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ORIGINAL RESEARCH ARTICLE

Effect of *Abutilon indicum* (L) Extract on Adipogenesis, Lipolysis and Cholesterol Esterase in 3T3-L1 Adipocyte Cell Lines

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Abstract *Abutilon indicum* (L) is an Indian traditional plant used for the treatment of diabetes and heart diseases. The present study is to evaluate the functional of *A. indicum* leaf extract as insulin like character to inhibit lipolysis and stimulates Adipogenesis activity. The ability of the *A. indicum* leaf extract in anti-obesity effect of Adipogenesis, lipolysis and cholesterol esterase functions can be predicted by using 3T3-L1 adipocyte cell lines. Substances were isolated from *A. indicum* leaves and the double filtered crude sample were used for Adipogenesis, lipolysis and cholesterol esterase activity using 3T3-L1 adipocytes at different concentrations. We used differential media-I, differential media-II and maintenance media (MM1) at concentrations of 20, 40, 60, 80, 100, 200 and 400 µg/mL respectively. In addition to the extract, there is a significance increase in glycerol release ($p < 0.001$) compared with crude and reference compounds. Cholesterol esterase activity predicts the IC_{50} = 27.11 µg/mL of orlistat positive control compare with IC_{50} = 8.158 µg/mL of crude extract. Based on the observation, *A. indicum* leaf extract can promotes lipolysis and differentiated adipocytes. It is potentially used as adjuvant in the treatment of Type 2 diabetes.

Keywords *Abutilon indicum* (L) · Adipogenesis · Lipolysis · Cholesterol esterase

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Introduction

Diabetes mellitus is a clinical syndrome that is epidemic in human populations around the world. And it is referring to a metabolic disease caused by an inability of the pancreas to produce insulin adequately to lower blood sugar levels and is a metabolic disorder. Obesity is a complex chronic disease involved in the storage of excess energy based on the diet, lifestyle, family inheritance and environmental factors [1]. Based on the sedentary lifestyle, people are not maintaining their healthy lifestyle due to several reasons that are often challenging to cause obesity [2]. However, many pharmaceutical industries are producing large quantity of anti-obesity drugs to manage obesity but have a serious adverse effect that leads to depression, anxiety, and cardiovascular risk. Based on our dietary content, many phytogenic substances must be used in diet that can be applied as alternatives to anti-obesity agents with less or no toxic side effects.

The accumulation of fat in the adipose tissue leads to an increase of adipocyte numbers and adipocyte size, which can disrupt the metabolism and result in obesity, type 2 diabetes, and cardiovascular diseases [3–5]. In insulin-sensitive individuals, glucose is not readily absorbed for

metabolism, increasing cholesterol levels [6]. Lipolysis, defined as the process of hydrolytic enzymes triglycerides (TG) into glycerol and free fatty acids, plays a critical role in the management of energy balance. Through this process, free fatty acids (FFA) are released into the blood stream with the purpose of going to other tissues and exerting different effects throughout the body or being re-esterified by the adipocyte [7].

The excessive cholesterol level leads to decrease lipolytic activity which regulates the hydrolysis of triglycerides. The triglycerides (TAG) can hydrolyze into diacylglycerol (DAG), then monoacylglycerol (MAG), along with fatty acid release in each step and finally MAG is converted into glycerol and fatty acids that can release into the circulation. Released fatty acids (FA) may be oxidized in muscle and glycerol may be used as precursor for gluco-neogenesis in the liver. Catecholamine stimulates lipolysis that can have impaired insulin sensitivity and anti-lipolytic effect that can enhance basal lipolysis in obesity [7, 8]. There is a precise cause of obesity and regional differences that alter remain elusive and effective agents for mobilization of these low-responding adipocytes are needed.

As a result, any chemical that inhibits lipid accumulation while increasing lipolysis in adipocytes is a potential anti-obesity substance. Adipocytes in the adipose tissues (ATs) can be exposed to a variety of internal and external stimuli, such as pro-inflammatory cytokines, free fatty acids, or lipopolysaccharide (LPS), and they are thought to express and secrete a variety of inflammatory factors, eventually leading to obesity inflammation [9]. Adipocyte differentiation and the quantity of fat accumulated have been implicated to the incidence and progression of obesity in several studies. As a result, inhibiting adipocyte differentiation is a therapy option for obesity. Adipogenesis is a multi-step process involving the expression of several genes. Metabolism derivatives can also regulate lipolysis [10]. Fatty acids are intermediate substances in synthesis of oleochemical products. Enzymatic technology of fatty acids production (also known as lipolysis) is now developing as potential substitution for the conventional production of fatty acid, i.e., thermal hydrolysis of triglyceride.

Lipolysis rates are finely controlled in response to changes in nutritional status via hormonal and pharmacological markers. Hyperplasia (an increase in the number of adipocytes) and hypertrophy (an increase in the size of adipocytes) are both factors that contribute to the development of obesity [10]. The activation of a controlled set of gene transcription events, including the over expression of adipogenic transcription factors such as CCAAT/enhancer-binding protein (C/EBP) α/β and peroxisome proliferator-activated receptor (PPAR) γ, is required for adipogenesis. Higher expression of adipocyte protein 2 (aP2), a hallmark of terminal adipocyte development, results as a result of this [11]. This lipolytic resistance has been associated to several changes in the lipolytic cascade.

