Anti-obesity activity of polyherbal formulation Divya-Medohar-Vati by inhibition of pancreatic lipase activity and triglyceride translocation through enterocytes

Acharya Balkrishna a, b, Anamica Singh a, Jagdeep Singh a, Subarna Pokhrel a, Kunal Bhattacharya a, Anurag Varshney a, b, *

a Drug Discovery and Development Division, Patanjali Research Institute, NH-58, Haridwar, 249405, Uttarakhand, India
b Department of Allied and Applied Sciences, University of Patanjali, Patanjali Yog Peeth, Roorkee-Haridwar Road, Haridwar, 249 405, Uttarakhand, India

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ABSTRACT

Background: Obesity is a modern-day epidemic affecting human lifestyle worldwide and originates from high fat-content food consumption. Traditional Ayurveda-based polyherbal medicine ‘Divya-Medohar-Vati’ (DMV) has been recommended as an anti-obesity formulation.

Methods: In the present study, we explored the anti-obesity activity of DMV using biochemical assay, simulated gastrointestinal tract (GIT) digestion model, and Caco-2-derived enterocyte model. We further correlated the observed biological activity of DMV with its phytochemical profile determined using the high-performance-liquid-chromatography method.

Results: Phytochemicals such as Gallic acid, Ellagic acid, Chebulic acid, Picroside I, and Arjungenin were identified in DMV. DMV and ‘Orlistat’ (positive control) showed half-maximal inhibitory concentrations (IC50) of 647.88 and 21.80 µg/ ml, respectively against porcine pancreatic lipase activity. Addition of DMV and Orlistat to full cream during simulated GIT digestion reduced the hydrolysis of triglyceride into long-chain Palmitic acids and glycerol molecules in obtained digestae. At the same time, both DMV and Orlistat stimulated the release of medium-chained Capric acids in full cream digestae. Treatment of enterocytes with DMV-induced a loss of cell viability (IC50 at 308.5 µg/ml). Translocation of re-esterified triglycerides by enterocytes grown on transwell plate following treatment with full cream digestae was inhibited after the addition of DMV and Orlistat.

Conclusions: Taken together, DMV showed good inhibition for pancreatic lipase activity, and hydrolysis of triglycerides modulating the release of free glycerol and dietary fatty acid (Capric acid and Palmitic acid) contents. DMV also reduces the translocation of triglycerides through the basal region of enterocytes. Hence, Divya-Medohar-Vati was found to be an effective anti-obesity polyherbal formulation.
Introduction

Obesity is an epidemic affecting the quality of human life (Chooi et al., 2019). According to World Health Organization (WHO) guidelines, a person’s daily fat intake should be between 20 and 35% (Lia et al., 2017). However, irregular dietary fat intake, metabolic- and storage-related issues may lead to obesity that may further escalate to additional health risks (Charlton, 2009; Jokinen, 2015; Polsky and Ellis, 2019). According to World Health Organization (WHO) guidelines, obesity is an epidemic affecting the quality of human life (Chooi et al., 2019). In a clinical setup, weight-loss therapies include lifestyle intervention, pharmacotherapy, and bariatric procedures (Patel and Stanford, 2018). However, many of these may have unwarranted health-related side effects (Patel and Stanford, 2018).

Dietary fat is consumed in triglycerides (TG) form containing fatty acids (FAs) in the 1 and 3 positions of the glycerol backbone. Bovine milk contains approximately 4.2% fat composed of 98% TG (Manson, 2008). Dietary fat intake, metabolic- and storage-related issues may lead to obesity that may further escalate to additional health risks (Charlton, 2009; Jokinen, 2015; Polsky and Ellis, 2019). In a clinical setup, weight-loss therapies include lifestyle intervention, pharmacotherapy, and bariatric procedures (Patel and Stanford, 2018). However, many of these may have unwarranted health-related side effects (Patel and Stanford, 2018).

