

Evaluation of Glucose Uptake-Enhancing Activity of *Dolichandrone falcata* Bark Extract in Insulin-Resistant HepG2 Cells

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Abstract

Dolichandrone falcata is a medicinal plant traditionally used for the management of various ailments and is considered a potential source of biologically active phytoconstituents. The present study was undertaken to evaluate the in vitro antidiabetic potential of the bark extract of *Dolichandrone falcata* by assessing its ability to enhance glucose uptake in insulin-resistant HepG2 cells. The bark was subjected to successive Soxhlet extraction using petroleum ether, chloroform and methanol, and the methanolic extract was selected for biological evaluation. Insulin resistance was induced in HepG2 cells using palmitic acid (0.2 mM). The glucose uptake-enhancing activity of the extract was assessed using the fluorescent glucose analogue 2-NBDG, and intracellular fluorescence was quantified as Mean Fluorescence Intensity (MFI) by flow cytometry. Metformin was used as the standard antidiabetic drug. The untreated HepG2 cells exhibited minimal 2-NBDG uptake, whereas metformin treatment produced a marked increase in glucose uptake, with approximately 78% of cells showing 2-NBDG positivity. Treatment with *D. falcata* methanolic extract at 100 µg/mL significantly enhanced glucose uptake, with approximately 64% of cells becoming 2-NBDG positive. A pronounced rightward shift in the 2-NBDG-FITC fluorescence profile further confirmed enhanced intracellular glucose accumulation following extract treatment. The glucose uptake response of the extract was close to that observed with metformin, indicating considerable glucose-utilizing activity. These findings suggest that the methanolic bark extract of *D. falcata* possesses promising antidiabetic and insulin-sensitizing potential, possibly through modulation of glucose transport and insulin-signaling pathways. Further studies are required to identify the active phytoconstituents and elucidate their precise molecular mechanisms.

Keywords: *Dolichandrone falcata*; antidiabetic activity; HepG2 cells; glucose uptake; 2-NBDG; flow cytometry; methanolic extract; insulin resistance.

Introduction

Nature always stands as a golden mark to exemplify outstanding phenomenon of symbiosis. Nature has provided a complete store house of remedies to cure ailments of mankind. The knowledge of drug has accumulated over thousands of year as a result of man's inquisitive

nature. Plants have been used for medicinal purposes long before recorded history (Jacqueline, 2002). Primitive men observed and appreciated the great diversity of plants available to him. The retrieval of knowledge on the use of medicinal plants has served as a valuable tool in the discovery of new drugs. Their usefulness can be in the form of food, shelter, religious, medicines etc. Herbs are witnessing a renewed global interest, signaling a revival of herbal knowledge.

The term medicinal plants were first used in 1967 in the context of the study of hallucinogenic plants. Knowledge of the beneficial properties of medicinal plants to treat diseases represents a valuable resource to preserve the biological and cultural diversity of different ethnicities (Heinrich et al., 2017). Medicines derived from plants are widely famous due to their safety, easy availability and low cost. Herbal medicines may include whole parts of plant or mostly prepared from leaves, roots, bark, seed and flowers of plants. They are administered orally, inhaled or directly applied in the skin. Medicinal herbs are more significant to the health of individual and community. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, phenolic compounds and many more. These natural compounds formed the foundations of modern prescription drugs as we know today. Use of plants based drugs for curing various ailments is as old as human civilization and is used in all cultures throughout history. The primitive man started to distinguish between useful and harmful are poisonous plants by trail and errors. A well defined herbal pharmacopoeia was developed by tribal people, which was based on information collected from local flora, religion and culture. The knowledge of medicinal plants was gradually developed and passed on from one individual to other, which foundation for traditional medicine throughout the world. However, plants derived medicines are far superior than the well defined drugs. For example, the quality and availability of raw materials is always a problem, the principles of handling are also unknown and also the quality control, i. e. standardization and stability are practically applicable but not too much easy. Herbal medicines are for superior than the synthetic drugs because they are naturally occurring, easily available without cost and have minimum side effects (WHO, 2002; Patwardhan et al., 2005). Majority of plants have medicinal properties, i. e. most pharmaceutical drugs are originally derived from plants (Newman & Cragg, 2016).

Table 1.1 Total percentage of plants used medicinally worldwide*

Country	Plant species	Medicinal plant species	Total Percentage (%)
China	26,092	4,942	18.9
India	15,000	3,000	20.0
Indonesia	22,500	1000	4.4
Malaysia	15,500	1,200	7.7
Nepal	6,973	700	10.0
Pakistan	4,950	300	6.1
Philippines	8,931	850	9.5
Srilanka	3,314	550	16.6
Philippines	8,931	850	9.5

Srilanka	3,314	550	16.6
Thialand	11,625	1,800	15.5
USA	21,641	2,564	11.8

*(Sadath Ali, 2008)

Need and scope for herbal therapy

As nature has bestowed the rich source of natural remedy, man's inquest nature of exploration has led to the development of new therapeutics in the field of medicines(Rates, 2001). The overwhelming problem of synthetics with severe adverse affects has raised question among the global health care sectors(WHO,1998). Hence herbal remedy has showed the ray of hope in patients and health care takers(Rates, 2001). To fulfill the basic needs of the patients it is quite necessary to follow the basic procedures for fulfilling the need and scope of herbal therapy viz Data collection from ancient scriptures and ethanobotanical survey, recompilation of data, collection and preservation of the herbs and plants, isolation, characterization, in vitro and in vivo pharmacological screening, clinical trials etc(Rates, 2001). Global care in this world of rich flora of plants can cater the needs of man only when the nature is preserved(Cunningham, 2001).