These genes include those encoding adipokines (adiponectin and leptin), lipid metabolizing enzymes (fatty acid binding protein (Fabp), lipoprotein lipase (LPL), fatty acid synthase (FAS) and insulin receptor (IR), all of which are up regulated by these transcription factors during adipocyte differentiation [12]. The FDA has only authorized orlistat (tetrahydrolip-statin, for long-term usage. Many studies have recently shown that natural compounds have anti-obesity action, implying that they might be used as anti-obesity treatments or supplements.

Due to the adverse effects of conventional medication, researchers have explored the possibility of developing natural anti-diabetic agents. Traditional herbal therapy well known to mankind has recently gained attention as being one of the oldest remedies. Herbs that have hypoglycaemic and antioxidant properties may also protect the organs affected by diabetes mellitus [13, 14].

A. indicum, a common name is Indian mallow (Kannada:Tutti; Telugu: Tutueabenda) belonging to the family Malvaceae is an Indian medicinal plant used in Ayurveda [11]. *A. indicum* is widely distributed in tropical and subtropical countries such as Indian subcontinents, China, Malaysia, Thailand, Indonesia etc. [15, 16]. The plant is an erect, many branched, perennial velvety-pubescent shrub with heart-shaped leaves with coarsely crenate margins. The plant can grow 1–2 m tall and leaves are arranged alternatively, it has orange-yellow flowers and petals are triangular-obviate, fruit is circular in shape consisting of 11–20 radiating hairy carpel's brown when dry [14]. Various parts of the plant have many medicinal properties and widely used for folk medicine to cure various diseases [17].

Herbal remedies such as roots, stems, leaves, flowers, and fruit contain some flavonoids, phenolic compounds, steroid, alkaloids, tannins, saponins, and glycosides that may be useful in treating inflammatory diseases, cardiovascular diseases, diabetes, muscular weakness, heart disease, bleeding disorders, and paralysis [18–20]. The *A. indicum* herb is currently accepted for folk and traditional medicine as the main source of diabetes; however, the cytotoxicity of plant extract has not been investigated for antidiabetic properties [21, 22]. Therefore, the main aim of this research work was to extract the crude *A. indicum* plant leaf extract and that could use to improve the responsiveness of adipocytes and lipolysis activity and potentially used as adjuvant in the treatment of diabetes.

Materials and Methods

Botanical Description

The leaves are 1.9–2.5 cm long, oval, acuminate, toothed, and sometimes subtrilobate. Yellow flowers with a peduncle that joins above the middle. Petioles 3.8–7.5 cm long, stipules 9 mm long, pedicels 2.5–5 mm long, axillary solitary, jointed near the top; calyx 12.8 mm length, divided in half, lobes oblong, apiculate, and corolla 2.5 cm diameter, yellow, emerging in the evening. The fruits are capsules with prominent and horizontally spreading beaks that are highly hairy. The stems are pubescent and robust, branching, and 1–2 m tall. The seeds are 3–5 mm long, reniform, tubercles or minutely stellate-hairy, black or dark brown, and reniform [23, 24].

Procurement of Plant Sample

The plant sample *A. indicum was* collected from "Krishnendra Botanical Plant Nursery "Lalbagh Bengaluru. Taxonomy identification: Accession number (UASB-4604) Dr A.N Sringeswara professor, Botanical Garden University of Agriculture Sciences, GKVK Bengaluru.

Methanol Extraction of the Herbal Product

A fresh green leaves of *A. indicum* were collected and washed under running tap water to remove dirt content as well with distilled water. The leaves were shade dried and powdered. The powdered leaf samples were sieved, weighed, and stored in an airtight container. The powder(5 g) was measured and mixed with 95% methanol (50 ml) in the dark at 28 °C for 24 h and shaken (150 rpm). The mixture was filtered through muslin cloth and Whatman (No.1) filter paper to obtain the extract [25]. Methanol was evaporated from the sample on a rotary evaporator at 50 mm high pressure at 50 °C. The evaporated leaf extract were thick and viscous materials and were kept in an air-tight bottle and stored in the freezer at 20 °C until they were analyzed [Fig. 1] [26].

