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ORIGINAL ARTICLE

An Edible Bioactive Fraction from *Rosa multiflora* Regulates Adipogenesis in 3T3-L1 Adipocytes and High-Fat Diet-Induced C57Bl/6 Mice Models of Obesity

H. V. Sudeep, K. Gouthamchandra, I. Ramanaiah, Amritha Raj, Shyamprasad K

R and D Center for Excellence. Vidya Herbs Pvt. Ltd., Bengaluru, Karnataka, India

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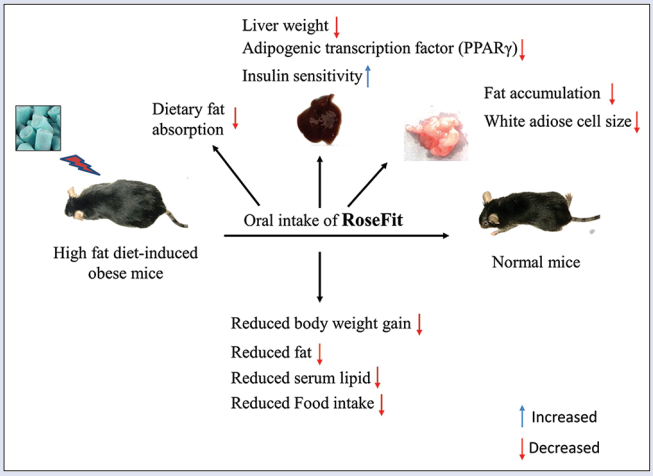
ABSTRACT

Objectives: Rose varieties are cultivated worldwide for ornamental flowers and have been greatly valued in the cosmetics and medicinal applications. Medicinal preparations from the different parts of the rose, including leaves, petals, and fruits, have been studied for various health benefits. Here, we have studied the efficacy of a standardized rose petal extract using the experimental models. **Materials and Methods:** The anti-obesity effect of a polyphenol-rich extract from the petals of *Rosa multiflora* var. platyphylla (RoseFit, 2%–3% isoquercetin) was studied using *in vitro* adipocyte differentiation model and *in vivo* diet-induced obesity model. **Results:** Treatment with 250 and 500 µg of rose fit dose-dependently reduced the differentiation of 3T3-L1 adipocytes and lipid accumulation thereof. The expression of adipogenic markers such as CCAAT/enhancer-binding protein-alpha and peroxisome proliferator-activated receptor gamma was markedly down regulated in the adipocytes treated with RoseFit. A 6-week treatment of high-fat diet (HFD) fed mice with RoseFit (100 mg and 200 mg/kg) significantly reduced the body weight, liver and fat pad weights, and adipocyte cell size of epididymal fat. Rose fit treatment also mitigated the hepatic inflammation significantly as compared to untreated HFD mice. **Conclusion:** Collectively, our findings suggest that the standardized rose petal extract may be used as a functional ingredient in food to increase the nutritional value and health-promoting effects.

Key words: Obesity, polyphenols, rose petals, weight loss

SUMMARY

- The present study provides experimental evidence on the efficacy of a composite rose petal extract in alleviating the diet-induced obesity
- The rose petal extract from *Rosa multiflora* standardized to 2%–3% isoquercetin (RoseFit) significantly downregulated the adipogenic markers such as peroxisome proliferator-activated receptor-γ and CCAAT/enhancer-binding protein alpha, thereby reducing the adipogenic differentiation *in vitro*
- Further, the extract dose-dependently exerted anti-obesity effects in diet-induced obesity model mice
- Oral treatment with RoseFit could significantly reduce the hepatic expression of adipogenic and inflammatory markers in HFD model mice
- Our results provide first ever experimental evidence on the anti-obesity effects of a composite rose petal extract. However, clinical studies are required to further demonstrate the efficacy of the extract.



Abbreviations used: Akt: Protein kinase B, C/EBPα: CCAAT/enhancer-binding protein alpha, Cox-2: Cyclooxygenase-2, FBG: Fasting blood glucose, H and E: Hematoxylin and eosin, HFD: High-fat diet, iNOS: Inducible nitric oxide synthase, IR-β: Insulin receptor beta, LCMS: Liquid chromatography mass spectrometry, NAFLD: Non-alcoholic fatty liver disease, NF-kB: Nuclear factor kappa-light-chain-enhancer of activated B-cells, OGTT: Oral glucose tolerance test, pAkt: Phosphorylated protein kinase B, PI3K: Phosphoinositide 3-kinase, PPARγ: Peroxisome proliferator-activated receptor gamma, PVDF: Polyvinylidene difluoride, RIPA: Radioimmunoprecipitation assay buffer, TNFα: Tumor necrosis factor alpha.

Correspondence:

Dr. H. V. Sudeep,
No. 14A, KIADB, R and D Center for Excellence,
Vidya Herbs Pvt. Ltd., Jigani Industrial Area, Anekal Taluk, Bengaluru - 560 105, Karnataka, India.
E-mail: research@vidyaherbs.com

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INTRODUCTION

Obesity represents a threatening health complication worldwide, with associated risk factors such as non-alcoholic fatty liver disease (NAFLD), type 2 diabetes, cardiovascular diseases, and various types of cancer.[1,2] Occurrence and progression of obesity are attributed to the genetic predisposition, lack of physical activities, and excess caloric intake. The excess fat stored as triglycerides (TG) in the adipose tissue contributes to the hyperplasia and hypertrophy of these cells.[3] Adipose tissue dysfunction in obesity causes disturbance in energy homeostasis leading to the risk of more serious complications.[4] Research in this area during

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the recent past has allowed for a greater understanding of the interplay between the dietary and genetic factors, metabolic relationship of cellular factors of different tissue origin, and identification of potential therapeutic targets.

Regulation of adipogenesis alongside the control of lipogenesis is one of the effective approaches to prevent or treat obesity.[5] Differentiation of adipocytes is mediated by the activation of CCAAT/enhancer-binding proteins (C/EBPs) and peroxisome proliferator-activated receptor-γ (PPARγ).[6] Evidence from previous studies demonstrate a correlation between the expression and transactivation of PPARγ and obesity.[7] Regulation of adipogenesis limits the expansion of white adipose tissue (WAT), thus reducing the progression of obesity.[8] Obesity is also associated with proinflammatory status and insulin resistance.[9] Experimental evidences suggest that over-activated inflammatory phenomenon results in reduced insulin sensitivity and glucose tolerance. Excess food intake during obesity leads to the initiation of inflammatory cascade.[10] The present-day knowledge of obesity and associated complications necessitates search for the therapeutic strategy which can exert the beneficial effects acting on multiple targets without adverse side effects.

