RESEARCH
O&G Forum 2024; 34 – 2s: 74 - 77

IN-VITRO ANTI LIPASE ACTIVITY OF POLYHERBAL SIDDHA FORMULATION ILAVANGATHI CHOORANAM

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Abstract
Obesity is a metabolic disorder that has emerged as an alarming condition worldwide. This results from the interaction of biological, psychological, and environmental factors that impact an individual's quality of life. Obesity leads to cause many chronic diseases like diabetes, hypertension and cardiovascular diseases. Pancreatic lipase (PL) is plays the major role in the absorption of dietary lipids. Inhibition of pancreatic lipase is one of the study to determine the anti obesity agents. In Siddha system of medicine, Ilavangathi chooranam (IVC) is widely used for dyslipidemia, atherosclerosis of heart and obesity etc by Siddha practitioners. Hence, we aimed to see whether Ilavangathi chooranam could be used as a potential source of new lipase inhibitors. An in vitro study, which was performed to check its anti-lipase activity, showed a potent lipase inhibition with IC50 of 273.21±4.18µg/ml compared to orlistat with IC50 of 0.3µg/ml. Finally this study suggests that the Ilavangathi Chooranam possess the best therapeutic action in the treatment of obesity.
Keyword: Ilavangathi chooranam, obesity, orliststat, pancreatic lipase, Siddha.

INTRODUCTION

Pregnancy and childbirth Obesity is a metabolic disorder that has emerged as an alarming condition worldwide. This results from the interaction of biological, psychological, and environmental factors that impact an individual's quality of life [1]. Nowadays obesity is the major concern as it leads to many chronic diseases like diabetes, hypertension, stroke and cardiovascular diseases [2]. This is often associated with excess calorie intake and low energy expenditure [3]. More over 2 billion adults worldwide were overweight or obese as of 2016, accounting for 44% of the adult population. Of these, more than 70% lived in low-income or middle-income nations [4]. Hence, it is essential to address the increasing rate of obesity to prevent the adverse health effects associated with it [5]. Better lifestyle plays main role in the drastic weght loss [6]. If a person has a BMI of at least 30 (or a BMI of at least 27 with concomitant diseases) and cannot reduce weight by lifestyle changes alone, pharmacotherapy is advised [7]. There are now four medications such as orlistat, naltrexone extended-release [ER]/bupropion ER, phentermine/topiramate controlled-release and liraglutide can be taken long-term (>12 weeks) to aid weight loss. These medications decrease appetite or reduce the absorption of fat [8]. But due to adverse effects of anti obesity medication people are looking for advanced and alternate obesity treatment from the natural medicine [9]. Pancreatic lipase (PL) plays the major role in the absorption of dietary lipids. Inhibition of pancreatic lipase is one of the study to determine the anti obesity agents [10].

Ilavangathi chooranam is the polyherbal Siddha formulation comprises 29 herbal drugs. It has the indication for soothagavayu (dysmenorrhoea), kazhichal (diarrhea), suram (fever), kai kaal erichal (peripheral neuritis), iruthaya adaippu (atherosclerosis of heart), moolam (piles) and perumbadu (menorrhagia) [11]. In general practice, this formulation is widely used in the treatment of cardiac diseases, obesity and dyslipidemia. To our knowledge, till date no prior research has done to evaluate the anti obesity activity of Ilavangathi chooranam. Therefore the current study attempts to explore the inhibitory activity of pancreatic lipase.

MATERIALS & METHODS:

Study drug:

The study drug Ilavangathi chooranam was purchased from the GMP certified pharma to evaluate the anti obesity activity.