Dietary fat is consumed in triglycerides (TG) form containing fatty acids (FAs) in the 1 and 3 positions of the glycerol backbone. Bovine milk contains approximately 4.2% fat composed of 98% TG (Manson, 2008). Dairy-origin FAs are composed of 4–16 carbon atoms with Palmitic acid representing ~30% of total FAs content (Mansson, 2008). TGs are hydrolyzed into free FAs and glycerol molecules by pancreatic lipase (PL) enzyme within the gastrointestinal tract (GIT) (Carrière et al., 2000). Free FAs present in the gut lumen is absorbed by brush-border enterocytes, resynthesized into TG, packed into chylomicrons, and transported into the systemic circulation. Currently, ‘Orlistat’, a hydrogenated derivative of lipstatin originating from Streptomyces toxytriciini is approved as anti-PL prescription medicine (Zhou et al., 2021). Orlistat blocks hydrolysis of TG into free FAs through inhibition of PL activity in GIT. However, a clinical study done for ‘Orlistat’ (XENDOS) showed 91% of patients developing GIT related incidences and 8% of individuals withdrew from the study due to other health-related complications (Patel and Stanford, 2018).

Polypehral formulation ‘Divya-Medohar-Vati’ (DMV) is composed of herbal components Amla (Emblica officinalis Gaertn.), Baheda (Terminalia belirica Wall.), Harad (Terminalia chebula Retz.), Guggul shuddha (Commiphora mukul (Hook. ex Stocks) Engl.), Shilajit shuddha (Asphaltum), KunKi (Picrorhiza kurroa Royle ex Benth.), Nisoth (Operculina turpethum (L.) Silva Manso), and Vayvidng (Embelia ribes Burn.f.) (Table 1). Some reported having anti-obesity activities (Chaudhari et al., 2012; Makihara et al., 2012). Caco-2 cells are immortalized human adenocarcinoma cells that transform into functionally active small intestinal enterocytes following 2–3 weeks’ differentiation (Arkursson et al., 2001).

The present study is directed towards understanding the anti-obesity activity of DMV using a simulated GIT digestion system consisting of mouth, stomach, and small intestinal phases, and Caco-2 cells transformed into enterocytes. Biochemical analysis was performed for studying its anti-PL activity. Physiochemical profiling of DMV was performed using High-Performance Liquid Chromatography (HPLC) methodology. The anti-obesity activity of DMV was correlated to the phytochemical constituent of DMV.

Materials and methods

Reagents

DMV (Batch No. A-MHV264) was sourced from Divya Pharmacy, Haridwar, India in the form of 500 mg tablets. Herbal composition and their quantities per 500 mg DMV tablets are mentioned in Table 1. Taxonomical identity of plants was performed at the Council of Scientific and Industrial Research—National Institute of Science Communication and Information Resources (CSIR—NISCAIR) (Government of India), Delhi, India, and each plant was issued an identification voucher number (Table 1). HPLC Standards for Chebulic acid, Picroside I, and Arjungenin were purchased from Natural Remedies, Bangalore, India. HPLC Standards for Gallic acid, Ellagic acid, and other HPLC grade reagents were purchased from Sigma Aldrich, St. Louis, MO, USA. Sodium chloride, ammonium nitrate, potassium phosphate, potassium chloride, potassium citrate (monohydrate), uric acid, lactic acid sodium salt (Sodium DL-lactate), Porcine gastric mucin-type II, hydrochloric acid, pepsin, calcium chloride (dihydrate), porcine bile salts, P-Nitrophenyl butyrate (PNPB) and Orlistat were purchased from Sigma Aldrich, USA. Porcine Pancreatic Lipase (PL) was purchased from MP Biomedicals, Illkirch-Graffenstaden, France. Glycerol and triglyceride detection kits were procured from Randox Laboratories Ltd., Crumlin, United Kingdom. Dulbecco’s Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), Antibiotics, Tryptsin-EDTA were purchased from Thermo Fisher Scientific Waltham, MA, USA. 0.45 μm polycarbonate membrane transwell plates were obtained from Corning Inc. Kennebunk, ME, USA.

