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EVALUATION OF ANTIDIABETIC POTENTIAL OF *WITHANIA SOMNIFERA* BY GLUCOSE UPTAKE ASSAY ON 3T3 F442A FIBROBLAST (ADIPOCYTE) CELLS

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ABSTRACT: Antidiabetic potential of leaf and root extracts of *Withania somnifera* from Nimuch, Lucknow, Mumbai and Karnataka regions of India were evaluated against 3T3F442A fibroblast (3T3 adipocyte) cell line using glucose uptake assay. Leaf extracts from all the four regions were active on the cell line tested. Samples from Mumbai region found to be more active when compared with other regions. Root extracts also gave good glucose promoting activity in the presence of insulin. Nimuch samples were mere active than Mumbai samples. Isolated fractions and standard Withaferin-A and Withanolide-A were active at 20 µg/ml. This concentration was higher than that for crude leaf extract from Mumbai region and crude extracts from Nimuch region. Crude extract of *Withania somnifera* have good antidiabetic potential indicating the synergistic effect of these extract on glucose uptake of 3T3 fibroblast in presence of insulin.

INTRODUCTION: Diabetic mellitus (DM) is one of the most common chronic diseases in nearly all countries. This disease is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels. Medicinal plants offer alternative remedies with tremendous opportunities. They not only provide access and affordable medicine to poor people but can also generate income, employment and foreign exchange for developing countries.

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Although, new insulin and oral hypoglycemic agents are the mainstay of treatment of diabetes, they have prominent side effects and fail to alter the course of diabetic complications. The high costs of some agents and potential for adverse effects have led several investigators to focus their attention on the traditional medicines. One third of the diabetic patients take alternative medications that they consider efficacious [1]. In India, indigenous remedies have been used in the treatment of diabetes mellitus since sixth century BC [2].

Withania somnifera commonly known as Indian ginseng/ Ashwagandha has been an important herb in the Ayurvedic and indigenous medical systems for over 3000 years. It is a small, woody shrub in the Solanaceae family. The roots of the plant are categorized as rasayanas, which are routed to promote health and longevity by augmenting defense against disease, arresting the ageing

process, revitalizing the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and creating a sense of mental well being. It is in use for a very long time for all groups and both sexes and even during pregnancy without any side effects. *W. somnifera* has been revealed as an immune stimulator in immune suppressed animal models [3] and also an immune regulator in immune inflammation animal models [4].

A variety of biologically active constituents including alkaloids, steroidal lactones (also known as Withanolide), saponins containing an additional acyl group, sitoindosides (Withanolide with glucose molecule at carbon 27), flavonoids, and tannins were identified, extracted, and isolated from *W. somnifera* by phytochemical examination [5, 6, 7]. Up to date, over 130 Withanolide are known and more than 40 withanolides, 12 alkaloids, and several sitoindosides were isolated from different parts (leaves, roots and cherries) of *W. somnifera* and their structures were elucidated [8, 9, 10]. Withanolides are found mainly in leaves and account for 0.001 to 0.5% dry weight of the plants depending on the different species [11, 12, 13].

Plants growing under different agro-climatic condition often show qualitative and quantitative variations in their phytoconstituents India's diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). Of these, about 15000-20000 plants have good medicinal value [14].

Modern investigations have demonstrated anti-stress (adoptogen) effect of *Withania somnifera*. Anti-stress drugs are useful in management of several stress related disorders including, diabetes. Hence hypoglycemic potential of test plants from various regions of India viz. Karnataka, Neemuch, Mumbai, and Lucknow was undertaken using Antidiabetic cell line 3T3 fibroblast (adiopocyte).

MATERIALS AND METHODS:

Extraction of the plant materials: Leaves and roots of test plants were cleaned, air dried and crushed into coarse powder with mortar and pestle. 10 g of powdered leaves and roots were extracted respectively with 160 ml and 250 ml of methanol for 36 hrs using Soxhlet apparatus. The extracts were concentrated on water bath to 20 ml and stored in properly labeled clean, dry, screw capped bottles until use [15].

