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Original Research Article

The Effects of Mulberry Leaves (*Morus alba L.*) Extract on Lipid Accumulation in Adipocytes 3T3-L1 Cells and *in Vitro* Cytotoxic Activity

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ABSTRACT

Diabetes mellitus is a disease that occurs when the pancreas cannot produce enough insulin or when the body cannot effectively use the insulin that is produced. Obesity is a major risk factor for diabetes, characterised by excessive lipid accumulation in adipose tissue. This study aims to determine the effects of a 70% ethanol extract from mulberry leaves (*Morus alba L.*) and its fractions on inhibiting lipid accumulation in 3T3-L1 adipocyte cell lines (preadipocytes derived from mouse embryonic fibroblasts). Mulberry leaves were macerated with 70% ethanol and partitioned with n-hexane and ethyl acetate. The extract of mulberry leaves and its fractions were first tested for cytotoxicity using the MTT assay method on 3T3-L1 cells. The 3T3-L1 cells were differentiated using differentiation agents. After differentiation, the cells were treated with samples of ethanol extract and its fractions at concentrations of 31.25, 62.5, 125, 250, 500, and 1000 ppm. The IC_{50} values obtained were 416 µg/mL for the ethanol extract, 49 µg/mL for the n-hexane fraction, 89 µg/mL for the ethyl acetate fraction, and 225 µg/mL for the water fraction. The results of the lipid accumulation test show that the ethyl acetate, n-hexane, and water fractions reduce lipid accumulation, but the ethanol extract increases lipid accumulation. According to these findings, the ethanol extract fractions from mulberry leaves can inhibit lipid accumulation in 3T3L1 adipocyte cells.

Keywords: *Morus alba L.*, Adipocyte 3T3-L1 cell, lipid accumulation, cytotoxicity

Introduction

Diabetes mellitus is a chronic disease that occurs when the pancreas can't produce enough insulin, or the body can't use the insulin it produces effectively. World Health Organization (WHO) reported that in 2014, around 8.5% of adults aged 18 years or older had diabetes, and in 2015, diabetes directly caused the deaths of 1.6 million people.[1] The prevalence of diabetes in Indonesia ranges from 1.5% to 2.3%. It is estimated that the Indonesian population over the age of 20 who have diabetes mellitus is 125 million people, with a percentage of 4.6%. In 2008, the prevalence of diabetes mellitus in the population over the age of 20 increased to 5.7%.[2]

Based on its aetiology, diabetes mellitus can be divided into type 1, type 2, gestational diabetes mellitus, and other types of diabetes.[3] Type 1 diabetes mellitus is also called *insulin-dependent diabetic mellitus* (IDDM), which is caused by impaired insulin production by pancreatic beta cells. While type 2 diabetes mellitus is called *non-insulin-dependent diabetic mellitus* (NIDDM). NIDDM is initially caused by a decrease in the sensitivity of the target tissue or resistance to insulin, often called resistance to insulin's metabolic effects. Other types of diabetes are gestational diabetes or diabetes caused by pregnancy.[4]

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Obesity is a major risk factor for chronic diseases, including type 2 diabetes mellitus. Obesity is a condition characterised by excessive accumulation of fat in adipose tissue. Subcutaneous fat strongly correlates with insulin resistance, so that obesity can cause insulin resistance, which is closely related to type 2 diabetes mellitus.[5] Fatty acid metabolites like glycerol, fatty acyl CoA, and ceramides are broken down faster when more fatty acids are delivered to muscle cells or when intracellular fatty acid metabolism slows down. These metabolites activate the serine or threonine kinase cascade, which causes amino acid serine or threonine phosphorylation on insulin receptor substrates (IRS-1 and IRS-2) by reducing the ability of insulin receptor substrates to activate PI3-Kinase. Thus, GLUT activity is reduced, and insulin receptor signalling is reduced.[6] Additionally, an increase in adipose mass in obesity can cause pathological changes in adipocyte hormones that regulate insulin sensitivity. Adiponectin is an adipokine that has insulinomimetic properties. In obese patients, adiponectin levels are low; administration of adiponectin improved insulin resistance in animal models. In the liver, adiponectin can improve insulin sensitivity, decrease fatty acid inflow, increase fatty acid oxidation, and reduce hepatic glucose output.[7]

Mulberry leaves (*Morus alba L.*) have been investigated as an efficacious medicinal plant with anti-inflammatory and antihyperglycaemic properties. The antihyperglycaemia effect of mulberry leaves has been proven in streptozotocin (STZ)-induced diabetic mice. Research has been conducted to develop the efficacy of mulberry leaves as a therapy for treating diabetes and hyperlipidemia.[8]Results of research conducted by Yang SJ (2014) mentioned that 70% of the ethanol extract from mulberry leaves and fruit has been used as an antioxidant and antidiabetic in cell culture models.[8] Antidiabetic in animal cell culture models because it contains several phenolic compounds, including 1-deoxycholic phenolic compounds, 1-deoxy-nojrimycin, rutin, quercetin, isoquercetin, and

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resveratrol. The administration of an ethanol extract from mulberry leaves did not affect the morphology and viability of 3T3-L1 adipocyte cells. All cells treated with ethanol extract had lower fat accumulation than the control cells, as shown by the low absorbance of the Oil Red O stain. Furthermore, 12 weeks of mulberry leaf extract intervention could reduce body weight, adiposity, and fat in the blood and serum, particularly in the liver, reduced by changes in lipogenesis and lipolysis.[9] Other research by Rosnani Nasution (2015) stated that the ethyl acetate extract from ethyl acetate extract of mulberry leaves in two-month-old mice can reduce mice's body weight.[10] Another research by Euna Park (2015) also stated that administering an aqueous extract of mulberry leaves for 6 days can increase the inhibitory activity of lipid accumulation in 3T3-L1 preadipocyte cells induced into adipose cells[10], preadipocyte cells induced into 3T3-L1 adipocyte cells, and reduce the mRNA expression levels of C/EBPα, β, and PPARγ.[11] In addition, research conducted by Hong Xu Li stated that administering flavonoid isolates from mulberry leaves with a concentration of 40 μm can inhibit fat accumulation in 3T3-L1 adipocyte cells by 2.1–36.6%.[12] Hence, based on these results, this study aimed to evaluate the effects of ethanol extract and fractions of mulberry leaves (*Morus alba* L.) on the buildup of fat in 3T3-L1 adipocyte cells. [13]

