Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

Study of Antidiabetic Properties of *Uvaria narum* Leaf Extract through Glucose Uptake and Glucose Transporter 4 Expression Studies in 3T3L1 Cell Line Model

Murad Alsawalha, P. B. Janardhana[1], K. R. Padma[2], Shiva Shankar Reddy[1], Abeer M. Al-Subaie[3], Srinivasa Rao Bolla[4], Vishnu Priya Veeraraghavan[5], Joel P. Joseph[1], Surapaneni Krishna Mohan[6]

Department of Chemical and Process Engineering Technology, Jubail Industrial College (JIC), P. O. Box 10099, Jubail Industrial City 31961, [6]Department of Medical Biochemistry, College of Applied Medical Sciences - Jubail (CAMSJ), Imam Abdulrahman Bin Faisal University, P. O. Box 4030, Jubail Industrial City, Al Jubail 35816, [3]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Imam Abdulrahman Bin Faisal University, P. O. Box 2435, Dammam 31441, [4]Department of Anatomy, College of Medicine, Imam Abdulrahman Bin Faisal University, P. O. Box 2114, Dammam 31451, Kingdom of Saudi Arabia, [1]Department of Biotechnology, Stellixir Biotech Private Limited, Bengaluru, Karnataka, [2]Sri Padmavati Mahila Visvavidyalayam, Padmavathi Nagar, Near West Railway Station, Tirupati, Chittoor (D.t), Andhra Pradesh, [5]Department of Biochemistry, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India

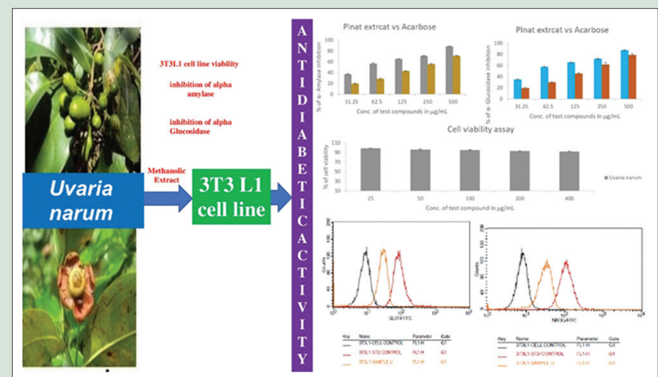
ABSTRACT

Background: *Uvaria narum* (UN) is known to have antipyretic, antimicrobial, anti-inflammatory and antimalarial properties. The antidiabetic properties of UN remains unexplored. The current study has been aimed at understanding the antidiabetic property of UN extract on an *in vitro* model using 3T3-L1 cell line. **Methods:** Methanolic extract of UN was prepared, and its cytotoxic effect on 3T3-L1 cells was assessed. Glucose uptake and glucose transporter 4 (GLUT4) translocation in 3T3-L1 cell line on treatment with the extract was evaluated against a standard drug, metformin. α-glucosidase and α-amylase inhibition activities of the extract were also assayed with acarbose as the standard drug. **Results:** Treatment with UN extract had no cytotoxic effect on the cells. UN extract showed a good percentage inhibition of α-amylase and α-glucosidase activities. UN extract showed 71.31% inhibition and the control drug Acarbose exhibited 88.54% inhibition in α-amylase activity. Furthermore, the extract showed 79.11% inhibition when Acarbose exhibited 87.35% inhibition in α-glucosidase activity. IC_{50} values were also determined. Further, on treatment with the extract, 75.49% of 3T3-L1 cells took up glucose and 70.67% had GLUT4 expression. **Conclusion:** UN extract enhances glucose uptake and GLUT4 expression, inhibits α-amylase and α-glucosidase activities, thereby demonstrating the antidiabetic properties *in vitro*.

Key words: 3T3-L1, antidiabetic activity, glucose transporter 4, *Uvaria narum,* α-amylase, α-glucosidase

SUMMARY

- *Uvaria narum* (UN) is a medicinal plant belongs to *Annonaceae* (custard apple) family and broadly disseminated in the foothills of Western Ghats
- The *in vitro* antidiabetic activities of UN leaf extract in 3T3-L1 cell line model have been studied
- We have performed biochemical studies such as α-amylase and α-glucosidase inhibition assays, glucose uptake, and glucose transporter 4 (GLUT4) expression studies in 3T3 L1 cell line, as part of *in vitro* antidiabetic activity
- Exposure to different concentrations of UN leaf extract for 24 h has not revealed any toxicity on 3T3 L1 cells
- methanolic leaf extract of UN has a major reduction effect on the activity of α-amylase enzyme and α-glucosidase enzyme
- Further on stimulation with plant extract, 75.49% of cells and 70.67% of cells displayed glucose uptake and GLUT4 expression in 3T3 L1 cells, respectively
- The UN leaves extract has shown promising antidiabetic activity and could be used as a potential source of antidiabetic agents.



