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Original research

An *in vitro* approach to evaluate the anti-adipogenic effect of *Myrica nagi* Thunb. Fruit extract on 3T3-L1 adipocyte cell line

Yash Prashar[a], Nilesh J. Patel[b,*]

[a] PhD Research Scholar, Ganpat University, Ganpat Vidyanagar, Mehsana-Gozaria Highway, Kherva, Gujarat, 384012, India
[b] Department of Pharmacology, Shree S.K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar, Mehsana-Gozaria Highway, Kherva, Gujarat, 384012, India

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ABSTRACT

Background: Obesity involves excess accumulation of body fat, leading to metabolic disorders. Although genetic factors involved in obesity have been identified, the influence of phytochemicals on the genes remains to be elucidated.

Aims: We aimed to (1) screen extracts of *Myrica nagi* Thunb. (Myricaceae) by estimating flavonoid, phenol, and tanin content; (2) evaluate enzyme inhibitory activities of the extracts; and (3) analyze the anti-adipogenic activity of the extracts in 3T3L1 cells.

Methods: We evaluated five extracts of *M. nagi* for their anti-obesity effect by phytochemical screening and enzyme inhibition assays. Furthermore, the extract with the highest inhibitory effect was characterized for its role in adipogenesis using 3T3-L1 cells.

Results: The methanolic extract of *M. nagi* (MEMN) showed the highest inhibitory activity against lipase, α-amylase, and α-glucosidase (IC_{50} values = 91 ± 1.0, 60 ± 4.25, and 100 ± 0.74 μg/mL, respectively). The flavonoid, phenol, and tanin content was highest in the MEMN, with 22.85 ± 1.20, 139.7 ± 11.53, and 1.79 ± 0.12 μg/mL, respectively. The extract also showed anti-oxidant activity with IC_{50} of 162.22 ± 0.65 μg/mL. Additionally, MEMN did not exert cytotoxicity against 3T3-L1cells; it inhibited adipogenesis and promoted lipolysis of 3T3-L1cells. MEMN at 160 μg/mL concentration reduced triglyceride accumulation in 3T3-L1 cells. Moreover, the extract downregulated PPAR-γ expression.

Conclusions: Overall, MEMN possesses potent anti-adipogenic and anti-obesity effects, and thus, can be an alternative herbal treatment for obesity.

1. Introduction

Obesity is a metabolic disorder with serious global effects. Obesity ensues when there is an abundance of dietary unsaturated fats and starch, and is characterized by expanded fat tissues (Spalding et al., 2008). A high fat diet, low physical activity, and excessive consumption of "junk food" lead to weight gain. However, the current treatment options have a low efficacy with various adverse effects. Hence, there is an urgent need to develop novel drugs to treat obesity with little or no adverse effects. The prolonged use of chemicals is harmful, and therefore, plant extracts and phytochemicals may offer an alternative and safer option.

Adipogenesis involves the differentiation of fibroblast-like preadipocytes into adipocytes with lipids, and it is promoted by hormones and other factors. Adipocytes help in maintaining the homeostasis because they control the storage of triacylglycerol (Spiegelman and Flier, 2001). Phenolic compounds are known to suppress adipogenesis and are useful in treating the associated metabolic disorders; currently, novel strategies are being developed for the treatment of obesity, particularly using phytochemicals, because of their non-toxic nature and better effectiveness (Chen et al., 2011). Numerous studies have shown that polyphenolic compounds, such as, chlorogenic acid (Cho et al., 2010), caffeic acid (Liao et al., 2014), and anthocyanin (Wu et al., 2016), can control adipogenesis by promoting lipolysis and apoptosis of adipocytes.

The differentiation of preadipocytes is regulated by various adipogenic factors, including cytokines, hormones, and other factors. Peroxisome proliferator-initiated receptor γ (PPAR-γ) plays a key role in adipogenesis by regulating lipid accumulation in adipocytes (Choi et al., 2016). It is a transcription factor that is abundant in adipose

* Corresponding author. Department of Pharmacology, Shree S.K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar, Mehsana-Gozaria Highway, Kherva, Gujarat, 384012, India.
E-mail addresses: yashprashar@gmail.com (Y. Prashar), nileshcology127@gmail.com (N.J. Patel).

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tissue and plays a central role in the function of mature adipocytes. PPAR-γ also plays a pivotal role in inflammatory response and cell differentiation. Obesity has been reported to induce a decline in the activity and expression of PPAR-γ (Motawi et al., 2017). This correlation appears to be strongly associated with the pathogenesis of obesity. A reduction in the PPAR-γ activity in 3T3-L1 adipocytes, when treated with plant extracts and phytocompounds, has been previously demonstrated (Ogawa et al., 2010).