Need for Herbal Medicine

Herbal medicine has been an integral component of traditional healthcare systems such as Ayurveda, Siddha, Unani, Traditional Chinese Medicine, and various indigenous healing practices across the world(WHO,2002). In recent decades, there has been a renewed global interest in herbal medicines due to their therapeutic potential, cultural acceptability, and perceived safety(Ekor, 2014). The growing burden of chronic diseases, including diabetes, cardiovascular disorders, cancer, inflammatory conditions, and neurodegenerative diseases, has highlighted the

Table 1.2 Natural products sources, therapeutic activity and their synthetic or semi synthetic analogs*

Sl. No	Source	Therapeutic activity	Phytoconstituents	Synthetic or synthetic analogs
1	Penicillin chrysogenum	Antimuscarinics	Atropine	Dicyclomine HCl, Hyoscinebutylbromide
2	Papaver somniferum	Antibiotic	Benzyle penicillin	Ampicillin, amoxicillin.
3	Camptotheca accumnata	Analgesic	Codeine	Nolorphine, mepiridine.
4	Cathranthus roseus	Antihypertensive	Teprotide	Captopril, enalapril, lisinopril.
5	Ephedra vulgaris	Cardiovascular	Digoxin	-
6	Aspargillus terrus	Anti-asthma	Ephedrine	Salbutamol, salmeterol.
7	Papaver somniferum	hypercholesterolemia	Lovastatin	Pravastatin.

8	Podophillum pettatum	Analgesic	Morphine	Heroine, naloxane, phthadine.
9	Cinchona succirubra	Anticancer	Podophyllotoxine	Etoposide, toniposide.
10	Tube curare	Hypotension and anticholinergic	Reserpine	- -
11	Taxus baccata	Neuromuscular blocking agent	Tubacurarine	Decamethoxium, soxamethorium.
12	Bathrops	Anticancer	Taxol	-
13	Rawolfia serpentina	Antimalarial	Quinine	Chloroquine, meploquinine, pamaquine,
14	Cathranthus roseus	Antihypertensive	Teprotide	Captopril, enalapril, lisinopril.

*(Sadath Ali, 2008)

limitations of conventional synthetic drugs, which are often associated with adverse effects, high costs, and long-term toxicity. Consequently, there is an increasing demand for safer, affordable, and more effective therapeutic alternatives, thereby emphasizing the need for herbal medicine(Patwardhan et al. , 2005).

Another significant need for herbal medicine arises from the emergence of drug resistance, particularly in antimicrobial therapy. The widespread and often indiscriminate use of synthetic antibiotics has led to the development of resistant strains of microorganisms, posing a serious public health challenge(WHO, 2014). Medicinal plants, with their complex mixtures of bioactive phytoconstituents, offer multi-target mechanisms of action that may reduce the likelihood of resistance development. This characteristic makes herbal medicines a promising source for novel therapeutic agents(Patwardhan et al. , 2005).

Additionally, herbal medicines play a crucial role in primary healthcare, especially in developing countries(WHO,2002), where a large proportion of the population relies on plant-based remedies due to limited access to modern medical facilities. The World Health Organization (WHO,2013) recognizes traditional medicine as an important component of healthcare delivery and encourages the integration of herbal medicines into national health systems, provided their safety, efficacy, and quality are scientifically validated. This recognition further underscores the need for systematic research and standardization of herbal medicines(Ekor, 2014).

Dolichandrone falcata

The genus *dolichandrone* belongs to the *Bignoniaceae* family(Kubitzki & Zjhra, 2004). The *Bignoniaceae* is a family of flowering plants in the order Lamiales commonly known as the bignonias or trumpet vines. It is not known to which of the other families in the order it is most closely related. Nearly all of the Bignoniaceae are woody plants, but a few are subwoody, either as vines or subshrubs. A few more are herbaceous plants of high- elevation montane habitats, in three exclusively herbaceous genera Tourrettia, Argylia, and Incarvillea. The family includes many lianas, climbing by tendrils, by twining, or rarely, by aerial roots. The largest tribe in the family, called Bignonieae, consists mostly of lianas and is noted for its unique wood

anatomy(Olmstead et al. , 2009). The family has a nearly cosmopolitan distribution, but is mostly tropical, with a few species native to the temperate zones. Its greatest diversity is in northern South America. The family has been covered in some major floristic projects, such as Flora of China, Flora Malesiana, and Flora Neotropica. It has not yet been covered in some others, such as Flora of Australia, and Flora of North America.

According to different accounts, the number of species in the family is about 810 or about 860. The last monograph of the entire family was published in 2004. In that work, 104 genera were recognized. Since that time, molecular phylogenetic studies have greatly clarified relationships within the family, and the number of accepted genera is now between 80 and 85.

In recent years, the *Dolichandrone falcata* is becoming very vital plant in medical and pharmaceutical industry(Kirtikar & Basu, 1935). *Dolichandrone falcata* is a small deciduous tree in the family Bignoniaceae(Plate 1.1). The plant is also called ‘medhshingi’ in Hindi and ‘mesasinghi’ in Sanskrit. It is endemic to India. Tree attains a height of 15–20 feet. Leaves are compound 2-6 inches long with 3-6 obovate or oval shaped leaflets. Flowers are white and fragrant. The flowering of this occurs in April–May. Despite of these applications this plant is yet to be worked out for its chemical composition. The plant has numerous medicinal uses like antiallergic, anti- inflammatory, antioxidant, antiestrogenic, anxiolytic, anticonvulsant, and anti parasitic(Nadkarni, 1954). This plant is also used in curing anemia, bloody diarrhea, and also as anthelmintic, analgesic, antiviral, and antifungal agents. The plant is used



Plate 1.1 *Dolichandrone falcata*

to prepare snake venom and also used in the treatment of liver disorder. The bark paste of *Dolichandrone falcata* is applied in case of fractures. The leaves of this plant are used as antioxidant, antiestrogenic and anti diabetic.

