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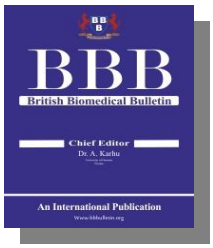

Research Article

Preliminary *in-vitro* Screening of Ayurvedic Formulations *Shuddhaguggulu* and *Triphalaguggulu* Adipocyte Differentiation and Induces Apoptosis in 3T3-L1 Cells and Pancreatic Lipase Inhibition in Obesity

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A R T I C L E I N F O

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A B S T R A C T

Objective: Plant-derived compounds have been used clinically to treat weight management (obesity) for many years as they also exert additional beneficial effects on various other disorders. The aim of the present study was to investigate the weight management activity of *Shuddaguggulu* and *Triphalaguggulu.*

Method: *Shuddhaguggulu* and *Triphalaguggulu* prepared as per "*Shodhana"* (Purification) mention in Ayurvedic formulary of India was investigated for lipase inhibition, adipogenesis in 3T3-L1 and adipolysis activity.

Result: The HPLC analysed *Shuddhaguggulu* and *Triphalaguggulu* tested, only *Shuddhaguggulu* at concentration of 125microgram/mL showed significant activity for lipase inhibition, adipogenesis and adipolysis compared to standard Simvastatin.

Conclusion: The in-vitro assessment suggest that the *Shuddhaguggulu* is potent, showed activity for lipase inhibition, adipogenesis, adipolysis and reveals that *Shuddhaguggulu* is potent enough to control obesity and may help in weight management. It was observed that SG can help in controlling obesity by reducing fat accumulation. The preliminary study has demonstrated weight management (Obesity) and future experiments viz. Clinical trials has to carry and outcome of the result will help to ploy-herbal supplement.

Introduction

According to history gaining weight was considered as the mark of good health, but now hard work, improper food habits; reduced energy intake has steered to lot of health hazards. Although the life style has been changed and people are well updated regarding health and nutrition, weight gain and obesity have been a major concern and a very big threat to human folks. WHO considers obesity is a chronic disease which is prevalent in developed and developing countries observed in children and adults[1]. Obesity being one of the major threat to mankind, which has led to discrimination specially in "The United States of America" especially in young women[2].

According to traditional reference cited in "*Charaka Samhita"* slim and healthy physique is a divine gift were as if a person gains excessive weight the treatment for reducing weight will be a very difficult task[3]. The experiment was carried out to set out a feasible plant derived extract as mentioned in the classical texts of "*Ayurveda*" can help to control, reduce and maintain weight in obese people.

Material & Methods

Preparation of *Shuddhaguggulu* (SG)

Commiphora weightii (Arn.) Bhandari was purchased from Gujarat medicinal plant growers society, *Emblicaofficinalis* Gaertn., *Terminalia-chebula* Retz, *Terminalia bellerica* (Gaertn.) Roxb. was obtained from approved vendors at Bangalore, botanical samples were authenticated by Botanist of The Himalaya Drug Company, Bangalore, India and voucher specimen (NPD/499/12, NPD/119/12, NPD/531/12 and NPD/59/12) can be archived.

The processing method for *Shuddhaguggulu*(SG) was as per AFI[4]. The obtained *Shuddhaguggulu*(SG) was tested for guggulusterone content according to the method mentioned in "The United State Pharmacopoeia" and found to have guggulusterone content 0.02% *w/w*.

Preparation of Triphalaguggulu(TG)

Triphalaguggulu was prepared as per the method mentioned AFI[4]and tablets were tested for guggulusterone content as per the method mentioned in USP[5]. The content of guggulusterone E & Z in each 500 mg was 0.02%*w/w*.

