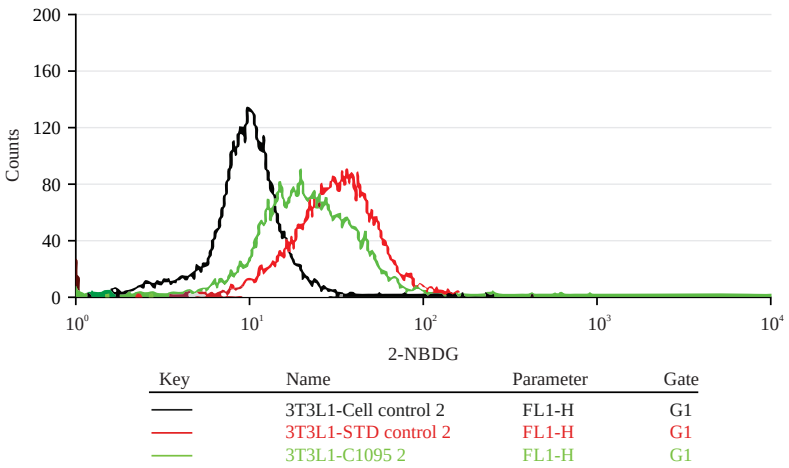


Fig. 3: Overlaid expression graph for the presence of fluorescent 2-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxyglucose (2-NBDG)

Untreated 3T3-L1 cells (black color line), standard drug-treated cells (metformin, 100 µM) (red color line) and 100 µg mL^{-1} of *Coleus forskohlii* treated cells (green color line)

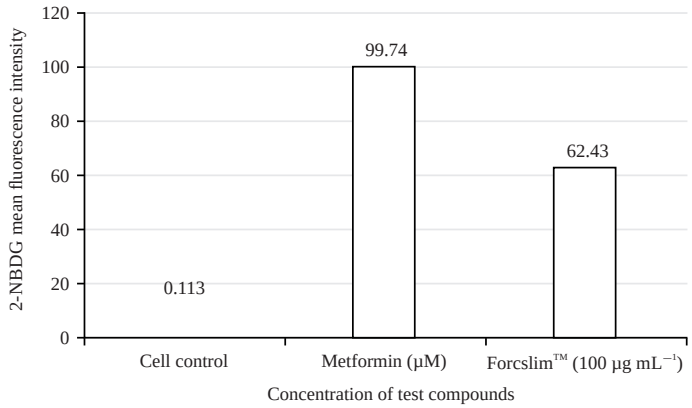


Fig. 4: Percentage of cells taken up the 2-(n-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxyglucose (2-NBDG) 100 µg mL^{-1} of Forsclim™ and 100 µm of Metformin

Data were represented as the Mean±Standard deviation of triplicate experiments (*p<0.05)