Phytochemical analysis

Standards for Gallic acid (potency-97.3%), Ellagic acid (potency-99.6%), Chebulic acid (potency-98.10%), Picroside I (potency-96.1%), and Arjungenin (potency-98.3%) were prepared in 99% methanol. 5 g of pulverized DMV sample was mixed in 20 ml of hydromethanolic solution. The mixture was sonicated for 30 min, centrifuged at 9000 rpm for 5 min and the supernatant was filtered through a 0.45 μm nylon filter.
The filtered solution was analyzed of Arjungenin, and Picroside I. Hydromethanolic solution was further diluted 10x for detection of Gallic Acid, Ellagic Acid, and Chebulic Acid. HPLC (Waters Corporation, Milford, MA, USA) equipped with Binary pump (1525), PDAD (2998) & AutoSampler (2707) was used for the analysis. Sunfire C18 (5 µm, 4.6 × 250 mm) column with binary gradient elution was used for chromatographic separation. Solvent A was prepared using water containing 0.1% orthophosphoric acid (pH 2.5 adjusted with triethylamine), and Solvent B contained acetonitrile. Gradient programming was set as 95% (0–3 min), 95–85% (3–10 min), 85–80% (10–20 min), 80–60% (20–25 min), 60–35% (25–30 min), 35% (30–35 min), 35–95% (35–39 min) and 95% (39–40 min) at a flow rate of 1.0 ml/min. 10 µl of test and standard solutions were injected into the column maintained at 35 °C. Measurements for Gallic acid, Ellagic acid, Chebulic acid, and Piperine were done at 254 nm and Arjungenin at 210 nm.

Anti-lipase activity

Five hundred milligram tablets of DMV were crushed into a fine powder using a clean mortar and pestle and weighed. For the assay, 8 µl of PL enzyme (24 µg), and 10 µl of 4 mM sodium deoxycholate were mixed in 50 mM Tris–HCl buffer (pH 8.0), 10 µl of Orlistat (0.02, 0.06, 0.2, 0.6, 2, 6 and 10 mg/ml prepared in ethanol) or, Medohar Vati (0.02, 0.06, 0.2, 0.6, 2, 6, 10 and 20 mg/ml were prepared in 50% DMSO) and added to the mixture. The solution was incubated at 37 °C for 30 min and the reaction was initiated by the addition of 5 µl of 2 mM PNPB (prepared in acetonitrile). The reaction was continued for 20 min and the final volume was calibrated to 200 µl using 50 mM Tris–HCl buffer. Reactions were terminated by heating at 96 °C for 5 min. The solution was cooled to RT and absorbance was measured at 405 nm using EnVision plate-reader (PerkinElmer, Waltham, MA, USA). Activity of negative control was also examined with and without inhibitor.

All the reactions were performed in triplicates and inhibitory activity (I) was calculated (Eq. (1)).

\[
\text{Inhibitory activity (I\%) } = \left[ \frac{(A - a) - (B - b)}{(A - a)} \right] \times 100
\]

Where ‘A’ is the activity without inhibitor; ‘a’ is the negative control without inhibitor; ‘B’ is the activity with inhibitor, and ‘b’ is the negative control with inhibitor. Based on the 1% the IC50 for DMV and Orlistat was calculated.

Simulated GIT digestion assay

For the study, DMV tablets were pulverized in a mortar and pestle and weighed. Two hundred and fifty milligrams (low), 500 mg (human equivalent dose), and 1000 mg (high) of powdered DMV were mixed in 20 ml of fresh full dairy cream. Orlistat (positive control) at the concentration of 500 mg/ml was mixed in 20 ml of fresh cream (Fig. 1). Full cream containing an equivalent amount of water and full cream alone was considered as starving and complete food models, respectively. All the food models went through simulated digestion in the mouth, stomach, and small intestine following the protocol by Deloid et al. (Deloid et al., 2017). Final digestaes containing 1.88 mg/ml of DMV and Orlistat was obtained. All the digestaes was diluted in DMEM media (without supplements) in a 1:3 ratio obtaining 1.88 mg/ml Penicillin, 100 µg/ml Streptomycin and 1% non-essential amino acids. For the triglyceride translocation assay, Caco-2 cells were plated on the apical side of a collagen-coated 12 chambered transwell plate in 1.5 ml complete DMEM media at the density of 3 × 10^5 cells/cm^2. 2.5 ml of complete DMEM media was added to the basal region of the transwell plate. First change of media was done on the 4th day after seeding and repeated every second day until 10 days. From day 10 onwards media was changed every day until 21 days. For the cell viability analysis, Caco-2 cells were seeded on a 12 well-plate at the density of 3 × 10^5 cells/cm^2. 21 days’ incubation and enterocyte transformation were performed as mentioned above.