Myrica nagi possesses medicinal properties and is globally used. Panthari et al. (2012) screened *M. nagi* leaf extract using various methods for its physico–chemical properties. The preliminary phytochemical analysis revealed the presence of carbohydrates, alkaloids, saponins, tannins, flavonoids, sterols and, triterpenoids.

The fruit and bark of *M. nagi* contain chemical compounds such as myricetin, myricitrin, quercetin, and specific glycosides, which are effective in treating metabolic disorders. *Myrica nagi* is primarily sourced for its fruits, and there is no study on its anti-obesity efficacy (Sood and shri, 2016). Hence, this study was undertaken to prove the anti-adipogenic effect of this plant *in vitro*.

The aim of our study was to explore the effect of *M. nagi* fruit extract on adipocytes and study the potential mechanisms underlying its efficacy. We first investigated the total flavonoid, phenol, and tannin content and the effect of the plant extract on various enzymes including amylase, glucosidase, and lipase. Furthermore, we studied the anti-oxidant, anti-obesity, and cytotoxic effects of the plant extract in 3T3-L1 cells. 3T3-L1 cells are a well-known *in vitro* model for studying the role of adipocytes during the treatment of obesity (Cho et al., 2008; Tang et al., 2003).

2. Material and methods

2.1. Chemicals

Glycerol standard, bioactive compounds, pancreatic lipase, α-glucosidase, α-amylase, DPPH, and ABTS were obtained from Sigma-Aldrich (MO, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), Oil Red O, trypsin, insulin, and dexamethasone were purchased from Sigma (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), Pen-Strep, and trypsin were procured from Invitrogen (Waltham, MA, USA). Fetal bovine serum (FBS) was obtained from Sijiqing (Hangzhou, China). The Mouse TG ELISA kit was purchased from Jingmei Biotech (Jiangsu, China). The Mouse ELISA kit PPAR-γ was from Cusabio Co. (Wuhan, China). All other reagents were of analytical grade and sourced from SD Fine Chemicals (Mumbai, India) and Spruce Enterprises (Ambala, India).

2.2. 3T3-L1 cell line

3T3-L1 preadipocyte cell line was purchased from American Type Culture Collection. This cell line was established from a 17–19-day-old Swiss 3T3 mouse embryo (Green and Meuth, 1974; Green and Kehinde, 1976). The cells were culture in DMEM supplemented with 10% FBS, penicillin (100 IU/mL), and streptomycin (100 μg/mL). The cultures were maintained in a humidified environment under 5% CO_2 at 37 °C.

2.3. Plant materials and extraction

The fruits of *M. nagi* were collected from Mandi District, Himachal Pradesh, India in July 2017. The plant material was authenticated by the National Institute of Science Communication and Information Resources (NISCAIR), Delhi, India (Ref. No. NISCAIR/RHMD/Consult//2017/3102-51-4). The healthy fruits were selected for further analysis.

The healthy fruits of *M. nagi* were dried in shade and pulverized to obtain a coarse powder. Finely powdered samples (500 g) were extracted using various solvents (hexane, chloroform, ethyl acetate, acetone, methanol, and acarbose) at 55–65 °C using the Soxhlet apparatus for 4 h. Marc thus obtained was dried and weighed before and after each extraction. The plant extract was filtered through a filter paper of diameter 120 mm (S and S, Dassel, Germany). The extraction was performed thrice; extracts were pooled and subjected to evaporation to dryness using a rotary evaporator (Heidolph Rotavapor, Model D-91126, Type Heizdab WB Eco; Heidolph, Schwabach, Germany). The extracts were then subjected to thin layer chromatography (TLC), phytochemical assays, and *in vitro* activity assays.

2.4. Preliminary phytochemical screening

The phytochemical screening of *M. nagi* extracts was performed for alkaloids, amino acids, carbohydrates, flavonoids, saponin, glycosides, and tannins as described in previous studies (Debiyi and Sofowora, 1978; Trease and Evans, 1989; Kokate et al., 2006; Sofowora, 1993).

2.5. Estimation of flavonoid content

The total flavonoid content in the solvent extracts (methanol, hexane, chloroform, acetone and ethyl acetate) was estimated following the method described by Jiao and Wang (2000). The flavonoid content in the extract is expressed as mg/g quercetin equivalent (QE).