Antidiabetic activity: Determination of antidiabetic potential of the samples was done by Glucose Uptake Assay on Rat cell line 3T3 F442A (3T3-Adipocyte) cells and Glucose content in the cell lysate was determined by DNSA method. The test samples were compared with standard Withaferin-A and Withanolide-A. All the reagents used were of analytical grade and spectrophotometer analysis was measured by ELISA plate reader (Thermo scientific Revelation MRX).

Preparation of Investigational Samples: The samples (100µg/ml) were prepared in sterile PBS (Phosphate Buffered Saline). After solubilisation, different concentrations viz. 10µg/ml, 20µg/ml, 40µg/ml and 80µg/ml were subjected to the cells by serial dilutions. All test samples were freshly prepared just before use. The above concentration range was finalized for the study after repeated pilot experiments.

Experimental Procedure: 3T3- Adipocyte cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% Foetal Calf Serum and were seeded into a 96-well plate and incubated for 24 hrs. After 24 hrs the cells were washed and supplemented with serum free DMEM to attain serum starved condition. The Media was replaced by 20 µl of a 2-deoxyglucose mix containing 130 µl of glucose-free DMEM. The test compounds were added into the respective wells in triplicates and incubated for a further 5 hours in with and without insulin condition. After the incubation, cells were observed under microscope. The Supernatant was discarded and the cell lysate was used to determine the Glucose content by DNSA method. The plate was read at 570 nm. The readings of the test samples were scored on the basis of comparison with the zero control. The Optical Density of the zero control is considered as 100% viability. Percent viability of the test substances is calculated in comparison with the control.

$$\text{Percent Increase} = \frac{t}{c} \times 100$$

where,

t - optical density of test substance
c - optical density of the Vehicle control

Statistical Analysis: Three separate experimental sets were performed. Difference between groups in experiments was analyzed for statistical significance by ANOVA ($p < 0.05$).

RESULTS:

Antidiabetic activity: The results obtained indicate significant glucose uptake. Among all the study extracts, it was found that glucose uptake was optimum in presence of insulin. The leaf crude extract showed highest percentage of glucose uptake (108.53%) in Mumbai region sample (WSM-L) when 10 µg/ml of sample was used. On increasing the dose concentration to 20µg/ml, 121.22% glucose uptake was measured in WSM-L sample whereas WSL-L sample also showed significant glucose uptake (103.06%) (**Fig. 1**). (**Table 1**)

The majority of extracts tested showed highly significant glucose uptake when 40 µg/ml of dose concentration was used where WSM-L sample indicated highest glucose uptake (158.21%) in presence of insulin (**Fig. 2**). However, 80 µg/ml dose concentration did not show any significant improvement in glucose uptake and the glucose uptake was declined in absence of insulin which could be due to the toxicity of increased dose.

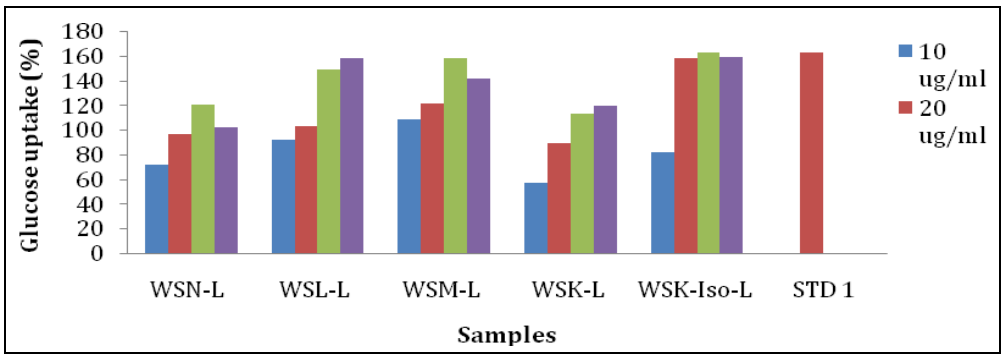

FIG. 1: ANTIDIABETIC POTENTIAL OF THE LEAF EXTRACTS OF TEST SAMPLES ON 3T3F442A FIBROBLAST (3T3 ADIPOCYTE) CELLS BY GLUCOSE UPTAKE ASSAY.