In Ayurveda *Dolichandrone falcata* has a long history of use by indigenous and tribal peoples all over the world[6], including India, for medicinal purposes and various pharmacological effects. The bark decoction is also given in the treatment of nodules. An aqueous extract of the fruit is used for abortion and bark is used as a fish poison. Infusion of powder is given internally in the treatment of acute rheumatism. In Ayurveda, the stem bark of *Dolichandrone falcata*

Seem is used to cure ulcers, pain, and epilepsy. Menorrhagia and leucorrhea are treated with bark juice of *Dolichandrone falcata* leaves paste is mixed with neem leaves and applied to curb swelling (Kirtikar & Basu, 1935; Nadkarni, 1954).

Materials and Methods

Collection of Plant Material

The *Dolichandrone falcata* plant was collected from the Gulbarga University campus, Kalaburagi, Karnataka, India. The taxonomic identity of the plant material was authenticated by the Department of Botany, Government College (Autonomous), Kalaburagi. A voucher specimen was deposited in the departmental herbarium (Voucher specimen No. GCAK-117).

Extraction of *Dolichandrone falcata* Bark

The shade-dried bark was powdered and sieved to obtain a uniform particle size of 22 meshes. The powdered material was subjected to successive sequential Soxhlet extraction using solvents of varying polarity, ranging from polar to non-polar, namely petroleum ether, chloroform, and methanol (40–60 °C). The extraction process was continued until the solvent became colorless. The obtained extracts were concentrated under reduced pressure and dried in vacuum desiccators. The dried extracts were subsequently subjected to phytochemical analysis for the detection of various secondary metabolites (Evans, 1996; Harborne, 1992; Indian Herbal Pharmacopoeia, 1996).

The different solvent extracts were designated as follows: *Dolichandrone falcata* petroleum ether extract (DFPE), *Dolichandrone falcata* chloroform extract (DFCE), and *Dolichandrone falcata* methanol extract (DFME).

In vitro Antidiabetic Potential (Glucose uptake assay) of *Dolichandrone falcate*

Cell lines and Reagents

1. Cell lines: HepG2-Human hepatocellular carcinoma cell line (NCCS, Pune)
2. Cell culture medium: DMEM glucose free medium
3. DMSO-Dimethyl Sulfoxide
4. PBS-Phosphate Buffered Saline
5. Metformin
6. Fetal Bovine Serum (FBS)
7. 2-NBDG- 2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl) amino)-2-deoxy-D-glucose

Cell Culture Medium

The cells were grown in **DMEM (high glucose)** supplemented with **10% fetal bovine serum (FBS)** and **1% penicillin–streptomycin**. The cultures were maintained at **37 °C in a humidified incubator with 5% CO₂**. For glucose uptake experiments, the cells were seeded and allowed to reach about **70–80% confluence**. Before treatment, the cells were **serum-starved for 6–12 hours** using **serum-free or low-serum DMEM** to reduce basal glucose uptake. After serum starvation, the cells were treated with different concentrations of the

methanolic extract of *Dolichandrone falcata*. Metformin was used as the standard antidiabetic drug. All treatments were prepared in low-serum DMEM.

After treatment, glucose uptake was measured using **2-NBDG**, a fluorescent glucose analogue. During this step, the medium was replaced with **glucose-free or low-glucose DMEM containing 2-NBDG**, and the cells were incubated to allow glucose uptake. The fluorescence inside the cells was then measured, which indicates the ability of the extract to enhance glucose uptake.

Glucose Uptake Assay

To assess the antidiabetic potential of the methanolic extract of *Dolichandrone falcata* by measuring its ability to improve glucose uptake in insulin-resistant HepG2 cells using the 2-NBDG assay. Glucose uptake into cells is a key indicator of insulin sensitivity. The fluorescent glucose analogue 2-NBDG is transported into cells via glucose transporters in a manner similar to glucose. The intracellular accumulation of 2-NBDG can be quantified using fluorescence measurement and is directly proportional to glucose uptake. HepG2 (human hepatocellular carcinoma) cells were procured from NCCS, Pune. The cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% antibiotic–antimycotic solution and maintained at 37 °C in a humidified incubator with 5% CO₂. Cells were sub-cultured at 70–80% confluence using Trypsin-EDTA. The methanolic extract of *Dolichandrone falcata* bark was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution. Working concentrations were prepared by diluting the stock with culture medium. The final DMSO concentration in all wells was kept below 0.1% to avoid solvent-induced cytotoxicity. To mimic the diabetic condition, insulin resistance was induced in HepG2 cells using palmitic acid. Cells were seeded in 96-well plates and allowed to attach overnight. The cells were then treated with palmitic acid (0.2 mM) for 24 h to induce insulin resistance by impairing insulin signaling and glucose uptake. After induction of insulin resistance, the cells were divided into the following groups: Normal control (untreated HepG2 cells), Insulin-resistant control (palmitate-treated cells), Standard drug group (metformin-treated cells), Test groups (palmitate-treated cells exposed to different concentrations of *Dolichandrone falcata* extract). Cells were incubated with metformin and plant extract for 24 h. After treatment, cells were washed with phosphate-buffered saline and incubated in glucose-free DMEM for 3 h to deplete intracellular glucose. Subsequently, cells were stimulated with insulin (500 nM) for 10 minutes to activate glucose transport pathways. Following insulin stimulation, cells were incubated with 2-NBDG (20 µM) in glucose-free medium for 30 minutes at 37 °C in the dark. Excess dye was removed by washing with PBS. The fluorescence intensity of 2-NBDG taken up by the cells was measured using a fluorescence microplate reader (excitation 485 nm and emission 535 nm). Fluorescence was detected in the **FITC channel**, and **10,000 cells** were recorded for each sample. The **Mean Fluorescence Intensity (MFI)** was calculated for each treatment group using flow-cytometry software. MFI represents the **average intracellular accumulation of 2-NBDG per cell** and thus serves as a quantitative measure of glucose uptake. The forward scatter (FSC-Height) versus side scatter (SSC-Height) dot plot was used to identify the main viable HepG2 cell population. A gate designated as R1 was drawn around the dominant cluster of cells to exclude