Digestae preparation for cell-based assays

For the cell-based studies, fresh digestaes were prepared following the protocol mentioned above immediately before the enterocyte treatments. Briefly, 500 mg of powdered DMV and Orlistat were mixed in 20 ml of fresh full dairy cream. Full cream containing an equivalent amount of water and full cream alone was considered as starving and complete food models, respectively. All the food models went through simulated digestion in the mouth, stomach, and small intestine following the protocol by Deloid et al. (Deloid et al., 2017). Final digestaes containing 1.88 mg/ml of DMV and Orlistat was obtained. All the digestaes was diluted in DMEM media (without supplements) in a 1:3 ratio obtaining working stock of complete food model, starvation model, DMV (625 µg/ml), and Orlistat (625 µg/ml) containing food models. Further dilutions of DMV and Orlistat were prepared in incomplete DMEM media as per requirement.

Cell viability analysis

Working DMV digestaes concentrations of 50, 100, 300, and 500 µg/ml were prepared in incomplete DMEM media. Enterocytes were incubated with the digestae containing media for a period of 24 h. Media containing digestaes with water was used as a negative control. At the end of the exposure period, enterocytes were treated with a 10 µg/ml concentration of Alamar blue™ for a period of 3 h. Fluorescence was measured at Ex. 560 nm/ Em. 580 nm wavelengths using PerkinElmer multiplate reader and cell viability percentage were calculated.

Free glycerol and fatty acid content analysis in digestae

Free glycerol content in the samples was analyzed using Rando detection kit and Monaco clinical chemistry analyzer (Rando Laboratories Ltd., Crumlin, United Kingdom) following manufacturer guidelines. Results obtained were presented as the percentage of free glycerol content in digestae.

FAs content was quantified using the GC-FID instrument. 200 mg of the digestae samples were added to 4 ml of n-Hexane containing 100 µl of 2 N methanolic potassium hydroxide. 0.5 ml of the mixture was diluted to 10 ml with double distilled MilliQ water and filtered using anhydrous sodium sulfate. The sample was injected into GC-FID 2010 Plus instrument (Shimadzu, Japan) and run through a Supelco SP™-2560 fused silica capillary column 75 m × 0.18 mm × 0.14 µm film thickness. The injector used in the study was 1177 Split/ Splitless Type, set at 250 °C with a split ratio of 1:100. Helium was used as a carrier gas at a flow rate of 1 ml/min. The oven was programmed at an initial temperature of 100 °C/min with a hold time of 1 min, followed by a 4 °C/min step increase till 150 °C and a hold time of 15 min. The final stepwise increase of oven temperature was performed at 3 °C/min till 230 °C with a hold time of 10 min. FA was presented as% of total FAs detected per sample.

Caco-2 cell culture and enterocyte transformation

Caco-2 cells were grown in high-glucose DMEM supplemented with 10% heat-inactivated Fetal Bovine Serum (FBS), 10 mM HEPES buffer, 100 IU/ml Penicillin, 100 µg/ml Streptomycin and 1% non-essential amino acids. For the triglyceride translocation assay, Caco-2 cells were plated on the apical side of a collagen-coated 12 chambered transwell plate in 1.5 ml complete DMEM media at the density of 3 × 10^5 cells/cm^2. 2.5 ml of complete DMEM media was added to the basal region of the transwell plate. First change of media was done on the 4th day after seeding and repeated every second day until 10 days. From day 10 onwards media was changed every day until 21 days. For the cell viability analysis, Caco-2 cells were seeded on a 12 well-plate at the density of 3 × 10^5 cells/cm^2. 21 days’ incubation and enterocyte transformation were performed as mentioned above.
Fig. 1. Schematic diagram for the simulated gastrointestinal digestion. Tri-phasic digestion of full cream food model with/without Divya-Medohar-Vati and Orlistat addition. Digestion was carried out through treatment with a panel of enzymes relevant representing each phase: Mouth- mucin and amylase in the mouth phase; Stomach- pepsin; and Small intestine- pancreatic lipase and bile salt. Images prepared with support from BioRender (http://bioRender.com).
Triglyceride formation and translocation analysis

Enterocytes were treated with starvation media (only water) digested, and full cream digestae containing water (control), Orlistat (100 μg/ml) and DMV (10, 30, and 100 μg/ml) mixed in incomplete DMEM media for a period of 24 h. At the end of incubation, basal media were transferred to 1.5 ml vials and stored at −80 °C till further analysis. Triglyceride analysis was done in the collected media using the Randox triglyceride assay kit and Randox Monaco clinical chemistry analyzer (Randox Laboratories Ltd., Crumlin, UK).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). Data were expressed as mean ± standard deviation (SD). Determination of significance between different treatment groups was done using one-way ANOVA followed by Dunnett’s post-hoc analysis. The results were considered to be statistically significant at a p-value < 0.05.

