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Anti - hyperglycemia activity by ethanolic extract *Aloe vera* of against mouse 3T3-L1 adipocyte cell line

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ABSTRACT

Several traditional medicinal plants have been investigated concerning their anti-obesity potential one such sources is *Aloe vera*. The medication is reported for different helpful properties like, love potion and counteractant, healing arsenic harming, tonic, restoring, diuretic, purgative, uterotonic, sterility in ladies, etc. These are medicationally very helpful especially in curing diabetes. The plants are explored for many different biopotential activities like antiulcerogenic, hypolipidemic, nephroprotective, mitigating, antidermatophytic, cancer prevention agent, antimicrobial and insecticidal exercises. In this study we performed phytochemical screening of various *Aloe vera* extracts to compare its bioactive potential. The control group of 700µg was compared with ethanolic extract of *Aloe vera* and observed that it reduced the expression of TNF α (Tumor necrosis factor) and Interleukin 6 (IL-6). It even increased the expression of adiponectin which revealed the presences of different phytochemicals are present in ethanol extract. The data created by this specific investigation gives pertinent pharmacognostic and phytochemical information required for appropriate distinguishing proof and verification of this specific species. The present study emphasis in finding the anti-hypergycemic activity of *Aloe vera* on cell lines and highlights the phytochemical and pharmacological aspects of *A. vera*.

Keywords: 3T3L1 Cell line, *Aloe vera*, Adipogenesis, antiulcerogenic, phytochemical.

1. INTRODUCTION

Aloe vera probably originated in northern Africa and believed to be from Sudan. Subsequently it was introduced in the Mediterranean region and other warm areas of the world. It is widely naturalized in temperate and tropical regions of Australia, Barbados, Belize, Nigeria, Paraguay and the United States [1]. The plant abundantly found in India,

Mexico, Pacific Rim countries, South America, Central America, the Caribbean, and many other regions. It is even widely cultivated throughout the world due to its various medicational and commercial benefits. Some species are cultivated for major commercial purposes, throughout the globe. In India, it is found in Rajasthan, Haryana, Punjab, Andhra Pradesh, Gujarat, Maharashtra and Tamil Nadu [2]. *Aloe vera* is a perennial, xerophytic, succulent plant with turgid green leaves with growth up to 60 to 100 cm length with spreading offsets. The outer protective layer is known as rinds which are made of is made of 15 to 20 cells followed by the centre layer, containing latex or yellow sap and finally the inward layer containing adhesive gel. The *A. vera* bears flower in summer, each flower being pendulous, with a yellow tubular corolla [3].

Its succulent property enables the species to survive in low rainfall areas, making it ideal for rockeries and other low-water use gardens. The species is hardy, although it is intolerant to very heavy frost or snow. During winter, *A. vera* has complex chemical ingredients. The main chemical components establish in Aloes can be classified into nine categories: anthraquinones, inorganic compounds, enzymes, vitamins, essential amino acids, non-essential amino acids, carbohydrates, fatty acids, and other miscellaneous chemicals. Among these constituents, anthraquinones are the most significant dynamic element of high clinical qualities. Blue light has been found to be beneficial for the accumulation of anthraquinones. *A. vera* also contains products of the isoprenoid pathway, which are carotenoids, steroids, terpenes and phytosterols [4]. Isoprenoids can be regarded as sensory molecules because they contribute to the color and fragrance of the products in which they exist, become dormant, during which little moisture is required.

Techniques based on DNA comparison suggest that *A. vera* is relatively closely related to *Aloe perryi*, a species that is endemic to Yemen. Comparative strategies, utilizing chloroplast DNA arrangement correlation and ISSR profiling have additionally recommended that *A. vera* is firmly identified with *Aloe forbesii, Aloe inermis, Aloe scobinifolia, Aloe sinkatana* and *Aloe striata.* [5].