Cell lines, Culture Medium Preparation and Treatment

The 3T3-L1 mouse pre-adipocyte cells were procured from NCCS Pune, India. Stock cells at a density of 20,000 cells/well were seeded in a 96 well plate and incubated for 24 h [27]. Furthermore, the cells were cultured in a Dulbecco's Modified Eagle's Media (DMEM) supplemented with 10% inactivated fetal bovine serum (FBS), penicillin (100 units/mL), streptomycin (100 µg/mL) in a humidified atmosphere of 10% CO_2 incubator at 37 °C. The cell was dissociation with cell dissociating solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The viability of the cells is checked with two days after the cells has reached the confluency (day 0), medium removal and addition were performed at the slowest pipetting speeds possible. On day 5, medium was replaced with 100 µl differentiation medium I (DM-I) with supplement of DMEM-97%, FBS-3%, Antibiotic–antimycotic-1%, Insulin-1 µg/ml, Isobutylxanthine-0.5 mM, and Dexamethasone-1 µM at different concentrations. On Day8, the medium was replaced with 100 µl Differentiation Medium II (DM-II) with the composition of DMEM-97%, FBS-3%, Antibiotic–antimycotic-1% and Insulin-1 µg/ml. On Day 10, medium was replaced with 100 µl of maintenance medium (MM) with the composition of DMEM-97%, FBS-3%, and Antibiotic–antimycotic-1%. Experiments were carried out on day 10–14 days at optimum differentiation state when cells as considered as mature adipocytes [28, 29].

Adipogenesis Assay

Various concentrations of *A. indicum* leaf extract were added together with DM-II slight modification to the protocol [30] presence or absence of insulin (1 µg/ml as a positive control). On Day 10, the cells were carefully washed twice with PBS and then fixed and dried with 0.5% formalin and rinse with 60% isopropanol with each well for 3 min before staining in Oil Red O solution. The cells were washed thrice with phosphate-buffered saline (PBS) to remove excess dye, then fixed with 4% formaldehyde for 30 min at room temperature. The cells were washed thrice more with PBS and stained with 0.5 µg/mL Oil red O for 15 min. Spectrophotometric quantification was done by dissolving the Oil Red O absorbed by lipid droplets with 60% isopropanol at 490 nm using a microplate reader [31]. The extent of adipogenesis was expressed as follows:

$$\text{Adipogenic activity (fold change)} = \frac{A_{Sample} - A_{Control}}{A_{Control}}$$

"Control" and "sample" refer to untreated cells and cells treated with extracts or insulin, respectively.

Glycerol Estimation and Lipolytic Activity

Lipolysis of adipocytes were quantified using glycerol quantification assay. On Day 14 maintenance medium (MM), the cells were cultured with 1% serum. On the day of the assay, medium was replaced with 100 µl DMEM without serum containing a range of α-Lipoic acid concentrations and various concentrations of test samples. Incubate for 5 h at 37 °C in 5% CO_2. Transfer 10µL of the appropriate standards and 10 µL of *A. indicum* leaf extract

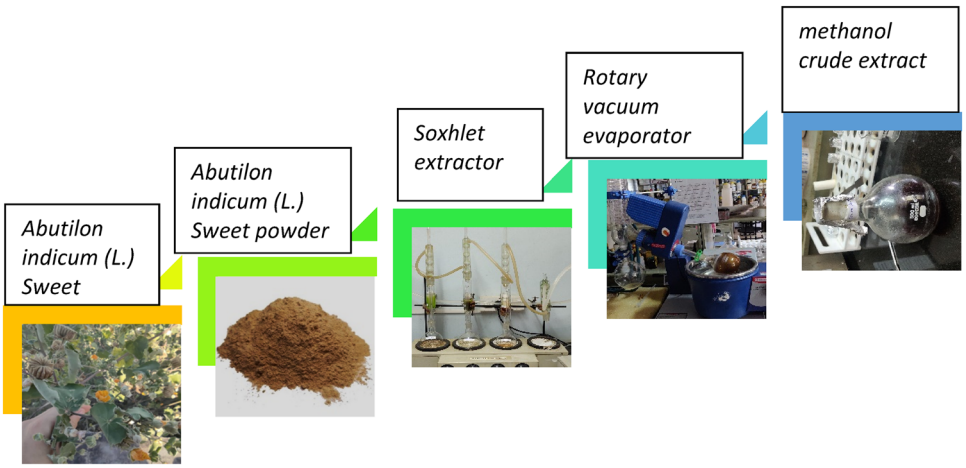

Fig. 1 *Abutilon indicum* methanolic sample processing

into separate wells of a 96 well plate. 100 µL of the Master Reaction Mix (Assay buffer-100 µl, Enzyme mix-2 µl, ATP-1 µl, Dye Reagent-1 µl) is added to each of the blank, standard, and various concentrations of the extract (25, 50, 100, 250 and 500 µg/ml) is mixed well using a shaker and incubated the reaction mixture for 20 min at room temperature. Glycerol released from triglycerides during the lipolysis was spectroscopically quantified at 570 nm using microplate reader upon incubation of the cultures with glycerol quantification reagent for 15 min at 37 °C. Increase in the amount of glycerol formed in the sample is directly proportional to the increase in lipolysis [30]. The extent of lipolysis was expressed in fold increase over untreated cells.

$$C = (A570)_{sample}/Slope$$

[where Slope = Slope determined from standard curve; C = Concentration of glycerol in sample (µM)].

Cholesterol Esterase Inhibition Assay

Hypercholesterolemia causes serious health problems such as coronary artery disease and atherosclerosis. Controlling plasma cholesterol levels, which are formed through biosynthesis and food intake, is one of the most important therapeutic techniques for effectively controlling chronic disorders. Inhibition of human cholesterol esterase, which is involved in the regulation of plasma cholesterol levels, is a key target in the treatment of hypercholesterolemia [32].