Abbreviations Used: UN: *Uvaria narum*, DMSO: Dimethyl sulfoxide, DMEM: Dulbecco's Modified Eagle's medium, FBS: Fetal bovine serum, D-PBS: Dulbecco's phosphate-buffered saline, GLUT4: Glucose transporter 4, FITC: Fluorescein isothiocyanate, PNPG-p-nitrophenyl-α-dglucopyran oside, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 2-NBDG: 2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxyglucose, SD: Standard deviation.

Correspondence:
Dr. Surapaneni Krishna Mohan,
Department of Medical Biochemistry,
College of Applied Medical Sciences - Jubail, Imam Abdulrahman Bin Faisal University, P.O.Box 4030, Jubail Industrial City, Al Jubail 35816, Kingdom of Saudi Arabia.
E-mail: skmohan@iau.edu.sa
DOI: 10.4103/pr.pr_7_19

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Diabetes mellitus (DM) is an endocrine metabolic disorder that manifests hyperglycemia (enhanced blood glucose level), hyperlipidemia (augmented lipid level), hyperaminoacidemia, and hypoinsulinemia (reduced insulin level) conditions.[1] Recurring hyperglycemia can lead to damage, dysfunction, and failure of different organs – the eyes, nerves, kidneys, and heart.[2,3] The number of diabetes cases have been spiraling with projections reaching >300 million cases by the end of 2025.[2-4]

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Alsawalha M, Janardhana PB, Padma KR, Reddy SS, Al-Subaie AM, Bolla SR, *et al*. Study of antidiabetic properties of *Uvaria narum* leaf extract through glucose uptake and glucose transporter 4 expression studies in 3T3L1 cell line model. Phcog Res 2019;11:304-9.

© 2019 Pharmacognosy Research | Published by Wolters Kluwer - Medknow

The onset of type 2 diabetes, the prevalent form of DM, is characterized by insulin resistance and could lead to other complications, including cardiovascular risk factors such as hypertension, dyslipidemia, and prothrombotic factors.[5-7] Existent therapeutic regimens known to have adverse effects on prolonged use.[5-7] Conventionally, ethnomedicinal plants have been known to possess phytochemicals that have therapeutic effects.[8,9] In the recent times, medicinal and nutraceutical herbs have been recognized for their holistic effects on the health of an individual.[10,11]

In diabetic patients, morbidity and mortality are due to the inability of target cells such as skeletal muscle cells, adipocytes, and liver cells to effectively use glucose from the blood.[12-14] In a healthy individual, insulin stimulation increases glucose transport in muscle cells by 3 or 4 folds. A major part of this outcome is related to the net translocation of the glucose transporter 4 (GLUT4) from the intracellular compartment to the cell surface, where it facilitates the uptake of glucose and its reduction.[12-14]

Uvaria narum (UN) is an ethnomedicinal plant that belongs to *Annonaceae* (custard apple) family and is found in the foothills of the Western Ghats and the Central Provinces of India. The leaves of the plant have been traditionally used to treat several diseases including diabetes.[15]

In this study, we investigated the *in vitro* antidiabetic activity of the leaf extract of UN, using biochemical assays and flow cytometric analyses, on 3T3-L1 cells.

MATERIALS AND METHODS

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 (D-PBS) (#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-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 (#EC800, Biotek).

Collection of plant material

Fresh leaves of UN were collected from local areas of Mangalore, Karnataka, India [Figure 1].

Preparation of extract

The shade-dried leaves were cut, pulverized, and stored in airtight containers. The leaves were extracted with methanol by the soxhlation process. The extract was concentrated using Rotary Evaporator (Hahn vapor, hs-2005 v) under reduced pressure at a temperature not exceeding 50°C. The filtrate was freeze-dried, and the extract was dissolved in DMSO for the assays

Figure 1: *Uvaria narum* – the climbing shrub

Cell culture

3T3-L1 cell line was procured from NCCS, Pune, and cultured in DMEM (high glucose) supplemented with 10% FBS, 10,000 units of Penicillin G, and 10,000 μg/mL streptomycin sulfate and 10 mM HEPES. The cultures were maintained at 37°C with 5% CO_2 in a humidified incubator.