Materials and reagents:

Lipase from Porcine Pancreas (Cat No: L3126)**,** p-nitrophenyl butyrate (Cat No: N9876)**,** Sodium Phosphate (Cat No: S0751) and Acetonitrile (Cat No: 1070310521) were purchased from Sigma -Aldrich Co. Analytical Weighing balance was purchased from Radwag labs, India**,** Pipettes (10ul, 200ul and 1ml) from ThermoFisher Scientific (USA), Microtips and Flat bottom 96well plate from Tarsons (India)**,** pH Meter was purchased from BioBee (India) and Microplate absorbance reader was purchased from ELX-800, BioTek (USA)

In-Vitro Anti Lipase Activity:

Pancreatic lipase activity was modified from the method previously reported by Kim et al [12,13]. About 5 mg of Porcine Pancreatic Lipase (PPL) was solubilised in 1 ml of 50mM sodium phosphate buffer (pH 8) and centrifuged at 5000 rpm for 10 min. About 10mM stock solution of substrate was prepared by solubilizing p-nitrophenol butyrate (p- NPB) in acetonitrile.

Assay reaction mixture (250µl) consists of 100µl of PPL, 50 µl of 0.2 M sodium phosphate buffer (pH 8), 50 µl of substrate and 50 µl of either distilled water (for Control) or Orlistat with 0.3ug/ml (Standard) or different concentrations of IVC extract (6.25, 12.5, 25, 50, 100, 200, 400ug/ml) prepared in Flat bottom 96well plate. Incubate the enzymatic reaction mixture for 30mins at 37°C. Lipase activity was determined by measuring the hydrolysis of p-NPB into p-nitrophenol. The p-NPB formed was measured at 415 nm using multi well plate reader (ELX800, BioTek, USA).

% Lipase inhibition activity was calculated by using the below formula:

% Lipase inhibition=Abs*of Control=Abs of Test sample/Abs of Control x 100

*Abs - Absorbence

RESULTS AND DISCUSSION

According to World Health Organization (WHO), obesity has become the global burden [14]. Obesity is the major risk factor for many life threatening diseases. The main cause for obesity is excess consumption of dietary fats and low physical activity [15]. Dietary fats comprised majority of triglycerides which gets absorption by lipase. There are three lipases responsible for the lipid digestion such as gastric, pancreatic and lingual lipase [16]. Among these, pancreatic lipase (triacylglycerol acylhydrolase) is an important enzyme involved in the absorption of dietary triglycerides which is secreted by the pancreas and that can hydrolyze 50 to 70 percent of the ingested fats [17]. Pancreatic lipase inhibition has been shown to reduce fat absorption, which is beneficial for controlling obesity [18]. Hence, pancreatic lipase inhibition is one of the effective studies used by researchers in newer anti obesity drug research [19].

In the current study, at a minimum concentration of 6.25µg/ml of the Ilavangathi chooranam extract showed the lipase inhibitory activity of 0.48±0.16% and at the maximum concentration of 400µg/ml of the IVC extract showed 65.04±0.87% of Lipase inhibitory activity (Graph 1). The obtained result of this study showed that IVC extract inhibited pancreatic lipase activity with IC_{50} of 273.21±4.18 µg/ml compared to orlistat with IC_{50} of 0.3µg/ml (Table 1& Chart 1). IVC extract was not more effective than positive control (orlistat). The Observations in Statistical data of Lipase enzyme inhibition study by ELISA Reader suggesting that, the test Compound IVC extract showed satisfactory Lipase enzyme inhibition activity on dose dependent manner. Presented values were the average of 2 independent individual experiments (N=2). Previous research has demonstrated the anti-lipase and anti-obesity properties of some ingredients of IVC. *Syzygium aromaticum* (L.) Merr & Perry extract reduced the lipogenesis and adipogenesis suggesting its potential in preventing obesity [20]. Cinnamon supplementation significantly reduces the body weight, body mass index, waist circumference and waist hip ratio as compared to the placebo groups in a study which was conducted in a group of Asian Indians [21,22]. Coriander seeds significantly reduced the Body Mass Index [23]. *Anethum graveolens* extract revealed the significant decrease in body weight and food intake [24]. The ethanolic extract of nutmeg exhibited a potent anti-lipase activity, which was very similar to that of orlistat [25]. Fenugreek seeds extract inhibit fat accumulation and ameliorate dyslipidemia in high fat diet-induced obese rats [26]. Fenugreek notably reduced the amount of fat that accumulated and body weight growth in obese mice on a high-fat diet [27]. Numerous studies demonstrated the anti obesity activity of black seeds. Reductions in body weight, adipose tissue mass, and serum fat levels were seen in animal models treated with black cumin seeds extracts or its active ingredients [28]. More than six studies demonstrated the effects of ginger in obesity treatment. Among this, four studies showed significant weight reduction in body weight [29,30]. Hence, this current study showed the anti obesity activity through pancreatic lipase inhibition. The bioactive compounds present in the ingredients of IVC responsible for the therapeutic effect of this formulation.

