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Jatupalathika herbal formula inhibits lipid accumulation and induces lipolysis in 3T3-L1 adipocytes

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ABSTRACT: Jatupalathika is composed of four dried fruit of *Terminalia bellerica* (Gaertn.) Roxb, *Terminalia chebula* Retz. var. chebula, *Terminalia arjuna* Roxb., and *Phyllanthus emblica* L. This research aimed to investigate the anti-obesity potential of Jatupalathika aqueous extracts on the inhibition of fat accumulation and the induction of lipolysis in 3T3-L1 adipocytes, compared with individual herbs in the formula, gallic acid, and ellagic acid at a series of concentrations of 10–100 μg/ml. The viability and proliferation of preadipocytes and differentiated adipocytes were analysed by the MTT technique. Lipid accumulation was measured by oil red O staining. The amount of triglycerides and glycerol released in the medium was analysed by a colorimetric method. Compared with the control, Jatupalathika extracts reduced fat accumulation (3.5 folds at 100 μg/ml) and triglyceride content (3 folds at 100 μg/ml), but increased glycerol release (4.5 folds at 10 μg/ml). These activities could be attributed to the synergy between gallic acid and other bioactive components in the formula. Moreover, Jatupalathika extract (100 μg/ml) decreased the % viability of both preadipocytes and differentiated adipocytes by $28.85 \pm 1.87\%$ and $32.36 \pm 2.66\%$, respectively. It could be concluded that Jatupalathika extract effectively reduced fat accumulation by the inhibition of lipogenesis and adipogenesis and the induction of lipolysis, which could be related to the induction of cell apoptosis or a reduction in cell proliferation. This herbal formula might potentially be used in the prevention and treatment of obesity. However, further investigations on mechanism of action, *in vivo* effects, and toxicity of the formula need to be done.

KEYWORDS: anti-obesity, Jatupalathika, 3T3-L1 adipocytes, lipogenesis, lipolysis

INTRODUCTION

Obesity, a chronic metabolic disorder caused by eating behaviours and an energy imbalance, has become a major health problem in humans worldwide. It is a risk factor associated with the progress of hyperlipidaemia, coronary heart disease, type II diabetes, hypertension, and metabolic syndromes [1, 2] as well as certain types of cancer and osteoarthritis [3]. Although several anti-obesity drugs have been approved and marketed, most of them have been withdrawn due to their risk of severe side effects, such as heart valve damage and psychiatric disorders [4]. Even orlistat, a gastrointestinal lipase inhibitor used to help lose weight due to its safety, also causes flatulence, urgent bowel movements, oily stools, and non-negligible side effects [5].

Alternative ways to manage obesity are uses of bioactive compounds from plants. Various herbs, such as *Fucus vesiculosis*, *Gymnema sylvestre*, *Pterocarpus marsupium*, *Commiphora mukul*, and *Antheraea perny*, were reported for the treatment of obesity [6, 7]. *Nepeta japonica* Maximiwicz was found to inhibit triglyceride accumulation and induce lipolysis in 3T3-L1 adipocytes [3]. Similarly, treatment of 3T3-L1 cells with *Momordica charantia* extract resulted in a decrease of lipid accumulation and intracellular triglyceride amounts as well as a reduction in preadipocyte proliferation [8]. In an *in vivo* study, an aqueous extract of *Cyperus rotundus* L. tubers exhibited a potent anti-obesity activity in rats fed with a high-fat diet [9].

Numerous dietary supplements or nutraceuticals for weight control, including vitamins, amino acids, single herbs, herb formulas, or a combination of these compounds, have been launched in pharmacy stores and on websites of online selling anti-obesity products with weight loss guarantee [10]. However, important limitations which have hindered the acceptance of using these products are their scientific verification and quality control.