In-vitro Adipolytic and Anti-adipogenic activity of samples in 3T3-L1 adepocytes

Chemicals and Reagents 3-(4, 5-dimethyl thiazol-2-yl)-5-diphenyltetra-zolium bromide (MTT), fetal bovine serum (FBS), phosphate buffered saline (PBS), Dulbecco's modified Eagle's Medium (DMEM) Dexamethazone, 3-iso butyl-1-methyl xanthine (IBMX), Insulin, oil red O and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl sulfoxide (DMSO), Propanol and Triglyceride estimation kit from E.Merck Ltd., Mumbai, India. Gallic acid, p-nitro-phenyl butyrate (Sigma, cat no N9876), crude porcine pancreatic lipase type II (Sigma, cat no L3126).

Cell lines and Culture medium 3T3 L1 cell lines were obtained from National Centre for Cell Science (NCCS), Pune, India. Stock cultures of these cell lines were cultured in DMEM supplemented with 10% inactivated fetal bovine serum, Penicillin (100 IU/mL), Streptomycin (100 mg/mL) and Amphoterecin-B (5 mg/mL) in a humidified atmosphere of 5% CO_2 at 37^0C until confluent. The cells were dissociated with 0.2% trypsin, 0.02% EDTA in Phosphate buffer saline (PBS) solution. The stock cultures were grown in 25 cm^2 tissue culture flasks and all cytotoxicity experiments were carried out in 96 and 24

well plates. (Tarsons India Pvt. Ltd., Kolkata, India)

Preparation of Test Solutions

For *in-vitro* studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/mL concentration and sterilized by filtration, serial two fold dilutions were prepared from this for carrying out cytotoxicity studies.

Cytotoxicity test for extracts were carried out in 3T3-L1 cells by MTT Assay[6].

In-vitro anti-adipogenic assay

The monolayer of the 3T3-L1 cells were trypsinized and re-suspended at $1x10^4$ cells/mL in DMEM with 10% FBS. 0.5 mL of cell suspension was seeded per well in 24 well plates (Tarsons India Pvt. Ltd., Kolkata, India), plate was incubated and allowed to attain the confluent monolayer. Growth medium was removed and followed by the addition of adipogenesis initiation media (DMEM with 10% FBS, 0.5 mm IBMX and 1µm Dexamethasone). The media, DMEM with 10% FBS, served as the negative control with subsequent media changes. The much care was taken while replacing media so as to avoid the disturbance of monolayer. The plates were incubated for 48 hrs at 5% CO_2 at 37°C, after incubation adipogenesis initiation media was replaced by adipogenesis progression media (DMEM with 10% FBS and 10µm/mL Insulin). The plate was incubated for 48 hrs at 5% CO_2 at 37°C and then adipogenesis progression media was removed. The cells were treated with various concentrations of test extracts, negative and positive controls in adipogenesis maintenance media (DMEM with 10% FBS) for 3 hrs. After 3 hrs, glycerol release in the cell supernatants was measured using a commercial Triglyceride estimation kit as per the manufacturer protocol.

In-vitro anti-adipogenicassay[7]

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The adipocytes formation was measured by oil red O staining method. At the end of the experiment, the cells were washed with PBS and fixed with formalin (10% formaldehyde, 90% PBS) and air-dried. Oil red O solution was added to each well and incubated at room temperature for 2 hours. After incubation, the plates were washed 3 to 4 times with plain water and allowed to air dry. Isopropanol (0.5 mL) was added to each well to extract Oil red O, then kept in orbital shaker for 5 min and the

sample was read at 510 nm in micro plate reader. The similar procedure was followed for cell control and adipogenesis control. Rate of adipocyte differentiation was evaluated by the measurement of fat formation compared against control.

In-vitro pancreatic lipase inhibition study of test extracts

Preparation of Test Solutions[8]

Standard drug and extract were prepared in phosphate buffer to final concentrations of 62.5-1000 µg/mL.

Pancreatic lipase preparation

The enzyme solutions were prepared immediately before use. For the in vitro assays, crude porcine pancreatic lipase type II (Sigma, cat no L3126) was suspended in tris-HCl buffer of pH 7.4.