Cytotoxicity assay

MTT assay was performed to test for the cytotoxic effect of the extract. MTT assay is a colorimetric assay that measures the reduction of yellow-colored MTT by mitochondrial succinate dehydrogenase to an insoluble, dark purple formazan product.[16,17] Reduction of MTT thus becomes a readout of the number of viable cells in the culture. 96 well plates were seeded with 3T3-L1 cells at an initial density of 20×10^4 cells per well. The cells were allowed to adhere overnight. Cells were then treated with varying concentrations of plant extract (25–400 μg/mL) for 24 h. Posttreatment, 0.5 mg/mL of MTT reagent was added to the cells and the cells were incubated at 37°C temperature for 2 h. Later, MTT reagent was removed and the formazan crystals were dissolved by adding 20 μL of DMSO. Absorbance at 570 nm was measured using microplate reader and percentage viability of the cells was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD of test at 570nm}}{\text{Mean OD of Untreated cells at 570nm}} \times 100$$

Glucose uptake assay

3T3-L1 cells were seeded into a six-well plate at an initial seeding density of 2×10^5 cells/well. Overnight culture of cells was washed with D-PBS and then treated for 2 h with 100 μg/mL of the extract or 100 μM the standard drug, metformin, as the control. The cells were then further incubated with 2-NBDG for 2 h. Cells were then trypsinized, washed with PBS, and resuspended in 0.5 ml of D-PBS. Cellular uptake of 2-NBDG was measured by flow cytometry (FACS Calibur, BD Biosciences) and the data were analyzed using Cell Quest Pro software.

Glucose uptake and glucose transporter 4 translocation studies

GLUT4 expression levels were analyzed using flow cytometry. 3T3-L1 cells were seeded in a six-well plate at an initial density of 2×10^5 cells/2 ml and were cultured overnight. The spent medium was aspirated, and the cells were treated with 100 μg/mL of UN extract or 100 μM of positive control, metformin, for 24 h. Posttreatment, the cells were washed with D-PBS, trypsinized, and resuspended in 0.5 ml of D-PBS. The cells were then incubated with Mouse Anti Glut4–FITC antibody (#NBP1-49533F, Novus Biologicals) for 30 min in the dark. Unbounded antibody was washed with D-PBS, and the cells were assessed for GLUT4 expression in a BD FACSCalibur Flow Cytometer in FL1 channel. The data were analyzed with Cell Quest Pro software.

α-amylase inhibition assay

The α-amylase inhibitory activity was estimated using a previously described method.[18] UN extracts of different concentrations ranging from 31.25 to 500 μg/mL in DMSO were prepared. About 100 μL of α-amylase and UN extract were mixed and incubated in microtubes for 10 min at 37°C. 100 μL of 1% soluble starch dissolved in buffer A was added to each microtube, and the mixture was incubated for 30 min at 37°C. 200 μL of dinitrosalicylic acid color reagent was added to arrest

the reaction, and the microtubes were then incubated at 100°C for 5 min. The samples were cooled to room temperature and 50 μL of the reaction mixture was transferred to the wells of 96-well microplate. This was further diluted with 150 μL of distilled water and the absorbance at 540 nm was measured in a microplate reader (EC800, Biotek). Percentage inhibition was calculated using the formula stated below, and the results were compared to the inhibitory action of the control drug, acarbose.

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

α-glucosidase inhibition assay

The α-glucosidase enzyme inhibitory activity of UN extract was carried out based on the procedure described by Shibano *et al.*[19] with slight modifications. UN extract of concentrations ranging from 31.25 to 500 μg/mL was prepared. These were mixed with 50 μL of 0.1M phosphate buffer (pH 7.0), 25 μL of 0.5 mM 4-nitrophenyl-α-D-glucopyranoside (dissolved in 0.1M phosphate buffer, with pH of 7.0), and 25 μL of α-Glucosidase (0.1 U/mL). The reaction mixture was incubated for 30 min at 37°C. The reactions were terminated with 100 μL of 0.2M sodium carbonate. Absorbance at 410 nm was read in the microplate reader (EC800, Biotek). The results were compared with the positive control, Acarbose. Percent inhibition of α-glucosidase was calculated using the below-stated formula. IC_{50} values were also determined.

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

Statistical analysis

IC_{50} values in enzyme inhibition assays were determined using linear regression graph (concentration versus percentage enzyme inhibition). All the experiments were conducted in triplicates, and the results are expressed as mean percentage inhibition ± standard deviation (n=3). Statistical significance was determined by one-way analysis of variance, followed by Bonferroni *post hoc* test for multiple comparisons, and $P < 0.05$ was considered statistically significant. All statistical analyses and IC_{50} values determination were carried out in GraphPad Prism (version 3.1) software (San Diego, CA).