Jatupalathika (JP) is a traditional formulation of four dried fruits of *Terminalia bellerica* (Gaertn.) Roxb, *Terminalia chebula* Retz. var. chebula, and *Terminalia arjuna* Roxb. of the Combretaceae family, and *Phyllanthus emblica* L. from Euphorbiaceae. Our search revealed that JP formulations, e.g., pills, capsules, and solutions, were launched on the herbal market and used for various indications such as detoxification, anti-ageing, cellulite reduction, body fat depletion, and weight loss. However, scientific information for the JP formula related to reducing fat accumulation and anti-

obesity still needs to be approved.

The pharmacological activities of the four individual herbs in the JP formula were investigated. *T. bellerica* (TB) extract has been reported to have anti-oxidation and hypoglycaemic effects, immunostimulating and anti-allergy activities, and the ability to lower total cholesterol, triglycerides, and uric acid [11]. Moreover, TB exhibited an increase in PPAR-α and PPAR-γ and induced the transportation of glucose into cells by increasing the adipocyte proliferation [12]. Bark and leave extracts of *T. arjuna* (TA) were reported on having pharmacological activities such as hypoglycaemic and anti-diabetic effects in mice. However, the active compounds responsible for these activities remain unknown [11]. The extracts from fruits, leaves, and bark of *T. chebula* (TC) have been used as herbal medicines for carminatives, astringents, and phlegm removal treatment, as an anti-oxidant and anti-diabetic, and to lower blood cholesterol levels [13]. One previous study reported that *P. emblica* (PE) slightly affected the differentiation of adipocytes and could induce glucose uptake into cells. However, the expression of glut-4 and transcription factors related to adipogenesis should be confirmed [14]. Interestingly, a clinical study comparing PE with simvastatin in a patient with hyperlipidaemia type II indicated that PE significantly lowered blood lipid in association with the antihypertensive drug [15]. Triphala is a traditional herbal formulation, commonly found in the herbal market, which consists of three of the four herbs in JP, TB, TC, and PE. The development of analytical techniques of Triphala for quality control was reported. Gallic acid, which was the major compound in Triphala, was generally used as a marker. However, the anti-obesity-related activity of JP has rarely been studied. Based on the literature review, we hypothesised that JP would have anti-obesity-related activities. This study aimed to determine the *in vitro* anti-obesity effects of JP formula compared with its individual herbs and its major components (gallic acid and ellagic acid). The scientific confirmation of the effect of JP extract on fat accumulation and lipolysis might be beneficial for the prevention and treatment of obesity.

MATERIALS AND METHODS

Chemicals and reagents

Dulbecco's modified Eagle medium (DMEM containing high glucose, L-glutamine, sodium pyruvate), insulin (INS), penicillin-streptomycin solution, dexamethasone (DEX), 3-isobutyl-1-methyl xanthine (IBMX), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide], gallic acid, ellagic acid, Oil Red O solution, phosphate-buffered saline (PBS), dimethyl sulphoxide (DMSO), triglyceride (TG), and glycerol assay kits were obtained from Sigma (MO, USA). Foetal bovine serum (FBS) was purchased from GIBCO (Invitrogen, NY, USA). All solvents and reagents were of analytical grade.

Cell culture

3T3-L1 cells, obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA), were grown in culture plates containing DMEM supplemented with 10% heat-inactivated FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin. The cultures were kept at 37 °C in a humidified chamber with 5% CO_2. The medium was changed every 2 days.

Differentiation of 3T3-L1 cells to adipocytes

The 3T3-L1 preadipocytes were plated in 24-well plates (3×10^4 cells/well) and cultured in DMEM containing 25 mmol/l glucose, 1 mmol/l sodium pyruvate, 4 mmol/l L-glutamine, 10% heat-inactivated FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37 °C in 5% CO_2. After cell confluence (day 0) for 2 days, the medium was removed and a differentiation medium, containing 0.5 mmol/l IBMX, 2.5 μmol/l DEX, and 10 μg/ml insulin INS, was added to induce cell differentiation. Test samples were added to the culture medium every 2 days. By day 12, more than 90% of the cells had differentiated into rounded cells with lipid droplets. Cells and culture medium were collected for further assays.