debris, dead cells, and cell aggregates, thereby ensuring that only healthy, single, metabolically active cells were included for further analysis. The gated R1 population was then analyzed by plotting SSC-Height against 2-NBDG-FITC fluorescence.

Glucose Uptake was calculated as

Glucose Uptake (%) = $\frac{\text{MFI of treated cells} - \text{MFI of control cells}}{\text{MFI of control cells}} \times 100$

Where:

MFI = Mean Fluorescence Intensity

All experiments were performed in triplicate. Data were expressed as mean $\pm$ SEM. Statistical significance was analyzed using one-way ANOVA followed by Dunnett's test. A p-value < 0.05 was considered statistically significant.

Statistical Analysis

All experiments were performed in triplicate, and results are presented as mean $\pm$ SD (n = 3). Statistical analysis was carried out using one-way ANOVA followed by an appropriate post-hoc test. Differences were considered statistically significant at $p < 0.05$ compared with the control group

Results and Discussion

the **histogram of 2-NBDG-FITC fluorescence intensity** in untreated HepG2 cells. A narrow, sharp peak is observed predominantly in the **lower fluorescence range**, confirming that most cells exhibit **minimal uptake of the fluorescent glucose analogue**. The limited right-side tail further indicates that only a small fraction of cells display moderate glucose uptake. Figure 4.7 shows the **flow cytometric dot plot of side scatter (SSC-Height) versus 2-NBDG-FITC fluorescence** in **untreated HepG2 cells**. The majority of events form a compact diagonal cluster in the **low-fluorescence region (10^1 range)**, indicating a **uniform population of viable cells with low intracellular glucose accumulation**. The low fluorescence intensity observed in both the histogram and dot plot of **untreated HepG2 cells** reflects **reduced glucose transport and utilization**, which is characteristic of the **insulin-resistant hepatic phenotype** of this cell line (RaJain, 2011, Aravinthan et al., 2014). HepG2 cells exhibit impaired insulin signaling and low basal GLUT activity under control conditions, closely mimicking the metabolic defects observed in type-2 diabetes. The compact distribution of events in the low-fluorescence region indicates that the cells were **viable and metabolically consistent**, but **unable to efficiently internalize glucose** in the absence of an insulin-sensitizing agent. Thus, the untreated HepG2 profiles

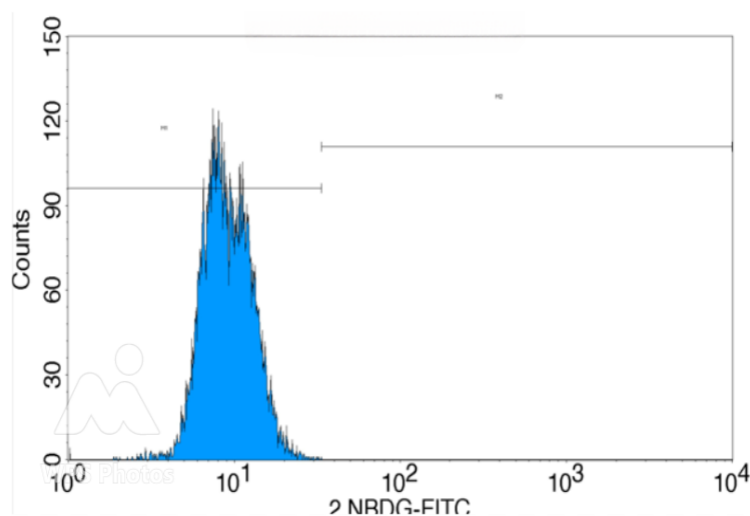


Fig ; Flow cytometric analysis of 2-NBDG uptake in untreated HepG2 cells

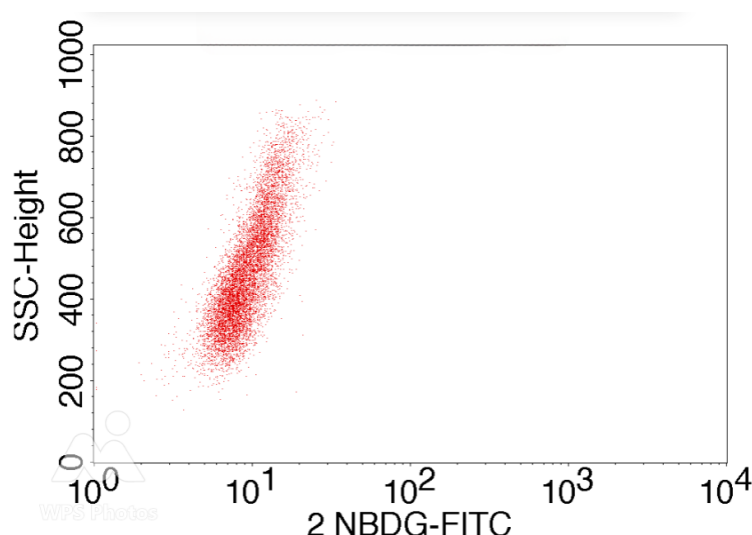


Fig : Flow cytometric dot plot showing SSC VS 2 NBDG-FITC florescence in untreated HepG2 cells.

confirm the presence of a **diabetic-like metabolic state**, validating the suitability of this model for evaluating antidiabetic activity through enhancement of cellular glucose uptake.