According to Pietsch and Gutschow, the pancreatic cholesterol esterase inhibition experiment was performed in triplicates [33]. Various concentrations of each compound/fraction were incubated with mixtures containing 100 µl of 5.16 mM taurocholic acid, 90 µl of 0.2 mM p-nitrophenyl butyrate in 100 mM sodium phosphate buffer diluted with100 mM NaCl, pH 7.0. The reaction was initiated by adding 5 µl of porcine pancreatic cholesterol esterase (1 µg/mL). After the incubation of 5 min at 25 °C, absorbance of the mixtures was measured at 405 nm. Orlistat was used as a positive control for this study.

Statistical Analysis

The Adipogenesis and lipolysis activity data were expressed as the mean ± standard deviation of the mean (mean ± SD). The difference values between each control, standard and samples were analyzed using a one-way analysis of variance (ANOVA) with graph prism. Values were considered statistically significant differences, which means sharing the different superscript letters when, and data were representative of three independent experiments (n = 3). Most of the experiments were performed in triplicate. Half maximal Inhibitory concentration IC_{50} is the concentration of the substance required to inhibit a biological process such as an enzyme, cell, cell receptor or microorganism by half. IC_{50} value is calculated using Graph Prism software version 5.0 by nonlinear regression analysis of % inhibition recorded for different concentrations of test substances/standard. For compounds showing < 50% inhibition, IC_{50} value is not calculated. The relative activity of the sample can be determined by comparing the IC_{50} value of sample with standard. Higher the IC_{50} value, lower will be the relative activity in comparison to standard and vice-versa.

Calculating percentage growth inhibition:

$$\%\,Inhibition = \frac{(OD\ of\ Control - OD\ of\ Sample)}{OD\ of\ Control} \times 100.$$

Results and Discussion

Effect of *A. indicum* leaf Extract During the Transformation of 3T3-L1 Preadipocytes to Adipocytes

The 3T3-L1 cells were treated with three different conditions MM, DM-I and DM-II respectively, together with *A. indicum* leaf extract. However, from day 0 to 10 days the 3T3-L1 pre-adipocyte were differentiated the cells by substantial accumulation of lipid which is a hallmark of matured adipocytes in Oil Red O staining. The Cell viability and concentration of lipid accumulation were than assessed with the incubation of different concentrations is measured at absorbance of 490 nm. We observed a concomitant decreased ($p < 0.05$) lipid accumulation in adipocytes after being treated with *A. indicum* and Dexamethasone for 10 days in a dose-dependent manner compared with untreated cells. The overall results show 17.11% (MM), 54.35% (DM-I), 46.75% (DM-II) as control groups and 9.8% (100 µg/mL), 17.8% (200 µg/mL), and 25.8% (400 µg/mL) respectively (Fig. 2). The results suggest that the cells at higher concentration of the test sample treated have taken up lesser oil red o stain suggesting the lesser differentiation compared to control.

Effect of *A. indicum* Leaf Extract in 3T3-L1 Preadipocytes for Glycerol Level and Lipolysis Effect

To investigate the lipolytic activity of *A. indicum* leaf are tested to analyze the release of triglyceride accumulation during the human pre-adipocyte/adipocyte differentiation in vitro, cells were cultured in Maintenance Medium (MM) for 10 days (n-10) in the absence/presence of 25%, 50%, 100%, 250%, and 500% *A. indicum* leaf extract solution. The total amount of lipid was determined (Fig. 3). Cells were treated with *A. indicum* leaf extract solution dose-dependent is significantly decreased triglyceride levels ($p < 0.05$) and increased the glycerol release level up to 70% compared to the control sample. As our studies show, treatment of adipocytes with 10% *A. indicum* leaf extract solution significantly ($p = 0.092$) increased the content of free glycerol to 9.2 mg/dL or 92 µg/ml (± 18) µg/ml as compared to control cells.

Cholesterol Esterase Assay Activity

The *A. indicum* leaf extract of cholesterol esterase assay inhibitory effect of the extract against bovine pancreatic cholesterol esterase. Orlistat was used as positive standard along with *A. indicum* leaf extract and the results shows good activity ranging from 3.69 to 57.74% inhibition in comparison to corresponding values of standard Orlistat ranges from 16.38 to 85.68% (Tables 1, 2), activity increasing with higher concentrations. IC_{50} value of the extract was found to be 8.158 µg/ml. These results indicate that the plant extract had a noticeable effect on the enzyme cholesterol esterase. The differences among the samples were found to be extremely significant as per analysis of variance ($p < 0.001$) (Fig. 4).