Pancreatic lipase inhibition by test samples

The inhibition of pancreatic lipase (PL) activity by the prepared test was measured using the spectro-photometric assay. PL was pre-incubated with each concentration of the tested material for at least 1 min at 37°C before adding the substrate. The volume was completed to 1 mL using the tris-HCl buffer before measuring the solution absorbance using spectrophotometer at 410 nm for minimum of 5 time points (1 - 5 min). The reaction maintained at 37°C was started by adding the p-nitro-phenyl butyrate (Sigma, cat no N9876) to the reaction mixture. The release of p-nitro-phenol was measured as the increase in absorbance measured at 410 nm, by an ultraviolet (UV) spectrophotometer (Systronics, India), against a blank that contain the same mixture but denatured enzyme. Enzyme activity was detected as an increase of the absorbance per minute.

Calculation

The percentage of residual activity of PL was determined for the tested extract/compound by comparing the lipase activity of PL with and without the tested material. The concentration required to give 50% inhibition (IC50) was determined for the tested extract. The percentage of residual activity of PL was determined for the tested extract/compound by comparing the lipase activity of PL with and without the tested material. The concentration required to give 50% inhibition (IC50) was determined for the tested extract.

Statistical Analysis

The results are expressed as mean S.E.M. the significant of various treatments was calculated using one way ANOVA and were considered statistically significant when $P< 0.05$.

Results

When TG and SG were tested against standard drug Simvastatin, it has been observed that SG shows activity for lipase inhibition, adipogenesis and adipolysis activity. The test sample SG showed potent activity when compared to that of TG, inhibition we compared with standard drug Simvastatin, It was concluded that SG was potent enough to inhibit.

Discussion

Obesity being a major problem across the globe and as per NIH claims that obesity climbing to epidemic levels and is associated with discrimination from society NIH is aware that it cannot solve this major public health problem. However, The NIH is seeking to exploit on recent scientific discoveries to boost new efforts towards further understanding the forces contributing to obesity and towards developing different

scientific based approach for prevention and treatment. The NIH also backs obesity related research at different levels that includes molecular and clinical based approach. Hence this case was taken up to understand the activity of SG and TG as both are proposed to help in weight management by different business groups which sell these products. This preliminary study suggests that SG at a concentration of 125 microgram/mL shows significant activity against gallicacid (Fig. 1) when compared to other levels of concentration, when the same samples were tested against adipolytic and anti- adipogenic against standard drug Simvastatin it was observed SG found to have potent activity by reducing fat accumulation (Fig. 2, Fig. 3).

Conclusions

Purification (*Shodhana*) an essential step mentioned in Ayurveda which involves various steps of processing mainly to remove foreign unwanted matter, reduce eradicate toxic substances, which in-turn potentiates the drug ,also enables for the better efficacy of the product. In this study we have tried to understand the activity of SG processed material intended to be used in different recipes mentioned in "*Ayurveda"* for different ailments. The outcome of *in-vitro* study Viz. adipogenic, adipolysis and pancreatic lipase activity reveals that SG is potent enough to control obesity and may help in weight management.

Weight management has been one of the major concerns by the people across the world, people are trying to maintain and control their weight by different means of food, medications and exercises in this category woman have been the major players and consumers in weight management program. Statins being one of the FDA approved OTC drug recommended for lipid lowering, but long time intake and abuse of this drug has certain implication on consumers has driven to oxalate nephropathy and chronic kidney disease[9-10], other side effects that has been observed involves abdominal discomfort[10] .Other drugs which were in European market was withdrawn by EMA (European Medical Agency) due to severe side effects are as follows long term use of Phentermine and its cessation leads to depression, Fenfluramine increased risk of pulmonary hypertension and heart valve defects, Rimonabant was discontinued due to concerns about neuropsychiatric side effects and Sibutramine was withdrawn due to long term potential risk for non-fatal myocardial infarction and stroke[11-13]. Understanding all these side effects of synthetic drugs a sincere investigation was carried in two combinations mentioned in traditional books of "*Ayurveda*" SG and TG. It was observed that SG can help in controlling obesity.

