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Evaluation of Anti-Obesity Properties of Few South Indian Medicinal Plants by Using Pancreatic Lipase Assay

Gude Bujjirao[1*], PK Ratnakumar[1], Ch. Satyanarayana[4], Khadeer Basha Shaik[2,3]

[1]Department of Botany, Andhra University, Waltair-530003, Visakhapatnam, Andhra Pradesh, India.
[2]Department of Human Genetics, College of Science & Technology, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India.
[3]Krisani Biosciences Private Limited, Jubilee Hills, Hyderabad-500 033, T.S., India.
[4]Department of Gen Surgery, Andhra Medical College, Visakhapatnam, Andhrapradesh, India.

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Abstract: Obesity and obesity-related complications are alarming threat to both in the developed and developing world because of obesity's many serious comorbidities put a large financial burden on the economy. As raised body weight leads to adverse metabolic effects on insulin resistance, blood pressure, cholesterol and triglycerides. Natural products, particularly medicinal plants, are believed to harbor potential anti-obesity properties through various mechanisms by preventing weight gain or promoting weight loss amongst others. The inhibitory activity of the selected medicinal plant extracts on pancreatic lipase was assessed using porcine pancreatic lipase (PPL) as an in vitro assay system. We report the results of in vitro anti-lipase assays of 10 plants grown in south indian region India. The extracts of *Achyranthes aspera Linn.*, and *Coffea canephora* showed up to 59% PPL inhibition. In addition to these findings, seven extracts from different plants were able to inhibit PPL in the range of 30-50%. The general composition of the most effective ethanol extracts was obtained in order to confirm their known chemistry reported by previous works.

Keywords: Obesity, South Indian Medicinal Plants, Pancreatic Lipase Assay

Introduction

Scientists and medical doctors compare obesity with a health time bomb. This is not surprisingly because of obesity's many serious comorbidities which also put a large financial burden on the economy. As raised body weight leads to adverse metabolic effects on insulin resistance, blood pressure, cholesterol and triglycerides. Overweight and obesity are strongly associated with a higher risk for multiple diseases (Bray GA 1998).

The world health organization has described obesity as one of today's most neglected public health problems, affecting every region of the globe (Pednekar MS *et al.,* 2006). Obesity in children and adolescents in gradually becoming a major public health problem in India (Popkin and Doak CM 1998). Totally 5% of the Indian population has been affected by obesity (Kumar NVRTP *et al.,* 2008). According to the National Family Health Survey (NFHS), the percentage of married women aged 15-49 years who are overweight or obese increased from 11% in National Family Health Survey-2 to 15% in National Family Health Survey-3. In south India the percentage of women who are overweight or obese is highest in Kerala (34%), followed by Tamil Nadu (24.4%), Andhra Pradesh (22.7%) and Karnataka (17.3%) (Kalra S, Unnikrishnan AG 2012).

***For Correspondence**
Bujjirao Gude,
Department of Botany,
Andhra University, Waltair-530003,
Visakhapatnam, Andhra Pradesh, India.

Most recent national data on obesity prevalence among U.S. adults, adolescents, and children show that more than one-third of adults and almost 17% of children and adolescents were obese in 2009–2010. More than 35% of U.S. men and women were obese, almost 41 million women and more than 37 million men aged 20 and over were obese in 2009–2010. (Cynthia L. Ogden *et al.,* 2009-2010). Which suggests the likelihood of worsening obesity trends in the future adult population. Therefore, it is essential to develop ways of preventing more people from becoming obese.