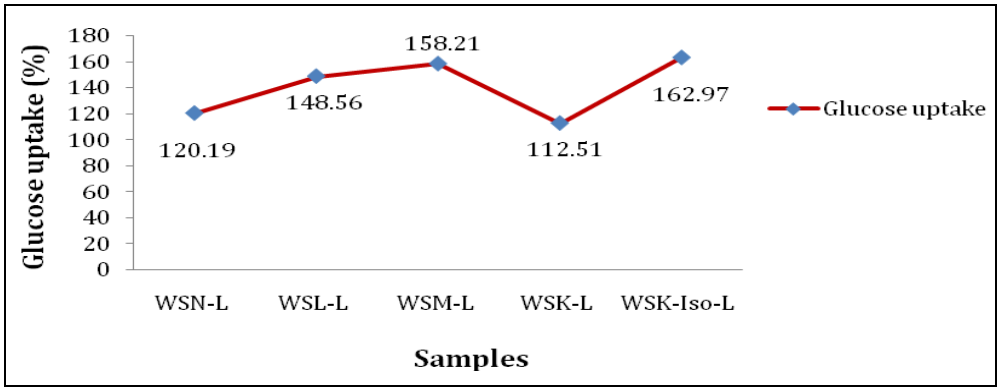

FIG. 2: GLUCOSE UPTAKE IN 40 µG/ML OF LEAF EXTRACT USED

The glucose uptake in 10 µg/ml was observed non significant in root crude extract of all four different regions except WSN-R sample which was measured 121.04% (**Table 2**). The increased dose (40µg/ml) showed improved glucose uptake as compared to 20 µg/ml dose concentration (**Fig. 3**). The root crude extract of Nimuch region (WSN-R) showed maximum glucose uptake i.e. 160.08 % (**Fig. 4**). The glucose uptake was declined in 80 µg/ml dose concentration of all tested root crude extract samples. Among all the studied extracts, the pronounced glucose uptake was shown by leaf extract as compared to vehicle control. The isolated Withaferin-A from leaf extract sample showed highly significant glucose uptake at 40 µg/ml dose concentration matching with standard glucose

uptake. The results obtained clearly indicate that the crude extract has synergistic effect in glucose uptake in presence of insulin. Therefore, it can be the crude extract of *W. somnifera* may have good antidiabetic potential.

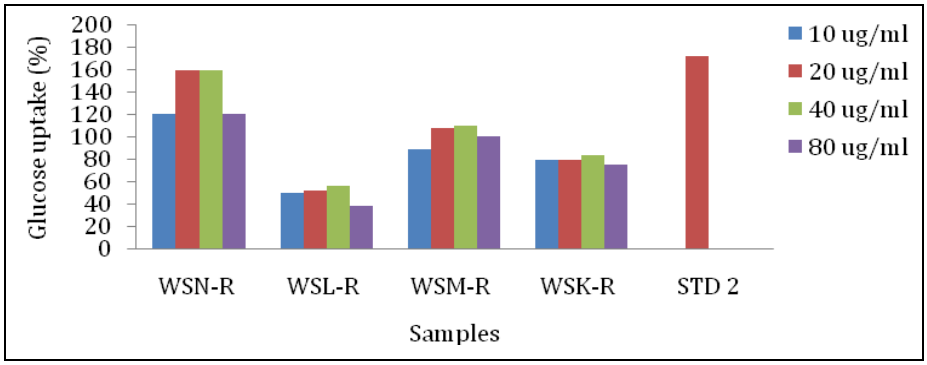

FIG. 3: ANTIDIABETIC POTENTIAL OF THE ROOT EXTRACTS OF TEST SAMPLES ON 3T3F442A FIBROBLAST (3T3 ADIPOCYTE) CELLS BY GLUCOSE UPTAKE ASSAY