Figure 4.8 shows the **forward scatter (FSC-Height) versus SSC-Height plot**, where the main cell population is clearly gated within region **R1**, ensuring that only **viable HepG2 cells** were included in the analysis. The **histogram of 2-NBDG-FITC fluorescence** in metformin-treated HepG2 cells. A pronounced right-shifted peak is observed in the **high-fluorescence region (M2)**. Quantitative histogram statistics show that **77.72% of the gated cell population** falls within the M2 region, indicating **high glucose uptake**, while only **22.78% of cells** remain in the low-uptake M1 region. The **geometric mean fluorescence intensity (53.45)** and **median**

fluorescence (51.40) further confirm a strong intracellular accumulation of the fluorescent glucose analogue. Figure 4.9 illustrates the **flow cytometric dot plot of side scatter (SSC-Height) versus 2-NBDG-FITC fluorescence** in **metformin-treated HepG2 cells**. A dense, well-defined population of events is observed, forming a diagonal cluster extending from the **10^1 to 10^2 fluorescence range**, indicating **substantial intracellular accumulation of 2-NBDG**. The distribution of SSC values confirms that the cells retained normal internal complexity and viability following metformin treatment. The strong enhancement of 2-NBDG uptake following metformin treatment reflects its established mechanism of action. Metformin activates **AMP-activated protein kinase (AMPK)**, which increases **GLUT-mediated glucose transport** and suppresses hepatic glucose production, thereby improving insulin sensitivity (Zhou et al., 2001; Foretz et al., 2014; Hardie et al., 2012). The high proportion of cells in the **M2 region (77.72%)** and the elevated **fluorescence intensity values** confirm that metformin not only increases the number of glucose-responsive cells but also enhances the **magnitude of**

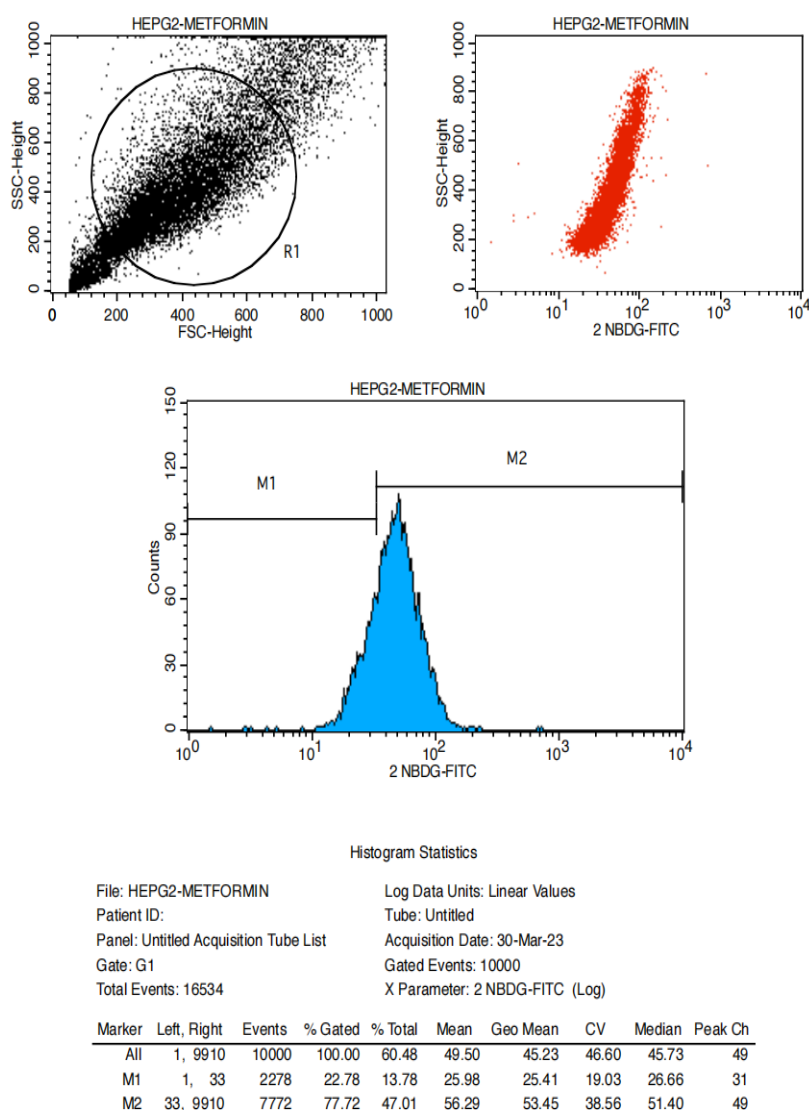


Fig ; Flow cytometric analysis of 2-NBDG uptake in metformin treated HepG2 cells

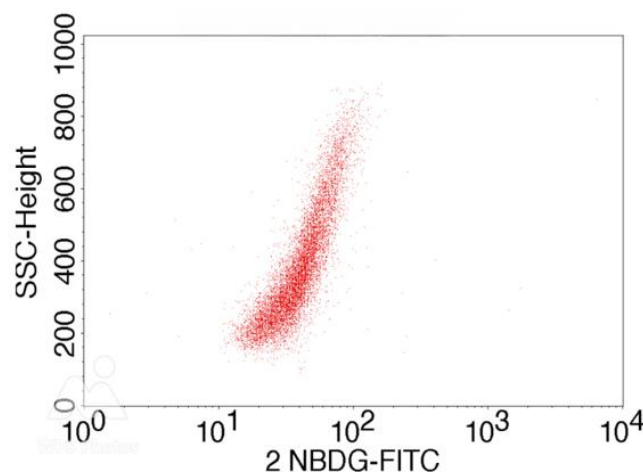


Fig ; Flow cytometric dot plot showing SSC VS 2 NBDG-FITC florescence in Metformin treated cells