Materials and Methods

Materials

Mulberry leaves (*Morus alba L.*) were obtained from BALITTRO, Bogor, West Java, Indonesia (-6.348635295980812, 106.68123793245827). 3T3-L1 cells were collected from Cell Culture Laboratory: LAPTIAB BPPT, DMEM F12, FBS (fetal bovine serum), FCS (fetal calf serum), Penstrep 1%, Fungizon 0.5%, Troglitazone 5 mM, Dexamethasone 0.25 uM, IBMx 0.5 mM, Insulin 1 ug/mL, PBS, Isopropanol 100%, Isopropanol 60%, Oil Red O 0.5%, Formaldehyde 4%, Tripan Blue. Rotary evaporator (Heidolph, Germany), conical **(**Corning Pentastar, USA), Flasks, and test tubes (Pyrex (USA.). Centrifuge (Hettich, Germany), Inverted microscope (Zeiss, Germany). The ELISA Reader (BioTek or Thermo (USA) and **CO_2** Incubator (Memmert, Germany). 96 Well Plate (Nunc, Denmark), hemocytometer (Assistant, (Germany). Micropipette (Eppendorf, Germany), volume pipette (TPP, Switzerland). Analytical balance (Kern, Germany). Filter paper: Whatman (UK), Biological Safety Cabinet **(**Heidolph, Germany). Crial Tank (Thermo Scientific Locator 8, USA), Autoclave (Wirayama Japan), and Oven (Memmert, Germany).

Methods

Plant material collection and identification

The plant sample was collected on 2nd March 2018 and identified using taxonomic keys and DNA barcoding at LIPI, Cibinong, and a voucher number 614/IPH.1.01/If.07 /III/2018 was assigned.

Loss of weight on drying

The purpose of the drying shrinkage measurements was to provide upper bounds on the quantity of compounds lost during the drying process.[17] The result was given in percentage (%) units. In this experiment, 1 g of mulberry leaf powder was placed inside a closed porcelain dish that had been marked and preheated to 105ºC for thirty minutes and then heated until constant weight. The drying shrinkage rate was measured using moisture-balancing equipment, and the percentage was computed after three replications.

Extraction and fractionation of Mulberry leaves (*Morus alba L.***)**

The dried powdered sample of *Morus alba* (315.69 g) was macerated in 1.5 L X 3 of 70% ethanol solvent for one hour while stirring. After a full day of standing, the mixture was filtered. The filtrate was collected and concentrated using a vacuum rotavapor at 40°C under reduced pressure to give a thick ethanolic extract of the plant leaves. About 20.06 g of the crude extract was dispersed in 50 mL of distilled water and successively extracted with 150 mL of n-hexane and ethyl acetate, respectively.[19] The fractions' filtrates were concentrated to dryness using a vacuum rotavapor at 40 °C and 100 mbar of pressure to obtain a thick ethanol extract of the plant sample.

Phytochemical screening of mulberry leaves powder and extracts

The mulberry leaves powder and crude ethanol extract were subjected to phytochemical screening according to established protocols described in Farnsworth's guidelines. The dried mulberry leaves were ground into a powder and then extracted with ethanol. It was filtered using Whatman No. 1 filter paper, and the filtrate was used for phytochemical analyses using established protocols to test for the presence of different plant secondary metabolites: alkaloids, flavonoids, saponins, terpenoids, etc.

MTT Cytotoxic Assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) technique

The cells were trypsinised to collect cells that were 80% confluent. The entire medium was removed using a pipette, and the culture was then twice cleaned with PBS. Trypsin was then uniformly applied to the culture's surface and incubated in a CO_2 incubator for three minutes. The cells were examined under an inverted microscope until they separated from the growing dish's bottom. The entire medium was added to halt trypsin action, and if any cells clumped together, they were resuspended. The cells that had separated from one another were placed into a fresh, sterile conical tube and centrifuged for five minutes at 1200 rpm. After discarding the supernatant, 1 mL of complete media was added to the cell precipitate and resuspended.[14]

Cell counting

To calculate the number of cells/mL, trypan blue staining and hemocytometer counting chambers (Germany) equipment were used to count the cells. After transferring the necessary number of cells into a second conical tube, the full medium was poured following the intended cell concentration of 10,000 cells per well.

Plating

The cells were plated into wells using 96 well plates. These cells were then used for treatment using test samples.

Sample and Preparations

Test samples consist of cells that have adhered to the plate's bottom and confluenced at a rate of around 80–90% (1000, 500, 250, 125, 62.5, 31.25 ppm). After re-incubating the cells in the incubator for a maximum of 24 hours, the incubator's conditions were examined near the end of the incubation period. The state of the cells in each treatment was recorded at the end of the incubation period.

MTT Administration

The MTT method was adapted from Agustini (2024). [30] After discarding the cell media, each well was filled with the MTT reagent after the cells had been cleaned with PBS. Each well was filled with MTT reagent and control media containing no cells, and the cells were cultured for four hours. The status of the cells was assessed using an inverted microscope. SDS, the stopper reagent, was introduced. After that, the plate was covered with aluminium foil and left to incubate overnight at room temperature in a dark area. Using a Biotek Microplate Reader (Germany) ELISA Plate Reader set to 570 nm, the absorbance of each well was measured.

Fat Accumulation Test.