Results

Phytochemical contents of herbal formulations play a major role in dictating their therapeutic application (Parasuraman et al., 2014). HPLC analysis of the DMV showed the presence of 6.31 μg/mg Gallic acid (Retention time (RT) 6.76 min), 5.65 μg/mg of Ellagic acid (RT 23.71 min), 25.01 μg/mg of Chebulic acid (RT 25.01 min), 0.091 μg/mg of Picroside I (RT 27.02 min), and 0.240 μg/mg of Arjungenin (RT 31.35 min) (Fig. 2). Based on the HPLC finding, each 500 mg tablet of DMV was found to contain 3.15 mg of Gallic acid, 2.82 mg of Ellagic acid, 12.50 mg of Chebulic acid, 45.5 mg of Picroside I, and 120 mg of Arjungenin.

DMV and Orlistat inhibited the pancreatic lipase activity in a dose-dependent manner. Half-maximal inhibitory concentration 50% (IC50) for DMV was observed at 647.88 μg/ml and for Orlistat at 21.80 μg/ml (Fig. 3A and 3B). Triglyceride hydrolysis within the simulated GIT digestion of fresh cream released free Capric acid (0.25 ± 0.43 g/100 g of total fat content) and Palmitic acid (46.10 ± 5.76 g/100 g of total fat content) molecules (Figs. 4A and 4B). Addition of varying concentrations of DMV to the full cream food model significantly elevated the levels of Capric acid content (0.94 mg/mL: 1.54 ± 0.08 g/100 g of total fat content; 1.88 mg/mL: 1.63 ± 0.27 g/100 g of total fat content; and 3.75 mg/mL: 0.88 ± 0.18 g/100 g of total fat content), while reducing the Palmitic acid content (0.94 mg/mL: 36.98 ± 8.15 g/100 g of total fat content; 1.88 mg/mL: 37.87 ± 7.59 g/100 g of total fat content; and 3.75 mg/mL: 41.76 ± 3.53 g/100 gm of total fat content) compared to the full cream digestae (Figs. 4A and 4B). Orlistat (1.88 mg/mL) addition to the full cream during simulated GIT digestion also significantly

![Fig. 2. Phytochemical constituents of Divya-Medohar-Vati (DMV). Phytochemicals identified in DMV were Gallic acid (Retention Time (RT) 6.76 min), Ellagic acid (RT 23.71 min), Chebulic acid (RT 25.01 min), Picroside I (RT 27.02 min) at 254 nm, and Arjungenin (RT 31.35 min) at 210 nm. Standards for each phytochemical were run in parallel to the samples for identification and quantification. The black color line represents the standard mix of phytochemicals and the blue color line represents phytochemicals identified in DMV.](image-url)

<table>
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<th>Sr. No.</th>
<th>Marker Compound</th>
<th>RT (min)</th>
<th>Result (μg/mg)</th>
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<td>3</td>
<td>Chebulic acid</td>
<td>25.01</td>
<td>5.240</td>
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<tr>
<td>4</td>
<td>Picroside I</td>
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<td>0.091</td>
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<td>5</td>
<td>Arjungenin</td>
<td>31.35</td>
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increased the level of Capric acid in full cream digestae (1.56 ± 0.10 g/100 g of total fat content) and reduced the free Palmitic acid content (33.34 ± 0.45 g/100 g of total fat content) in the digestae (Figs. 4A and 4B). Hydrolysis of triglycerides also released one free molecule of glycerol (Balkrishna et al., 2021). Hence, inhibition of triglyceride hydrolysis/digestion in the GIT can be directly correlated to the free glycerol content in the digestae. Addition of DMV to the fresh cream food model significantly reduced the free-glycerol content in the digestae. 0 mg/mL: 100 ± 0.94 mg/mL; 10.38 ± 16.73%; 1.88 mg/mL: 8.39 ± 5.41%; and 3.75 mg/mL: 4.08 ± 6.72% (Fig. 4C). The addition of Orlistat to full cream also significantly (p-value < 0.001) reduced the glycerol content (14.20 ± 3.41%) in the digestae (Fig. 4C).