Preparation of herbal test samples

Dried fruits of *T. bellerica* (TB), *T. arjuna* (TA), *T. chebula* (TC), and *P. emblica* (PE) were cleaned and ground into powder. TB, TA, TC, and PE at a ratio of 1:1:1:1 by weight were mixed to prepare the Jatupalathika (JP) formula. 80 g each of JP, TB, TA, TC, and PE were macerated in 200 ml distilled water for 4 h with occasional stirring and, then, sonicated. The supernatant was collected and filtered. The filtrate was dried in a hot air oven at 40 °C for 2 days. The aqueous solution of JP, TB, TA, TC, and PE extracts, gallic acid (GA), and ellagic acid (EA) of different concentrations (10, 25, 50, 100 μg/ml) and isoproterenol (Isop) (10 μM) were prepared by dissolving each powder in distilled water. Test solutions of JP, TB, TA, TC, PE, GA, EA, and Isop were assayed and compared with the control (medium with cells, without test sample).

Determination of gallic acid (GA) and ellagic acid (EA) by HPLC analysis

HPLC analysis of GA and EA in JP, TB, TA, TC, PE extracts was performed. The HPLC system consisted of a LC-20AT pump, a SPD-20A UV detector equipped with a SPD-20A system controller, and a SIL-10ADVP sample injector fitted with a 20 μl sample loop (Shimadzu, Kyoto, Japan). The chromatographic separations were performed on a C18 column (250 mm × 4.6 mm i.d., 5 μm, 250 Å) (ACE®, Scotland). The mobile phase was water-acetonitrile (90:10, v/v) containing 1.0%

v/v orthophosphoric acid, pH 3.00. All separations were performed isocratically at a flow rate of 1 ml/min. The column temperature was maintained at room temperature (27 ± 2 °C). The peaks were determined using a UV detector set at a wavelength of 210 nm. Standard solutions of GA and EA were prepared in the mobile phase at concentrations between 1–100 μg/ml. The dried extracts were dissolved in the mobile phase. Standard solutions or test samples were injected directly into the system after filtering through a filter paper and a 0.45 mm membrane filter (Millipore, MA, USA). Identification of the components was based on comparing retention times of the peaks of the reference standards. Quantification of GA and EA was accomplished using the calibration curve of pure standards ($R^2 = 0.999$).

Cell viability assay

The viability of 3T3-L1 adipocytes was assayed by the MTT technique according to the method of Mosmann [16]. For post-confluent preadipocytes, cells (3×10^4 cells) were seeded in each well of a 24-well plate and grown at 37 °C in a humidified chamber with 5% CO_2 for 24 h. Test solutions (100 μl) were added to the cultures, and cell viability was assayed 24 h after the addition. For the viability of differentiated cells, post-confluent preadipocytes were treated with the test solutions (100 μl) in a differentiation medium every 2 days for 12 days; after that, the viability was assayed. Then, 5 mg/ml MTT solution in phosphate-buffered saline (PBS, pH 7.4) (10 μl) was added to each well. After 30 min, the culture medium was discarded, and a DMSO:ethanol (1:1) solution (200 μl) was added and mixed. The absorbance was measured at 595 nm using a microplate reader (Eon, BioTek Instruments, USA). % Viability was calculated as follows:

$$\%\,\text{viability} = \left[\left(\text{Abs}_{\text{sample}}\right) / \left(\text{Abs}_{\text{control}}\right)\right] \times 100$$

Determination of lipid accumulation by Oil Red O staining assay

The lipid content in the adipocytes was determined using the Oil Red O staining assay as previously described [3]. Post-confluent preadipocytes (3×10^4 cells) were grown as mentioned in the viability assay. Adipocytes were cultured in the differentiation medium and treated with either test samples or a vehicle (control) for 12 days with the medium changed every 2 days. On day 12, cells were washed with PBS solution and fixed in fresh 10% formaldehyde for 1 h. After that, cells were washed with 60% isopropanol, stained with 0.6% Oil Red O dye in 60% isopropanol for 15 min, and then washed with distilled water 3 times. Red stained fat droplets were extracted from cells by adding isopropanol (100 μl) to each well and mixing. The absorbance at 500 nm was measured using a microplate reader to calculate the fat accumulation.