2.6. Estimation of total phenols and tannins

The phenolic content in all the solvent extracts and supernatant was determined according to the method described by Makkar et al. (1993) and Makkar (2003). The tannin content was determined based on the results obtained for phenolic content using the following formula:

$$Tannin\ \ content = Total\ \ phenolic\ \ content - Non\ tannin\ \ phenol\ \ content$$

2.7. In vitro assay to estimate the inhibition of α-amylase, α-glucosidase, and lipase

The inhibition of α-amylase activity by the solvent extracts of *M. nagi* was estimated based on the method described by Oboh et al. (2012). The IC_{50} values were calculated, and it indicated the efficacy of each extract. The α-glucosidase inhibition assay was performed based on the method described by Dong et al. (2012). The activity of the pancreatic lipase enzyme was determined using the method described by Yuniarto et al. (2015).

2.8. Separation of bioactive constituents by thin layer chromatography (TLC)

The standard TLC-based analytical procedure proposed by Stahl (1969) was used to analyze the methanolic extract of *M. nagi* (MEMN) for the isolation and identification of secondary metabolites. The mobile phase consisted of a mixture of butanol, acetic acid, and water at a ratio of 4:2:1 (*v/v/v*). The spots corresponding to different compounds were detected using iodine chamber (Wagner and Bladt, 1996). The spots were identified based on the method proposed by Harborne (1973).

2.9. Evaluation of in vitro anti-oxidant activity of the plant extract

The antioxidant activity of MEMN was performed using the H_2O_2 method reported by Ilhami et al. (2005). The antioxidant activity of the extract was calculated using the following formula:

$$\%\ \boldsymbol{inhibition} = [(\boldsymbol{A}0 - \boldsymbol{A})/\boldsymbol{A}] \times 100$$

where, A0 is the absorbance of control and A is the absorbance of the test/standard.

Table 1
Quantitative analysis of various solvent extracts of *Myrica nagi*.

Assay (μg/mL)	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol
Total flavonoids	2.85 ± 1.20	10.85 ± 1.20	18.85 ± 1.20	14.85 ± 1.20	22.85 ± 1.20
Total phenolics	13.7 ± 11.53	50.7 ± 11.53	80.7 ± 11.53	115.7 ± 11.53	139.7 ± 11.53
Total tannins	0.79 ± 0.12	0.29 ± 0.12	0.22 ± 0.12	1.00 ± 0.12	1.79 ± 0.12

Note: values are expressed as mean ± standard deviation, n = 3.

2.10. MTT assay to determine the cytotoxic effect of plant extract on 3T3-L1 cells

For the cytotoxicity assay, 32 mg/mL stock solution was prepared using DMSO. The following two-overlay dilutions were prepared: 320 and 10 μg/mL, using DMEM for the treatment. 3T3-L1 cells were separated by treating with PBS solution containing 0.2% trypsin, 0.02% EDTA, and 0.05% glucose. Furthermore, 50 000 cells/well were seeded in a 96-well plate and incubated under 5% CO_2 for 24 h at 37 °C. The cytotoxic effect of MEMN was determined using the procedure reported by Sathyamurthy et al. (2018). The inhibition rate of cell proliferation was calculated using the following formula:

$$\%\ Inhibition = [(OD\ of\ control - OD\ of\ sample)/OD\ of\ control] \times 100$$

where, OD is optical density.

2.11. Anti-obesity assay

3T3-L1 cells were separated by treating with phosphate buffer saline (PBS) solution containing 0.2% trypsin, 0.02% EDTA, and 0.05% glucose, followed by centrifugation. Thereafter, 20 000 cells/well were seeded in a 96 well plate and incubated under 5% CO_2 for 24 h at 37 °C.

Thirty-two milligrams of the sample was diluted in 1 mL of DMSO to prepare a stock solution of 32 mg/mL concentration. The stock solution was further diluted to prepare solutions of 160, 40, and 10 μg/mL concentrations using DMEM; these solutions were then used to treat 3T3-L1 cells. Untreated cells were considered as the control. Trypsinized 3T3-L1 cells were treated with different concentrations of MEMN. Triglyceride content was estimated by Oil red O staining following the method reported by Roh and Jung (2012). Furthermore, triglycerides secreted into the culture medium after the treatment of cells for 8 days were measured using the Mouse TG ELISA kit (Jingmei Biotech).

2.12. Measurement of PPAR-γ expression

Trypsinized 3T3-L1 cells were seeded in a six-well plate at a volume of 2 mL and treated for 8 days with different concentrations of MEMN. PPAR-γ expression was determined using the Mouse PPAR-γ ELISA kit (Cusabio Co., Wuhan, China).

2.13. Statistical analysis

Statistical analyses were performed using Graph pad prism version 8.0. The results are expressed as mean ± standard deviation from three replicates. The differences between the treated groups and control with a p of < 0.05 were considered statistically significant.