RESULTS

α-Amylase activity inhibition

Inhibition of α-amylase activity by UN extract was tested against the control drug acarbose. UN leaf extract inhibited α-amylase activity by 19.60% at 31.25 μg/mL and 71.31% at 500 μg/mL concentration. The IC_{50} value of the extract was found to be 253.61 μg/mL. The control drug acarbose showed 37.15% inhibition of α-amylase activity at 31.25 μg/mL and 88.54% at 500 μg/mL concentration. The IC_{50} value of acarbose was found to be 40.66 μg/mL [Table 1 and Figure 2].

Inhibition of α-glucosidase activity

UN extract exhibited 19.74% inhibition of α-glucosidase activity at 31.25 μg/mL and 79.11% at 500 μg/mL, respectively. Its IC_{50} was found to be 208.60 μg/mL. The control drug acarbose showed 34.96% inhibition in α-glucosidase activity at 31.25 μg/mL and 87.35% at 500 μg/mL concentrations, respectively. Its IC_{50} was found to be 43.53 μg/mL [Table 2 and Figure 3].

Cytotoxicity assay

3T3-L1 cells were treated with various concentrations (25 μg–400 μg/mL) of UN extract and were assayed for their cytotoxic effect. The extract did not show any cytotoxic effect. The concentrations of the extract used and the respective percent cell viability were tabulated and plotted [Table 3 and Figures 4, 5].

Glucose uptake and glucose transporter 4 expression analysis

GLUT4 expression on UN extract treatment was analyzed using flow cytometry. UN extract (100 μg/mL) treatment increased GLUT4 expression. Metformin (100 μM) was used as the control. Flow cytometric analysis revealed that 70.67% of 3T3-L1 cells treated with UN extract expressed GLUT4 and 99.97% of metformin-treated cells expressed GLUT4 [Figures 6 and 7].

Glucose uptake studies

2-NBDG, a fluorescent deoxyglucose analog, was used to detect the glucose uptake in 3T3-L1 cells cultured in the presence of antidiabetic drugs. Flow cytometric analysis of the UN extract treated 3T3-L1 cells

Table 1: Inhibition of α-amylase activity by *Uvaria narum* extract

Sample	Concentration (μg/mL)	Percentage inhibition of enzyme activity	IC_{50} (μg/mL)
Acarbose	31.25	37.15±0.84	40.66±4.22
	62.50	56.85±1.55	
	125.00	65.31±0.88	
	250.00	71.14±0.73	
	500.00	88.54±0.55	
Uvaria narum extract	31.25	19.60±1.3	253.61±3.54
	62.50	28.70±2.19	
	125.00	42.72±0.91	
	250.00	55.68±1.81	
	500.00	71.31±0.84	

Table 2: Inhibition of α-glucosidase activity by *Uvaria narum* extract

Sample	Concentration (μg/mL)	Percentage inhibition of enzyme activity	IC_{50} (μg/ml)
Acarbose	31.25	34.96±0.64	43.53±0.40
	62.50	57.53±1.35	
	125.00	65.56±0.53	
	250.00	72.12±0.81	
	500.00	87.35±1.00	
Uvaria narum extract	31.25	19.74±1.70	208.60±7.25
	62.50	29.79±1.00	
	125.00	45.72±1.70	
	250.00	61.88±5.20	
	500.00	79.11±2.65	

Table 3: Percent viability of the cells treated with various concentrations of *Uvaria narum* extract

Treatment	Percent viability
Control (no treatment)	100±0.9
Uvaria narum extract (μg/mL)	
25	98.51±0.56
50	96.12±1.80
100	95.02±1.11
200	93.24±0.13
400	92.24±0.20
Metformin (100 μM)	93.83±1.25

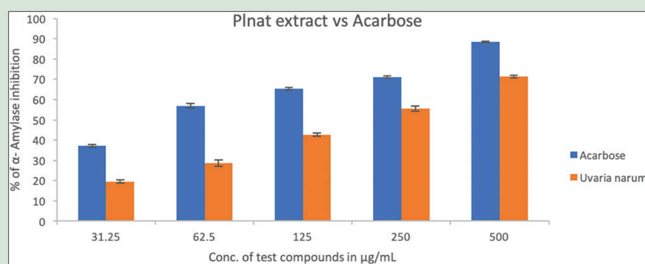


Figure 2: Inhibition of α-amylase against different concentrations of *Uvaria narum*; the IC_{50} value of the extract was 253.61 ± 3.54 µg/mL. Data were presented as mean ± standard deviation