Determination of cellular triglyceride (TG) content

Total cellular TG content was determined by a coupled enzyme assay. Post-confluent preadipocytes (3×10^4 cells) were grown and cultured in the differentiation medium treated with test solution as described in the viability assay. The culture of differentiated adipocytes treated with test solution at day 12 was washed gently with PBS solution twice, and then lipid extraction buffer (100 μl) was added to the cells. The plates were sealed, incubated at 90 °C for 30 min, cooled to room temperature, and then shaken for 1 min to mix the solution. TG content in the cloudy solution was analysed using a commercial TG assay kit according to the manufacturer's instructions. The colorimetric product, which was proportional to the TG content, was measured by absorbance at 570 nm using a microplate reader to calculate the TG content.

Determination of glycerol content

Lipolysis was assessed by the measurement of glycerol content released into the medium according to the manufacturer's instructions. Post-confluent preadipocytes (3×10^4 cells) were grown and cultured in the differentiation medium. The differentiated adipocytes in serum-free medium were treated with the sample extracts for 24 h. The medium (10 μl) was collected and mixed with a master reaction mix (100 μl) in a 96-well plate. Plates were sealed and incubated at room temperature for 20 min. The colorimetric product, which was proportional to the glycerol content, was measured by absorbance at 570 nm using a microplate reader to calculate the glycerol content.

Statistical analyses

Statistical analyses were conducted by one-way ANOVA with a post-hoc test, and $p < 0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Preparation of herbal test samples

JP is a herbal mixture composed of TB, TA, TC, and PE fruit powder. The powder of individual herbs or the herbal mixture of JP was macerated in distilled water. JP aqueous extract of approximately 4% (w/w of dried weight) was obtained. The physical appearance of JP was a brown viscous extract. The percentage yields of TB, TA, TC, and PE were 3.20, 3.44, 3.08, 2.97% (w/w of dried weight), respectively; and the physical appearance was a greenish brown viscous extract.

Determination of GA and EA by HPLC analysis

GA and EA in JP and the four fruit extracts (TB, TA, TC, and PE) in the JP formula were determined by HPLC analysis. The retention time of GA and EA was approximately 4.4 and 3.9 min, respectively. By comparing retention times of the extract peaks to the standard GA peak (Fig. 1), the GA content in the

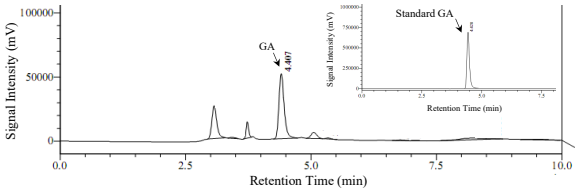

Fig. 1 HPLC chromatogram of Jatupalathika (JP) extract showing gallic acid (GA) as the major constituent.

Table 1 Determination of gallic acid in Jatupalathika and its four fruit extracts by HPLC analysis.

Extract	Gallic acid (mg/g of extract)
Jatupalathika (JP)	211.56 ± 0.121
Terminalia bellerica (Gaertn.) Roxb (TB)	207.48 ± 0.145
Terminalia arjuna Roxb. (TA)	181.66 ± 0.041
Terminalia chebula Retz. var. chebula (TC)	198.16 ± 0.058
Phyllanthus emblica L. (PE)	306.14 ± 0.053

extracts was calculated, and the results are presented in Table 1. The results indicated that GA was one of the major polyphenolic compounds of JP and the four fruit extracts. The JP extract contained GA content of 211.56 ± 0.121 mg/g of extract. Among the four fruit extracts, PE contained the highest GA content of 306.14 ± 0.053 mg/g of extract. However, the EA content in all extracts was below the detection limit of the HPLC analysis and could not be clearly identified in the chromatogram.