Cell Plating

3T3-L1 cells were extracted from flasks between 80% and 90% confluent. The number of cells was counted using a hemocytometer stained with trypan blue to assess cell sufficiency, and the number of cells/mL was calculated. After that, a culture medium was added to the cells to get the required cell count. The plating process was carried out in 24 well plates, with up to 10,000 cells per well. The cells were divided into 6 different concentration ranges: 1000, 500, 250, 125, 62.5, and 31.25 μg/mL, with a maximum volume of 250 uL in each well. After that, 31 cells were cultured for 48 hours at 37 ℃ in a 5% CO_2 incubator. After 24 hrs, the media was changed using DMEM F12 + FBS 10% + Penstrep 1% + Fungizon 0.5%.[15,16]

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Test sample treatment and Oil Red O staining
Differentiated cells were treated with the assay on day 12. In the first step, the medium in each well was discarded and washed using PBS. After appropriate dilution with assay medium, the cells were treated with the extract according to the desired concentration. The plate was incubated for 2 hours at 5% CO_2 and temperature 37℃ in an incubator. After 2 hours, the plate was removed from the incubator. The medium was discarded and washed twice with PBS. After that, 4% formaldehyde was added and incubated for 1 hour at room temperature. Formaldehyde was applied to fix the cells. Then, after 1 hour, the formaldehyde contained in the well was removed and rewashed using PBS. The well was discarded and rewashed using PBS for 1 time. [30]

Data analysis
The data was analyzed using GraphPad Prism Ver.10, 2012, and the IC50 value was determined by linear regression of log concentration against the percentage of inhibition of proliferation.

Results and Discussion

The weight loss results on drying showed that the mulberry leaves' average drying shrinkage was 8.67%. Table 1 displays the drying shrinkage rate measurement findings for powdered mulberry leaves simplisia (*Morus alba L.*). Also, the extract yield of the plant sample was 43.04 g, which is 13.63%.[18] The fractions yields from 20.06 g crude extract were 0.67 g (0.21%) and 5.68 g (1.80%) for n-hexane and ethyl acetate, respectively, and aqueous fraction was 8.66 g with a yield of 2.74%. The moisture content of the ethanol extract of mulberry leaves revealed a 10.6971±1.2908%. The water content result of mulberry leaves ethanol extract (*Morus alba L.*) is presented in Table 3. Due to the concentrated nature of this mulberry leaf ethanol extract, a maximum water concentration of 10% is required for acceptable results. The result of the phytochemical screening of the plant extract is presented in Table 4. The result showed that flavonoids, saponins, terpenoids, and essential oils are present.

Table: Determination of Drying Shrinkage Rate

No of measurements	% drying shrinkage
1	9.72
2	8.52
3	7.78
Average % drying shrinkage	8.67

Table 2: Extraction and partitioning results of 70% ethanol extract of mulberry leaves

Extract/Phase	Weight of extract obtained (g)	Yield (%)
Ethanolic extract	43.03	13.63
n-hexane	0.67	0.21
Ethyl acetate	5.68	1.80
Ethanol-water	2.74	2.74

Table 3: Measurement results of moisture content of 70% ethanol extract of mulberry leaves

No. of Measeurement	% level water
1	10.788
2	11.940
3	9.3633
Average % level water	10.6971
SD	1.2908

Table 4: Phytochemical screening results of 70% ethanol extract of mulberry leaves

Chemical	Result (+ / -)
Alkaloids	-
Flavonoids	+
Steroids / Triterpenoids	+
Saponins	+
Tannins	-
Essential oil	+
Quinones	-
Coumarin	-

+:Detection/Positive; -:Undetection/Negative

The concentration of the test sample treatment against 3T3-L1 adipocyte cells in the fat accumulation test was established using the IC_{50} value of the extract from the cytotoxicity test findings. This is to demonstrate that the extract is not cytotoxic to normal to prevent 3T3-L1 adipocyte cells from proliferating.[19,20] An IC_{50} value of 416 µg/mL was found for the ethanol extract, 49 µg/mL for the n-hexane fraction, 89 µg/mL ppm for the ethyl acetate fraction, and 255 µg/mL for the water fraction (Figure 1). For each extract, the IC_{50} values were calculated to ascertain the precise quantities that can impede the 3T3-L1 cell growth, as shown in Figure 2 and Table 1.
Deoxynojrimycin (DNJ), a flavonoid found in mulberry leaves, was used as a positive control agent in the fat accumulation assay. Yang demonstrated that the flavonoid deoxynojrimycin (DNJ) is an anti-obesity agent that blocks preadipocyte development.[21, 22] Still, the test of mulberry leaves (*Morus alba L.*) mixed in 70% ethanol extract on 3T3-L1 adipocyte cells did not show a decrease in the buildup of lipids. Each test concentration's uptake (1000, 500, 250, 125, 62.5, and 31.25 ppm) (Figure 3) indicates that it is higher than the uptake in the control. This may be because the mulberry leaf samples utilised in the study were taken from a location different from Yang's research site, which could have affected the secondary metabolite concentration and produced different findings. The lipid accumulation test graph for mulberry leaves (*Morus alba L.*) extracted in 70% ethanol is shown in Figure 4. Staining was done on 3T3-L1 adipocytes treated with ethanol extract from mulberry leaves (*Morus alba L.*) using the Oil Red O staining method to observe the lipid droplets accumulated in the adipocytes. The staining results demonstrated that the number of stained lipid droplets at each test concentration was almost comparable to that of the control. The results are presented in Figure 4. The results showed that the ethyl acetate fraction stopped 3T3-L1 adipocyte cells from making fat at 1000, 250, 1250, 62.5, and 31.25 ppm concentrations (Figure 5). However, lipid buildup increased at a concentration of 62.5 ppm, as evidenced by higher absorption than the control. This could result from several things, such as improper cell washing techniques that left Oil Red O residue in the wells after staining, which could impact the subsequent absorption. From the figure, the number of fat droplets stained at each concentration was lower, and the resulting colour was not as intense. The findings of 3T3-L1 adipocyte cells treated with 70% ethanol extract ethyl acetate fraction using the Oil Red O staining method are shown in Figure 6. A study by Chiung-Huei Peng *et al.* found that 2.5% of the anthocyanins in mulberry leaves water extract can lower visceral fat.[23] These anthocyanins include cyanidine-3-glucoside, cyanidine-3-rutinoside, pelargonidin-3-glucoside, and pelargonidin-3-rutinoside. In the phytochemical content test, the mulberry leaves water fraction (*Morus alba L.*) contained flavonoids. Since anthocyanins are a known form of flavonoid, it is assumed that the water portion of mulberry leaves produces the suppressive effect of fat accumulation because of their influence (Figure 7). The figure also demonstrates that the aqueous fraction of mulberry leaf extract inhibited 3T3-L1 adipocyte cells' ability to accumulate fat at 31.25, 62.5, 125, 250, 500, and 1000 ppm. The reduced uptake of each sample compared to the uptake generated by the control indicates this outcome. Additionally, it was observed that the 3T3-L1 adipocyte cells' fat droplets absorbed less colour than the control at each test concentration when stained with Oil Red O. This suggests that as lipid deposition declines, less lipid was stained (Scheme 8).[24] Similarly, the n-hexane fraction was also subjected to lipid accumulation tests. Lipid accumulation decreased at 31.25, 62.5, and 25 ppm concentrations, as