As the main component of adipose tissue, adipocytes and the study of their biochemical and morphological characteristics have received great attention over the years. Numerous in vitro cellular models have been established, and among these models, the 3T3-L1 cell line is one of the most commonly employed preadipose cell models. It is a substrain of 3T3 cells, which is derived from disaggregated 17- to 19-day Swiss 3T3 mouse embryos through clonal isolation and is chosen based on its inclination to accumulate triacylglycerol lipid droplets. The high risk and prevalence of obesity have led to the exploration of various prevention and treatment strategies. Previous anti-obesity strategies mainly focused on the suppression of adipocyte hypertrophy, which is one of the three major events (together with adipocyte hyperplasia and angiogenesis) involved in the development of obesity. Many models and techniques are being used to evaluate and understand adipocyte biology [6]. 3T3-L1 is a pre-adipose cell line which was originated from the clonal expansion of murine Swiss 3T3 cells and contains only a single cell type. Several investigations use 3T3-L1 cells because it helps in identifying key molecular markers including transcription factors and various pathways during pre-adipocyte differentiation [7]. Pre-adipocytes contain less amount of lipid droplets accumulated, but four days after induction they start to accumulate lipids that grow in size and number over the differentiation time [8]. Cytotoxicity investigation of medicinal plants only considers a

single cell type, metabolic pathway or enzyme, which reduces the possibility of identifying an antidiabetic compound. In vitro assays also only measure acute or immediate effects but does not consider the effect after chronic exposure to these bioactive compounds. Moreover, both in vitro and in vivo studies demonstrated that resveratrol has great potential in the management of obesity [9]. Resveratrol supplementation is capable of relieving the harmful effects induced by a high-calorie diet such as reducing the rodent's body weight gain, adipose tissue depots, plasma triglycerides and increasing their survival and motor function [10]. It is important to emphasize that in this study, in vitro cytotoxicity assays using Chang liver cell line is of low concordance with human liver toxicity, thus predictions of the clinical hepatotoxicity potential will be unreliable. To maximize the predictive capacity of this pre-anti-diabetic cytotoxicity screening, different endpoints are assessed to more comprehensively characterize the nature of the plant *Aloe vera* from extracts.

2. MATERIALS AND METHODS

2.1. Plant Collection

Fresh, healthy, and young *Aloe vera* plants were collected from Saliyamangalam (10.7821° N, 79.2756° E) at Thanjavur district, Tamilnadu, India. The freshly collected specimen was carefully brought to lab and preserved for further screening process.

2.2. Extract preparation

The *Aloe vera* plants were cleaned and dried in shades. The dried leaves were reduced to fine particles using dry blender and was sieved through 20 µ mesh sieve. The fine powdered *Aloe vera* was dissolved to ethanol and extract was prepared using soxhlet apparatus. The extraction was carried out for 24 hrs. at room temperature. The concentrate were separated and assembled at 45 °C utilizing rotating vacuum evaporator. The extract obtained was vacuum dried and used for further screening.

2.3. Phytochemical analysis tests

The phytochemical analysis of ethanolic extract of *Aloe vera* was performed using Khandelwal (2002) and Kokate (2005) [11, 12] method with slight modifications. The screening was conducted to evaluate the secondary metabolites such as Tannin, Phlobatannins, Saponin, Flavonoids, Steroids, Terpenoids, Triterpenoids, Alkaloids, Carbohydrate, Protein, Anthraquinone, Polyphenol and Glycoside.

2.4. Cell Culture

The 3T3-L1 cell line was obtained from Kings Institute of Preventive Medicine and Research, Guindy, Chennai. The cells were grown in culture flasks containing DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS. Upon reaching confluence, the cells were detached using trypsin-EDTA solution and used for subculture and the pH was adjusted to 7.4. The final volume was created up to one metric capacity unit with water. The medium was sterile filtered (0.22 µm) and stored at 4°C. The cells were grown in 96-well tissue culture (TC) plate in Dulbecco's Minimum Essential Medium (MEM) with Trypsin-phosphate-verseneglucose (TPVG) solution, 10% New Born Calf Serum (NBCS) (Gibco-Invitrogen), 100 U/mL of penicillin (Gibco-Invitrogen) and 100 µg/mL of streptomycin (Gibco-Invitrogen). The cells were incubated in CO2 incubator (Haier Electric Co., Ltd.,) at 37oC in 95% humidified atmosphere enriched by 5% CO2 and sub-cultured once in every 3-4 days.