Cell viability assay

The 3T3-L1 cell line, generally used as a model of adipocyte differentiation and fat accumulation, was used in this investigation. The differentiation of preadipocytes into adipocytes is an important step to obtain mature adipocytes, which possess the capability to take up, synthesise, and store lipids [17]. The viability of 3T3-L1 preadipocytes and differentiated

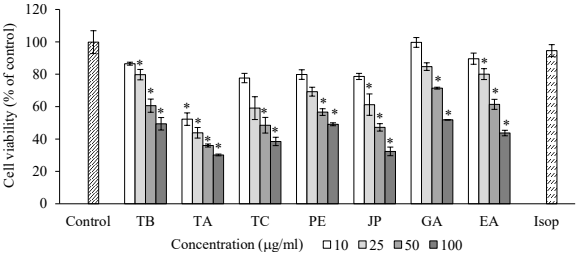

Fig. 2 Effect of *T. bellerica* (TB), *T. arjuna* (TA), *T. chebula* (TC), *P. emblica* (PE), Jatupalathika (JP) extracts, gallic acid (GA), ellagic acid (EA), and isoproterenol (Isop) on cell viability of 3T3-L1 adipocytes. Values are expressed as mean ± SD ($n = 3$), $^{*}p < 0.05$ as compared with the untreated control.

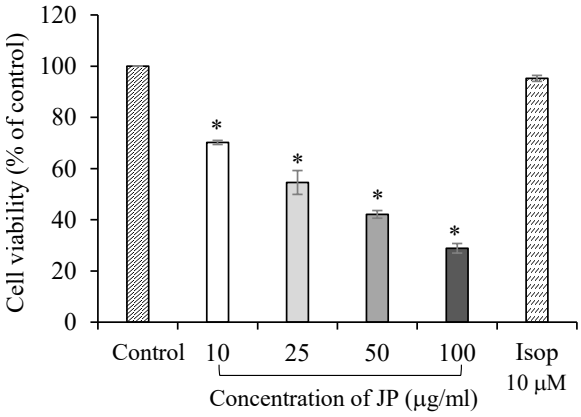

Fig. 3 Effect of Jatupalathika (JP) extract and isoproterenol (Isop) on cell viability of 3T3-L1 preadipocytes. Values are expressed as mean ± SD ($n = 3$), $^{*}p < 0.05$ as compared with the untreated control.

adipocytes treated with JP extract, four herbal ingredients of JP formula (TB, TA, TC, and PE), GA, and EA was determined using the MTT assay, and the results were compared with the control. The mitochondrial reduction of MTT to formazan is directly proportional to the living cell number [16]. The effect of the tested samples on the % viability of differentiated adipocytes is shown in Fig. 2. At the concentration of 10 μg/ml, JP extract did not cause any alteration in viable cell numbers of differentiated adipocytes, while JP at 25–100 μg/ml showed an inhibitory effect on cell viability in a dose-response relationship with the lowest % viability of 32.36 ± 2.66 (at 100 μg/ml). TB at 25–100 μg/ml, TA at 10–100 μg/ml, and TC and PE at 50 and 100 μg/ml significantly decreased the adipocyte viability in a dose-dependent manner. These individual herbal extracts at 100 μg/ml exhibited the lowest percentages of viable cell number of 30.16 ± 0.54%, 38.51 ± 2.59%, 49.11 ± 0.90%, and 49.40 ± 3.85% for TA, TC, PE, and TB, respectively. For the major compounds in JP extract and the four individual plants, GA at 10 and 25 μg/ml and EA at 10 μg/ml did not affect cell viability. However, GA and EA at the concentration of 100 μg/ml significantly reduced the viability of differentiated adipocytes by 51.83 ± 0.05% and 43.69 ± 1.70%, respectively. This was similar to the results for the four individual herbs and the JP extract. Isoproterenol did not cause any alteration in the viable cell number. The effect of JP extract on cell viability of 3T3-L1 preadipocytes was also investigated. As shown in Fig. 3, JP at 10–100 μg/ml decreased preadipocyte viability in a dose-dependent manner with the lowest viability being 28.85 ± 1.87% (at 100 μg/ml).