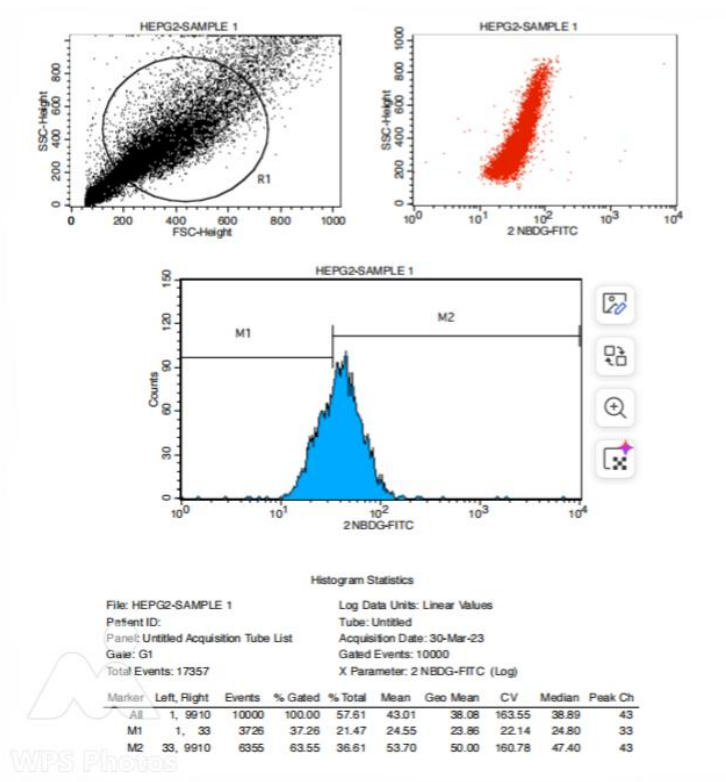


Fig ; Flow cytometric analysis of 2-NBDG uptake in methanolic extract treated HepG2 cells.

glucose uptake per cell. This effect is critical for reducing hepatic glucose output and improving glycemic control in type-2 diabetes (Saltiel and Kahn, 2001).

Figure 4.10 presents the **histogram of 2-NBDG-FITC fluorescence** in *Dolichandrone falcata* plant extract treated HepG2 cells. A pronounced right-shifted peak is observed in the **high-fluorescence (M2) region**. Histogram statistics show that **63.55% of the gated cells** were present in the M2 region, indicating **high glucose uptake**, whereas **37.26% of cells** remained in the low-uptake M1 region. The **geometric mean fluorescence intensity (50.00)** and **median**

fluorescence (47.40) further confirm strong intracellular glucose accumulation in plant extract treated cells. Figure 4.11 shows the **flow cytometric dot plot of side scatter (SSC-Height) versus 2-NBDG-FITC fluorescence** in methanolic extract treated HepG2 cells. The cell population forms a dense diagonal cluster extending from the 10^1 to 10^2 fluorescence range, indicating **substantial intracellular accumulation of the fluorescent glucose analogue**. The SSC distribution confirms that the cells retained normal internal complexity and viability following methanolic extract treatment. It represents the **forward scatter (FSC-Height) versus SSC-Height dot plot**, where the main population of viable HepG2 cells is clearly gated within the **R1 region**, ensuring that only intact cells were included in the analysis. The fluorescent glucose analogue **2-NBDG** is transported into cells via **glucose transporters (GLUTs)** and becomes intracellularly trapped after phosphorylation, making it a reliable marker of **cellular glucose uptake** (Zou et al., 2005; Blodgett et al., 2011). HepG2 cells normally exhibit **low basal glucose uptake** due to their **insulin-resistant phenotype** (Rains and Jain, 2011; Aravinthan et al., 2014).