Biosafety evaluation of DMV in the enterocytes showed a concentration-dependent loss of cell viability (Fig. 5A). The half-maximal concentration (IC_{50}) value for DMV was evaluated at 308.5 μg/mL (Fig. 5A). For demonstrating the uptake, biotransformation of fatty acids into triglycerides, and systemic translocation by enterocytes, we added the fresh cream digestae w/wo DMV and Orlistat to Caco-2 transformed enterocytes grown on transwell plates (Fig. 5B). The transformed enterocytes treated with fresh cream digestae translocated 55.08 ± 3.6 fold (p < 0.01) more triglyceride into basal media compared to the enterocytes treated with starvation (water only digestae) media (Fig. 5C). Treatment of enterocytes with DMV and Orlistat (30 and 100 μg/mL) containing full cream digestae showed a significant (p < 0.01) reduction in the basal region media triglyceride content (Orlistat: 1.09 ± 0.46 fold; DMV 30 μg/mL: 3.68 ± 0.65 fold; DMV 100 μg/mL: 1.11 ± 0.60 fold) (Fig. 5C). DMV produced a concentration-dependent reduction in basal media triglyceride content (Fig. 5C).

Overall, results showed an anti-obesity effect for the DMV and Orlistat. Both DMV and Orlistat inhibited porcine PL activity. DMV treatment also reduced the long-chain saturated Palmitic acid content in the fresh cream digestae while increasing the levels of medium-chain Capric acid. This was accompanied by reduced translocation of triglycerides through enterocytes layer into the basal media in transwell culture.

**Discussion**

GIT tract is a primary site for the hydrolysis of triglycerides into free fatty acid and glycerol molecules. These are further emulsified by the bile salt making them polar and ready for uptake by enterocytes for further processing and systemic translocation for storage and energy metabolism (Lowe, 2002). Consumption of dietary fats along with reduced physical activity leads to over-accumulation of fatty acids in the liver and adipocytes causing obesity. Obesity can lead to the development of other metabolic diseases such as inflammation, steatosis, type-2 diabetes, hypertension, atherosclerotic dyslipidemias, and disorders of blood coagulation and fibrinolysis (Balkrishna et al., 2020).

Earlier studies have shown anti-obesity and anti-lipase activities for herbal components of DVM (Makihara et al., 2012; Patil et al., 2012; Yu et al., 2009). Harad (Terminalia chebula Retz.) reduced lipogenesis through reduction of lipogenic enzyme expression (Subramanian et al., 2021a). Terminalia chebula, Embelia ribes, and Picrorrhiza kurroa show anti-lipase activities (Patil et al., 2012). These anti-obesity activities are associated with the phytochemical constituents of the herbs. Identified phytochemicals from the different herbal components of DMV are listed in Table 2. Flavonoids present in the Emblica officinalis Gaertn. have been found to inhibit lipid synthesis and enhancement of their degradation in rats with hyperlipidemia (Anila and Vijayalakshmi, 2002). Similarly, Embelin phytochemical present in Embelia ribes Burm. f. have been reported to modulate lipid metabolism in obese Wistar rats (Chaudhari et al., 2012). Phytochemicals present in DMV were identified as Gallic acid, Ellagic acid, Chebulic acid, Picroside I, and Arjunigen. Gallic acid and Ellagic acid have been found to function as inhibitors for pancreatic lipase activity (Rahim et al., 2015) (You et al., 2012). While, Chebulic acid, Picroside, and Arjunigen modulate lipid metabolisms (Shengule et al., 2018; Subramanian et al., 2021a; Xu et al., 2020).