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shown in Figure 9. On the other hand, lipid buildup increased at concentrations of 12.5, 50, and 100 ppm. It was characterised by higher uptake at the test concentration compared to the uptake generated by the control (Figure 9). Oil Red O staining was also carried out to determine the number of lipid droplets that accumulated in 3T3-L1 adipocyte cells following treatment with n-hexane fraction. The findings demonstrated that, at the test concentrations, the colour absorbed by lipids was not significantly different from the control (Figure 10).

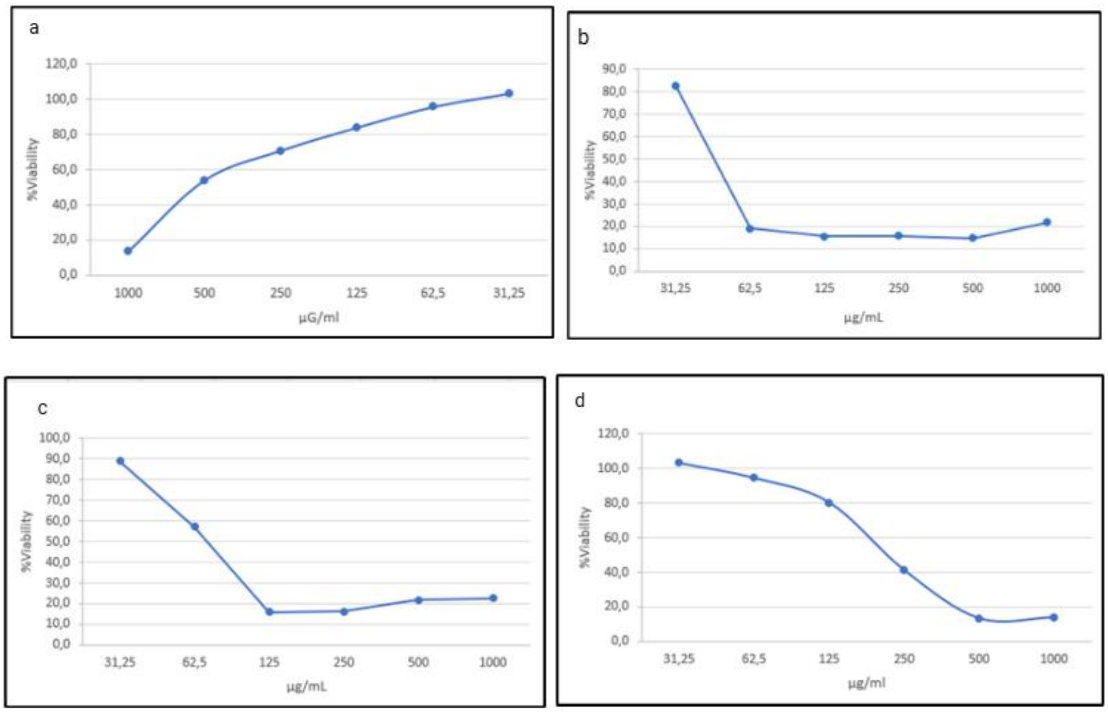

Figure 1: Relationship between log concentration of (a) ethanol extract, (b) n-hexane fraction, (c) ethyl acetate fraction, (d) aqueous fraction with % inhibition of proliferation.