Nevertheless, weight loss drugs have been developed as a solution to obesity, the associated side effects pose public health concern.[11] On the contrary, traditional herbal medicine with a long history of use could be effective alternative for appetite suppression and weight loss.[12] Experimental and clinical evidences suggest that abundant natural dietary and herbal products have been reported for their potential in exerting weight loss effects.[13]

Rose (Fam. *Rosaceae*) is a popular ornamental plant cultivated in abundance throughout the world for its cosmetic and medicinal objectives.[14] The flowers of this plant comprise 1.3% of saponin; petals consist of methionine sulphoxide. The volatile oil fraction from the petals consists of geraniol, citronellol, phenylethanol, and nerol. It also contains 15% tannins. The whole plant produces quercetin, kaempferol, and cyanidin. The rose hip (fruit) is a good source of Vitamin C, malic acid, and citric acid. Described in the systems of traditional medicine such as Ayurveda, medicinal preparations from rose have been implicated in maintaining good heart health and managing high blood pressure and blood disorders.

Experimental and clinical evidence suggest that rosehips from different *Rosa* species have potent therapeutic applications such as treatment to liver diseases, renal dysfunction, inflammation, obesity, hyperlipidemia, and cancer.[15] Previously flower extract of *Rosa damascena* was reported to have beneficial effects on the animal model of high-fat diet-induced NAFLD.[16] It is further reported that ethanolic extract of *R. damascene* has profound anti-lipase activity *in vitro*.[17] Gholamhoseinian *et al.* documented the lipid-lowering effect of *R. damascena* extract on lipid profile in a rabbit model of hyperlipidemia.[18] In another study, hydroalcoholic extract of *R. centifolia* was demonstrated to have significant inhibitory effect on the key enzymes of lipid metabolism such as β-Hydroxy β-methylglutaryl-CoA (HMG CoA) reductase and diacylglycerol acyltransferase.[19,20] Ochir *et al.* reported the inhibitory effects *R. gallica* petal extract on the digestive enzymes *in vitro*.[21] Considering the previous reports, it is adequate to study the possible protective role of rose flowers from different varieties against metabolic diseases comprehensively.

In this study, the effect of a standardized petal extract from *Rosa multiflora* (RoseFit) on *in vitro* adipogenesis was examined. Further, the weight loss attributes of the extract were demonstrated in obesity model mice.

MATERIALS AND METHODS

Plant extract

RoseFit™ is a standardized rose petal extract of *R. multiflora* var. platyphylla. RoseFit containing 2%–3% isoquercetin was procured from Phytochemistry Department, R and D Center, Vidya Herbs Pvt Ltd., Bangalore, India. The plant identification and authentication were performed at Biomedicinal Research Laboratory, Vidya Herbs Pvt Ltd., Bangalore, India (VH/18/RPE/06).

Chemicals and reagents

Isoquercetin (high-performance liquid chromatography [HPLC] grade 93%), Porcine pancreatic lipase, p-nitrophenyl butyrate, Dulbecco's Modified Eagle Medium (DMEM), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetraolium bromide (MTT), bovine pancreatic insulin, dexamethasone (DXM), and 3-isobutyl-1-methylxanthine (IBMX) were bought from Sigma-Aldrich. Antibodies against C/EBPα, PPAR-γ, Cox-2, C/EBPα, tumor necrosis factor alpha (TNF-α), inducible nitric oxide synthase (iNOS), and nuclear factor kappa B (NF-kB) were procured from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

High-performance liquid chromatography analysis of RoseFit

HPLC was performed on a Shimadzu LC2030 C Prominence-i (Japan) system. Five microliter of the sample was injected into the Kinetex XBC-18 column (100 Aº, 250 mm × 4.6 mm, pore size: 5 µm Gradient separation was performed using acetic acid (0.5%) and acetonitrile solution as the mobile phase [Table 1]. All solutions were degassed and filtered through 0.45 µm pore size filter. The column was maintained at 27°C throughout analysis and the ultraviolet detector was set at 350 nm. Seventy percent methanol used as a diluent for assay by HPLC analysis, and the total liquid chromatography run time was 20 min. The retention time (RT) of isoquercetin was confirmed by the injection of corresponding reference standard separately using the chromatographic conditions as above.

Lipase inhibition assay

The lipase inhibitory effect of RoseFit was determined by the method described elsewhere with minor modifications.[22,23] Briefly, the reaction mixture contained porcine pancreatic lipase solution (pH 6.8), tris buffer, and different concentrations of Rose Fit. The mixture was allowed to stand at 37°C for 15 min. Later, 5 µL of 10 mM p-NPB in dimethyl formamidesubstrate solution was added and following incubation at 37°C for 30 min, at the absorbance was read at 405 nm using a microplate reader. Percentage inhibition was calculated using the following formula:

Inhibition % = 100 − (B − b/A − a × 100)

where "A" and "B" corresponds to the activity without and with inhibitor, respectively; "a" and "b" blank readings without and with inhibitor, respectively.

Table 1: Gradient program of mobile phase for high-performance liquid chromatography analysis

Time (min)	Pump A concentration	Pump B concentration
0.01	82	18
13.0	81.5	18.5
15.0	82	18
20.0	Stop	

Cell culture

3T3-L1 mouse adipocytes used in this study were bought from National Center for Cell Sciences, Pune. The cells were grown in a 5% CO_2 humidified atmosphere, supplemented with DMEM containing 10% fetal bovine serum (GIBCO).

Cell viability (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetraolium bromide assay)

The viability of cells in the presence or absence of RoseFit was determined using MTT assay. Briefly, 3T3-L1 cells (5×10^3/well) were cultured in 96-well plates and allowed to attach for 16 hat 37°C in the CO_2 incubator. The cells were then incubated with various concentrations (0.2–2 mg/mL) of RoseFit . After 24 h, medium was discarded and added 20 µL of 5 mg/mL solution of MTT in phosphate buffered saline, incubated at 37°C for 4 h. The formazan crystals were dissolved in DMSO and absorbance read at 563 nm the formazan concentration is proportional to the viable cell number.

Differentiation of 3T3-L1 adipocytes

3T3-L1 cells were until confluence, in a 5% CO_2 supplied humidified incubator. After reaching 100% confluence, the cells were allowed to remain in the same medium two more days. Later, the cells were differentiated using Mix, Dex and insulin (MDI) cocktail media (10% FBS supplemented DMEM media containing 1 mM DXM, 0.5 mM IBMX, and 5 mg/mL. After 2 days, MDI media was replaced with insulin (5 mg/mL)-containing medium and incubated for 2 days. The cells were then maintained for 6 days in DMEM with every 2 day-media change. By this time, most of the cells differentiate into mature adipocytes. During the 8-day differentiation, cells were exposed to 250 µg and 500 µg/mL RoseFit from day 4.