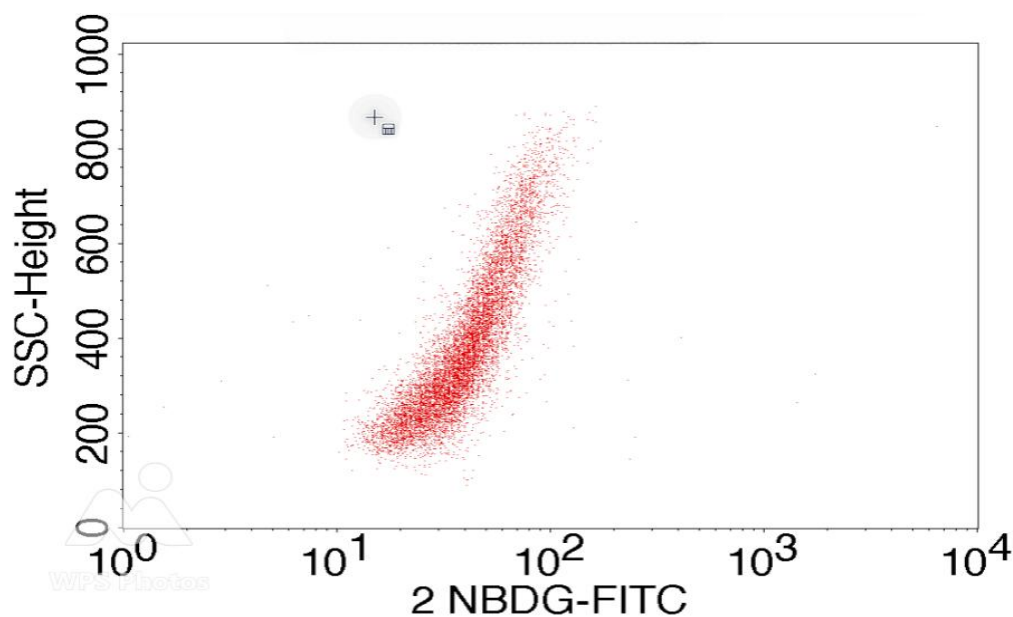


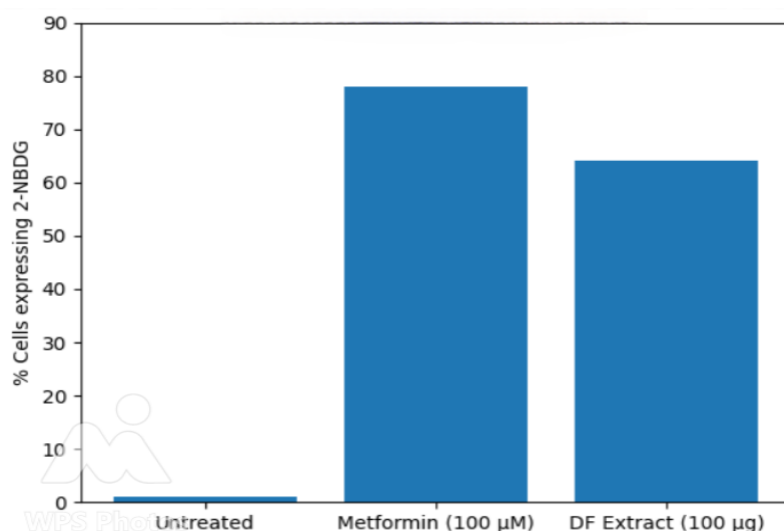
Fig ; Flow cytometric dot plot showing SSC VS 2 NBDG-FITC florescence in Methanolic extract treated cells

The marked rightward shift in fluorescence intensity observed in methanolic extract **treated cells** demonstrates that the test sample significantly **enhances glucose uptake**. The high proportion of cells in the **M2 region (63.55%)** and the elevated fluorescence intensity values indicate that methanolic extract not only increases the number of glucose-responsive cells but also enhances the **amount of glucose internalized per cell**. The magnitude of glucose uptake induced by methanolic extract approaches that of the standard drug **metformin**, suggesting that methanolic extract contains bioactive compounds with **insulin-mimetic or insulin-**

sensitizing properties. These effects may be mediated through **activation of AMP-activated protein kinase (AMPK)**, up-regulation of **GLUT transporters**, or improvement of insulin signaling pathways (Zhou et al., 2001; Hardie et al., 2012; Foretz et al., 2014). Since impaired hepatic glucose uptake is a major contributor to **hyperglycemia in type-2 diabetes** (Saltiel and Kahn, 2001), the ability of methanolic extract to markedly increase 2-NBDG uptake in HepG2 cells provides strong **mechanistic evidence for its antidiabetic potential.**

Figure 4.12 shows the **quantitative bar graph of 2-NBDG–positive HepG2 cells**, representing cellular glucose uptake under different treatments. **Untreated HepG2 cells** exhibited negligible 2-NBDG positivity ($\approx 1\text{--}2\%$), confirming **low basal glucose uptake**. In contrast, **metformin (100 μM)** treatment produced a marked increase in glucose uptake, with approximately **78% of cells expressing 2-NBDG**. *Dolichandrone falcata* extract (100 μg) also significantly enhanced glucose uptake, with about **64% of cells becoming 2-NBDG positive.**

Figure 4.13 presents the **overlay histogram of 2-NBDG-FITC fluorescence** for untreated, metformin-treated, and methanolic extract–treated HepG2 cells. The **untreated population**



(black curve) is confined to the **low-fluorescence region ($\sim 10^1$)**,

Fig ; Effect of Metformin and test sample on glucose uptake in HepG2 cell

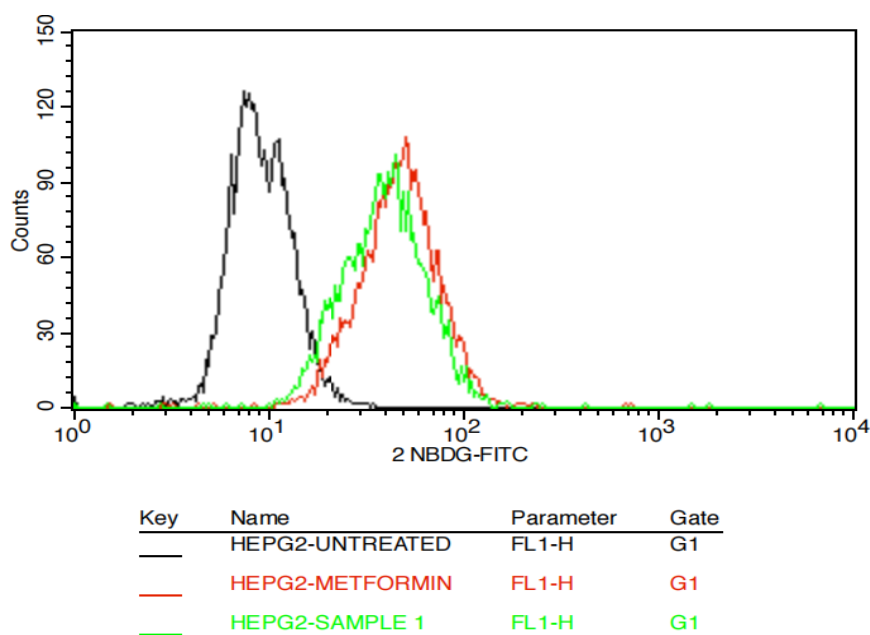


Fig :