Discussion

The use of herbal medicine as a complementary and alternative treatment for diabetes mellitus has been practiced for centuries [34]. Plants contain a diversity of

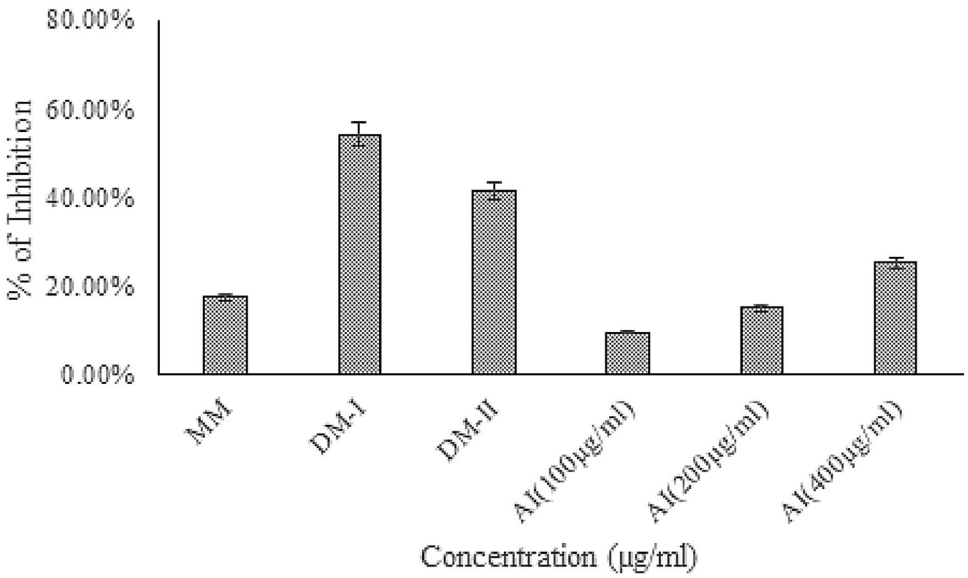

Fig. 2 Effect of *A. indicum* leaf extract as adipogenesis assay. Y-axis indicates the percentage of inhibition of Adipogenesis ± SEM. Preadipocytes (20,000 cells/well) were induced to differentiate with various concentrations of *A. indicum* extracts with/without insulin (100 µg/mL) prior to Oil Red O assay. *Note*: maintenance medium—(MM), differentiation medium—(DM-I&DM-II), *A. indicum*–(AI)

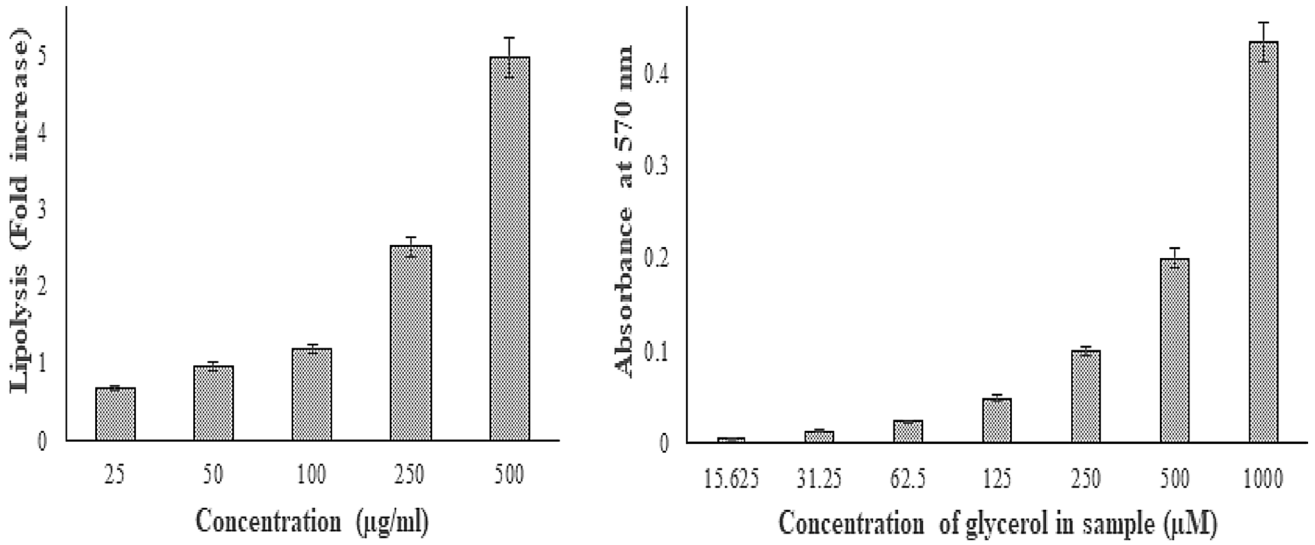

Fig. 3 Effects of *A. indicum* leaf extract solution on triglyceride accumulation during preadipocyte/adipocyte differentiation. **a** Lipolysis activity, **b** Glycerol estimation based on the cell viability of preadipocytes incubated with different amounts of *A. indicum* leaf extract solutions (25%, 50%, 100%, 250%, and 500%) is shown relative to untreated control cells sets. Results are depicted as mean ± SD. Significant differences are marked with an asterisk (*for $p < 0.0001$)

Table 1 Estimation of adipogenesis assay by methanol extract of *A. indicum* leaf