Determination of lipid accumulation

Oil red O staining was used to measure the lipid accumulation in the cells. The cultured 3T3-L1 cells (0.2×10^6/well) were treated with 250 and 500 µg of RoseFit in MDI cocktail medium for 10 days. During differentiation, the media were changed with fresh media every 2 days. The cells after differentiation were fixed in 10% formaldehyde and stained with a filtered Oil Red O solution (60% in aqueous 2-isopropanol)[24] for an hour . Isopropanol (3 mL per well) was added to dissolve the stained lipid droplets. The intracellular lipids were quantified by reading the absorbance at 500 nm in a microplate reader (MultiskanEX, Thermofischer Scientific).

Animals

Male C57Bl/6 mice (12-week-old) were supplied by Biogen Laboratory Animal facility, Bengaluru, Karnataka, India (Reg. No. 971/PO/RcBiBt/S/2006/CPCSEA). Mice were maintained in air-conditioned rooms under controlled humidity (30%–70%) and temperature (22°C ± 3°C) and with a 12 h light/dark cycle. During the 7 days of acclimatization, the animals were provided with commercially available rodent diet and water *ad libitum*. The animal study protocols were approved by the Institutional Animal Ethics Committee (VHPL/PCL/IAEC/13/18).

Experimental design

The animals were grouped as follows: Group 1 animals served as normal control mice were provided with normal diet. Group 2–4 were fed an high fat diet (HFD) (D12451, Research Diet, USA) for 14 weeks. After 8 weeks, the mice in Group 3 and 4 were administered orally with low (100 mg/kg/day) and high (200 mg/kg/day) doses of RoseFit along with HFD. The extract was dissolved in physiological saline and administered to the respective group of mice through oral gavage. The animals were monitored daily for feed intake, whereas the body weights were recorded weekly. After 14 weeks, all the mice were euthanized by inhalant anesthetic overdose. The animals were exposed to 5% gaseous isoflurane in the induction chamber. Following blood collection by heart puncture, serum was separated for the analysis of cholesterol, TG, high-density lipoprotein-c (HDL-c), and low-density lipoprotein-c (LDL-c) using a biochemical analyzer (RandoxRX Imola, Co Antrim, UK). Liver was harvested, weighed, and homogenized in tissue lysis buffer for further analysis. The epididymal and mesenteric fat pads were weighed and photographed. The epididymal fat tissue was lysed in the tissue lysis buffer for further analysis.

Histopathological examination

Hematoxylin and eosin (H and E) staining and Oil red O staining were used to examine the histology of paraffin-embedded sections of liver samples. The gonadal fat pads were processed similarly and stained (H and E). The slides were observed under the microscope (Leica, Germany). The images were photographed. Adipocyte size was determined at × 100 magnification using a microscope.

Western blotting

The cell lysates and tissue homogenates were analyzed using western blotting. The samples were measured for total protein using Bradford assay and aliquots of protein samples (50–100 µg) were resolved in 10%–12% SDS-PAGE and transferred on to Polyvinylidene difluoride membrane. Following 1 h blocking in 5% skimmed milk at the room temperature, appropriately diluted primary antibodies were added on to the membrane and kept overnight at 4°C. The membranes were then probed for 1 h with HRP-conjugated secondary antibody (1:5000 dilution) at the room temperature. The protein bands were detected on ImageQuant™ LAS 500 (GE Health-care Life Sciences) and the relative expression of proteins with reference to GAPDH was quantified using Image J software (version 1.46, National Institutes of Health, Bethesda, Maryland).

Statistical analysis

GraphPad Prism (Version 5.0) (San Diego, CA) was used to analyze the data. All the data were subjected to one-way analysis of variance followed by Tukey *post-hoc* test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Species of *Rosa* genus are cultivated in the many parts of the world for the attractive, scented flowers of various colors. Interestingly, fruits, parts of the rose plant such as leaves, flowers, and fruits have been valued for their medicinal benefits as well. RoseFit is a bioactive extract from the petals of *R. multiflora*, standardized to 2%–3% isoquercetin. Here, we have studied the weight loss effects of RoseFit using *in vitro* and *in vivo* models. RoseFit has a high phenolic content (60%) and flavonoids (9%). Fingerprint analysis of RoseFit by LCMS method showed the presence of anthocyanins and flavonoid derivatives in RoseFit [Supplementary File 1]. In the present study, we have reported the potent anti-obesity effects of RoseFit, owing to the presence of rich phenolic content.

Quantitative analysis of isoquercetin in RoseFit

Figure 1 shows the HPLC chromatogram of standard isoquercetin and RoseFit. The RT of isoquercetin reference standard was found to be 10.350. Rose fit chromatogram confirmed the presence of isoquercetin with RT at 10.320 without any interference. Furthermore, the sample

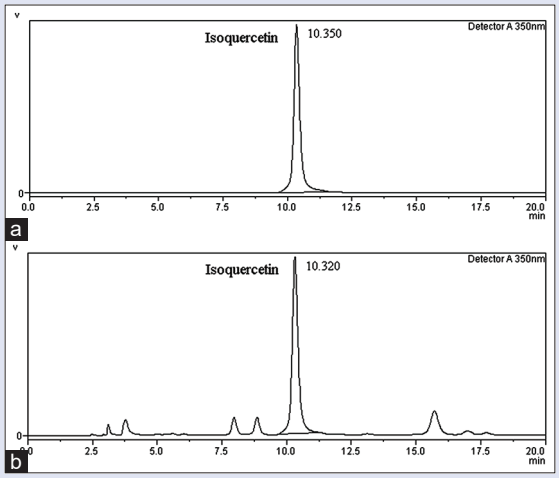

Figure 1: High-performance liquid chromatography chromatogram of (a) Isoquercetin reference standard and (b) RoseFit

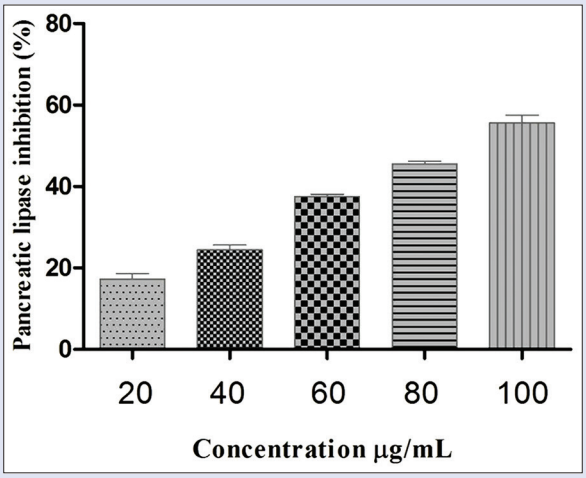

Figure 2: Effect of RoseFit on pancreatic lipase activity. The values are mean ± standard error of the mean of three independent experiments

chromatogram was found to be overlaid with the standard chromatogram. The chromatographic parameters such as column efficiency and peak symmetry were done to the standard solution according to the ICH guidelines. The theoretical plates and the tailing factor were found to be 70,653 and 1.154, respectively, indicated that column efficiency was satisfactory. HPLC analysis revealed the presence of 2%–3% isoquercetin in RoseFit.