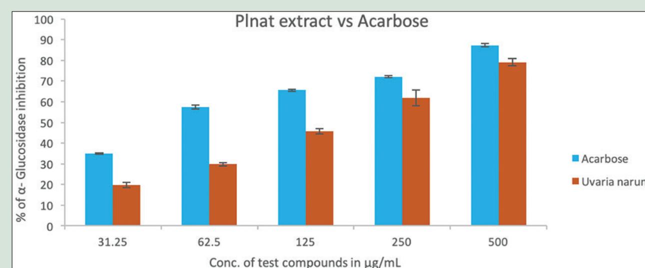


Figure 3: Inhibition of α-glucosidase against logarithm of different concentrations of *Uvaria narum*; the IC_{50} value of the extract was 208.60 ± 7.25 µg/mL. Data were presented as mean ± standard deviation

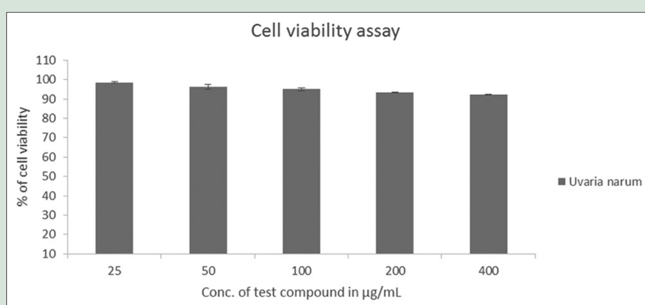


Figure 4: The effect of *Uvaria narum* on 3T3L1 cell line viability as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay method. Each bar graph represents percentage viability of 3T3L1 cell line against 25, 50, 100, 200, and 400 µg/mL concentrations of *Uvaria narum* extract after 24h exposure. The data were shown as mean ± standard deviation of triplicate experiments

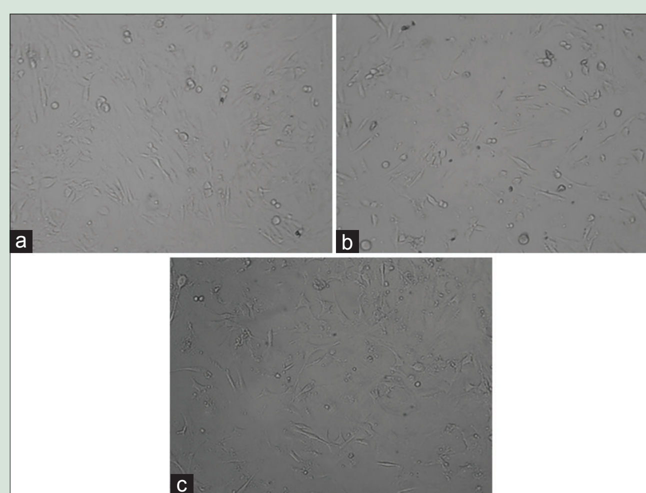


Figure 5: Images of 3T3 L1 cell line in inverted light microscopy after the exposure to *Uvaria narum* extract. From "A" to "C" where (a) control (untreated cells), (b) Standard metformin drug, and (c) 400µg concentration of *Uvaria narum* extract. After incubation of 24 h *Uvaria narum* extract displayed no toxicity

showed that 75.49% had taken up 2-NBDG. Furthermore, 99.98% of the cells treated with the control drug, metformin, had taken up 2-NBDG [Figures 8 and 9].

DISCUSSION

The major enzymes that metabolize carbohydrates in the small intestine are pancreatic α-amylase and α-glucosidase enzymes, which convert polysaccharides to monosaccharides. Antidiabetic drugs reduce the action of these enzymes and regulate the postprandial blood glucose level in type 2 diabetic patients. The mechanisms of control of glucose levels vary with the drug. Some drugs stimulate insulin secretion or synthesis by the pancreatic beta-cells, others help in regenerating damaged pancreatic beta-cells, still others increase insulin sensitivity triggering glucose uptake by fat and muscle cells, and certain others modify the activity of enzymes pertinent to glucose metabolism to reduce the absorption of sugars from the gut.[20,21] Contemporary antidiabetic drugs that specifically inhibit the activity of enzymes include acarbose, voglibose, and miglitol. However, usage of these drugs can have adverse effects such as flatulence and abdominal bloating.[22]

Natural compounds from ethnomedicinal plants that do not manifest such effects could be used to treat type 2 diabetes.[23] Such medicinal plants play a vital role in herbal medicine, particularly in treating ailments such as diabetes.[20,21,24,25] UN, an ethnomedicinal plant that belongs *Annonaceae* (Custard apple) family is a primary plant with several varieties, many of which have been traditionally used for medicinal and ethnobotanical purposes.[26-28] In this study, we investigated the antidiabetic properties of UN.