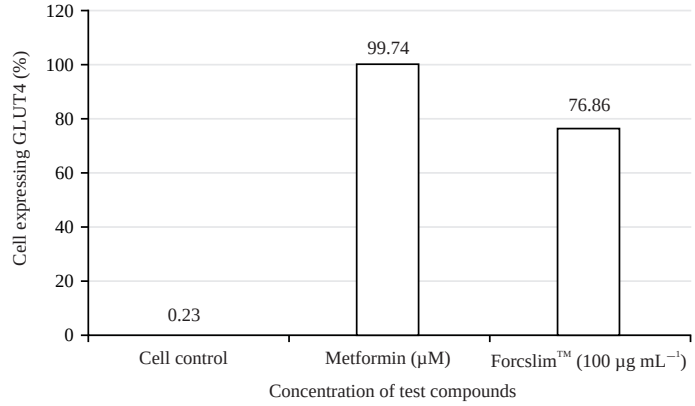
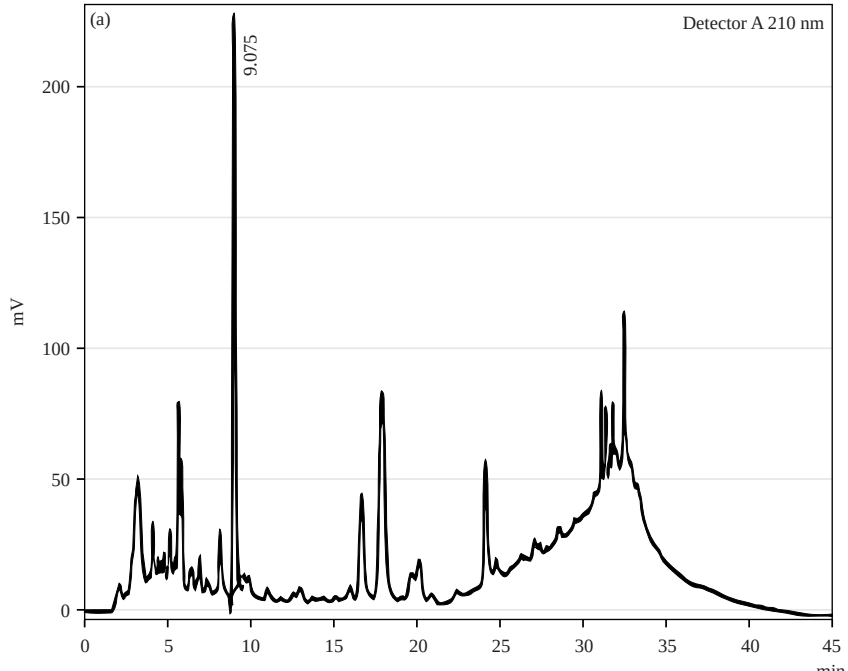
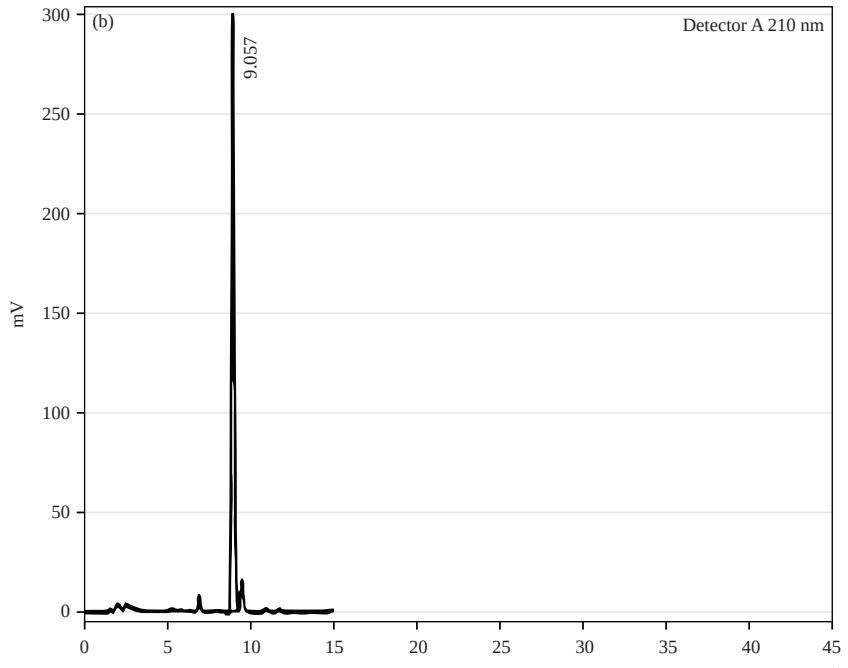


Fig. 5: Glucose transporter 4 (GLUT4) expression upon exposure of 3T3-L1 cells to 100 µg mL^{-1} of Forcslim™ and 100 µM Metformin

Data were represented as the Mean±Standard deviation of triplicate experiments (*p<0.05)



Peak#	Name	Ret. Time	Area	Area (%)	Height
1	*C. forskohlii* (Forcslim™)	9.075	2265053	100.000	220693
Total			2265053	100.000	220693



Peak#	Name	Ret. Time	Area	Area (%)	Height
1	Forskolin	9.075	3070406	100.000	300434
Total			3070406	100.000	300434

Fig. 6(a-b): Determination and quantification of, (a) HPLC spectra for *Coleus forskohlii* (Forcslim™) and (b) HPLC spectra for standard forskolin

Table 1: Effect of percentage inhibition of α-amylase activity by Forcslim™

Sample	Concentration (μg mL^{-1})	Inhibition (%)	IC_{50} (μg mL^{-1})
Acarbose	50	37.10±0.84	117.00
	100	55.85±1.55	
	150	64.30±0.88	
	200	70.14±0.73	
	250	87.54±0.55	
Forcslim™	50	19.50±1.30	177.47
	100	27.70±2.19	
	150	41.72±0.19	
	200	55.58±1.81	
	250	70.31±0.84	

Results were presented as Mean±Standard deviation of triplicate experiments (*p<0.05)

α-amylase activity inhibition: Inhibition of α-amylase activity by *C. forskohlii* (Forcslim™) was tested against the control drug acarbose. Forcslim™ inhibited amylase activity by 19.50% at 5 0 μg mL^{-1} and 70.31% at 250 μg mL^{-1}. The IC_{50} value of Forcslim™ was found to be 177.47 μg mL^{-1}. The control drug, acarbose, inhibited α-amylase activity by 37.10% at 50 μg mL^{-1} and 87.54% at 250 μg mL^{-1}. The IC_{50} value of acarbose was found to be 117 μg mL^{-1} (Table 1).