Effect of RoseFit on pancreatic lipase activity

Excess caloric intake is one of the main reasons for obesity.[25] Pancreatic lipase converts a major part of dietary fats in to fatty acids and monoglycerides. Pancreatic lipase inhibition is key approach and a number of natural lipase inhibitors of plant origin have been reported.[26] In this study, we have used different concentrations of RoseFit for potential inhibitory effects against pancreatic lipase *in vitro*. RoseFit showed a strong lipase inhibitory effect with IC_{50} of 88.43 µg/mL. The results were comparable to orlistat (IC_{50} 11.05 µg/mL) [Figure 2]. The inhibitive capacity of RoseFit in less concentration, against the lipase activity might be entirely concurrent with their total phenolic compounds. McDougall and co-workers have reported that the lipase inhibitory activity might due to the phenolic compounds found in some medicinal plant such as gallic acid, catechin, ellagic acid, quercetin and resveratrol, and anthocyanin.[27,28]

RoseFit downregulates adipogenic markers in 3T3-L1 cells

Figure 3a shows the cell viability of 3T3-L1 cells exposed to the various concentrations of RoseFit (0.25–2 mg/mL). RoseFit was found less cytotoxic to the cells up to 1 mg/mL concentration. However, the cell viability was reduced by 24.52% and 66.89% at 1 and 2 mg/mL concentrations of RoseFit, respectively. There were no significant effects of RoseFit on cell viability up to 0.5 mg/mL. We have used the non-toxic concentrations of the extract for further efficacy evaluation in 3T3-L1 cells.

Further, we have examined the effect of RoseFit on adipogenesis in 3T3-L1 adipocytes. RoseFit at 500 µg/mL concentration noticeably inhibited the lipid accumulation in cells ($P < 0.05$) [Figure 3b]. These findings indicate that RoseFit could regulate adipocyte differentiation in 3T3-L1 cells possibly due to the presence of higher polyphenol content. To gain further insights into the anti-adipogenic mechanism of RoseFit-mediated anti-adipogenic effect, the levels of key proteins in differentiating 3T3-L1 cells were examined. Western blot analysis of cellular proteins clearly demonstrated that RoseFit at 250 µg and 500 µg markedly suppressed PPARγ and C/EBPα expression in concentration-dependent fashion [Figure 3c]. It has been reported that PPARγ and C/EBPα family of transcription factors plays a pivotal role in the regulation of the transcriptional events occurring during adipocyte differentiation.[29,30] The expression of PPARγ is low in 3T3-L1 preadipocytes, and it rises dramatically during adipocyte conversion.[31] In this study, we have used a synthetic glucocorticoid DXM cocktail for inducing adipogenesis which also induces the expression PPARγ and C/EBPα. RoseFit significantly suppressed adipogenic markers in 3T3-L1 cells. The results from *in vitro* studies prompted us to study the effects of RoseFit in HFD model mice.

RoseFit markedly reduces body weight of high fat diet-induced C57Bl/6 mice

Here, we have selected the male C57Bl/6 mice as the HFD model based on the previous reports indicating their higher feeding efficiency and susceptibility to diet-induced obesity.[32,33] The HFD-fed mice showed an obviously higher body weight gain throughout the treatment period ($P < 0.001$) compared to normal mice. Interestingly, RoseFit exhibited significant reduction in the body weight of HFD mice after 2 weeks of administration of extract ($P < 0.001$). Figure 4 shows the changes in body weight of mice during the treatment period. Interestingly, there was a significant decrease in the food intake in RoseFit-treated groups compared to HFD mice ($P < 0.001$).

RoseFit treatment reduces lipid accumulation in liver

The mean liver weights and liver weight to body weight ratio of HFD-fed C57Bl/6 mice are presented in Figure 5a. The mean liver weight of HFD mice was higher compared to the control animals ($P < 0.05$). A 6-week RoseFit treatment led to the dose-dependent reduction in the liver weight of HFD mice ($P < 0.01$ at 200 mg/kg dose) compared to the untreated obese mice. Similar decreasing trend was observed in the liver to body weight ratio of RoseFit treatment groups compared to HFD group. However, the data were not significant.

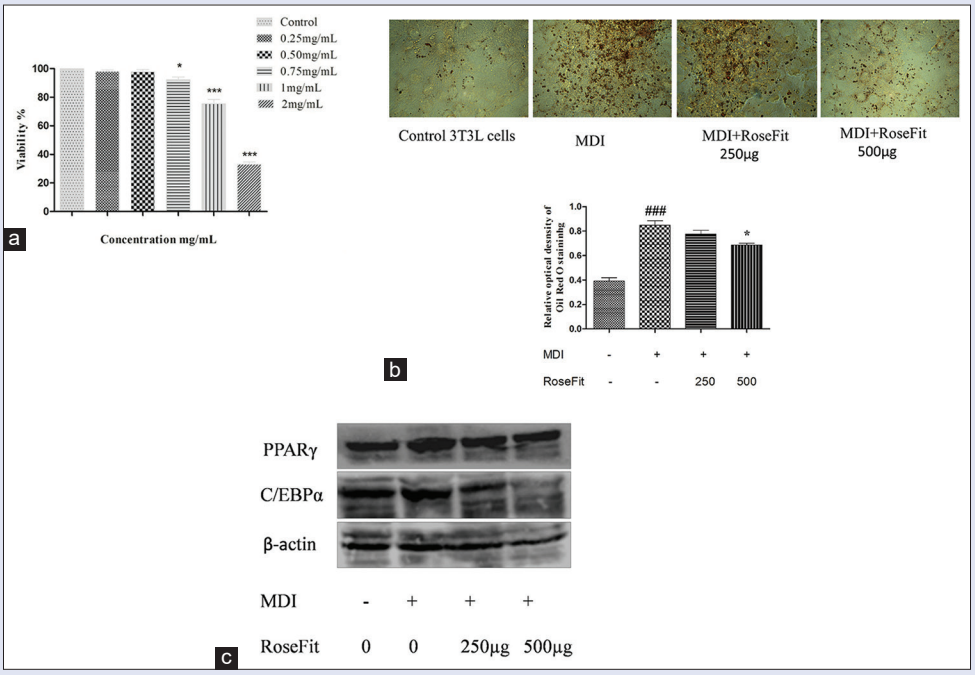

Figure 3: Effect of RoseFit on 3T3-L1 adipocyte differentiation. (a) Cytotoxicity of 3T3-L1 cells by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetraolium bromide assay (*P < 0.05, ***P < 0.001 vs. control) (b) representative images of Oil Red O staining and measurement of lipid droplets (###P < 0.001 vs. control; *P < 0.05 vs. MDI control) (c) expression of peroxisome proliferator-activated receptor gamma and CCAAT/enhancer-binding protein alpha during the differentiation in 3T3-L1 cells. Peroxisome proliferator-activated receptor gamma and CCAAT/enhancer-binding protein alpha protein expressions were analyzed by Western blot analysis. Beta-actin was used as a loading control. Data were analyzed by the one-way analysis of variance followed by Tukey's test. Expressed as mean ± standard deviation (n = 3)