2.5. MTT cell viability assays

The MTT assay was performed by using Mossman T (1983) [13] method to test the cytotoxic effect of the *Aloe vera* ethanolic extract. It is a colorimetric assay in which the MTT reduction is measured in presence of substrate tetrazolium dye named as 3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide by mitochondrial succinate dehydrogenase, forming an insoluble, dark purple formazan product. The monolayer of cell culture was trypsinized and the cell count was adjusted to 1.0 x 105 cfu/ml. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once and 100µl of *Aloe vera* ethanolic extract with different concentrations (100, 200, 250,500 and 1000µg/ml) was added to each well. The plates were then incubated at 37oC for 3 days in 5% CO2 atmosphere, and microscopically examined at the end of 6, 12, 24 and 36 hours and result aws recorded. After 72 hours, the test solutions in the wells were discarded and 50 µl of MTT in HBSS-PR was added to each well. The plates were gently shaken and incubated for 3 hours at 37oC in 5% CO2 atmosphere. The supernatant was removed and 50 µl of propanol was added and therefore the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader BMG LABTECH- at the wavelength of 540nm. The percentage growth inhibition was calculated using the formula given below:

% Cytotoxicity = (1-Abs test/Abs Control) × 100

Table 1. Qualitative analysis of Phytochemicals analysis *Aloa vera* ethanolic extract

Sr. No.	Phytochemical analysis	Ethanol	Methanol
1	Tannin	++	+
2	Phlobatannins	-	-
3	Saponin	+	+
4	Flavonoids	++	+
5	Steroids	+	-
6	Terpenoids	+	+
7	Tritrpenoids	+	-
8	Alkaloids	+	+
9	Carbohydrate	+	-
10	Protein	-	+
11	Anthroquinone	+	-
12	Polyphenol	++	+
13	Glycoside	+	+

Indications: "+" means positive activity, '-' means negative activity

2.6. Glucose uptake

The test was performed according to Alonso-Castro et al, 2008 [16] with some minute changes for standardization process. 3T3-L1 preadipocytes were cultured in Dulbecco's Modified Eagle's Medium (DMEM) under standard tissue culture conditions. 3T3-L1 cells were differentiated using a mixture of 500 µM IBMX, 1µg/mL Insulin and 1µM dexamethasone with 10% FBS for first three days and subsequently subjected to 10nM Insulin and 10% FBS every two days for 10-14 days. Cells from early passage numbers were used for the experiments. The treatment was continued for fourteen days. Cells were treated with 700µg ethanol extract of *Aloe vera* plant for fourteen days by replacing the media after every 48 hrs. The Protein expression of TNF α (Tumor necrosis factor) (cell signaling protein (cytokine) involved in systemic inflammation and is one of the cytokines that make up the acute phase reaction). To determine the effects of quercetin or isorhamnetin on cell viability during adipocyte differentiation period of 8 days, MTS cell viability assay was performed on 3T3-L1 cells treated with 0 to 25 µM quercetin or 0 to 10 µM isorhamnetin, and non-differentiated cells maintained solely on PEM. Data were collected on days 1, 3, 5, 7, 8 and 14. The cell viability results are expressed as the percentage

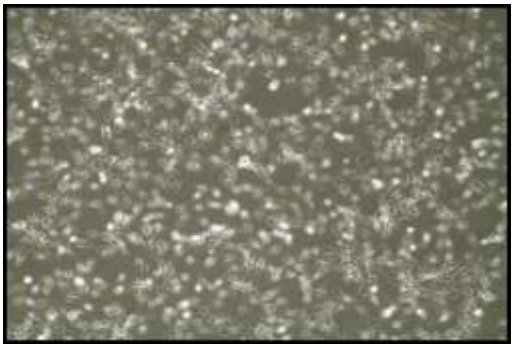

Fig 1. 3T3 L1 pre-adipocytes

test cells surviving compared to control cells. Treatments were performed in triplicates. [16]

2.7. Statistical analysis

Statistical analysis was performed by one way Analysis of Variance (ANOVA) followed by Duncan's multiple range test (DMRT) using Software Package for the Social Science (SPSS) software package version 15.00. Results were communicated as Mean Standard Deviation for p values <0.05 and considered significant for analysis of percent inhibition of cell growth.