Several approaches have been implied for the treatment of obesity targeting at specific mechanisms, which include lipase inhibition, suppressive effect on food intake, stimulatory effects on energy expenditure, inhibition of adipocyte differentiation and the regulatory effect on lipid metabolism [Yun JW 2011]. Amongst the approaches available, pancreatic lipase, the main lipolytic enzyme secreted by the pancreas, is greatly involved in the digestion of triglycerides. In fact, pancreatic lipase is responsible for the hydrolysis of 50-70% of total dietary fats [Martins F *et al.,* 2009] and pancreatic lipase inhibition is one of the most widely studied mechanisms for the determination of the potential efficacy of

natural products as anti-obesity agents [Birari R.B. and Bhutani K.K. 2007]. Therefore, pancreatic lipase inhibitors are considered to be valuable therapeutic agents for treating diet-induced obesity in humans.

The most common anti-obesity drug is Orlistat; a hydrogenated derivative of lipstatin derived from *Streptomyces toxitricini*, a potent inhibitor of gastric, pancreatic and carboxyl ester lipase and it has been proved to be affective for the treatment of human obesity by 35 percent reduction in fat absorption (Sharma *et al.,* 2005). Management of hyperlipidaemia without any side effect is still a challenge to the medical system (Xie *et al.,* 2007). For instance, consumption of synthetic drugs leads to hyperruricemia, diarrhea, nausea, gastric irritation, flushing, dry skin and abnormal liver function (Kumar *et al.,* 2008). While, plant products are considered to have less toxic and side effects than synthetic ones.

Naturally occurring phytochemicals present an exciting opportunity for the discovery of newer anti-obesity agents. Some have already been identified as lipase inhibitors, e.g. licochalcone A, which has been isolated from the roots of *Glycyrrhiza uralensis* (Won SR *et al.,* 2007), platycodin D from the fresh roots of *Platycodon grandiflorus* (Zhao HL *et al.,* 2004), dioscin from *Dioscorea nipponica* (Kwon CS *et al.,* 2003), phenolic constituents from the leaves of *Nelumbo nucifera* (Ono Y *et al.,* 2006), and other components from other kinds of herbs. However, it remains more searches for finding more efficacious lipase inhibitors from traditional herb sources is needed. In the present study, we have screened Chloroform, Ethyl acetate, Hexane and Methanol extracts of various plants for their anti-lipase activity to find safer and cheaper medicines in prevention and control of obesity.

Materials and Methods

Plant materials:

All the plant materials selected for the present study were collected from several locations of Andhra Pradesh, during the flowering periods of these plants. The collected plants were identified taxonomically, by Prof. S.B. Padal, Department of Botany, Andhra University, and voucher specimens were deposited at the Herbarium Museum of the Department of Botany, Andhra University. The plant parts were cleaned of residual soil and air-dried at room temperature. They were then ground to a fine powder using a laboratory mill and passed through a 24-mesh sieve to generate a homogeneous powder. The powder was then stored in a temperature-controlled, dry and dark storage room until extraction (Bustanji Y *et al.,* 2011a)

Extraction:

The selected medicinal plants were collected and dried with active ventilation at ambient temperature (25±1°C) and coarsely powdered. The powdered plant material was extracted with successive solvent extraction ranging from non-polar to polar (Hexane, Chloroform, Ethyl Acetate and Methanol) using soxhlet hot extraction process and concentrated at 45°C in a rotator vacuum evaporator (*HEIDOLPH* Hei-VAP PRECISION Rotary Evaporator, Germany). The final extracts were stored at −20°C.

Table 1:

S.No.	Plant Name	Family	Part Used
1	*Morinda citrifolia*	*Rubiaceae*	Fruit
2	*Allium sativum*	*Amaryllidaceae*	Bulb
3	*Centella asiatica*	*Mackinlayaceae*	Leaf
4	*Coffea canephora*	*Rubiaceae*	Seed
5	*Cyperus rotundus Linn.*	*Cyperaceae*	Aerial Part
6	*Bauhinia purpurea*	*Fabaceae*	Leaf
7	*Camellia sinensis*	*Theaceae*	Leaf
8	*Achyranthes aspera Linn.*	*Amaranthaceae*	Leaf
9	*Salacia reticulata*	*Celastraceae*	Aerial Part
10	*Zingiber officinale Linn.*	*Zingiberaceae*	Tuber

Preparation of Extract for *In Vitro* Assay

The plant extracts (Hexane, Chloroform, Ethyl acetate and Methanol) were initially dissolved in Dimethyl sulfoxide (DMSO) to give a concentration of 5.0mg/ml (Stock Solution). 20µl of stock solution was used in the reaction mixture to give the final concentration of 100µg/ml.