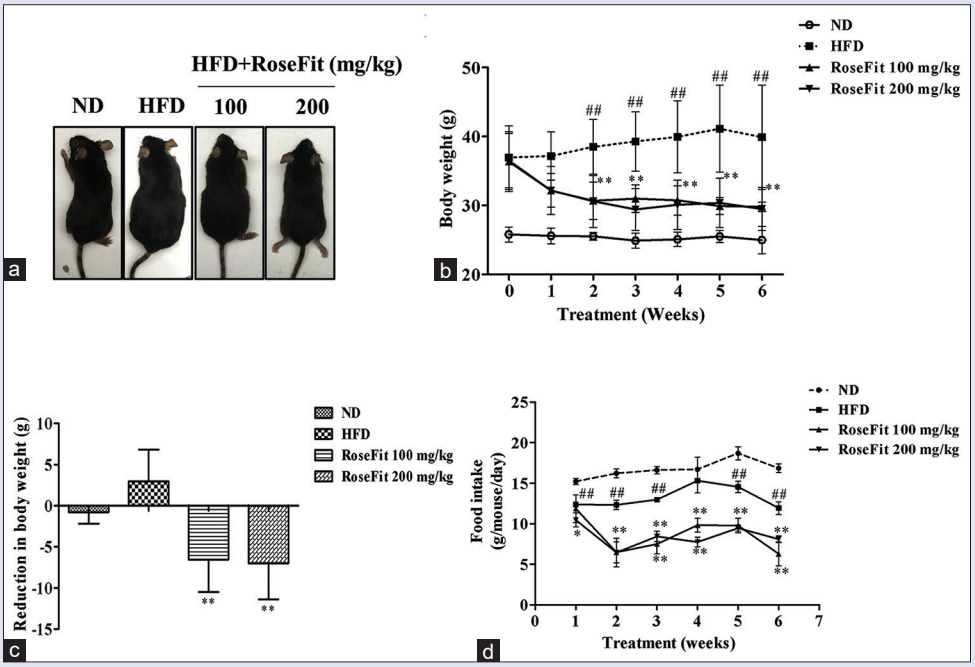

Figure 4: Effect of RoseFit on the body weight during the treatment. (a) Images of representative mice from each group, (b) Weekly body weight measurements during the treatment, (c) mean change in body weight after treatment period and (d) Daily food intake. Data are expressed as mean ± standard deviation (n = 6 per group). ##P < 0.001 vs. control; **P < 0.001 vs. High-fat diet group. ND: Normal diet group, HFD: High-fat diet group

The results of liver H and E staining are shown in Figure 5b. Mice in the control group showed a normal liver architecture, whereas in HFD model group, there was a marked presence of lipid droplets. The presence of macrovesicles was clearly seen in the liver of HFD-fed mice, a characteristic feature of fatty liver. 100 and 200 mg/kg RoseFit treatment restored the normal liver histology in HFD-mice. Oil red O staining

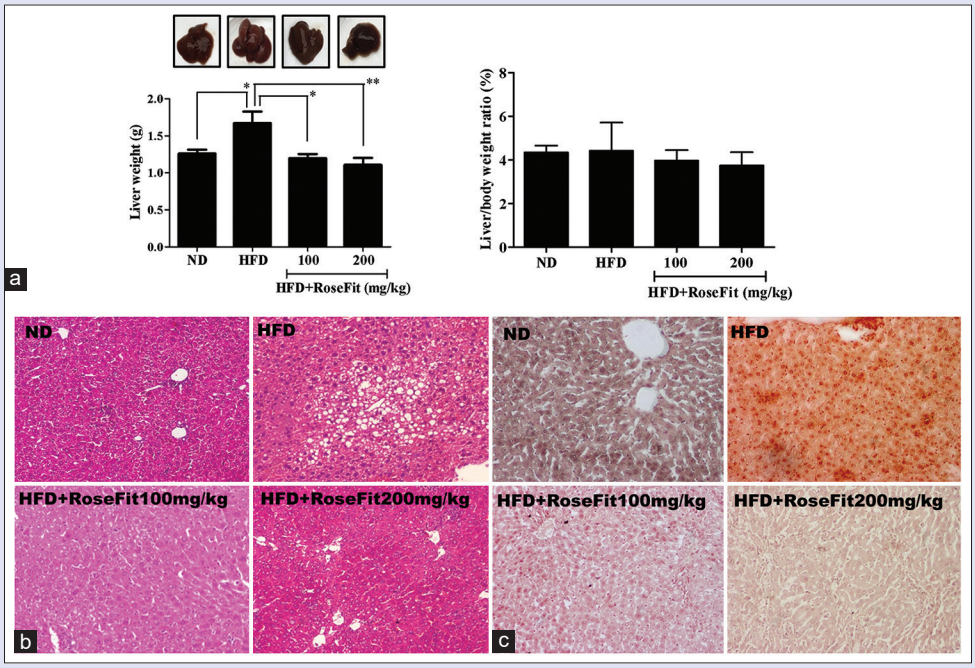

Figure 5: Effect of RoseFit on liver weight and morphology. (a) Liver weights and liver to body weight ratio of treatment groups; representative histological images of liver sections stained with (b) H and E (×100) and (c) oil red O staining (×100)