Sample	Conc. (µg/ml)	OD @ 490 nm				SD	SEM
		n = 1	n = 2	n = 3	Mean OD		
Control	0	0.055	0.049	0.052	0.052	0.003	0.002
Oil red	14.06	0.076	0.071	0.077	0.075	0.003	0.002
	28.13	0.095	0.098	0.092	0.095	0.003	0.002
	56.25	0.187	0.191	0.189	0.189	0.002	0.001
	112.5	0.256	0.249	0.247	0.251	0.005	0.003
	225	0.555	0.558	0.551	0.555	0.004	0.002
	450	0.895	0.890	0.896	0.894	0.003	0.002
	900	1.748	1.741	1.746	1.745	0.004	0.002
Abutilon indicum	0	0.145	0.149	0.151	0.148	0.003	0.002
	100	0.134	0.131	0.135	0.133	0.002	0.001
	200	0.126	0.124	0.120	0.123	0.003	0.002
	400	0.113	0.107	0.109	0.110	0.003	0.002

Table 2 Estimation of glycerol calibrant in lipolysis assay by methanol extract of *A. indicum* leaf

Sample	Conc. (µM)	OD @ 570 nm			SD	SEM	
		n = 1	n = 2	n = 3	Mean OD		
Control	0	0.000	0.000	0.000	0.000	0.000	0.000
Glycerol	15.625	0.005	0.004	0.005	0.005	0.001	0.000
	31.25	0.013	0.012	0.013	0.013	0.001	0.000
	62.5	0.024	0.025	0.024	0.024	0.001	0.000
	125	0.049	0.048	0.050	0.049	0.001	0.001
	250	0.099	0.099	0.098	0.099	0.001	0.000
	500	0.201	0.204	0.202	0.202	0.002	0.001
	1000	0.433	0.433	0.432	0.433	0.001	0.000

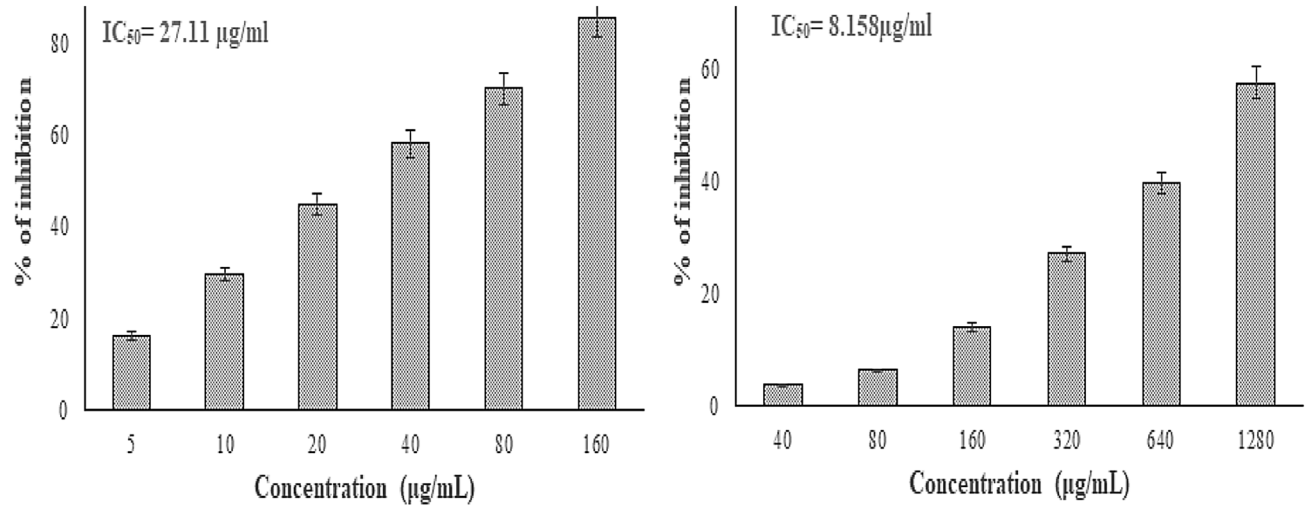

Fig. 4 Cholesterol esterase inhibition by methanol extract of *A. indicum* leaf

antioxidants, including tocopherols, carotenoids, ascorbic acid, flavonoids, tannins and flavonols, which may explain the effectiveness of plants as diabetes remedies. There are a few synthetic antioxidants available. However, their toxicity limits the margin of safety for these compounds [35].

We have screened the extracts related to lipolytic activity both in vitro resulting in a powerful lipolytic effect. Insulin is capable of inducing adipogenesis, suppressing lipolysis, and stimulating the uptake of glucose and free fatty acids in adipose besides liver and muscle tissues. A number of enzymes and proteins associated with diabetes are used in this strategy to study the anti-diabetic properties of leaf extract compounds [36].

The cell viability and concentration of lipid accumulation were than assessed with the incubation of different concentrations is measured at absorbance of 490 nm. We observed a concomitant decreased ($p < 0.05$) lipid accumulation in adipocytes after being treated with *A. indicum* and Dexamethasone for 10 days in a dose-dependent manner compared with untreated cells. Although antilipolysis has "insulin-like" properties, it can cause weight gain because it prevents adipocytes from mobilising lipids. As a result, "insulin-like" plant extracts with modest lipolytic activity or anti-adrenaline induced lipolytic activity might be a superior diabetic treatment [37]. Moreover, we also found that *A. indicum* (L) leaf extract have an antiadipogenic effect during the differentiation of 3T3-L1 preadipocytes. These results suggest that *A. indicum* (L) leaf extract is a good candidate for the development of functional ingredients that can help reduce the high rates of death from cardiovascular diseases associated with obesity [38].