3. RESULTS AND DISCUSSIONS

The phytochemical analysis of ethanolic and methanolic extract of *Aloe vera* reveals that *Aloe vera* is rich in secondary metabolites. In the ethanolic extract flavonoids, tannins and polyphenol are majorly found whereas the methanolic extract shows fairly presence of the metabolites (Table 1). The study revealed that the ethanolic extract of *Aloe vera* provided maximum number of metabolites providing some release for its bioactivity. The methanolic extract also revealed the presence of metabolites but they lack some as compared to ethanolic extract. The secondary metabolites in plant plays major role in the survival of the plant in its environment. There are several studies which prove that different solvent extracts of *Aloe vera* constituent of different phytochemical like steroids, triterpenoid, Anthraquinone glycosides, proteins etc. In previous study it was observed that ethyl acetate extract showed moderately better phytochemical composition as compared to ethanolic extract. These constituent plays an important role and deficiency of any one constituent may lead to abnormal developments in the body [14]. They serve a variety of ecological functions leading to ultimate enhancing the plants during stress. In addition the metabolites may be responsible for the bio-potential effects in plants on an array of health related measures. Medicinal plants serve great importance in curing some severe diseases supposedly without any side effects and used as alternative to allopathic medicines across the world [15].

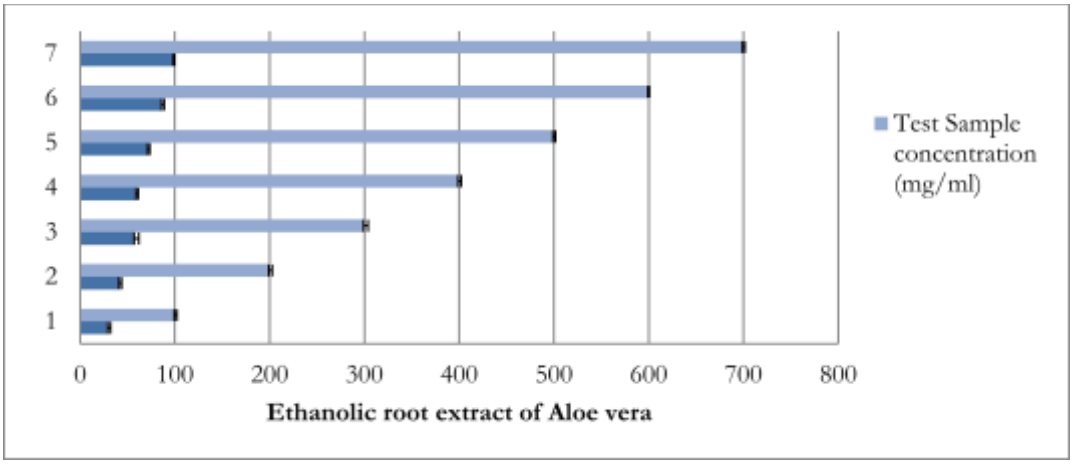

Fig 2. Cell cytotoxicity Assay

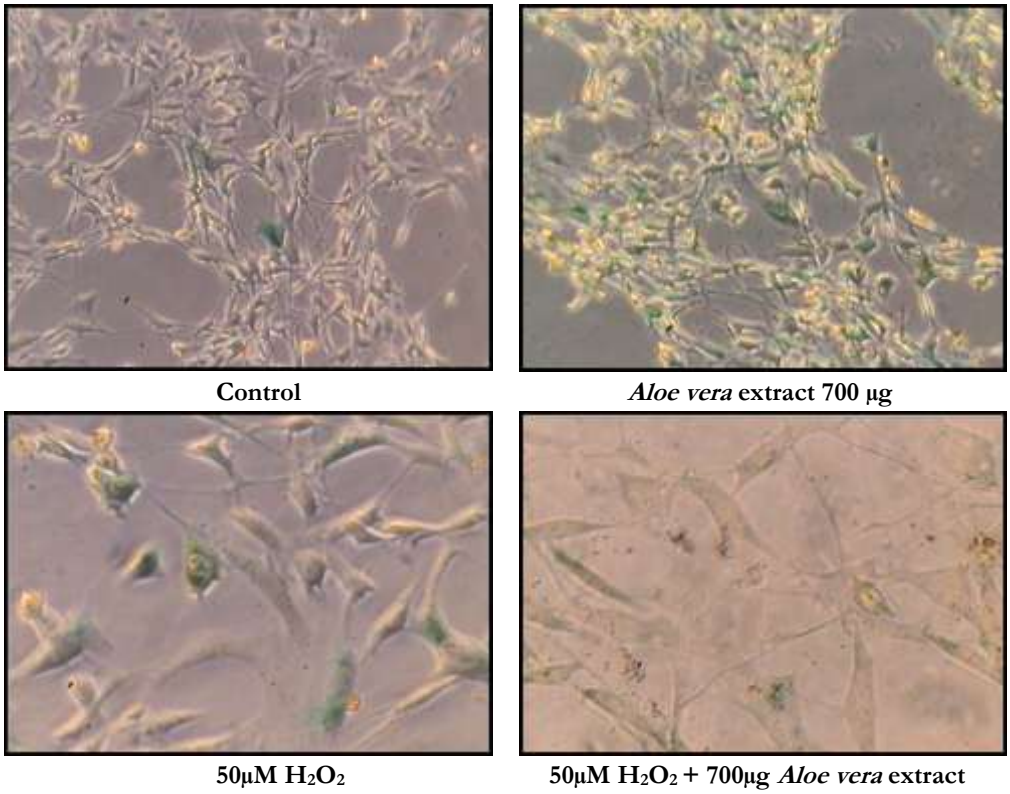

Fig 3. Oxidative Stress induced by Hydrogen peroxide (H2O2) in 3T3 L1 adipocyte cells