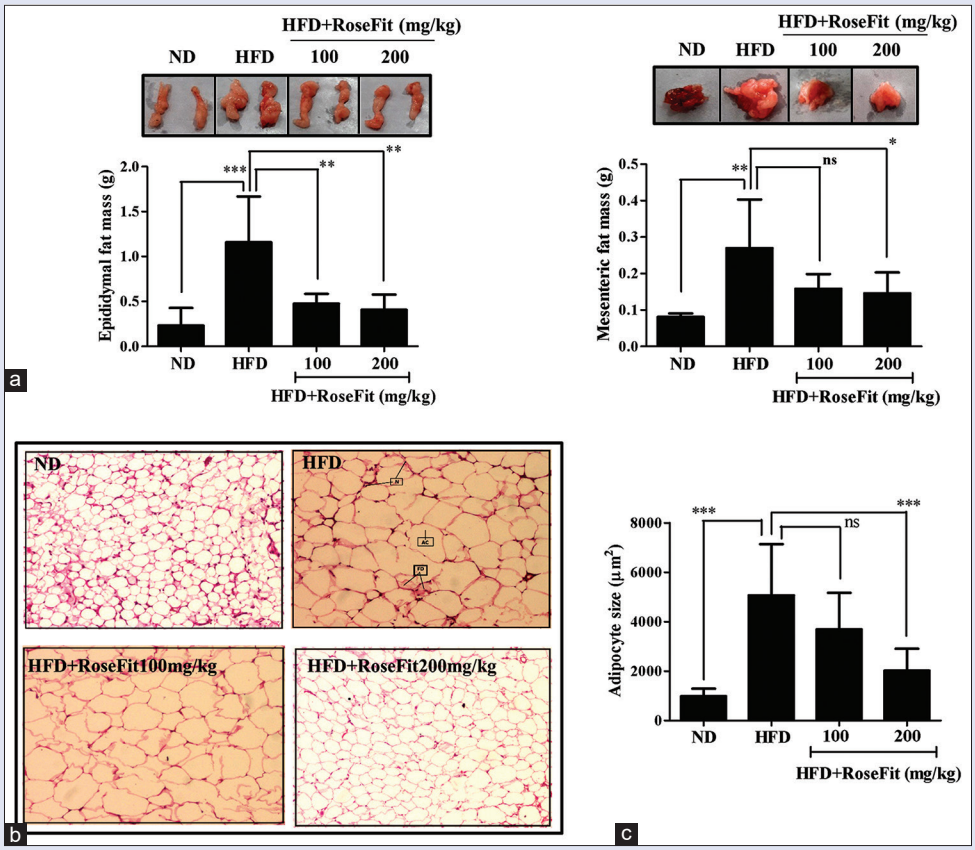

Figure 6: Effect of RoseFit on fat depots in high fat diet-induced C57Bl/6 mice. (a) Epididymal and mesenteric fat pad weights, (b) paraffin sections of epididymal white adipose tissue (H and E staining), (c) Average adipocyte cell area of epididymal fat. Data are expressed as mean ± standard deviation ($n = 6$ per group). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$

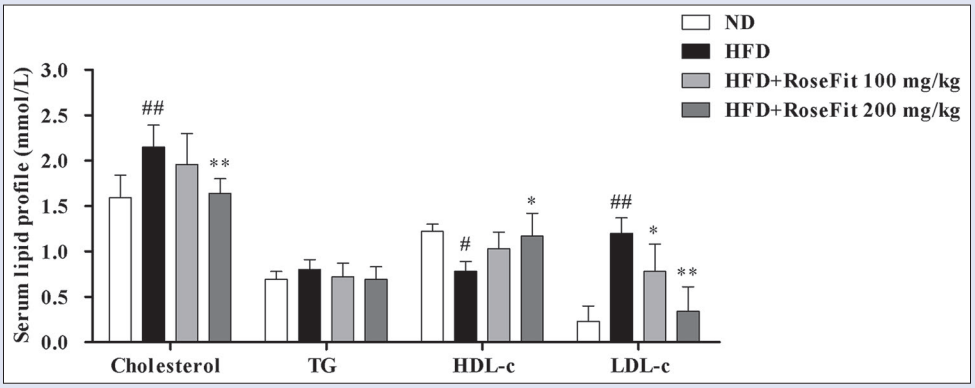

Figure 7: Effect of RoseFit on serum lipid profile of high-fat diet-induced obese C57Bl/6 mice. Data are expressed as mean ± standard deviation ($n = 6$ per group). ##$P < 0.001$ versus Normal group (ND); *$P < 0.01$ and **$P < 0.001$ versus high fat diet group

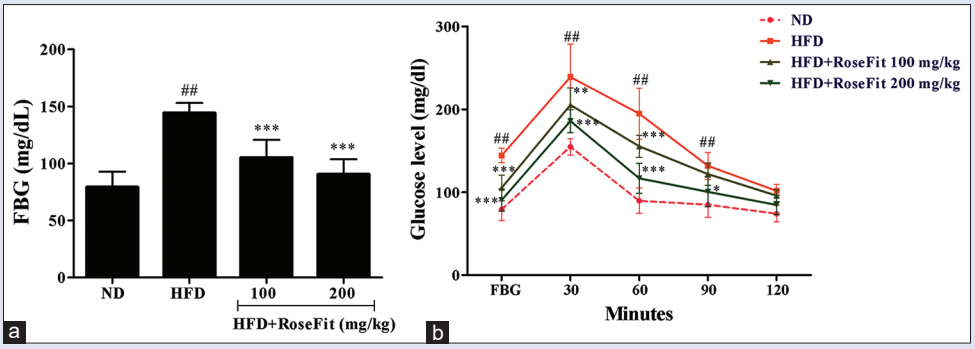

Figure 8: Effect of RoseFit on blood glucose level. (a) Fasting blood glucose, (b) Time course changes in blood glucose levels during OGTT. Data are expressed as mean ± standard deviation ($n = 6$ per group). ##$P < 0.001$ versus normal group (ND); *$P < 0.05$, **$P < 0.01$ and **$P < 0.001$ versus high-fat diet group

further confirmed the accumulation of fat in HFD-fed mice [Figure 5c]. In the HFD-fed group, prominent red fat vacuoles stained by oil red O were observed. RoseFit treatment markedly reduced the hepatic accumulation of lipids in obese mice.

RoseFit reduces fat pad weight and morphology

The adipose tissue weights increased significantly in HFD group compared to normal animals [Figure 6a]. There was a dose-dependent reduction of fat pads in the RoseFit-treated groups. RoseFit (200 mg/kg) markedly decreased the epididymal and mesenteric fat weights in HFD-fed mice. In order to investigate the effect of RoseFit on the morphology of WAT in HFD mice, histological evaluation was performed and observed in a microscopic view with H and E staining [Figure 6b]. Adipocyte hypertrophy was clearly observed in HFD group compared to normal diet-fed mice ($P < 0.001$). RoseFit treatment at 100 and 200 mg/kg showed remarkable decrease in the adipocyte size. RoseFit at 200 mg/kg exhibited significant reduction in the adipocyte size as compared to HFD group ($P < 0.001$) [Figure 6c].

RoseFit ameliorates altered serum lipid profile of high fat diet mice

The HFD fed C57Bl/6 mice showed higher lipid profile compared to normal animals [Figure 7]. Obese mice treated with low and high doses of RoseFit exhibited remarkable improvement in the lipid profile. The HFD mice treated with 200 mg/kg RoseFit showed significantly lower serum total cholesterol, LDL-c ($P < 0.001$), and higher HDL-c ($P < 0.001$) relative to the untreated HFD group. RoseFit treatment also decreased the serum TG of HFD mice; however, the data were not significant.

RoseFit improves glucose tolerance in high fat diet-induced C57Bl/6 mice

Figure 8a shows the effect of RoseFit on fasting blood glucose (FBG) and glucose tolerance. As expected, HFD significantly elevated the FBG in mice compared to the control group ($P < 0.01$). However, a significant dose-dependent reduction in the FBG was observed in RoseFit-treated groups ($P < 0.001$). As evident from OGTT, the glucose tolerance of HFD-fed mice was markedly impaired as compared to normal diet-fed group [Figure 8b]. The blood glucose levels were markedly higher in HFD group than those of normal mice ($P < 0.001$). 100 and 200 mg/kg RoseFit treatment significantly restored the blood glucose level in HFD mice ($P < 0.001$).