The effect of *A. indicum* leaf extract in 3T3-L1 preadipocytes for glycerol level and lipolysis effect: To investigate the lipolytic activity of *A. indicum* leaf are tested to analyze the release of triglyceride accumulation during the human pre-adipocyte/adipocyte differentiation in vitro. Our studies show, treatment of adipocytes with 10% *A. indicum* leaf extract solution significantly ($p = 0.092$) increased the content of free glycerol to 9.2 mg/dL or 92 µg/ml (± 18) µg/ml as compared to control cells. Some pathological diseases, such as diabetes mellitus, insulin resistance, obesity, and fatty liver, include dysregulation of lipid metabolism as a fundamental characteristic. *A. indicum* (L) leaf extract is capable of inhibiting lipid and carbohydrate accumulation in adipocytes and also has the potential to inhibit an enzyme associated with fat absorption [39].

Atherogenic dyslipidemia is caused by different metabolic abnormalities including increased cholesterol synthesis, increased production of triglyceride-rich lipoproteins, and increased HDLcatabolism. It is believed that among these abnormalities, the pivotal role is played by increased hepatic production of lipoproteins. Availability of triglycerides within hepatocytes is an important factor influencing synthesis of very low-density lipoprotein (VLDL) [18, 40]. Triglycerides are provided from de-novo synthesized or extra hepatic fatty acids. Adiposetissuederivedfreefattyacidisthelargestextrahepaticsourceof fatty acid for triglyceride synthesis. Fatty acids are released from adipose tissue through a highly regulated process named lipolysis. Although a variety of factors are involved in regulation of lipolysis, in physiologic situation insulin and catecholamine's are the main anti-lipolytic and prolipolytic agents, respectively [41].

Adipose tissue is an important organ and site for lipid storage. Which carryout several important physiological processes. there are two type of classifications adipose

Table 3 Estimation of glycerol released in lipolysis assay by methanol extract of *A. indicum* leaf

Sample	Conc	OD @ 570 nm	Mean Abs		Glycerol Conc. (µM)	Glycerol Conc. (µg/ml)	SD	SEM
		n = 1	n = 2	n = 3				
Abutilon indicum (µg/mL)	25	0.003	0.004	0.003	0.003	17.83	1.605	0.0006	0.0003
	50	0.004	0.004	0.005	0.004	20.33	1.830	0.0006	0.0003
	100	0.005	0.005	0.006	0.005	22.83	2.055	0.0006	0.0003
α-Lipoic acid (µm)									
	250	0.011	0.012	0.011	0.011	37.83	3.405	0.0006	0.0003
	500	0.022	0.021	0.021	0.021	62.83	5.655	0.0006	0.0003

Table 4 Estimation of cholesterol esterase inhibition assay by methanol extract of *A. indicum* leaf

Sample	Conc. (µg/ml)	OD @ 405 nm						SD	SEM	IC50 (µg/ml)	
		n = 1	% Inhibition	n = 2	% Inhibition	n = 3	% Inhibition	Mean inhibition			
Control	0	0.832	0.00	0.836	0.00	0.828	0.00	0.00	0.00	0.000	**27.67**
Orlistat	5	0.696	16.35	0.689	17.58	0.695	16.06	16.66	0.81	0.467	
	10	0.585	29.69	0.582	30.38	0.589	28.86	29.65	0.76	0.439	
	20	0.459	44.83	0.462	44.74	0.466	43.72	44.43	0.62	0.356	
	40	0.348	58.17	0.350	58.13	0.342	58.70	58.33	0.31	0.181	
	80	0.248	70.19	0.244	70.81	0.251	69.69	70.23	0.56	0.326	
	160	0.119	85.70	0.121	85.53	0.123	85.14	85.46	0.28	0.163	
Control	0	0.832	0.00	0.836	0.00	0.828	0.00	0.00	0.000	0.000	**839.8**
Abutilon indicum	40	0.785	5.65	0.780	6.70	0.788	4.83	5.73	0.936	0.541	
	80	0.763	8.29	0.769	8.01	0.770	7.00	7.77	0.678	0.391	
	160	0.700	15.87	0.703	15.91	0.705	14.86	15.54	0.596	0.344	
	320	0.594	28.61	0.590	29.43	0.596	28.02	28.68	0.706	0.408	
	640	0.491	40.99	0.487	41.75	0.495	40.22	40.98	0.765	0.441	
	1280	0.345	58.53	0.339	59.45	0.342	58.70	58.89	0.489	0.282	