A reduction in the number of viable adipocytes due to treatment with JP, TA, TC, PE, TB extracts, GA, and EA may contribute to the induction of cell apoptosis

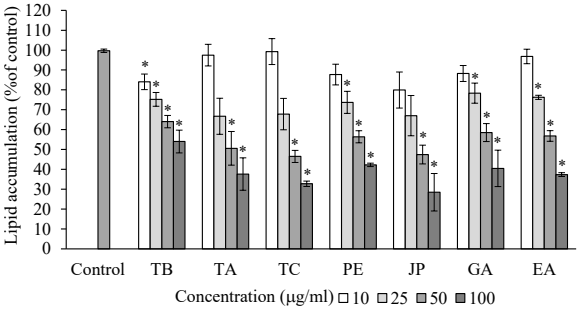

Fig. 4 Effects of *T. bellerica* (TB), *T. arjuna* (TA), *T. chebula* (TC), and *P. emblica* (PE), Jatupalathika (JP) extracts, gallic acid (GA), and ellagic acid (EA) on lipid accumulation in 3T3-L1 adipocytes by Oil Red O staining technique. Values are expressed as mean ± SD ($n = 3$), $^{*}p < 0.05$ as compared with the untreated control.

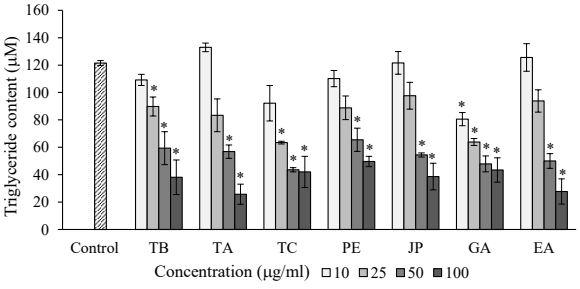

Fig. 5 Effects of *T. bellerica* (TB), *T. arjuna* (TA), *T. chebula* (TC), and *P. emblica* (PE), Jatupalathika (JP) extracts, gallic acid (GA), and ellagic acid (EA) on triglyceride content in 3T3-L1 adipocytes. Values are expressed as mean ± SD ($n = 3$), $^{*}p < 0.05$ as compared with the untreated control.

or the suppression of cell viability and proliferation. Moreover, the JP extract affected both differentiated adipocytes and preadipocytes in the same way. These could be potential mechanisms for reducing adipogenesis.

Lipid accumulation assay

The adipocyte volume mainly depends on the accumulation of triacylglycerols. The differentiated adipocytes were stained with Oil Red O solution and lipid contents in 3T3-L1 adipocytes undergoing adipogenesis were measured spectrophotometrically. The untreated differentiated adipocytes contained many lipid droplets with the Oil Red O dye staining, indicating lipid accumulation. The accumulation was significantly reduced by higher doses of JP, the four individual herb extracts, GA, and EA. The effects of the tested samples on intracellular lipid accumulation in 3T3-L1 adipocytes are shown in Fig. 4. The inhibition of lipid accumulation was observed by the treatment of JP at 50 and 100 μg/ml, TB at 10–100 μg/ml, TA and TC at 50, 100 μg/ml, PE at 25–100 μg/ml, and GA and EA at 25–100 μg/ml with the maximum inhibition at 100 μg/ml. JP extract exhibited the highest % inhibition of lipid accumulation of about 71.50 (28.50 ± 9.43% of control, 100 μg/ml), which was higher than that of the four individual herbs with the maximum % inhibition being; TC 67.25 (32.75 ± 1.35% of control) > TA 62.35 (37.65 ± 8.16% of control) > PE 57.73 (42.27 ± 0.86% of control) > TB 45.99 (54.01 ± 5.72% of control). These results were similar to those for GA 67.25 (32.75 ± 1.35% of control), and EA 62.35 (37.65 ± 8.16% of control).