RoseFit downregulates hepatic expression of adipogenic transcription factors

We have examined the hepatic expression of adipogenic proteins to gain more insights into the underlying mechanism of RoseFit-mediated weight loss effects [Figure 9]. The hepatic levels of PPARγ were significantly higher in the HFD mice compared to normal animals ($P < 0.01$). The RoseFit extract dose-dependently suppressed the expression of PPARγ. RoseFit was particularly very effective

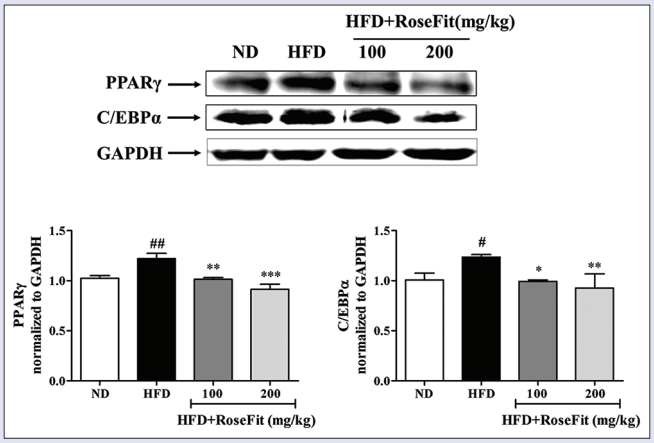

Figure 9: Effect of RoseFit on hepatic protein expression of adipogenic factors. Peroxisome proliferator-activated receptor gamma, CCAAT/enhancer-binding protein alpha protein expression levels were detected by Western blot analysis. Expression levels were normalized to GAPDH. Values are mean ± standard deviation #$P < 0.05$ and ##$P < 0.01$ versus normal control group; *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ versus high-fat diet group

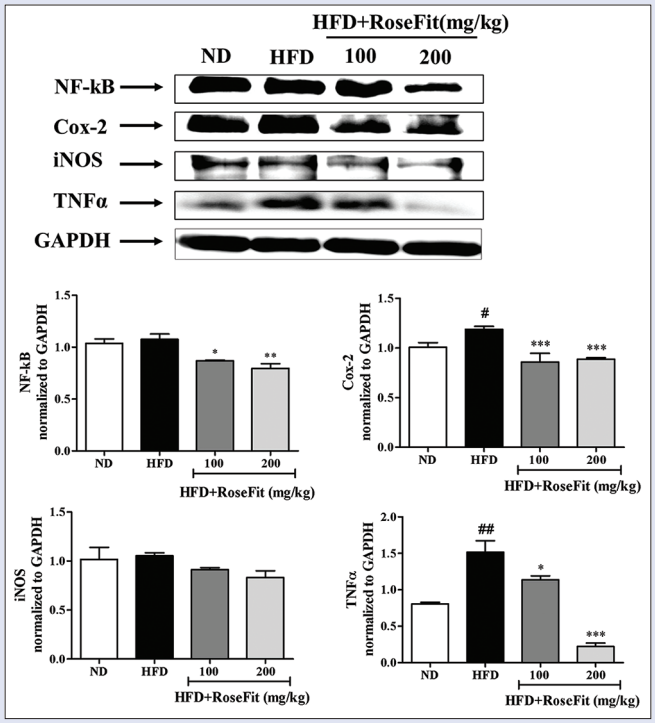

Figure 10: Effect of RoseFit on the expression of hepatic inflammatory proteins in high-fat diet mice. Nuclear factor kappa, Cox-2, Inducible nitric oxide synthase and tumor necrosis factor alpha protein expression levels were detected by the Western blot analysis. Expression levels were normalized to GAPDH. Values are mean ± standard deviation. #$P < 0.05$ and ##$P < 0.01$ versus normal control group; *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ versus high-fat diet group

at the dose of 200 mg/kg ($P < 0.001$). Similarly, the increase in C/EBPα expression level was decreased significantly in RoseFit-treated mice ($P < 0.01$).

Lipid metabolism is largely regulated in the liver.[34] Activation of PPARγ facilitates the transcription of lipogenic transcription factors and the upregulation of lipogenesis results in increased hepatic TG.[35,36] Hepatic expression of PPARγ is often correlated to the fat accumulation in conditions such as obesity and diabetes.[37] In our study, the hepatic levels of PPARγ were substantially reduced by RoseFit in HFD mice. Further, the expression of another key lipid metabolic enzyme C/EBPα is downregulated by the RoseFit at the tested doses.

RoseFit reduces the levels of inflammatory proteins in the liver of high-fat diet mice

The effect of RoseFit on the inflammatory proteins in the liver was evaluated by Western blot analysis. HFD-fed mice significantly upregulated NF-kB, Cox-2, iNOS, and TNFα compared to the normal group. However, 100 and 200 mg/kg RoseFit treatment significantly reduced the inflammatory protein expression in the liver of obese mice [Figure 10]. Energy homeostasis is hindered during obesity that leads to the initiation of inflammatory response.[10,38] Inflammatory mediators such as cytokines and chemokines are up-regulated through activation of NF-kB.[39] Our results clearly suggest that RoseFit effectively suppressed the hepatic inflammation induced by HFD.

CONCLUSION

Collectively, this study concludes that RoseFit-containing 2%–3% isoquercetin might possibly exert its effect by reducing the dietary fat absorption through lipase inhibition. Second, the extract may also regulate adipogenesis and mitigates the hepatic inflammation. RoseFit, a first-of-its-kind extract from rose petals can be explored as a key functional ingredient in health supplements to improve the metabolic health and well-being.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Finucane MM, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, *et al.* Global burden of metabolic risk factors of chronic diseases collaborating group (Body Mass Index). National, regional and global trends in body-mass index since 1980: Systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9·1 million participants. Lancet 2011;377:557-67.
2. Malik VS, Willett WC, Hu FB. Global obesity: Trends, risk factors and policy implications. Nat Rev Endocrinol 2013;9:13-27.
3. Kopelman PG. Obesity as a medical problem. Nature 2000;404:635-43.
4. Kanneganti TD, Dixit VD. Immunological complications of obesity. Nat Immunol 2012;13:707-12.
5. Gregoire FM, Smas CM, Sul HS. Understanding adipocyte differentiation. Physiol Rev 1998;78:783-809.