Dietary fatty acid contents also play a major role in obesity. Medium-chain fatty acids (MCFAs) are absorbed more efficiently than long-chained fatty acids (LCFAs). MCFAs are transported directly to the liver through the portal vein and metabolized in mitochondria without the requirement of carnitine transport (Papamandjaris et al., 1998). LCFA are metabolized by the enterocytes and transported to the liver and adipocytes using chylomicrons (Papamandjaris et al., 1998). In our study, DMV and Orlistat addition to the fresh cream promoted the release of MCFA Capric acid and reduced the release of LCFA Palmitic acid and glycerol molecules. This is an important finding as higher MCFAs to LCFAs ratio has been found to mitigate the LCFA stimulated non-alcoholic steatosis and associated autophagy (Wang et al., 2017). Lipases show stereoselectivity towards MCFA containing triglycerides (MCTs) compared to LCFA containing triglycerides (LCTs) (Deckelbaum et al., 1990). In an emulsion phase, MCTs show higher mobility and solubility compared to LCTs making them more available for hydrolysis.
by pancreatic lipase (Deckelbaum et al., 1990). At the same time, other physiological conditions such as pH, salinity, solubility, the orientation of fatty acids in the lipid-water micelle, bile salts, and colipases assisted opening the lid-domain in active lipases also affect the pancreatic lipase activity (Lowe, 2002). Hence, it might be possible that at the highest tested concentration of DMV, any of the optimal physiological conditions for lipase activity might be altered reducing its orientation towards the MCTs hydrolysis.

In the enterocytes grown on transwell plates, the addition of DMV and Orlistat to fresh cream digestae inhibited the translocation of triglycerides to the basal medium. An important step in the lipid digestion process is the diffusion-based uptake of hydrolyzed free fatty acids and glycerol molecules by enterocytes present in the small intestine. Here the absorbed fat and glycerol are rapidly re-esterified into triglycerides within the endoplasmic reticulum and released in the systemic circulation (Langhans et al., 2011). Hence, reduced availability of LCFAs due to inhibition of PL activity may lead to reduced FA absorption and re-esterification into triglycerides (Wu et al., 2013).

The current study illustrates the anti-lipase activity of DMV along with systemic translocation inhibition of resynthesized triglycerides to the basal medium.

**Fig. 4. Fatty acids and glycerol quantification in full cream digestae.** Hydrolysis of triglyceride content present within full cream digestae w/wo Divya Medohar Vati (DMV) and Orlistat by pancreatic lipase was analyzed through the release of (A) MCFA Capric acid, (B) LCFA Palmitic acid, and (C) glycerol molecules. All the experiments were performed in triplicate. Statistical analysis was performed using one-way ANOVA followed by Dunnett’s post-hoc test. p-value ** < 0.01 (Negative Control versus Treatment).
through the intestinal barrier using Caco-2 cells transformed enterocytes monolayer model (Costa and Ahluwalia, 2019). However, it is important to discuss the underlying limitations of the study before extrapolating the results to humans. Nonetheless, Caco-2 cells-based enterocyte monoculture does not envisage several underlying biological complexities that may influence the behavior and functionality of pancreatic lipase and enterocytes promoting or demoting triglyceride digestion process and its translocation through the actual intestinal barriers. Some of these complications include the presence of a thick mucus layer covering the enterocytes, inter-and intracellular...
interactions between the epithelium and the stroma, the presence of a live and dynamic microbiota population, and presence of optimum conditions for the enzymes to function like pH and temperature (Li et al., 2013). Hence, the study provides a direction to move forward towards more complex cell- and animal-based study models that may more realistically represent the pharmacoekinetics and toxicological perspective of herbal formulations like DMV.

Conclusion

Taken together, it can be concluded that DMV showed credible anti-PL and anti-obesity activity, which is controlled by its phytochemical composition. DMV selectively modulated the ratio between the MCFA (Capric acid) and LCFA (Palmitic acid), a function that needs further exploration. Additionally, the reduced bioavailability of free fatty acids and glycerol molecules in the food digesta led to reduced triglyceride re-esterification and release from the enterocytes. Hence, Divya-Medohar-Vati showed promising anti-obesity activity as a polyherbal formulation.

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Author agreement

We, the undersigning authors declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons, who satisfied the criteria for authorship, but are not listed.

We further confirm that the order of authors listed in the manuscript has been approved by all of us. We understand that the Corresponding Author is the sole contact for the editorial process. He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

CRediT authorship contribution statement


Declaration of Competing Interest

The test article was sourced from Divya Pharmacy, Haridwar, Uttarakhand, India. Acharya Balkrishna is an honorary trustee in Divya Yog Mandir Trust, Haridwar, India, that governs Divya Pharmacy, Haridwar. In addition, he holds an honorary managerial position in Patanjali Ayurved Ltd., Haridwar, India. Besides, providing the test article, Divya Pharmacy was not involved in any aspect of this study. All other authors have no conflict of interest to declare.

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