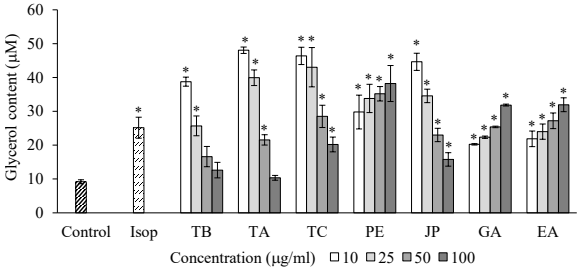

Fig. 6 Effects of *T. bellerica* (TB), *T. arjuna* (TA), *T. chebula* (TC), *P. emblica* (PE), Jatupalathika (JP) extracts, gallic acid (GA), ellagic acid (EA), and isoproterenol (Isop) on glycerol release in 3T3-L1 adipocytes. Values are expressed as mean ± SD ($n = 3$), $^{*}p < 0.05$ as compared with the untreated control.

Lipid accumulation based on the triglyceride (TG) content of 3T3-L1 cells was determined by a colorimetric assay. The effects of the tested samples on intracellular TG content in 3T3-L1 adipocytes are shown in Fig. 5. The result showed that JP at 50 and 100 μg/ml, TB and TC at 25–100 μg/ml, TA and PE at 50 and 100 μg/ml, GA at 10–100 μg/ml, and EA at 50 and 100 μg/ml caused a decrease in TG content in adipocytes, with the maximum inhibition seen at 100 μg/ml. TG deposition in adipocytes was decreased following treatment with JP extract by about 24.15 ± 5.72 μM, with a maximum % inhibition of 80.12. Similarly, TG content from the treatment of TA, TB, TC, and PE was approximately 25.76 ± 7.33, 38.13 ± 12.63, 42.01 ± 11.37, and 49.64 ± 3.76 μM, respectively, with the maximum % inhibition being 78.80, 68.62, 65.42, and, 59.14, respectively. Both GA and EA significantly decreased the TG content to 19.93 ± 8.90 and 27.75 ± 9.21 μM with the highest % inhibition of 83.60 and 77.16, respectively.

Our findings indicate that JP extract significantly reduced lipogenesis via the inhibition of fat accumulation and TG content in adipocytes. Nevertheless, GA and EA reduced fat accumulation less than JP extract. These results indicate that GA may be partially responsible for this activity together with other phytocompounds in the formula. Although EA has beneficial effects on fat and triglyceride reduction within adipocytes, its impact might be low due to the

low or negligible amount of EA in the extracts. For TG content, GA may be responsible for this activity since it caused a reduction in TG content similar to the JP extract. The four individual herbs in the JP formula also exhibited inhibition of fat accumulation and a decrease in TG content in adipocytes, but their effects were lower than that of the JP extract. This investigation indicated that the four individual herbs in the JP formula have a synergistic effect in the reduction of lipogenesis. Moreover, these may be partially due to a decrease in cell numbers resulting from the inhibition of cell viability and the proliferation of adipocytes. For further study, a positive control such as Troglitazone, a known PPAR γ agonist, might be used to clarify the related mechanism of action for fat accumulation [18].