Our results showed that methanolic extract of UN effectively inhibited α-amylase and α-glucosidase activities. These inhibitory effects were estimated with acarbose as the standard drug. Furthermore, UN had no cytotoxic effects on the cells. The implications of this study corroborate the previous studies on inhibitory effect of other natural compounds on α-amylase and α-glucosidase activities.[29-31]

Cellular uptake of glucose from blood plays a crucial role in the reduction of DM. This is generally mediated by GLUT4 in the cell. On stimulation with antidiabetic drugs, GLUT4 is translocated from their intracellular sites to the cell surface. Insulin induces the translocation of GLUT4 through phosphatidylinositol-3-kinase pathway.[32] PKB/Akt-mediated stimulation of glucose transport by insulin has also been reported in rat adipocytes and L6 muscle cells.[33] Metformin is one of the standard antidiabetic drugs that enhance glucose uptake by inducing the translocation of GLUT4. This drug is known to act through AMP-activated protein kinase pathway.[34] In the current study, metformin was used as the positive control for glucose uptake and GLUT4 expression studies. Of all the 3T3-L1 cells treated with UN extract, 75.49% took up 2-NBDG (a glucose analog) and 70.67% expressed GLUT4. These findings suggest that the leaf extract of UN could enhance cellular glucose uptake by inducing GLUT4 translocation. However, further studies must be performed to understand the exact mechanism of action.

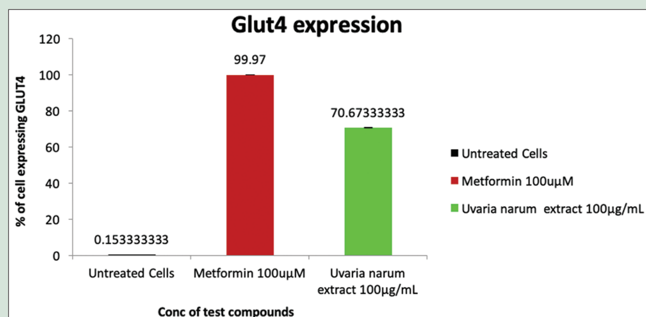


Figure 6: Glucose transporter 4 expression upon exposure of 3T3L1 cells to 100 μg/mL of plant extract and 100 μM of metformin. Each bar graph represents the % of cells expressing glucose transporter 4. Data were represented as mean ± standard deviation of triplicate experiments

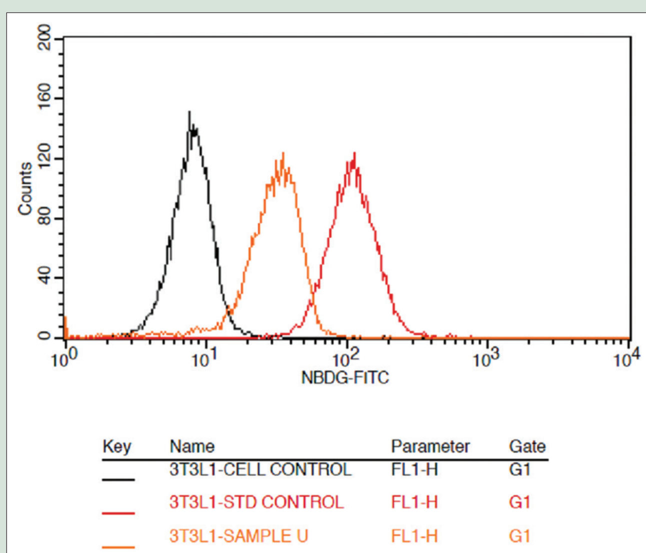


Figure 8: Overlaid expression of given untreated 3T3L1 cells (black color line) and standard drug-treated cells (metformin 100 μM) (red color line) and test compound-treated cells (orange color line) against the 2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxyglucose stain

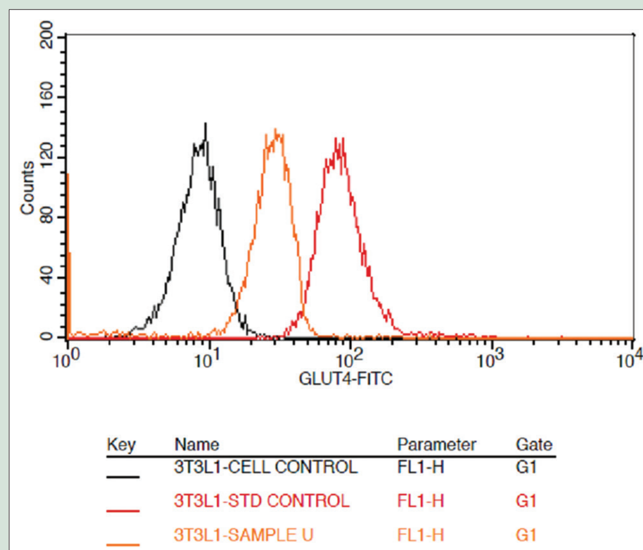


Figure 7: Overlaid expression of glucose transporter 4 in untreated 3T3L1 cells (black color line) and standard drug-treated cells (metformin 100 μM) (red color line) and test compound-treated cells (orange color line)