3. Results

3.1. Phytochemical screening of plant extract

Both qualitative and quantitative analyses of the plant extracts were performed. Percentage yield of plant extracts with different solvents was as follows: hexane, 5.12%; chloroform, 6.05%; ethyl acetate, 7.18%; acetone, 6.23%; and methanol, 8.83%. Furthermore, acetone and methanol plant extracts showed the presence of maximum number of compounds. The results were positive for the presence of alkaloids, glycosides, flavonoids, steroids, amino acids, carbohydrates, tannins, and phenols. Of the nine compounds detected using various tests, eight compounds, except alkaloids, were present in the acetone and methanol extracts.

3.2. Quantitative analysis of the solvent extracts

Quantitative analysis was carried out based on the qualitative results. Only flavonoid, phenol, and tannin content was estimated. The results of the quantitative analysis correlated with the qualitative analysis results. Methanol and acetone extracts showed a high content of all compounds. The analysis was performed using the standard, such as gallic acid and quercetin (Table 1).

3.3. Enzyme inhibition assay

3.3.1. α-Amylase inhibition assay

Enzyme inhibition assays were carried out to determine the inhibitory activity of all solvent extracts on three different enzymes (α-amylase, α-glucosidase, and pancreatic lipase). The experiments were performed in triplicate and at five different concentrations (10, 50, 100, 250, or 500 μg/mL); the IC_{50} value of each solvent extract was calculated. The results were compared with those of the standard chemicals. Based on the results, MEMN was found to be the most effective in inhibiting the enzyme activities (Table 2).

The plant extracts effectively inhibited the α-amylase activity. The inhibition rate was estimated using maltose as the standard. Chloroform and acetone showed the highest IC_{50} value of 180 and 150 μg/mL, respectively. Both ethyl acetate and methanol extracts showed the highest inhibitory activity at a very low concentration compared with the other solvent extracts. Hexane extract showed a moderate activity

Table 2
Enzyme inhibition assay using different solvent extracts of *Myrica nagi*.

Assay	IC_{50} values of different solvents (μg/mL)				
	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol
α-Amylase	100 ± 1.58	180 ± 2.64	80 ± 3.45	150 ± 1.85	60 ± 4.25
α-Glucosidase	300 ± 0.74	551 ± 0.34	246 ± 0.77	251 ± 1.71	100 ± 0.74
Pancreatic lipase	111 ± 1.00	245 ± 0.65	98 ± 0.52	100 ± 0.22	91 ± 1.00

Note: values are expressed as mean of triplicates ± standard deviation.

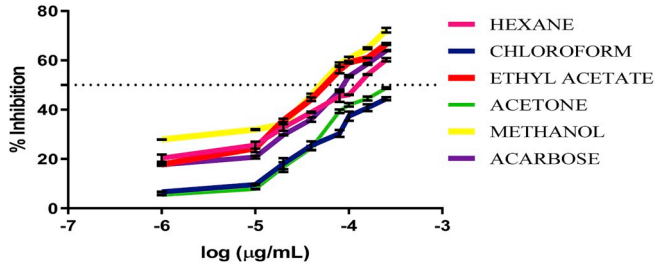

Fig. 1. Effect of various solvent extracts of *Myrica nagi* on the inhibition of α-amylase enzyme. The IC50 value is indicated with a dotted line.

toward α-amylase (Fig. 1).

3.3.2. α-Glucosidase inhibition assay

The α-glucosidase inhibition assay was carried out to determine the inhibitory activity of the solvent extracts by comparing with that of an appropriate standard. The results showed low activity of all solvent extracts, determined based on the IC_{50} values. Fig. 2 represents the percentage of inhibition activity of the α-glucosidase enzyme.

3.3.3. Pancreatic lipase inhibition assay

The IC_{50} values of ethyl acetate and methanol extracts of 98 and 91 µg/mL, respectively, revealed that they effectively inhibited the pancreatic lipase activity (Fig. 3).

Among all extracts, MEMN showed effective activity at a relatively lower quantity.

3.4. TLC finger printing

The TLC finger printing analysis of the MEMN showed the best results with the toluene:ethyl acetate solvent system at a ratio of 7:3 for methanolic extracts. The TLC plates showed the best result (Fig. 4, Table 3) when scanned and visualized with iodine.

3.5. Cytotoxic assay

The results of the cytotoxic assay revealed that the inhibitory activity of MEMN was concentration dependent. With an increase in the concentration of the plant extract, there was an increase in cell death (Table 4).