The effect of the JP extract and its herbal mixtures on lipolysis was evaluated by the measurement of glycerol released into the culture medium of 3T3-L1 adipocytes using a colorimetric assay. Isoproterenol was used as a positive control to induce lipolysis resulting in glycerol and free fatty acid releases [19]. The effects of the tested samples on glycerol release from 3T3-L1 adipocytes are shown in Fig. 6. JP extract at 10–50 μg/ml, similar to TA (10–50 μg/ml), TC (10–100 μg/ml), TB (10 and 25 μg/ml), and PE (10–100 μg/ml), significantly increased glycerol release. The maximum contents of detected glycerol were as follows: JP (44.63 ± 2.53 μM, 10 μg/ml), TA (48.05 ± 0.95 μM, 10 μg/ml), TC (46.38 ± 2.55 μM, 10 μg/ml), TB (38.77 ± 1.34 μM, 10 μg/ml), and PE (38.22 ± 5.32 μM, 100 μg/ml). However, glycerol releases following treatment with JP, TB, TA, and TC extracts at higher concentrations tended to decline. This may be due to the inhibitory effect of the extracts on cell viability. All concentrations of GA and EA (10–100 μg/ml) significantly increased glycerol release with the maximum amount of 31.86 ± 0.28 μM and 31.95 ± 0.28 μM (100 μg/ml), respectively. For isoproterenol, the elevated glycerol content of 25.16 ± 3.10 μM was detected.

Fat accumulation is determined by the balance between lipogenesis and lipolysis. Lipogenesis includes the syntheses of fatty acids and triglycerides taking place in the liver and adipose tissues, while adipogenesis involves the differentiation of preadipocytes into mature fat cells [20]. The inhibition of adipocyte differentiation is an important strategy to control obesity since an increase in adipose mass is caused by both adipocyte hypertrophy and adipocyte hyperplasia [21].

In this study, we found that JP extract and the four herbal components, TB, TA, TC, and PE, had anti-obesity activity related to lipogenesis, adipogenesis, and lipolysis in 3T3-L1 adipocytes. However, the mechanisms pertaining to these effects, such as the expression of key adipogenic transcription factors including peroxisome proliferator-activated receptor gamma (PPAR γ) and CCAAT/enhancer-binding protein (C/EBP α) [21], still need to be explored. Further evaluation of toxicity and *in vivo* experiments is necessary to be undertaken to support its therapeutic uses.

Various strategies have been used for weight management and obesity, such as monitoring of food intake and calories, increased exercise, and behavioural changes involving diet. The inhibition of lipogenesis, the stimulation of lipolysis, and the induction of adipocyte apoptosis result in a reduction in adipose tissue mass. A reduction of carbohydrate or fat absorption and the inhibition of preadipocyte proliferation and differentiation have been assumed to account for the mechanisms of anti-obesity [22]. Many investigations have indicated that novel substances from natural sources affect adipocyte differentiation and gene regulation associated with obesity [23]. The potential of herbal formulations presenting anti-obesity activities was also reported. KBH-1, mixtures of *Polygala tenuifolia*, *Curcuma longa*, and *Saururus chinensis* [24], and SH21B composed of seven herbs [25] inhibited lipid accumulation in 3T3-L1 adipocytes and in mice with HFD-induced obesity. Although the mechanisms of action of many traditional herbal medicines are not clear, several herbal formulas are more efficacious than an equivalent dose of their individual components alone [26]. In some cases, the isolated phytocompounds may be effective only in combination with other components in the mixture due to synergistic effects [27]. Developing an effective anti-obesity agent derived from herbal formulations is therefore a tremendous challenge for researchers.

CONCLUSION

In conclusion, our results demonstrated that the Jatupalathika extract efficiently inhibited fat accumulation by the inhibition of adipogenesis, lipogenesis, and the induction of lipolysis in 3T3-L1 adipocytes in a dose-response relationship. Jatupalathika extract was more effective than an equivalent dose of its individual herbal ingredients and its major components, the gallic acid and the ellagic acids. These findings suggested that Jatupalathika formula. This herbal formula might potentially be used in the prevention and treatment of obesity.

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