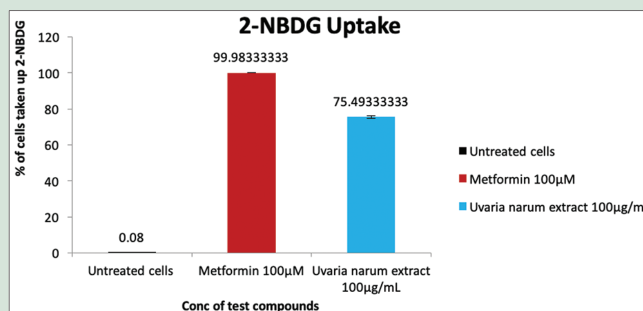


Figure 9: Each bar graph represents the % of cells taken up the 2-(*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxyglucose in the presence of 100 μg/ml of *Uvaria narum* and 100 μM of metformin. The data were represented as mean ± standard deviation of triplicate experiments

CONCLUSION

The methanolic extract of UN inhibited the activity of the enzymes α-amylase and α-glucosidase, enhanced glucose uptake, and GLUT4 translocation in 3T3-L1 cells. These results were comparable to the action of acarbose and metformin. Further, the extract had no cytotoxic effect on the cells. In conclusion, UN extract has potential antidiabetic applications. Further work must be done to identify lead molecules from this plant.

Acknowledgements

All the authors acknowledge the services rendered by Stellixir Biotech Pvt. Ltd, Bengaluru, India, in carrying out the *in vitro* cell line work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Dwivedi C, Daspaul S. Antidiabetic herbal drugs and polyherbal formulation used for diabetes: A review. J Phytopharmacol 2013;2:1-7.
2. Gnanu N, Divagar M, Juliet L. Evaluation of *in vitro* anti diabetic activity of seendhil herbal formulation. Asian J Pharm Clin Res 2013;1:91-3.
3. Chakrabarti R, Rajagopalan R. Diabetes and insulin resistance associated disorders: Disease and the therapy. Curr Sci 2002;83:12.
4. Zimmet P. Globalization, coca-colonization and the chronic disease epidemic: Can the doomsday scenario be averted? J Intern Med 2000;247:301-10.
5. Sharma G, Kumar S, Sharma M, Upadhyay N, Kumar S, Ahmed Z, *et al.* Anti-diabetic, anti-oxidant and anti-adipogenic potential of quercetin rich ethyl acetate fraction of *Prunus persica*. Pharmacogn J 2018;10:463-9.
6. Gray RS, Fabsitz RR, Cowan LD, Lee ET, Howard BV, Savage PJ. Risk factor clustering in the insulin resistance syndrome. The Strong Heart Study. Am J Epidemiol 1998;148:869-78.
7. Durga RK, Karthikumar S, Jegatheesan K. Isolation of potential anti bacteria and antioxidant compounds from *Acalypha indica* and *Ocimum basilicum*. Afr J Plant Sci 2009;4:163-16.
8. Fan C, Wang W, Wang Y, Qin G, Zhao W. Chemical constituents from *Dendrobium densiflorum*. Phytochemistry 2001;57:1255-8.
9. Cousins MM, Adelberg JW. *In vitro* plant and organ culture of medicinal and neutriceutical species in laboratory and industrial scales. Acta Physiol Plant 2009;31:961-7.
10. Cragg GM, Newman DJ, Snader KM. Natural products in drug discovery and

development. J Nat Prod 1997;60:52-60.