Table 1: Invitro porcine pancreatic lipase inhibition activity of the IVC extract

Porcine Pancreas Lipase inhibition activity-SU	
Drug conc (µg/ml)	**% Lipase inhibition±SD**
Control	0.00
Orlistat-0.3µg/ml	
LVC-6.25	0.48±0.16
LVC-12.5	2.48±0.20
LVC-25	12.74±0.77
LVC-50	21.44±0.09
LVC-100	33.35±1.02
LVC-200	41.82±1.15
LVC-400	65.04±0.87
IC50 concentration = 273.21±4.18ug/ml	

Graph 1: Overlaid Scatter graph showed the PPL inhibitory activity of IVC extract

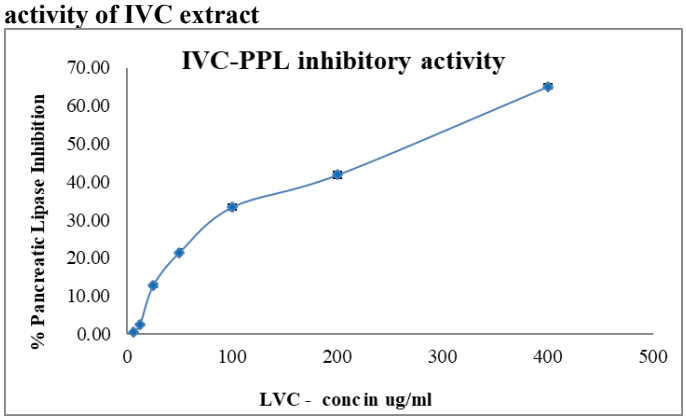
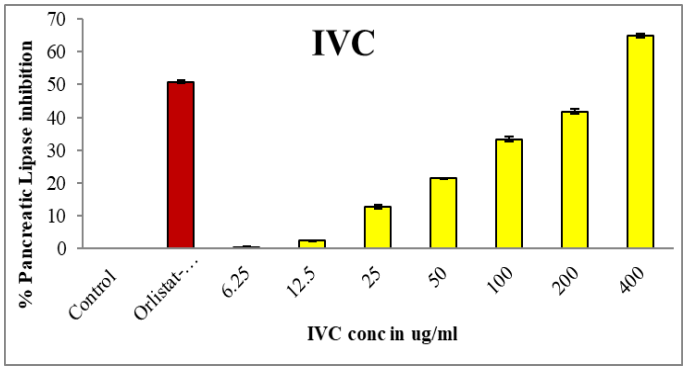


Chart 1: Inhibition of PPL activity by SU extract. Orlistat was used as a Positive control



CONCLUSION

In the treatment of obesity, pancreatic lipase inhibition is one of the methods apart from amylase and glucosidase enzyme activities to study the effect of natural products anti-obesity activity. This study showed the effective inhibition of pancreatic lipase at higher concentrations. Further studies like alpha

amylase and alpha glucosidase enzyme inhibitory activities need to be employed to prove the anti-obesity activity invitro level of Ilavangathi chooranam.

ACKNOWLEDGEMENT
Authors are very thankful to National Institute of Siddha, Chennai and Inbiotics, Nagercoil for their help and support throughout the study.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

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