But exhaustive clinical trials have to carry and the outcome of the data will help in using this poly-herbal supplement.

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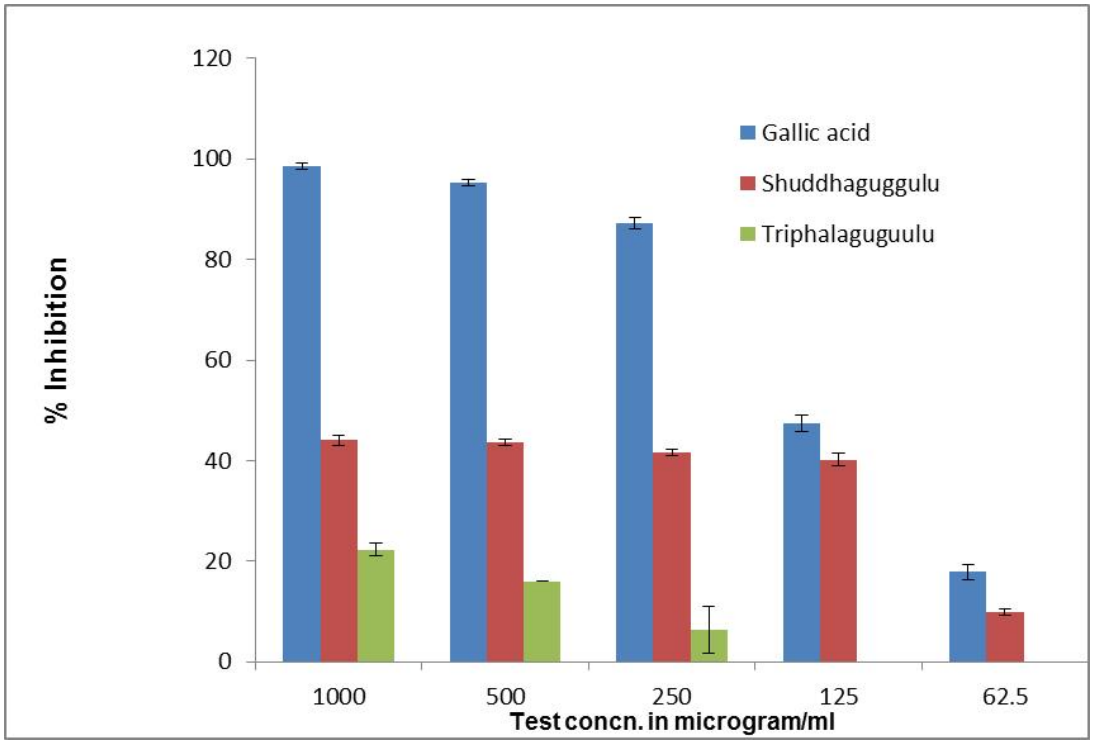

Fig. 1: Pancreatic lipase inhibitory activity of test samples against Gallic acid