6. Cristancho AG, Lazar MA. Forming functional fat: A growing understanding of adipocyte differentiation. Nat Rev Mol Cell Biol 2011;12:722-34.
7. Shiomi Y, Yamauchi T, Iwabu M, Okada-Iwabu M, Nakayama R, Orikawa Y, *et al.* A novel Peroxisome proliferator-activated receptor (PPAR)α agonist and PPARγ antagonist, Z-551, ameliorates high-fat diet induced obesity and metabolic disorders in mice. J Biol Chem 2015;290:14567-81.
8. Xue Y, Xu X, Zhang XQ, Farokhzad OC, Langer R. Preventing diet-induced obesity in mice by adipose tissue transformation and angiogenesis using targeted nanoparticles. Proc Natl Acad Sci U S A 2016;113:5552-7.
9. Grundy SM. Obesity, metabolic syndrome, and cardiovascular disease. J Clin Endocrinol Metab 2004;89:2595-600.
10. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, *et al.* Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. Nat Med 2005;11:183-90.
11. Kang JG, Park CY. Anti-obesity drugs: A review about their effects and safety. Diabetes Metab J 2012;36:13-25.
12. Chandrasekaran CV, Vijayalakshmi MA, Prakash K, Bansal VS, Meenakshi J, Amit A. Review article: Herbal approach for obesity management. Am J Plant Sci 2012;3:1003-14.
13. Sun NN, Wu TY, Chau CF. Natural dietary and herbal products in anti-obesity treatment. Molecules 2016;21:E1351.
14. Mishra RP, Arshad M, Sami A. Antibacterial properties of *Rosa indica* (L.) stem leaves and flowers. J Pharm Biomed Sci 2011;4:1-3.
15. Mármol I, Sánchez-de-Diego C, Jiménez-Moreno N, Ancín-Azpilicueta C, Rodríguez-Yoldi MJ. Therapeutic applications of rose hips from different *Rosa species*. Int J Mol Sci 2017;18:1137.
16. Davoodi I, Rahimi R, Abdollahi M, Farzaei F, Farzaei MH, Memariani Z, *et al.* Promising effect of *Rosa damascena* extract on high-fat diet-induced nonalcoholic fatty liver. J Tradit Complement Med 2017;7:508-14.
17. Gholamhoseinian A, Shahouzehi B, Sharififar F. Inhibitory effect of some plant extract on pancreatic lipase. Int J Pharmacol 2010;6:18-24.
18. Gholamhoseinian A, Shahouzehi B, Joukar S, Iranpoor M. Effect of *Quercus infectoria* and *Rosadamascena* on lipid profile and atherosclerotic plaque formation in rabbit model of hyperlipidemia. Pak J Biol Sci 2012;15:27-33.
19. Thilagavathy A, Dsouza S, Shriraksha A, Singh KG. Anti-inflammatory and antihypercholesterolemic activity of *Rosa centifolia*, *Coriandrum sativum* and *Cynodondactylon*. Int J Pharmaceutical Sci Res. 2019Feb; 10:966-972.
20. Kondo H, Hashizume K, Shibuya Y, Hase T, Murase T. Identification of diacylglycerol acyltransferase inhibitors from *Rosa centifolia* petals. Lipids 2011;46:691-700.
21. Ochir S, Nishizawa M, Park BJ, Ishii K, Kanazawa T, Funaki M, *et al.* Inhibitory effects of Rosa gallica on the digestive enzymes. J Nat Med 2010;64:275-80.
22. Kim YS, Lee YM, Kim H, Kim J, Jang DS, Kim JH, *et al.* Anti-obesity effect of *Morus bombycis* root extract: anti-lipase activity and lipolytic effect. J Ethnopharmacol 2010;130:621-4.
23. Kim YS, Lee YM, Kim J, Sohn E, Kim CS, Lee YM, *et al.* Inhibitory activities of *Cudraniatricuspidata* leave on pancreatic lipase *in vitro* and lipolysis *in vivo*. eCAM 2012;8:14-21.
24. Ramírez-Zacarías JL, Castro-Muñozledo F, Kuri-Harcuch W. Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O. Histochemistry 1992;97:493-7.
25. Lu Y, Zhang C, Bucheli P, Wei D. Citrus flavonoids in fruit and traditional Chinese medicinal food ingredients in China. Plant Food Hum Nutr 2006;61:57-65.
26. Yajima H, Noguchi T, Ikeshima E, Shiraki M, Kanaya T, Tsuboyama-Kasaoka N, *et al.* Prevention of diet induced obesity by dietary isomerized hop extracts containing isohumulones, in rodents. Int J Obes (Lond) 2005;29:991-7.
27. Lei F, Zhang XN, Wang W, Xing DM, Xie WD, Su H, *et al.* Evidence of anti-obesity effects of the pomegranate leaf extract in high-fat diet induced obese mice. Int J Obes (Lond) 2007;6:1023-9.
28. McDougall GJ, Kulkarnni NN, Stewart D. Berry polyphenols inhibit pancreatic lipase activity *in vitro*. Food Chem 2009;115:193-9.
29. Rosen ED, Hsu CH, Wang X, Sakai S, Freeman MW, Gonzalez FJ, *et al.* C/EBP alpha induces adipogenesis through PPARgamma: A unified pathway. Genes Dev 2002;16:22-6.
30. Tang QQ, Otto TC, Lane MD. Commitment of C3H10T1/2 pluripotent stem cells to the adipocyte lineage. Proc Natl Acad Sci USA 2004;101:9607-11.
31. Tontonoz P, Hu E, Spiegelman BM. Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. Cell 1994;79:1147-56.
32. Chu DT, Malinowska E, Jura M, Kozak LP. C57BL/6J mice as a polygenic developmental model of diet-induced obesity. Physiol Rep 2017;5:e13093.
33. Almind K, Manieri M, Sivitz WI, Cinti S, Kahn CR. Ectopic brown adipose tissue in muscle provides a mechanism for differences in risk of metabolic syndrome in mice. Proc Natl Acad Sci 2007;104:2366-71.
34. Corey KE, Kaplan LM. Obesity and liver disease: The epidemic of the twenty-first century. Clin Liver Dis 2014;18:1-18.
35. Fraulob JC, Souza-Mello V, Aguila MB, Mandarim-de-Lacerda CA. Beneficial effects of rosuvastatin on insulin resistance, adiposity, inflammatory markers and non-alcoholic fatty liver disease in mice fed on a high-fat diet. Clin Sci (Lond) 2012;123:259-70.
36. Fernandes-Santos C, Carneiro RE, de Souza Mendonca L, Aguila MB, Mandarim-de-Lacerda CA. Pan-PPAR agonist beneficial effects in overweight mice fed a high-fat high-sucrose diet. Nutrition 2009;25:818-27.
37. Lee YK, Park JE, Lee M, Hardwick JP. Hepatic lipid homeostasis by peroxisome proliferator-activated receptor gamma 2. Liver Res 2018;2:209-15.
38. Ehses JA, Perren A, Eppler E, Ribaux P, Pospisilik JA, Maor-Cahn R, *et al.* Increased number of islet-associated macrophages in type 2 diabetes. Diabetes 2007;56:2356-70.
39. Debnath M, Sarkar S. Obesity induced metaflammation: Pathophysiology and mitigation. J Cytokine Biol 2016;1:104.