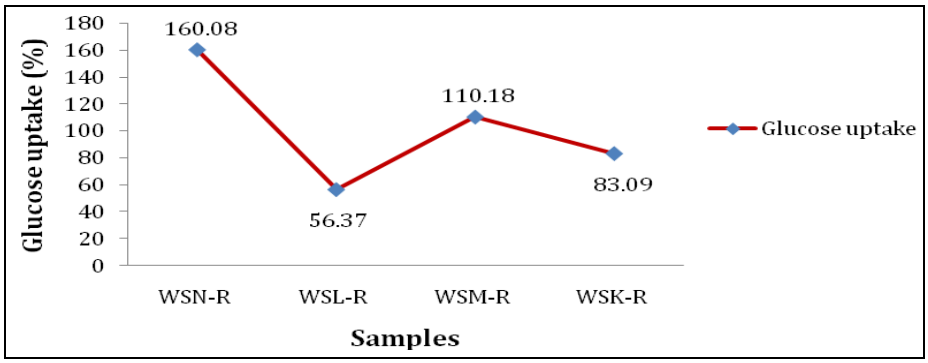

FIG. 4: GLUCOSE UPTAKE IN 40 µG/ML OF ROOT EXTRACT USED

TABLE 1: ANTIDIABETIC POTENTIAL OF THE LEAF EXTRACT OF TEST SAMPLES ON 3T3F442A FIBROBLAST (3T3 ADIPOCYTE) CELLS BY GLUCOSE UPTAKE ASSAY

Concentrations	10µg/ml		20µg/ml		40µg/ml		80µg/ml	
Insulin	Absent	Present	Absent	Present	Absent	Present	Absent	Present
WSN-L	7.32 ±0.27	71.30 ±2.68	15.13 ±1.03	96.17 ±1.10	21.17 ±0.87	120.19 ±0.74	20.86 ±0.22	102.31 ±0.84
WSL-L	30.52 ±0.50	92.14 ±2.26	34.51 ±1.46	103.06 ± 3.77	45.91 ±2.06	148.56 ±0.44	40.77 ±3.32	158.37 ±0.70
WSM-L	40.72 ±1.49	108.53 ±2.51	41.59 ±1.43	121.22 ±3.99	50.06 ±2.62	158.21 ±1.71	40.83 ±2.03	141.41 ±0.62
WSK-L	5.52 ±0.59	56.71 ±2.28	8.24 ±0.86	89.14 ±1.68	15.65 ±1.05	112.51 ±0.48	10.22 ±0.85	119.15 ±0.84
WSK-Iso-L	38.89 ±0.26	81.86 ±2.48	58.98 ±1.90	158.24 ±3.22	63.43 ±0.59	162.97 ±1.38	59.21 ±0.79	159.09 ±0.78
WS-Std 1	NA		60.72 ± 1.59	162.34 ± 5.80	NA		NA	

TABLE 2: ANTIDIABETIC POTENTIAL OF THE ROOT EXTRACTS OF TEST SAMPLES ON 3T3F442A FIBROBLAST (3T3 ADIPOCYTE) CELLS BY GLUCOSE UPTAKE ASSAY.

Concentrations	10µg/ml		20µg/ml		40µg/ml		80µg/ml	
Insulin	Absent	Present	Absent	Present	Absent	Present	Absent	Present
WSN-R	44.73 ±1.70	121.04 ±4.87	53.55 ±1.31	159.57 ±4.71	58.54 ±1.41	160.08 ±1.22	40.33 ±4.44	120.77 ±0.47
WSL-R	10.19 ±1.08	50.13 ±0.90	10.36 ±1.13	52.24 ±1.65	17.88 ±0.71	56.37 ±0.88	5.17 ±0.16	38.15 ±0.20
WSM-R	35.28 ±2.51	88.97 ±1.62	41.90 ±2.34	108.41 ±2.41	51.19 ±1.97	110.18 ±0.49	40.60 ±1.57	100.26 ±0.42
WSK-R	20.03 ±1.67	79.66 ±2.16	21.80 ±1.12	79.65 ±1.27	29.23 ±1.94	83.09 ±0.64	20.52 ±0.52	75.20 ±0.20
WS-Std 2	NA		68.16 ±2.91	172.62 ± 0.88	NA		NA	