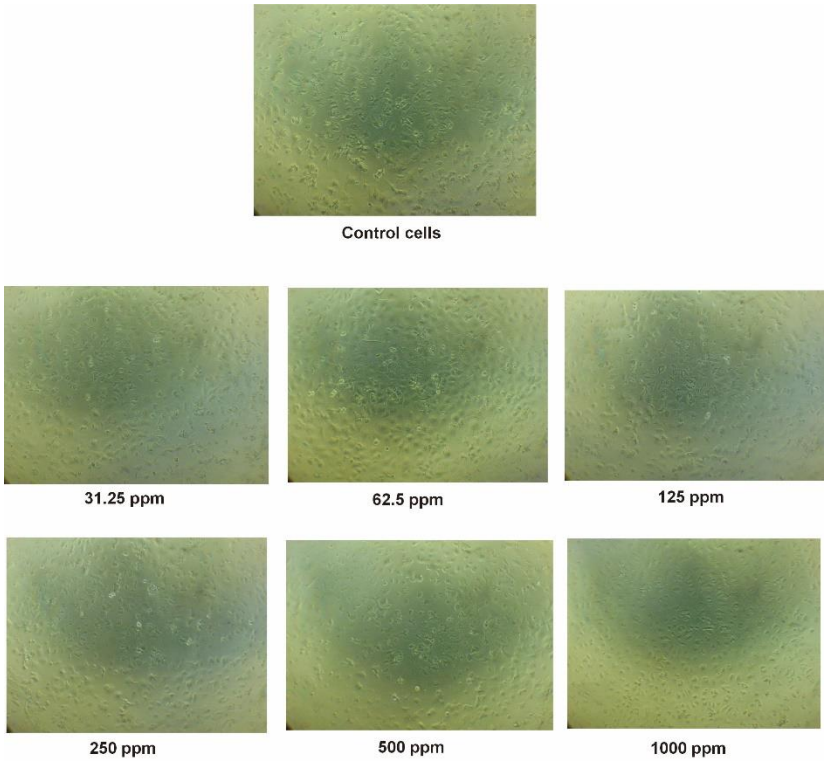

Figure 2: MTT assay results of 70% ethanol extract of mulberry leavess

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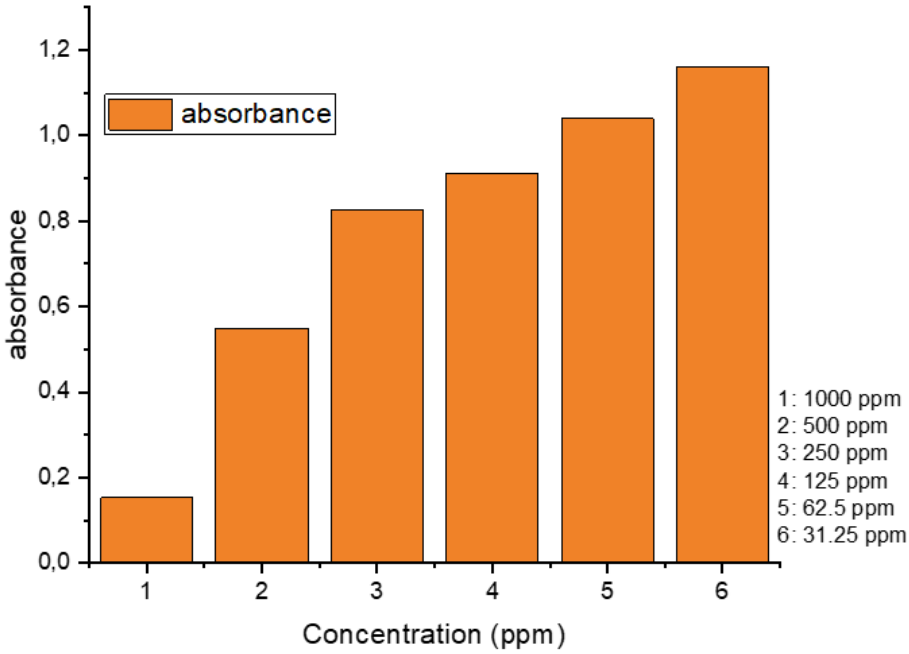

Figure 3: Lipid accumulation profile of 3T3-L1 adipocyte cells with mulberry leaves ethanol extract

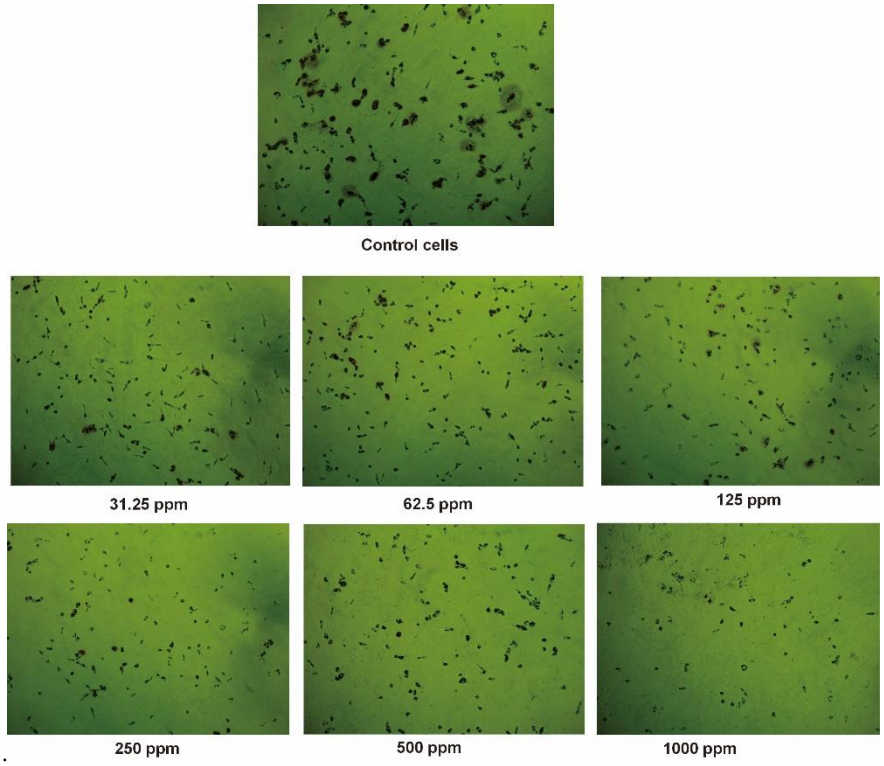

Figure 4: 3T3-L1 adipocyte cells with mulberry leaves ethanol extract and Oil Red O staining