Overlaid 2-NBDG-FITC fluorescence histograms of HepG2 untreated (black), metformin-treated (red), and methanolic extract–treated (green) cells

indicating minimal glucose uptake. The **metformin-treated population (red curve)** shows a strong **rightward shift toward $\sim 10^2$** , demonstrating a substantial increase in intracellular glucose accumulation. Importantly, the **Sample-1 treated population (green curve)** also exhibits a **pronounced rightward shift**, closely overlapping with the metformin profile, although slightly lower in intensity. Overall, glucose uptake followed the order:

Metformin (100 μ M) > DF extract (100 μ g) » Untreated

The significant increase in 2-NBDG uptake following **metformin treatment** confirms its well-known mechanism of action. Metformin activates **AMP-activated protein kinase (AMPK)**, which increases **GLUT-mediated glucose uptake** and suppresses hepatic glucose production, thereby improving insulin sensitivity (Zhou et al., 2001; Foretz et al., 2014; Hardie et al., 2012).

Importantly, *Dolichandrone falcata* extract produced a **glucose uptake response nearly comparable to metformin**, as evidenced by both the bar graph and the overlay histogram. The strong rightward shift of the green curve demonstrates that methanolic extract markedly enhances **cellular glucose transport**. This indicates that the phytoconstituents present in the extract likely possess **insulin-mimetic or insulin-sensitizing activity**, possibly via **AMPK activation, GLUT up-regulation, or antioxidant-mediated restoration of insulin signaling** (Kahn et al., 2005; Hardie et al., 2012). Since impaired hepatic glucose uptake is a major contributor to **hyperglycemia in type-2 diabetes** (Saltiel & Kahn, 2001), the ability of the DF extract to restore glucose uptake to levels approaching that of metformin provides **strong mechanistic evidence for its antidiabetic potential**. Thus, the combined quantitative and flow-cytometric data clearly demonstrate that *Dolichandrone falcata* extract **significantly enhances glucose uptake in HepG2 cells**, validating its promise as a **natural antidiabetic agent**. These findings are in agreement with several previous studies reporting glucose-uptake–

enhancing effects of medicinal plant extracts in hepatic and peripheral cell models. **Momordica charantia** (bitter melon) has been shown to stimulate glucose uptake in liver and muscle cells by promoting **GLUT-4 translocation and AMPK activation** (Tan et al., 2008; Shih et al., 2009). Similarly, **Trigonella foenum-graecum** (fenugreek) seed extract significantly increases glucose uptake in adipocytes and muscle cells through flavonoids and saponins that modulate glucose transporter activity and insulin signaling (Broca et al., 2004; Vijayakumar et al., 2005). **Cinnamon** (*Cinnamomum cassia*) extracts have also been reported to possess insulin-mimetic properties by stimulating glucose transport and enhancing insulin receptor signaling (Khan et al., 2003; Anderson et al., 2004). Furthermore, several medicinal plants are known to exert **metformin-like effects** by activating **AMP-activated protein kinase (AMPK)**, a key regulator of cellular energy metabolism. **Berberine**, an alkaloid isolated from *Berberis* species, has been shown to significantly increase glucose uptake in HepG2 cells through **AMPK activation**, closely resembling the mechanism of action of metformin (Zhou et al., 2008; Turner et al., 2008). Likewise, extracts of **Gymnema sylvestre** have been reported to enhance insulin sensitivity and glucose utilization by increasing glucose transporter expression and improving insulin signaling (Shanmugasundaram et al., 1990; Persaud et al., 1999). Similarly, **Ocimum sanctum** (holy basil) has been shown to improve glucose uptake and insulin sensitivity by modulating glucose transporters and intracellular metabolic pathways (Hannan et al., 2006; Jamshidi and Cohen, 2017).

The strong glucose uptake–stimulating activity observed for *Dolichandrone falcata* in the present study is therefore consistent with these well-documented effects of other antidiabetic medicinal plants. The significant rightward shift in 2-NBDG fluorescence and the high proportion of glucose-positive HepG2 cells (approximately 63–65%) indicate that the bioactive phytoconstituents of *Dolichandrone falcata* may act through similar molecular pathways, such as activation of AMPK, enhancement of GLUT-1 and GLUT-4 transporter activity, or improvement of insulin-signaling cascades.

Overall, the similar glucose uptake patterns of *Dolichandrone falcata* extract and metformin suggest that this plant may improve insulin sensitivity and glucose utilization. These findings support its traditional use in managing metabolic disorders. However, further studies are needed to identify the active compounds and understand how they work.

Conclusion

In antidiabetic activity the HepG2 liver cells showed *dolichandrone falcata* extract markedly enhanced glucose uptake compared to untreated cells, showing effects comparable to metformin and indicating its potential to improve glucose utilization and manage hyperglycemia. Overall, these findings suggest that *Dolichandrone falcata* possesses promising antidiabetic properties, warranting further studies to validate its efficacy and identify the active bioactive compounds.

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