The MTT assay was performed to test for cytotoxicity: is a quantitative and reliable colorimetric test that is based on the enzymatic reduction of yellow water-soluble MTT dye by mitochondrial succinate dehydrogenase to form a purple formazan product (insoluble in water) which measures viability, proliferation and the activation of cells. The MTT assay measures cell membrane integrity by determining mitochondrial activity through an enzymatic reaction to the reduction of MTT to formazan. The effects of plant extracts on insulin action as well as insulin-mimetic activity can thus be assessed by measuring basal- and insulin-stimulated glucose uptake in muscle and adipocytes cell lines such as L6 and 3T3-L1. These cell lines have been used as a model for insulin-regulated glucose transport. Once glucose is taken up by the myocytes and adipocytes, it can either be oxidized to carbon dioxide and water, stored as glycogen or fat. Glycogen storage is regulated by glycogen synthase and the impairment of glycogen synthase activity has been reported to be one of the earliest defects in skeletal muscle seen in T2DM [17].

The *Aloe vera* ethanolic extract showed the highest percentage of cell viability at the tested concentration as indicated by both MTT and crystal violet assays, which could indicate that the extract is both non-toxic and may possess active principle(s) with mitogenic effect or is able to induce the expression of growth-stimulating factors. The glucose utilization observed in the ethanolic extract of *Aloe vera* was comparable to that of insulin, and therefore the result reported in this study using L6 and 3T3-L1 differentiated cells established that the *Aloe vera* ethanolic extract can improve glucose uptake in muscle and fat cells respectively. This increase in glucose utilization in the L6 and 3T3-L1 differentiated cells suggests that the *Aloe vera* ethanolic extract possesses insulin-mimetic activity but the underlying mechanism needs to be established.