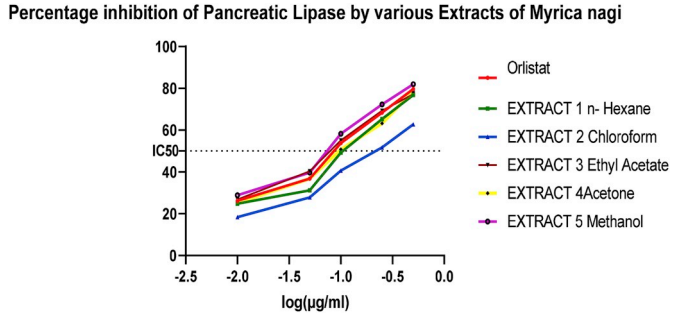

Fig. 3. Effect of various solvent extracts of *Myrica nagi* on the inhibition of pancreatic lipase enzyme. The IC50 value is indicated with a dotted line.

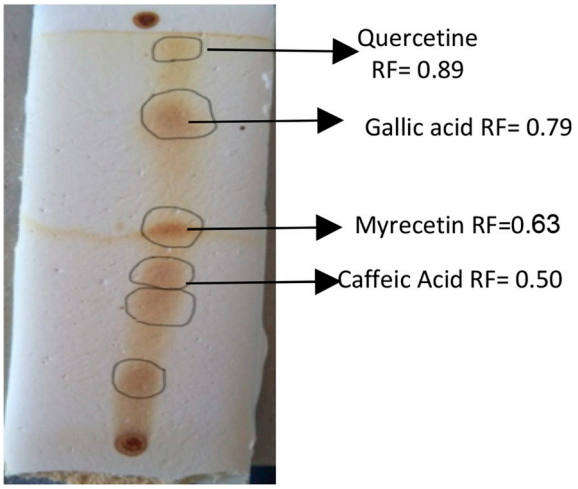

Fig. 4. Thin layer chromatography finger print showing different compounds in the methanolic extract of *Myrica nagi*. RF: retention factor.

3.6. Antioxidant activity of the MEMN

The antioxidant assay results showed that the IC_{50} value of MEMN was 162.22 ± 0.65, which was different from that of ascorbic acid, at 78.31 ± 0.72. These results indicate that the plant extract had a high free radical-scavenging activity (Table 5).

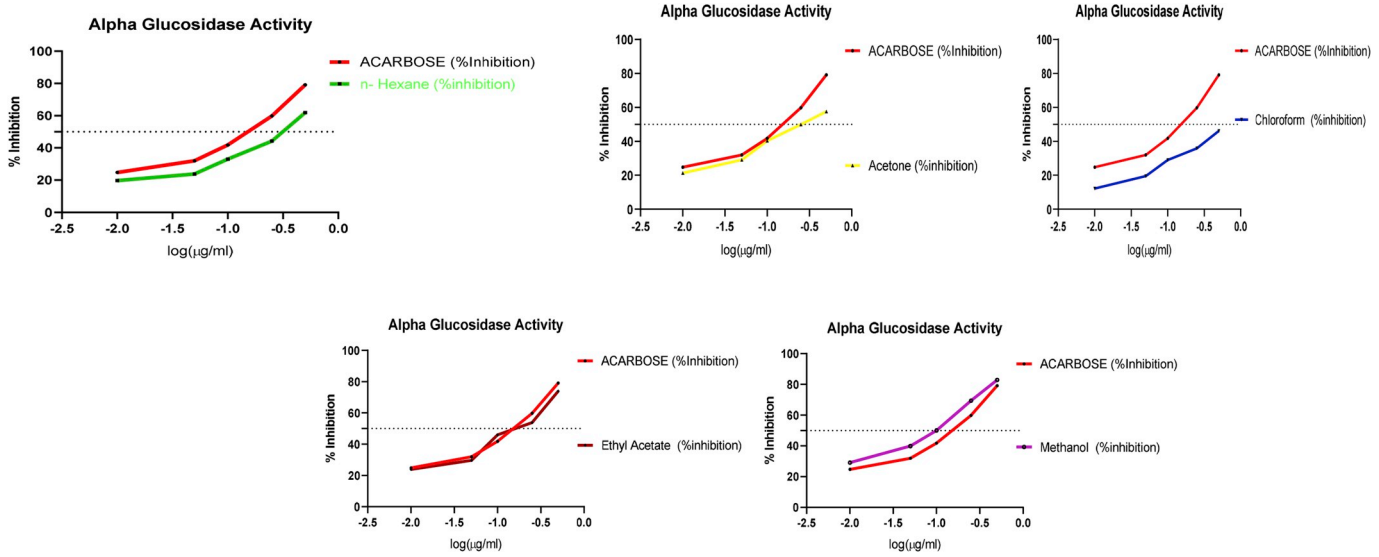

Fig. 2. Effect of various solvent extracts of *Myrica nagi* on the inhibition of α-glucosidase enzyme. The IC50 value is indicated with a dotted line.