tissue one is (WAT) white adipose tissue and second is (BAT) brown adipose this tissue arose from resident mesenchymal progenitor cells that were present in adipose tissue. The lipid storage and metabolization was carried out by adipocytes in different ways. During fasting BAT accumulate nutrient-derived triglycerides and release them by Lipolysis whereas WAT oxidize their lipid stores in an elegant heat-producing pathway. White adipose tissue for the majority of fat present in adults and is the site for energy homeostasis, endocrine action and insulin signaling. Brown adipose tissue is main responsible for thermo genesis [42]. White adipose tissue is essential for energy homeostasis by storing excess energy and releasing lipids in response to energy and often associated with development of obesity and type 2 diabetes [43]. It contributes to the overall metabolic deficit observed in obesity. From Various medicinal plant Phyto natural compounds have shown promise to regulate white adipose tissue activity and enhance the lipolytic and catabolic potential of white adipose tissue [44]. The Phyto and fungal constituents such as flavonoids are secondary metabolites which are the most common group of polyphenolic compounds in the human diet. These flavonoids shown to induce browning of white adipocytes and brown adipose tissue as well to increase energy expenditure and improve glucose and lipid metabolism [45].

Based on literature and their studies on the effect of various extracts at the level of adipose tissue on diets with the natural products. With the use of in vitro models on adipocyte cells, we have focused on testing the effect directly on pre-adipocytes in cell culture methods and we studied their lipolytic and inhibitory effects on adipogenesis. Our objective was *A. indicum* crude leaf extract used to evaluate and screened to induce lipolysis directly on mature Preadipocytes to adipocytes in vitro [46, 47]. The 3T3-L1 cells were treated with three different conditions MM, DM-I and DM-II respectively, together with *A. indicum* leaf extract. Substantial accumulation of lipid

which is a hallmark of matured adipocytes in Oil Red O staining. We observed a concomitant decreased ($p < 0.05$) lipid accumulation in adipocytes after being treated with *A. indicum* and Dexamethasone for 10 days in a dose-dependent manner compared with untreated cells show 17.11% (MM), 54.35% (DM-I), 46.75% (DM-II) as control groups and 9.8% (100 µg/mL), 17.8% (200 µg/mL), and 25.8% (400 µg/mL) respectively [48, 49]. To study and investigate the lipolytic activity of *A. indicum* leaf extract by analyze the release of triglyceride accumulation during the human prea-dipocyte/adipocyte differentiation in vitro, cells were cultured in Maintenance Medium (MM) for 10 days (n-10) in the absence/presence of 25%, 50%, 100%, 250%, and 500% *A. indicum* leaf extract solution. The total amount of lipid was determined (Fig. 3). Cells were treated with *A. indicum* leaf extract solution dose-dependent is significantly decreased triglyceride levels ($p < 0.05$) and increased the glycerol release level up to 70% compared to the control sample. The lipolytic and anti-audiogenic effects arising from *A. indicum leaf* extract have medicinal properties in the treatment of cardiovascular diseases because it directly affects one of the most important risk factors for the development of CVD and obesity [50, 51]. This preliminary research work may help to identify and studying the active principles and effects in vivo. Diabetes mellitus (DM) prevalence continues to rise alarmingly despite intensive research. In the management and treatment of Diabetes mellitus, it is increasingly important to explore alternative remedies such as traditional phototherapy by using *A. indicum* leaf extract.

Conclusion

The results of the present study reported that the crude methanol extract of *A. indicum* was able to promote Adipogenesis and lipolysis effect that can hydrolyze triglycerides into glycerol and fatty acids. The adipogenesis effect of crude extract with DM-I, DM-II and MM media of 100 µg/mL is treated with adipocyte cells with increased inhibition of adipocyte cell function. The lipolytic effect of also observed about the concentration of glycerol release while treating of 100 µg/mL of crude extract and differential media concentrations shows increasing lipolytic activity that shows the increase concentration of glycerol release. And the cholesterol esterase activity is also observed with the treatment of Orlistat antidiabetic drug along with crude extract shows the significant effect ($p < 0.001$) with IC50 = 27.11 µg/mL of positive control and IC50 = 8.158 µg/mL of crude extract. In conclusion, the methanolic crude extract of *A. indicum* shows the potential inhibitor for adipogenesis and lipolysis activity in vivo and confirming the *A. indicum* anti-diabetic potential in vivo. As well further these crude extract needs to study on animal models to evaluate as a traditional medicine to control type 2 diabetes and cardiovascular diseases.

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Availability of Data and Materials Authors declare that all generated or analyzed data are included in the article.

Declarations

Conflict of interest Authors declares that he has no conflict of interest.

Ethical Approval and Consent to Participate The overall research is not used animals and is only in vitro based study.

Consent for Publication Authors stated that there is no informed consent in the article.

Human and Animal Rights No Human cell are used only animals cell lines are use for the studies. Animal cell line are used Sigma Aldrich [ATCC-CL-173].

Research Resource Identifiers (RRID) Product category: Animal cells
Organism: Mus musculus, mouse
Classification: Eukaryota, Animalia, Metazoa, Chordata, Vertebrata, Tetrapod
Cell type: fibroblast
Morphology: fibroblast Tissue Embryo
Applications 3D cell culture
Product format: Frozen Storage conditions Vapor phase of liquid nitrogen

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