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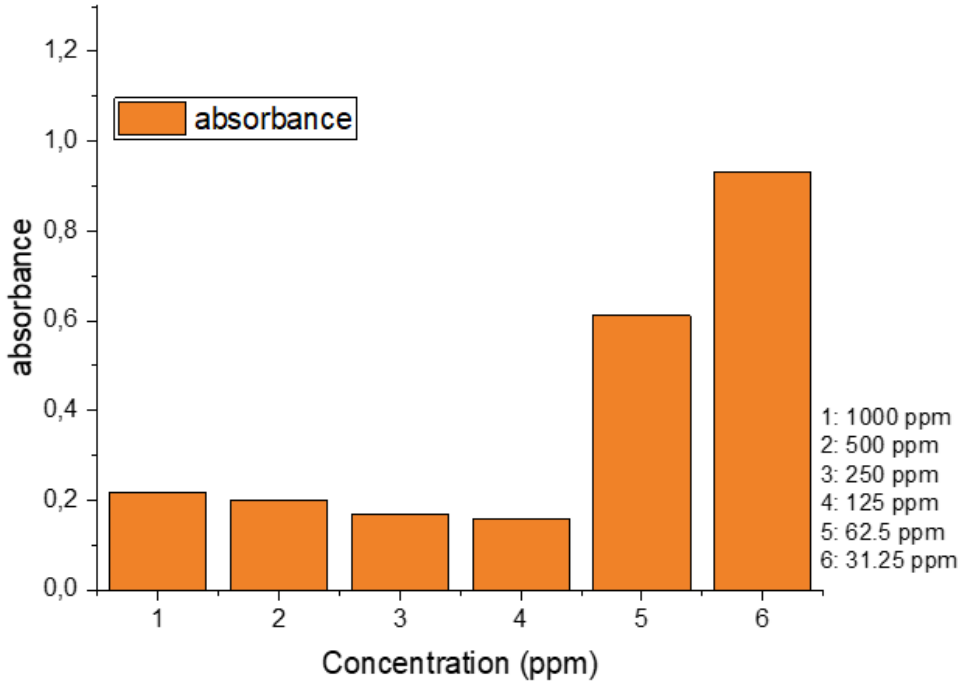

Figure 5: Lipid accumulation of 3T3-L1 adipocyte cells with ethyl acetate fractionation of mulberry leaves

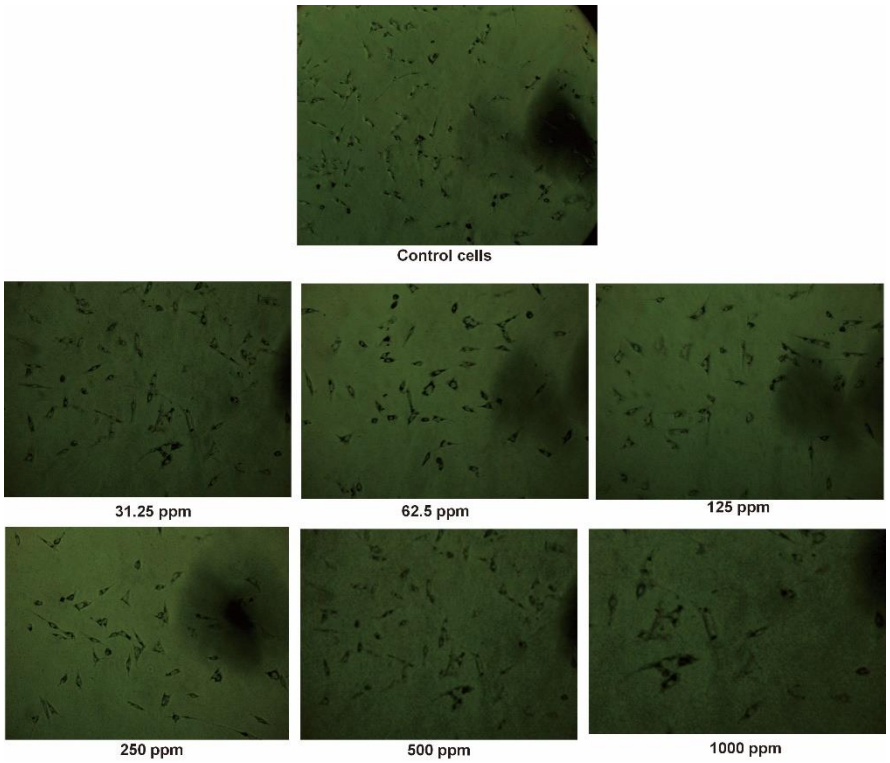

Figure 6: 3T3-L1 adipocyte cells with ethyl acetate fractionation of mulbery leaves and stained with Oil Red O

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Table 5: Calculation of IC_{50} values for each extract is done to determine exactly what concentrations can inhibit the growth 3T3-L1 cells

Tested Compound	Linear Regressions equations	IC_{50} (µg/mL)	Interpretations
Ethanol extract	y = -2,2153x + 10.802; R^2 = 0.9700	416	*No potentially*
N Hexane fractionation	y = -1.3925x + 7.3544; R^2 = 0.8870	49	*Very Strong*
Ethyl acetate fractionation	y = -1.7507x + 8.403; R^2 = 0.7138	89	*Strong*
Water fractionation	y = -2.5808x + 11.033; R^2 = 0.9825	255	*No potentially*