As a natural phenol rich in the plant mulberry (Morus alba L.), oxyresveratrol is similar in structure with resveratrol and has been identified to hold various biological functions similar to those of resveratrol. In recent years, the anti-obesity effect of resveratrol has been well established and many studies demonstrated that resveratrol was effective in lowering body weight and adiposity in rodents fed a high-calorie diet by inducing favorable changes in specific genes and proteins expressions. Adiponectin expression and early markers of differentiation were rescued in Lo glucose by pulsing with NAM. Because we found that total NAD levels were already rising in 3T3-L1 adipocytes on Day 3 of the pulse with 25mM glucose or 42mM pyruvate, it is possible that NAD+ was limiting in the Lo glucose cells, preventing differentiation from proceeding. These data are consistent with NAM and were required for the development of ppar-γ mRNA expression in umbilical cord-derived mesenchymal stem cell differentiation in vitro in 4 mM glucose.

3T3-L1 adipocytes have traditionally been grown and differentiated in DMEM media containing 25mM glucose, a supraphysiological concentration. We wanted to learn if high levels of glucose were required for adipocyte differentiation and maturation to occur. To test this, cells were cultured in media containing either 4mM (Lo) or 25mM (Hi) glucose during their proliferation (P) phase, differentiation (D) phase, and maturation (M) phase (as indicated in. Maximal expression of GLUT4 at day 9 post-differentiation required Hi glucose during the 3-day D phase [18]. 25mM glucose in either the Por M-phases was not required. In contrast, adiponectin expression was optimal when cells were pulsed with Hi glucose during either the D-phase or the M-phase. These data suggest that the role of glucose in adipocyte development and gene expression may impact multiple signaling pathways during the developmental program. Glucose-dependent gene expression was also observed during differentiation of primary stromal vascular cells isolated from subcutaneous fat from mice indicating that the glucose requirement was not unique to the transformed 3T3-L1 cells.

Glucose is used for the de novo lipogenesis in rodent adipose tissue [19]. In the present study 1a-hydroxylase mRNA was expressed in the 3T3-L1 cells. Similar to the VDR mRNA, 1a-hydroxylase expression appears to peak early during the differentiation process at the 6 hour point. The addition of the vitamin D metabolites after initiation of differentiation is associated with a decrease in the 1a-hydroxylase gene expression. However a peak in expression was noted at the same time point of 6 h in the cells treated with 1, 25-D and 25-D similar to the control group. It is known that 1, 25-D is a negative regulator of 1a- hydroxylase and upregulates 24-hydroxylase activity at an endocrine level in the body which could potentially explain a similar negative effect in its expression on addition of 1,25-D at the tissue specific level [20]. Cellular viability and proliferation are considered to be important functional characteristics of healthy and actively growing cells. Therefore an increase in cell viability indicates cell proliferation in a non-toxic environment while a decrease in cell proliferation indicates cell death due to toxicity.

4. CONCLUSION

Nature has talented us with bountiful plants having restorative ethics which can fix a few ailments. Currently, diabetes mellitus is a severe threat to human well-being across the world due to the rapidly increasing incidence of diabetes. New effective bioactive drugs are in need, as plants do harbour and are proven to have potential antidiabetic activity than the present hypoglycemic medicines used in clinical

therapy. In this study, in vitro cytotoxicity, glucose uptake, and anti-adipogenic activities in vitro cell line from ethanolic extract of *Aloe vera* possessed the potential antibacterial activity, antioxidant and anticancer activity and it could be used as natural source for treating human diseases. Free radicals/ROS/RNS are produced mainly from diverse sources in the biological system causing a cascade of oxidation events leading to disruption of cellular membranes and attacking other major cell organelles. A cancer prevention agent as a rule works by hindering this procedure of oxidation brought about by these flimsy substances. To compare to control group of 700µg from ethanolic extract of *Aloe vera* reduced the expression of TNF α (Tumor necrosis factor) and Interleukin 6 (IL-6). To compare to control group of 700µg from ethanolic extract of *Aloe vera* increased the expression of adiponectin.

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6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

7. SOURCE/S OF FUNDING

No source of funding

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