Determination and quantification of forskolin in *Coleus forskohlii* root extract: The determination and quantification of Forskolin were carried out by High-Performance Liquid Chromatography (HPLC). The HPLC chromatogram for diterpenoid Forskolin in forskohlii Root extract has been shown in Fig. 6a coleus root extract and Fig. 6b reference standard. The HPLC chromatogram shows that the ethanolic root extract of *C. forskohlii* contains 10% forskolin. The retention time of Forskolin in reference standard and sample is found to be same. The results concluded that a higher amount (10%) of forskolin was present in the ethanolic root extract of *C. forskohlii*.

DISCUSSION

Diabetes is considered as a serious health issue and continues to be one of the main causes of death globally. Although there are several therapeutic drugs available in medicine for the treatment of diabetes, many were reported to have various side effects and unaffordable[24]. One of the essential enzymes in the human digestive system is the α-amylase enzyme which converts starch to monosaccharide and causes blood glucose to rise[25]. Alpha amylase interacts with large polysaccharides (starch) at their internal bands. The alpha amylase inhibition has been suggested as an effective method for managing diabetes. Though biguanides, sulfonylureas, thiazolidinedione and other synthetic therapeutic agents have been developed in contemporary medicine to treat diabetes, there are still no effective medications to manage diabetic complications[26].

The discovery of safer hypoglycemic drugs is possible from traditional medicinal plants[27]. One of the most important sources for finding novel therapeutically useful chemicals for the development of drugs against the most prevalent and widespread disease, diabetes mellitus, is *C. forskohlii*. The leaves of *C. forskohlii* have been reported to have various pharmaceutical applications, including in diabetes[28]. The extract of *C. forskohlii* has been found to attenuate/reduce the hypoglycaemic action through a hepatic CYP2C-mediated mechanism[29].

In the present study, the *C. forskohlii* (Forcslim™) extract was evaluated for antidiabetic activity by employing standard *in vitro* techniques such as α-amylase enzyme inhibition, Glucose uptake and GLUT4 Translocation studies. Besides being of great importance in therapeutic treatments, medicinal plants also exhibited cytotoxic potential because of the production of various chemical substances for defense purposes[30]. As the identification of toxicity is a crucial prerequisite for the integration of medicinal plants into public health programs, the cytotoxic potential of *C. forskohlii* root extract was investigated in this study using MTT colorimetric assay, a commonly used *in vitro* method for cytotoxic testing. Interestingly, the extract showed 90-99% cell viability on 3T3-L1 cell lines with 25 to 400 μg mL^{-1} concentration.

The present study has employed various biochemical and cell-based assays to identify the potential mechanisms of probable antidiabetic actions of extract prepared from *C. forskohlii* root. Experiments on cellular uptake of glucose revealed that the extract has the capability to induce glucose utilization by 3T3L1 cell lines. The Forcslim™ showed 62.43% of 2-NBDG uptake and the metformin-treated cells showed 99.74% of the highest cellular uptake of 2-NBDG. The result implies that the extract of Forcslim™, functions similar to metformin by increasing glucose uptake in the liver. Metformin is an oral hypoglycemic medication that belongs to the biguanide class of drug. Its hypoglycemic effect is achieved by activating AMP-activated protein kinase (AMPK)

in the liver, which may result in various pharmacologic effects including glucose inhibition, lipid synthesis and enhanced insulin sensitivity[31,32]. Based on this, *C. forskohlii* extract may also act by activating the insulin signaling cascade, stimulating GLUT 2 and facilitating glucose translocation into the cell, potentially leading to improved homeostasis.