Table 3
Results of thin layer chromatography.

Plant extract	Detection reagent used	Obtained Rf value	Inference (compound detected)
Myrica nagi	iodine	0.89	Quercetin
		0.79	Gallic acid
		0.63	Myricetin
		0.50	Caffeic acid

Table 4
Result of the cytotoxicity assay using the MTT method.

Compound name	Conc. μg/mL	OD at 590 nm	% Inhibition	IC_{50} μg/mL
Control	0	0.781	0	
Myrica nagi	10	0.729	6.66	1280
	20	0.665	14.85	
	40	0.615	21.25	
	80	0.584	25.22	
	160	0.551	29.49	
	320	0.498	36.26	
	640	0.455	43.16	
	1280	0.399	50.12	

Table 5
Antioxidant activity of MEMN, with ascorbic acid as the standard.

Plant	Concentration (μg/mL)	%Inhibition (%)	IC_{50} (μg/mL)
Ascorbic acid	10	19	78.31 ± 0.72
	50	36	
	100	58	
	250	68	
	500	80	
MEMN	10	12	162.22 ± 0.65
	50	30	
	100	38	
	250	52	
	500	60	

Values are mean ± SD with n = 3.

3.7. Anti-obesity assay

The results of the anti-obesity assay showed high triglyceride accumulation (41.46 mg/dL) at a concentration of 10 μg/mL MEMN. The accumulation of triglycerides reduced with the increase in concentration; for instance, at 40 and 160 μg/mL MEMN, the amount of triglycerides accumulated was 29.27 and 14.63 mg/dL, respectively (Table 6).

3.8. Effect of the MEMN on intracellular triglyceride content

Triglyceride content was determined by oil red O staining to confirm the results of the qualitative analysis (Fig. 5). As shown in Fig. 6, the MEMN treatment significantly reduced triglyceride accumulation. The triglyceride content in 3T3-L1 adipocytes was 14.63% after the treatment with 160 μg/mL MEMN compared with that in control cells. These results indicate the anti-obesity role of MEMN in developed adipocytes.

Table 6
Results of triglyceride accumulation in 3T3L1 cell lines.

Sample (μg/mL)	Conc. μg/mL	Abs 540 nm	Triglycerides (mg/dL)
Myrica nagi	10	0.094	41.46
	40	0.106	29.27
	160	0.116	14.63

3.9. Effects of the MEMN on PPAR-γ expression

As shown in Fig. 7, the PPAR-γ expression was significantly reduced at a concentration of 160 μg/mL. These results further confirm that the MEMN has an inhibitory effect on 3T3-L1 preadipocytes.

4. Discussion

Adipogenesis begins with the differentiation of multi-potent mesenchymal cells into preadipocytes, followed by their differentiation into adipocytes. It was previously considered that the number of fat cells do not increase in humans. However, it was later discovered that the number of fat cells can increase or decrease depending on the physiological condition of a person (Gregoire et al., 1998). Hence, research on adipogenesis has been the focus of recent studies in order to treat obesity and related disorders. The anti-obesity activity of *M. nagi* extract has not been explored in this context; hence, the aim of our study was to explore the role of different constituents in *M. nagi*, considered to possess anti-adipogenic and anti-obesity effects. Previous studies on different species of *Myrica* (*M. esculenta*) have demonstrated that the phytoconstituents, such as myricetin, gallic acid, caffeic acid, and quercetin, present in the plant extract have the anti-adipogenic effect (Sood and Shri, 2016).

Here, the MEMN was used to explore the anti-obesity effect of *M. nagi* in 3T3-L1 adipocyte cells. The *in vitro* study indicated that the antioxidant properties of the extract can be attributed to the presence of various bioactive compounds, such as phenolic acids, flavonoids, and tannins. Furthermore, we evaluated the role of *M. nagi* extract in the inhibition of various enzymes, such as α-glucosidase, α-amylase, and pancreatic lipase. The inhibition of these enzymes can been considered one of the important factors in the treatment of obesity.

Enzymes play a major role in various biological processes in the body. α-Amylase is one of the important enzymes involved in the hydrolysis of α-linked polysaccharides, glucose, and starch. α-Glucosidase is responsible for the hydrolysis of carbohydrates, whereas, pancreatic lipase converts triglycerides into monoglycerides. Our results showed that the MEMN inhibited α-amylase, α-glucosidase, and pancreatic lipase activities at a concentration (IC_{50}) of 60, 100, and 91 μg/mL, respectively. The MTT assay demonstrated that the MEMN at 10–160 μg/mL concentrations had no significant cytotoxic effect on 3T3-L1 preadipocytes. Furthermore, the results indicated that the MEMN reduced the differentiation of 3T3-L1 preadipocytes *in vitro*.