Lipase inhibitory activity:

Pancreatic lipase preparation: The enzyme solutions were freshly prepared immediately prior to use. Crude porcine pancreatic lipase type II (Sigma, USA) was prepared with 0.1ml of porcine pancreatic lipase (200Units/ml in 0.1 M potassium phosphate buffer, pH 6.8) to achieve a concentration of 200 unit/ml. Porcine PL was selected due to its high homology to the human enzyme (85% homology) and similar enzyme kinetics and behavior (Lowe ME, Rosenblum JL, Strauss AW (1989). Cloning and characterization of human pancreatic lipase cDNA. J. Biol. Chem., 264: 20042-20048).

Pancreatic lipase Assay: Inhibition of lipase by the extracts given was determined using a modified assay described by Smeltzer, *et al.,* (1992). Briefly, a suspension containing 1% (v/v) of triolein, and 1% (v/v) Tween 40 in 0.1 M phosphate buffer (pH 8) was prepared

and emulsified. Assays were then initiated by adding 800µl of the triolein emulsion to 200µl of porcine pancreatic lipase and 200µl of extract. The final concentration of DMSO was fixed and did not exceed 2.0%. The percentage of residual activity of PL was determined for each extract by comparing the lipase activity of PL with and without the extract. The concentration required to give 50% inhibition (IC50) was determined for each tested extract. Orlistat, a known inhibitor of PL, was used as a positive control in the assay mixture. The contents were mixed and the absorbance measured immediately at 450nm and designated as T0. The test tubes were incubated at 37°C for 30 min and at the end of the incubation; the absorbance at 450nm was recorded and designated as T30. The variation in absorbance = [A450 (T0) - A450 (T30)] was calculated for both control and the treatment and the % inhibition was calculated using the formula:

% inhibition = ([_A450Control - _A450Extract] / _A450Control) × 100

All assays were triplicated and the calculated inhibition percentages were the mean of 3 observations.

Results and Discussion

Hexane, Chloroform, Ethyl acetate and Methanol extracts of 10 selected plants were prepared and investigated at a concentration of 100µg/ml using pancreatic lipase inhibitory activity and the results are shown in Table 2. Pancreatic Lipase (PL), the principal lipolytic enzyme synthesized and secreted by the pancreas, which plays a key role in the efficient digestion of triglycerides. PL is responsible for the hydrolysis of 50–70% of total dietary fats [Mukherjee M. 2003]. PL inhibition is one of the most widely studied mechanisms for the determination of the potential efficacy of natural products as anti-obesity agents. A Complementary and Alternative Medicine (CAM) approach, along with pharmacological and non-pharmacological management, seems to provide an effective and safe tool to help combat obesity. Phytochemicals identified from traditional medicinal plants present an exciting opportunity for the development of newer therapeutics [Birari and Bhutani 2007]. Previous reports have screened for biologically active agents derived from natural herbal sources for their anti-PL activity [Sharma N et al., 2005]