The MTT cytotoxicity test is a popular technique for assessing how plant extracts affect cells. Mulberry leaves were extracted in this study using 70% ethanol and then fractionated using n-hexane and ethyl acetate. According to the test results, the ethanol extract's IC_{50} value was 416 µg/ml; however, the IC_{50} values of the n-hexane and ethyl acetate fractions were much lower at 49 µg/ml and 89 µg/ml, respectively. This suggests that the n-hexane fraction has a greater capacity for cytotoxicity than the ethanol extract. This fits with other research about how lipophilic chemicals in the n-hexane fraction might be better at stopping the growth of fat cells and cancer cells.[31] Following the cytotoxicity test, different amounts of ethanol extract and its fractions were applied to the differentiated 3T3-L1 cells. According to the test results, lipid buildup was decreased by the ethyl acetate, n-hexane, and water fractions, while the ethanol extract caused an increase. This result defies the original theory, which predicted that ethanol extract would be able to reduce lipid buildup. The results could be explained by the fact that other studies have demonstrated the potential lipid-promoting properties of some phytoconstituents in ethanol extract.[32] From a molecular standpoint, several of the chemicals in ethanol extract might be responsible for the increased lipid buildup. Often found in ethanol extracts, phenolic and flavonoid chemicals can change how adipocyte cells use fats, making it easier for fats to build up. However, the decrease in lipid accumulation in 3T3-L1 cells can be explained by the fact that n-hexane and ethyl acetate fractions containing lipophilic chemicals are more likely to block lipid production and improve fat oxidation.[33] Cytotoxicity test was used to determine the ability of a substance to stop the growth of cells. The plant sample's half-maximal concentration (IC_{50}) effect on 3T3-L1 cell development, a cytotoxic test was performed using 3T3-L1 adipocyte cells and mulberry leaves ethanol extract.[25] The MTT (3-(4,5-dimethylthiazolyl-2,5-diphenyltetrazolium bromide) method was employed in this cytotoxic test, and it determines the number of living cells that remain after being exposed to the test substance. The MTT method is renowned for being quick, simple, and accurate. Because the reading time is simultaneous and only fully living cells are assessed, the number of cells using the MTT method is accurately calculated. The enzyme succinate dehydrogenase, produced in the mitochondria of living cells, reduces tetrazolium salt to make purple formazan crystals, which may be detected at 570 nm using an ELISA reader. Since cells contain a relatively constant amount of succinate dehydrogenase, the amount of formazan produced indicates how many cells are alive.[26] Diabetes and cardiovascular disease are more common among those who are obese. Adipose tissue hyperplasia, as well as hypertrophy, are the two leading causes of obesity. During adipogenesis, preadipocytes become adipocytes and build up fat in adipose tissue. Yang's research also demonstrated that the ability of 3T3-L1 adipocyte cells to increase can be inhibited by 70% ethanol extract from mulberry leaves.[27] A lipid accumulation test was done on ethyl acetate, n-hexane, water, and 70% ethanol extract mulberry leaves against 3T3-L1 adipocyte cells using the Oil Red O staining method.[28] The 3T3-L1 adipocyte cells' decreased Oil Red O colour, suggesting that the cells had been treated with ethyl acetate, n-hexane, water, and 70% ethanol extracts of mulberry leaves (*Morus alba L.*) to decrease lipid formation.[29] Overall, the test findings show that the mulberry leaves extract's n-hexane and ethyl acetate fractions have more ability than the ethanol extract to prevent lipid buildup. This study offers fresh perspectives on the potential applications of mulberry leaves in developing treatments for metabolic disorders associated with obesity.[30,33]

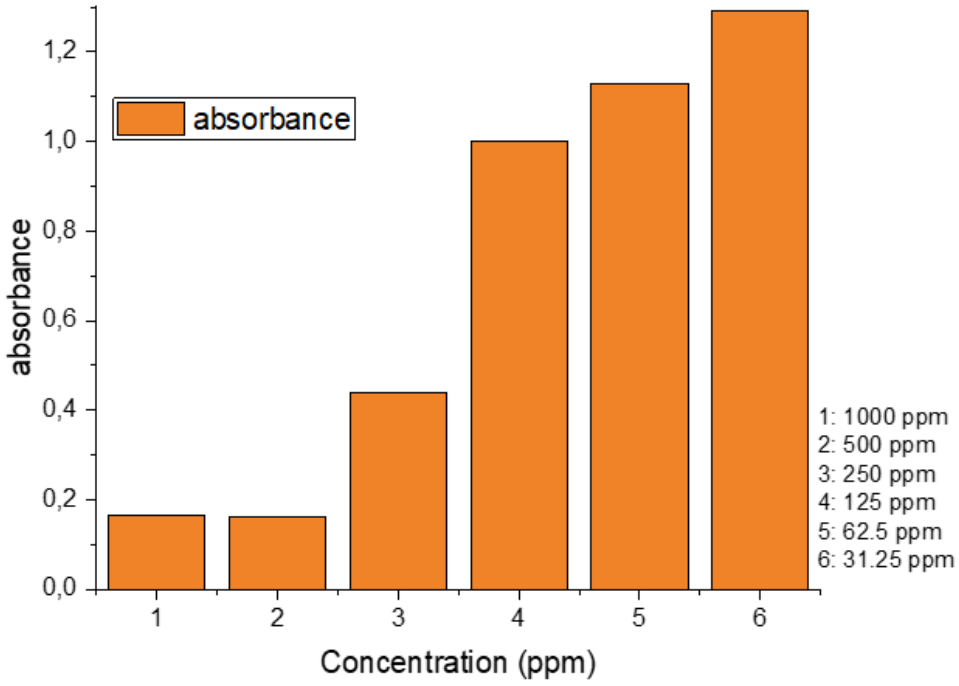

Figure 7: Lipid accumulation of 3T3-L1 adipocyte cells with water fractionation of mulberry leaves

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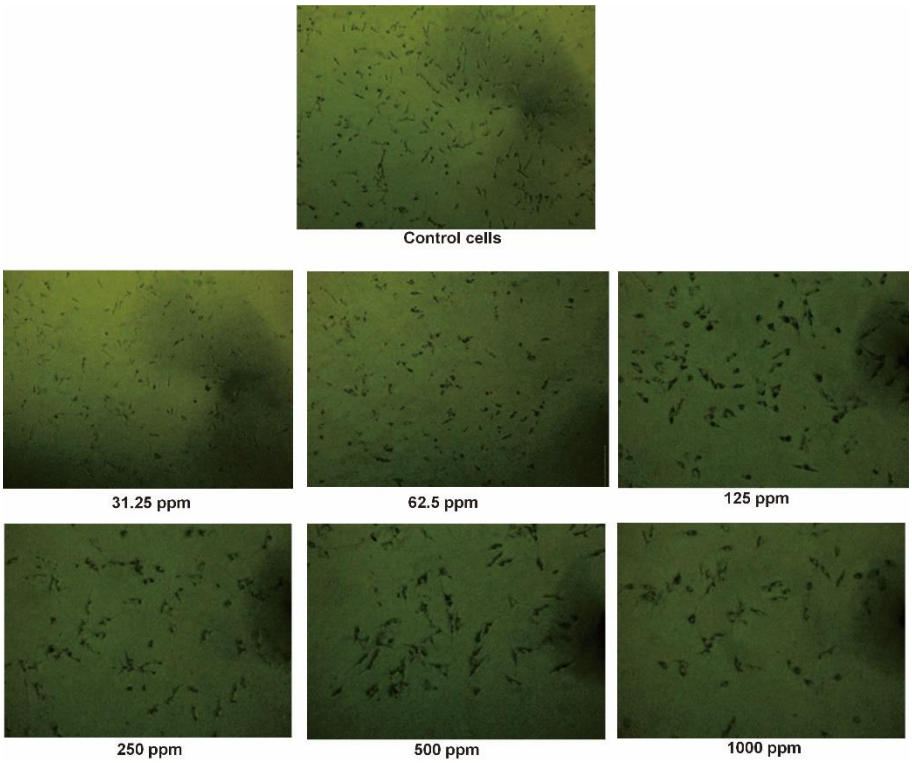