3T3-L1 cell line is developed from murine Swiss 3T3 cells (Green and Meuth, 1974). Moreover, the use of various agents during cell culture causes increased lipid droplet accumulation and glucose transporter 4 (GLUT4)-mediated glucose uptake (Vishwanath et al., 2013). 3T3-L1 cell line is one of the well-studied models for investigating the changes associated with the differentiation of preadipocytes to adipocytes. It has been reported that 3T3-L1 preadipocytes in a characterized adipogenic solution undergo differentiation. Around 24 h after treatment with MDI (3-isobutyl-1-methylxanthine, methylisobutylxanthine, or dexamethasone), 3T3-L1 cells undergo division (also known as mitotic clonal development) followed by terminal differentiation. In the present study, our goal was to evaluate whether *M. nagi* extracts suppress the differentiation of 3T3-L1 preadipocytes into adipocytes by influencing the mechanisms associated with the cell cycle and differentiation. The phytoconstituents in *M. nagi*, including triterpenoids and phytosteroids, not only influence the G1 cell cycle, but also affect adipocyte differentiation and lipid aggregation in 3T3-L1 cells. Triterpenoids and phytosteroids are auxiliary plant metabolites with various functions. The inhibitory effect of triterpenoids and phytosteroids can be attributed to the membrane cholesterol content (Popov, 2002; Zhang et al., 2015).

Furthermore, the accumulation of triglycerides was reduced in 3T3-L1 cells treated with MEMN in the present study. Lipid digestion in adipocytes can be attributed to various factors. As the MEMN reduced

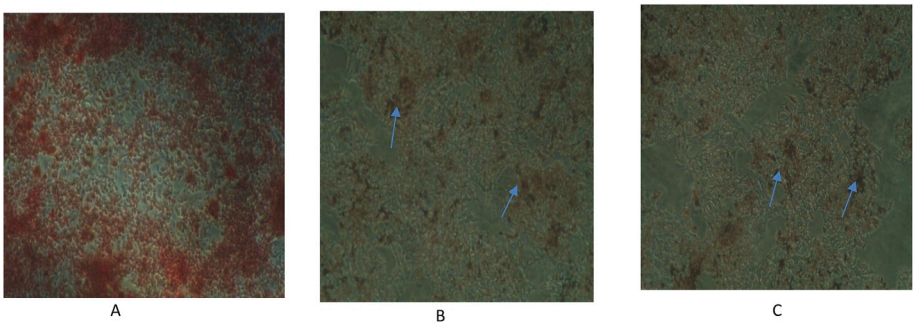

Fig. 5. Effect of the methanolic extract of *Myrica nagi* (MEMN) on lipid droplet formation as measured by Oil Red O staining. A: MEMN (10 μg/mL); B, Control; C: MEMN (160 μg/mL). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

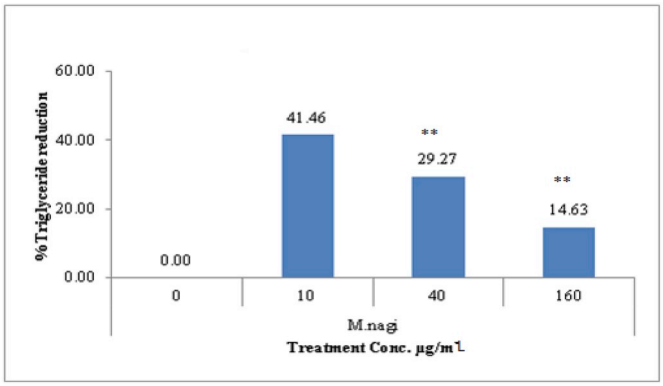

Fig. 6. Reduction in triglyceride concentration after treatment of 3T3-L1 cells with methanolic extract of *Myrica nagi* (MEMN).