11. Takazawa K, Noguchi T, Hosooka T, Yoshioka T, Tobimatsu K, Kasuga M. Insulin-induced GLUT4 movements in C2C12 myoblasts: Evidence against a role of conventional kinesin motor proteins. Kobe J Med Sci 2008;54:E14-22.
12. Hargreaves M. Exercise increases skeletal muscle GLUT4 gene expression in patients with type 2 diabetes. Diabetes Obesity Metab 2012;14:768-71.
13. Bryant NJ, Govers R, James DE. Regulated transport of the glucose transporter GLUT4. Nat Rev Mol Cell Biol 2002;3:267-77.
14. Govind P. Medical plants against liver diseases. Int Res J Pharm 2010;2:115-21.
15. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
16. Patel S, Gheewala N, Suthar A, Shah A. *In vitro* cytotoxicity activity of *Solanum nigrum* extract against Hela cell line and vero cell line. Int J Pharm Sci 2009;1:38-46.
17. Apposotolidis E, Kwon YI, Shetty K. Inhibitory potential of herb, fruit and fungi- enriched cheese, against key enzymes linked to type 2 diabetes hypertension. Inn Food Sci Emerg Technol 2007;8:46-54.
18. Shibano M, Kitagawa S, Nakamura S, Akazawa N, Kusano G. Studies on the constituents of *Broussonetia* species. II. Six new pyrrolidine alkaloids, broussonetine A, B, E, F and broussonetinine A and B, as inhibitors of glycosidases from *Broussonetia kazinoki* sieb. Chem Pharm Bull 1997;45:700-5.
19. Tiwari AK, Rao JM. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Curr Sci 2002;83:30-8.
20. Tiwari BK, Pandey KB, Abidi AB, Rizvi SI. Therapeutic potential of Indian medicinal plants in diabetic condition. Ann Phytomed 2013;2:37-43.
21. Kuppusamy A, Muthusamy U, Thirumalaisamy SA, Varadharajan S, Ramasamy K, Ramanathan S. *In vitro* (α-glucosidase and α-amylase inhibition) and *in vivo* antidiabetic property of phytic acid (IP6) in streptozotocin- nicotinamide-induced type 2 diabetes mellitus (NIDDM) in rats. J Complement Integr Med 2011;8. Doi:10.2202/1553-3840.1483.
22. Gupta PD, Amartya D. Diabetes mellitus and its herbal treatment. Int J Res Pharm Biomed Sci 2012;3:706-12.
23. Rana CS, Ballabha R, Tiwari JK, Dangwal LR. An ethnobotanical study of the plant resources in the Nanda Devi Biosphere Reserve (a world heritage site), Uttarakhand, India. J Ethnobiol Tradit Med 2013b; 120:591-601.
24. Rana CS, Tiwari JK, Dangwal LR, Gairola S. Faith herbal healer knowledge document of Nanda Devi Biosphere Reserve, Uttarakhand, India. Indian J Tradit Knowl 2013a; 12:208-14.
25. Poretsky L. Principles of Diabetes Mellitus. 2nd ed. New York: Springer; 2009. p. 3.
26. Jaiswal V. Culture and ethnobotany of Jaintia tribal community of Meghalaya, North East India – A mini review. Indian J Tradit Knowl 2010;9:38-44.
27. Lohitha P, Tulasi C, Rao A, Swathi S, Swetha W, Sowmya M, *et al.* Evaluation of *in vitro* anthelmintic activity of *Litsea glutinosa* bark Ethanolic and aqueous extract. Int J Pharm Sci 2010;2:612-14.
28. Mogale MA, Lebelo SL, Thovhogi N, de Freitas AN, Shai LJ. α-amylase and α-glucosidase inhibitory effects of *Sclerocarya birrea* [A.Rich. Hochst] subspecies caffra (sond) Kokwaro (*Anacardiaceae*) stem-bark extracts. Afr J Bot 2011;10:15033-9.
29. Scott LJ, Spencer CM. Miglitol: A review of its therapeutic potential in type 2 diabetes mellitus. Drugs 2000;59:521-49.
30. Bischoff H. Pharmacology of alpha-glucosidase inhibition. Eur J Clin Invest 1994;24 Suppl 3:3-10.
31. Shepherd PR, Withers DJ, Siddle K. Phosphoinositide 3-kinase: The key switch mechanism in insulin signalling. Biochem J 1998;333(Pt 3):471-90.
32. Tanti JF, Grillo S, Grémeaux T, Coffer PJ, Van Obberghen E, Le Marchand-Brustel Y. Potential role of protein kinase B in glucose transporter 4 translocation in adipocytes. Endocrinology 1997;138:2005-10.
33. Ueki K, Yamamoto-Honda R, Kaburagi Y, Yamauchi T, Tobe K, Burgering BM, *et al.* Potential role of protein kinase B in insulin-induced glucose transport, glycogen synthesis, and protein synthesis. J Biol Chem 1998;273:5315-22.
34. Lee JO, Lee SK, Kim JH, Kim N, You GY, Moon JW, *et al.* Metformin regulates glucose transporter 4 (GLUT4) translocation through AMP-activated protein kinase (AMPK)-mediated Cbl/CAP signaling in 3T3-L1 preadipocyte cells. J Biol Chem 2012;287:44121-9.