Furthermore, these findings were correlated with GLUT4 expression studies, which demonstrated the ability of *C. forskohlii* root extract to induce GLUT4 translocation. The GLUT4 expression on the cell surface was assessed by flow cytometry after 3T3L1 cells were treated for 24 hrs with 100 μg mL^{-1} of *C. forskohlii* root extract and 100 μM of the standard drug metformin. After the incubation, GLUT4 translocation was induced by metformin and *C. forskohlii* root extract at a rate of 99.74 and 76.86% of cells, respectively. This suggests that the ability of Forcslim™ to induce GLUT4 translocation may have caused cellular uptake of 2-NBDG in 3T3L1 cells.

One of the main targets in the treatment of diabetes conditions is considered to be carbohydrate-degrading digestive enzymes. These enzymes help break down the complex carbohydrates in the diet by reducing them to simpler sugar molecules, increasing their concentration in the bloodstream. In patients with diabetes mellitus, inhibition of these digestive enzymes can lower blood glucose levels[33]. Thus, this study investigated the inhibitory effect of *C. forskohlii* root extract (Forcslim™) on α-amylase enzyme. Acarbose was used as the control drug. Our results showed that ethanolic root extract of *C. forskohlii* (Forcslim™) inhibited α-amylase with an IC_{50} value of 177.47 μg mL^{-1}. At the same time, the control drug, acarbose, inhibited α-amylase with an IC_{50} value of 117 μg mL^{-1}. The α-amylase inhibiting drugs currently in the treatment of type 2 diabetes mellitus are mainly acarbose, miglitol and voglibose, which are the current drugs[34]. It has been reported that long-term use of these synthetic drugs can result in clinical side effects[35] and there is evidence suggesting the potentially helpful impact of a vast variety of medicinal herbs in the management of diabetes[36]. Aside from their efficiency, herbal therapies appear to have few side effects and offer a cost-effective alternative to ingested commercial hypoglycemic medications. The World Health Organization (WHO) suggested in 1990 that extensive studies be conducted on the positive benefits of these plants[37]. Therefore, using natural resources like *C. forskohlii* root extract (Forcslim™), which has no toxicity and side effects, is advisable for the management of hyperglycemic conditions.

The decrease in diabetes mellitus depends critically on the cellular uptake of glucose from the blood. Normally, GLUT4 serves as a cell's mediator for this. The GLUT4 is translocated from its intracellular locations to the cell surface in response to stimulation by anti-diabetic medications. Through the phosphatidylinositol-3-kinase pathway, insulin stimulates the translocation of GLUT4[38]. One of the common diabetes medications, metformin, increases glucose absorption by causing the translocation of GLUT4. The AMP-activated protein kinase pathway is recognized to be the drug's mode of action[39]. The chemical composition of Forcslim™ was found to be dominated by diterpenoids like Forskolin, 1-deoxy forskolin and 1, 9-dideoxy forskolin of which Forskolin is the major compound. The extract was standardized by HPLC analysis. The 10% forskolin content of the standardized extract of *C. forskohlii* (Forcslim™) contributed to the antidiabetic activity. Previous studies have reported that Forskolin stimulates glucose-induced insulin secretion in the *in vitro* model[40]. These findings suggest that Forcslim™ could enhance cellular glucose uptake by inducing GLUT4 translocation.

CONCLUSION

The possible antidiabetic activity of an ethanolic extract of *Coleus forskohlii* (Forcslim™) has been evaluated through *in vitro* cellular assays. When compared to the standard drugs metformin and acarbose, flow cytometry studies on glucose uptake and GLUT4 expression in the 3T3-L1 cell line, as well as colorimetric assays on α-amylase enzyme inhibition, yielded promising results with a high percentage of inhibition. These results suggested that Forcslim™ is a potential indicator for sensitizing insulin secretion and it strongly inhibits the production of glucagon. Furthermore, current results proved that Forcslim™ can be used as a new synergetic drug to control diabetes mellitus.

SIGNIFICANCE STATEMENT

This study aims to explore the antidiabetic activity of the ethanolic extract of Forcslim™, which contains 10% forskolin. As there are few reports available on the *in vitro* antidiabetic activity of *Coleus forskohlii*, this study elucidates the molecular mechanism behind the anti-diabetic properties of *Coleus forskohlii*, particularly the ethanolic extract of Forcslim™. The study concludes that Forcslim™ is a potential indicator for sensitizing insulin secretion and it strongly inhibits the production of glucagon and confirms the use of Forcslim™ as an antidiabetic in traditional medicine. The future investigations of Forcslim™ as a source of natural product that has the potential to be developed as a medicinal element in the prevention and treatment of diabetes and associated disorders in humans.

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