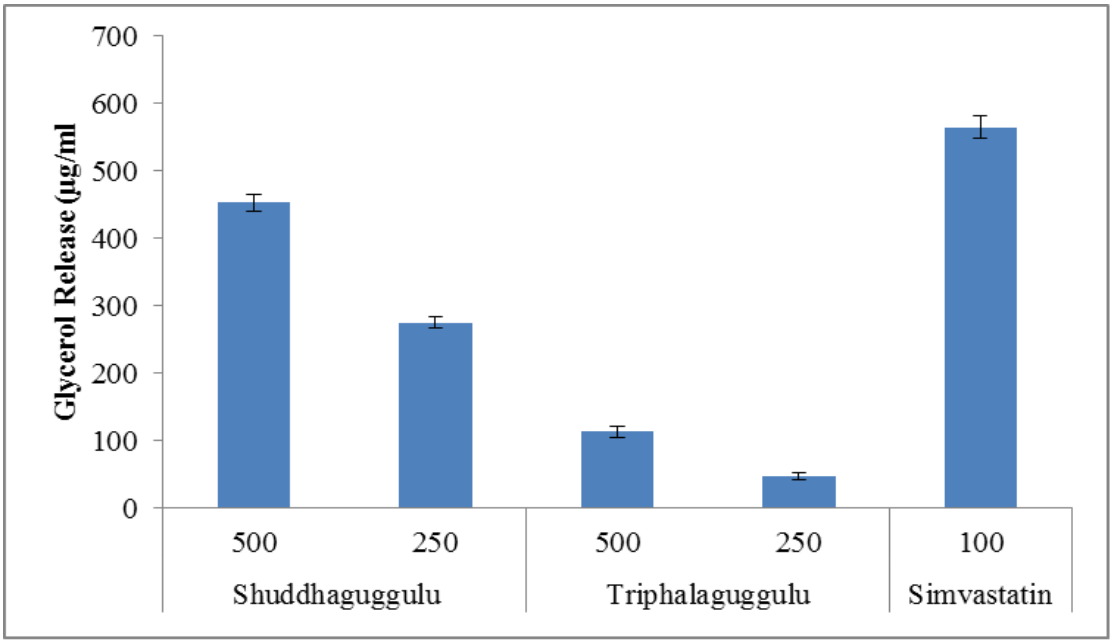

Fig. 2: *In vitro* adipolytic activity of test extracts in 3T3 L-1 cells

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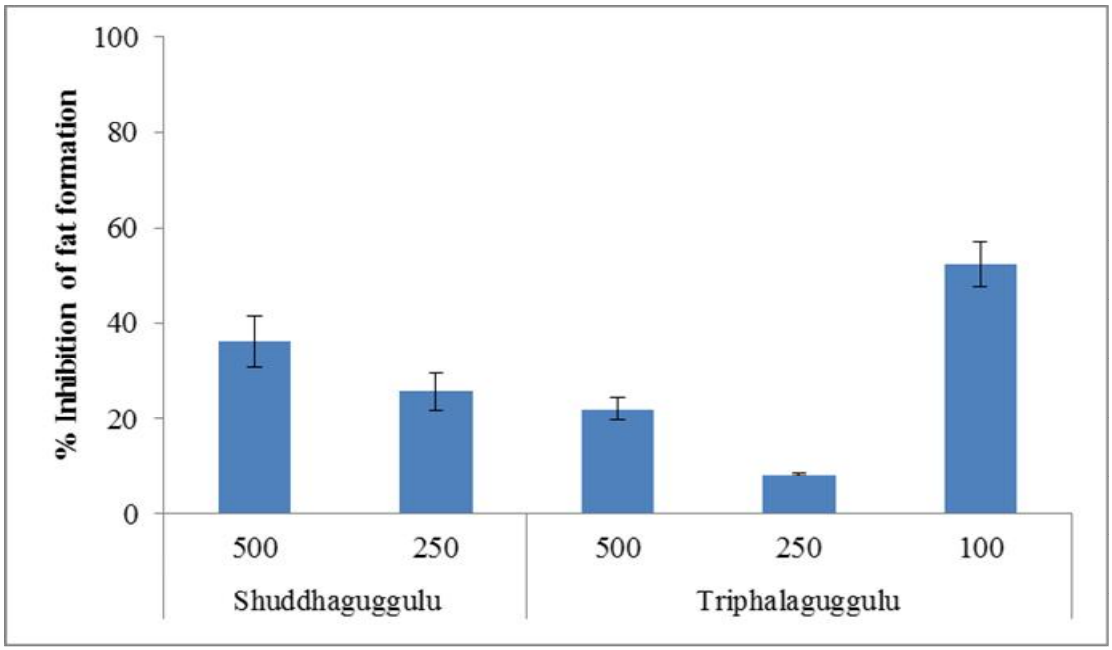

Fig. 3: *In vitro* anti-adipogenic activity of test samples in 3T3 L-1 cells