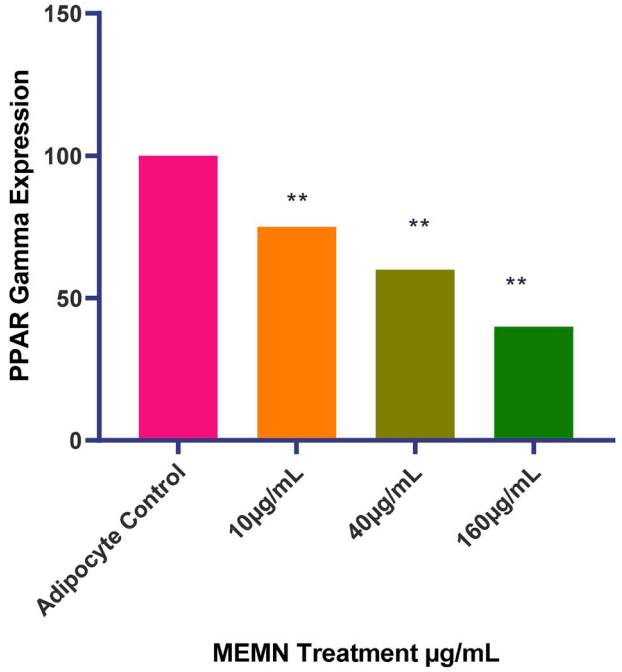

Fig. 7. Expression of PPAR-γ in 3T3-L1 cells after treatment with methanolic extract of *Myrica nagi*. $^{**}p < 0.001$.

lipid aggregation and triglyceride content during the differentiation of 3T3-L1 cells, the role of the MEMN in lipogenesis was investigated.

Various diseases including obesity are related to gene expression; in humans, PPAR-γ expression is linked with adipogenesis. PPAR-γ is expressed in the adipose tissue, and it has a role in controlling inflammation through macrophages. The upregulation or down-regulation of PPAR-γ expression leads to obesity (Lazar, 2007). Currently, synthetic agonists of PPAR are used for dyslipidemia. The main molecular mechanism by which the anti-inflammatory activities of PPAR-γ occur is via interference with proinflammatory transcription factors including STAT, NF-κB, and AP-1 (Stienstra et al., 2007). The MEMN at a concentration of 160 μg/mL inhibited the expression of PPAR-γ, indicating the effectiveness of the plant extract at the molecular level. Our results also indicated that the phytoconstituents extracted from *M. nagi* are effective in regulating gene expression.

Further studies using mammalian models are needed to establish the role of MEMN for successful pharmaceutical intervention on reverse cholesterol transport, blood glucose, and related conditions associated with increased lipogenesis.

The association between obesity and inflammation increases the levels of proinflammatory biomarkers such as cytokines and C-reactive protein (CRP) in obese individuals. Besides TNF-α, other adipokines are also produced by the adipose tissue. Resistin, adiponectin, leptin, and monocyte chemoattractant protein-1 (MCP-1) are a group of proteins produced by the adipose tissue. Furthermore, it is important to note that when the levels of MCP-1 and resistin increase in obesity, the secretion of adiponectin decreases (Kadowaki and Yamauchi, 2005; Yu and Ginsberg, 2005). Hence, these markers can be of great interest for future studies.

The present study findings promote the use of herbal extracts in the treatment of obesity. The usage of herbal extract results in less adverse effects with considerable efficacy. The usage of herbal drugs in synergy has been found to be more effective than using them as individual constituents. *Myrica nagi* extract has a broader range of applications as it possesses a number of bioactive components with a wide therapeutic index. The MEMN showed better efficacy even at a lower dosage, and it can be an effective approach toward the treatment or prophylaxis of obesity.

The anti-adipogenic effect of herbal extracts has been demonstrated; for instance, the effects of *Zingiber officinale* plant extract has been studied in mice induced with obesity using the gold thioglucose method. The results revealed that both methanol and acetate extracts resulted in effective weight loss, whereas, the glucose level was normal compared with that in the control mice. An increase in insulin level was observed in the treated mice, and this was attributed for the weight loss in obese mice (Goyal and Kadnur, 2006). Lei et al. (2007) evaluated the effect of pomegranate plant leaf extract on high fat diet-induced obese mice. There was a decrease in weight and the other evaluated parameters in mice treated with leaf extract for 5 weeks.

The present study provides direct evidence that *M. nagi* induces lipolysis. The flavonoids and phytosteroids present in the plant extract are responsible for its anti-obesity activity. Our findings suggest that under *in vivo* conditions, combinations of phytoconstituents exhibit anti-adipogenic effect. The clinical effect of such combinations should be explored.

5. Conclusions

Several studies support that obesity-induced inflammation is associated with the over expression of PPAR-γ. The findings of the present study demonstrated that the MEMN has potential anti-obesity activity because of its role in the downregulation of PPAR-γ expression. Our results demonstrated that pharmacological targeting of adipose tissue is a promising strategy for the development of novel drugs for obesity. We investigated the effects of *M. nagi* extracts on various enzymes and differentiation of 3T3-L1 cells to adipocytes, based on lipid accumulation and gene expression assays. The results confirmed that the *MEMN* is safe and beneficial in the prevention and management of obesity.

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CRediT authorship contribution statement

Yash Prashar: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Writing - original draft, Writing - review & editing. **Nilesh J. Patel:** Supervision, Validation, Visualization, Project administration, Resources.

Declaration of competing interest

The authors declare that they have no conflicts of interest to disclose.

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