Figure 8: 3T3-L1 adipocyte cells with water fractionation of mulbery leaves and stained with Oil Red O

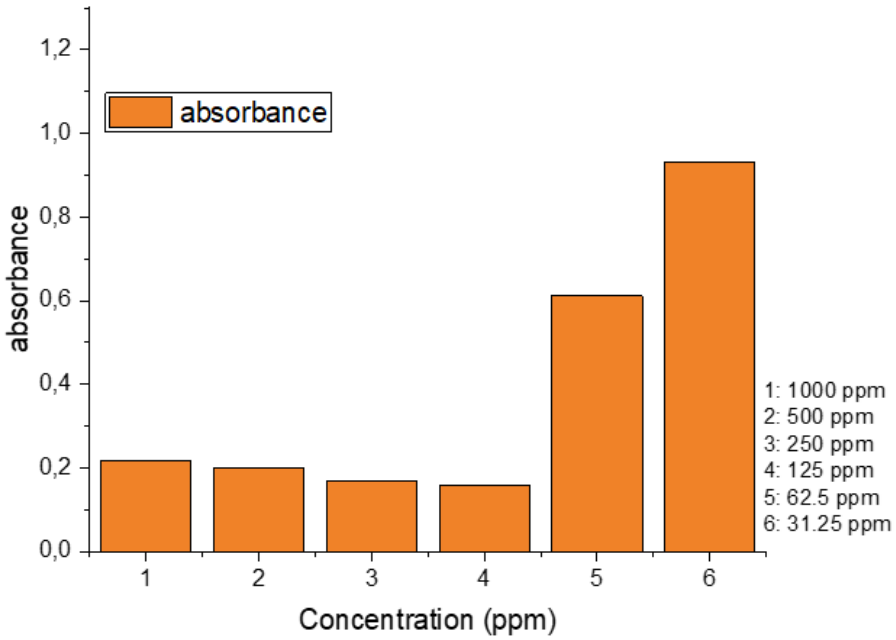

Figure 9: Lipid accumulation of 3T3-L1 adipocyte cells with N-Hexane fractionation of mulberry leaves

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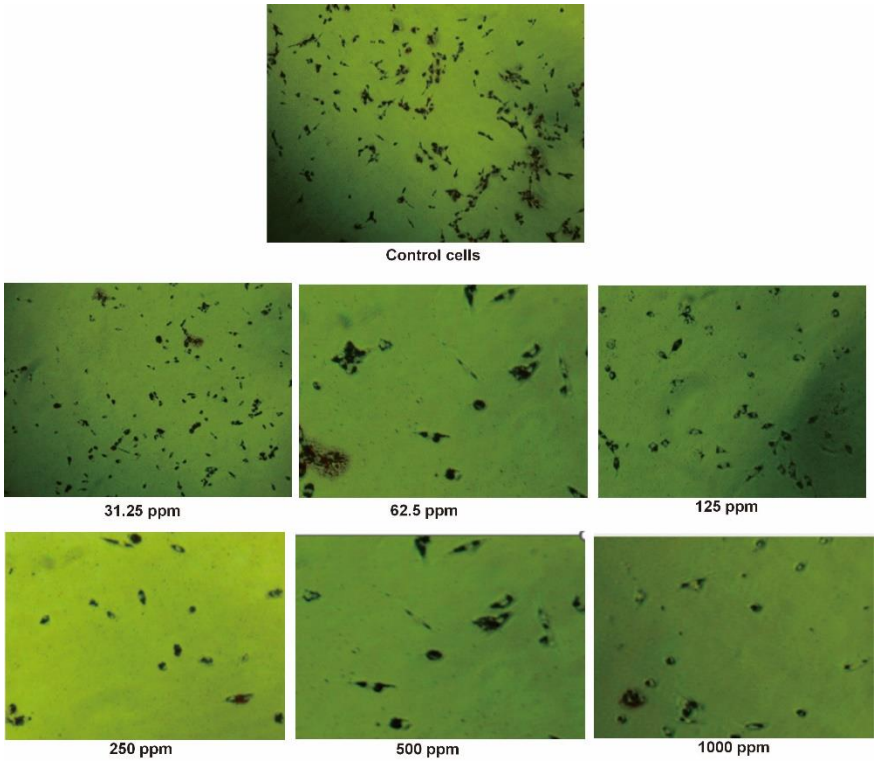

Figure 10: 3T3-L1 adipocyte cells with N-Hexane fractionation of mulbery leaves and stained with Oil Red O

Conclusion

The MTT test on 3T3-L1 cells showed that the fraction exhibits varying inhibitory activities in the following order: n-hexane (49 µg/mL) > ethyl acetate (49 µg/mL) > aqueous fraction (255 µg/mL) and ethanol fraction (416 µg/mL), respectively. The study showed that extract and fractions of mulberry leaves (*Morus alba L.*) can potentially reduce lipid accumulation in 3T3L1 adipocyte cells.and would be a useful alternative in managing obesity, especially in diabetes patients.

Conflict of interest

The authors have no conflicts of interest regarding this investigation**.**

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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