All the value represent (Mean ± S.D) derived from three replicates. The results are statistically significant ($p<0.05$)

DISCUSSION: Leaf extracts from all the four regions were active on 3T3F442A fibroblast (3T3 adipocyte) cells using Glucose Uptake Assay. Samples from Mumbai being active at >10 ug /ml and that from Lucknow at concentration above 20 gm/ml, in the presence of insulin. Root extracts gave good glucose promoting activity at 40ug/ml, in the presence of insulin as compared to vehicle control.

Nimuch samples being active at lower concentration (above 10ug/ml) than Mumbai samples (above 20ug/ml).

Isolated fractions and standard Withaferin A and Withanolide A were active at 20 ug/ml indicating superiority of Mumbai leaf samples (crude extract) and Nimuch root samples which are active at lower concentration.

The results obtained clearly indicate that the crude extract has synergistic effect in glucose uptake in presence of insulin, therefore it could be the crude extracts of *Withania somnifera* that have good antidiabetic potential. Though Mumbai and Lucknow samples are evolutionarily related [16], no clear relationship could be drawn between either genetic similarity and antidiabetic potential.) or environmental condition and antidiabetic potential (Environmental condition at Mumbai and Nimuch are quite different though the samples showed almost equal antidiabetic potential).

The search for a less expensive new drug for diabetes treatment which has more potential and has no side effects is the need of the day [17]. A large number of medicinal plants have been used in the treatment of diabetes in different parts of the world. WHO[18] has recommended the use of plants as an alternative therapy for diabetes. Evaluation of the antidiabetic potentials of these plants becomes necessary to provide scientific proof and justify their uses on ethnomedicines. In vitro antidiabetic activity of five different medicinal herbs used in Chinese medicinal formulae have been evaluated on mouse adipocytes 3T3 cell lines among others[19]. The tested herbs viz. *Fructus corni*, *F. Schisandrae, Rhizoma alismatis* showed significant stimulatory effects on the glucose uptake of 3T3 L1 cells.

Use of animal models for hypoglycemic activity studies seems to be preferred method using cultured cells for glucose uptake. The hypoglycemic activity of ethanolic root extracts of *Withania somnifera* was tested on streptozotocin treated diabetic sprayul rats over a period of three weeks and prominent hypoglycemic activity was reported in lowering blood glucose in type I diabetic model [20].

Hypoglycemic and hypolipidaemic effect of *Withania somnifera* root and leaf extract on Alloxan induced diabetic albino wistar strain rats has also been reported [21]. Antidiabetic effect of *Withania coagulans* aqueous fruit extracts evaluated [22] using SDZ induced diabetic male albino wistar rat and attributed the same to the high concentration of Mg^{+2} along with Ca^{+2}, since high concentration of Mg and lower concentrations of K are known to play a vital role in diabetic management [23, 24] and Ca^{+2} are known to activate insulin gene expression via Calcium Responsive Element binding protein responsible for exocytosis of stored insulin [25]. Antidiabetic activity of purified Withanolides obtained from hydroalcoholic extracts of dried berries was also reported[26]. Similarly, purified Withanolides of *W. somnifera* revealed significant inhibition of post prandial rise in hypoglycaemia post sucrose load in SDZ induced diabetic rats [27].

Hypoglycemic activity of several other medicinal plants using induced diabetic animal models has also been demonstrated by many workers. The methanolic stem bark extracts of *Adansonia digitata* has shown significant results in lowering blood sugar level in streptozocin induced diabetic wistar rats[17]. Same animal models were used again to demonstrate antidiabetic properties of *Securine gavirosa* leaf extracts[17].

CONCLUSION: In this study, the crude extract of *Withania somnifera* have good antidiabetic potential indicating the synergistic effect of these extract on glucose uptake of 3T3 fibroblast in presence of insulin.

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