Table 2: Medicinal plant solvent extracts and their activity percentile

S.No.	Plant Name	Hexane 100µg/ml Activity %	Chloroform 100µg/ml Activity %	Ethyl acetate 100µg/ml Activity %	Methanol 100µg/ml Activity %
1	*Morinda citrifolia*	0.9	2.71	7.23	11.3
2	*Allium sativum*	20.81	10.85	11.76	20.81
3	*Centella asiatica*	15.45	20.12	46.6	4.52
4	*Coffea canephora*	24.88	3.61	59.41	40.6
5	*Cyperus rotundus Linn.*	24.4	18.09	1.3	19
6	*Bauhinia purpurea*	49.77	21.71	4.97	11.76
7	*Camellia sinensis*	13.12	20.36	11.31	13.12
8	*Achyranthes aspera Linn.*	53.39	48.86	11.31	28.05
9	*Salacia reticulata*	43.43	35.74	33.48	15.83
10	*Zingiber officinale Linn.*	39.36	38.46	43.43	11.76

In this study, we have screened ten medicinal plants for their potential as PL inhibitors. The results are summarized in Table (2). Lipase inhibition is expressed by the concentration of extract residues that inhibits 50% of the enzymatic activity (IC50 value). Three of these plant extracts *Coffea canephora, Achyranthes aspera Linn.* and *Zingiber officinale Linn.* Highly inhibit the PL in a dose-dependent manner, with an IC_{50} range between 40-60% at 100µg/ml concentration. Orlistat, a pancreatic lipase inhibitor, showed an IC50 value of 0.65µg/ml. On the other hand, the extracts of remaining 7 plants showed mild to moderate lipase activity, with IC50 range between 10-30% at 100µg/ml concentration, and are considered active. Our results showed that *Coffea canephora* and *Achyranthes aspera* Linn. The most potent inhibitory action (IC50 =100µg/ml). *Coffea canephora* was previously reported to have an antioxidant activity (Udaya Prakash NK et al., 2014), which was suggested to be attributed to its content of phenolic compounds including some identified flavonoid constituents. The present study reports the anti-PL activity of seven other active plants (namely *Coffea canephora, Achyranthes aspera Linn. and Zingiber officinale Linn.*). Our results suggest that these plants could serve as crude drugs for the treatment of hyper triglyceridemia. However, further studies are needed, using animal models, to verify the inhibitory activities of these plants in vivo and also identification of active chemicals responsible for the claimed results. In addition, we are currently developing methods to isolate, identify and characterize the potentially

phytoactive compounds in these medicinal plants.

Conclusion

Natural products prepared from traditional medicinal plants have always presented an exciting opportunity for the development of new types of therapeutic leads. Our results suggest that role of south Indian medicinal plant extracts for treating and controlling Obesity and its related complications out of 10 selected medicinal plants according to literature reports of folklore citations and screened for their claims, most of them were found to be potentially active against PL. However, further investigations are necessary to verify the inhibitory activities of these plants under in vivo conditions and its chemical responsibility. Moreover, these promising active plants are considered of value as a starting material for further isolation, identification and characterization of phytoactive compounds for the purpose of developing anti-obesity functional agents.

References

1. Bray GA. Obesity: a time bomb to be defused. Lancet. 352(9123), 1998, 160-1.
2. Pednekar, M.S., Gupta, P.C., Shukla, H.C., et al., 2006. Association between tobacco use and body mass index in urban Indian population: implications for public health in India. BMC Public Health 6, 70.
3. Popkin, B. M. & Doak, C. M. (1998) The obesity epidemic is a worldwide phenomenon. Nutr. Rev. 56:106-114.
4. Kumar NVRTP., Mohanta GP., Manna PK., Manavalan R., (2008). "Body mass index-a diagnostic tool to assess obesity," Indian Journal of Pharmacy Practice. 2: 81–83.
5. Sharma N., Sharma Vinay K., Seo S-Y. Screening ofsome medicinal plants for anti-lipase activity. J.Ethnopharmacol. 2005; 97:453-456.
6. Birari R. B., Bhutani K. K. Pancreatic lipase inhibitorsfrom natural sources: Unexplored potential. Drug Discov. Today 2007; 12:879-889.
7. Kalra S, Unnikrishnan AG. Obesity in india: the weight of the nation. J Med NutrNutraceut. 1, 2012, 37-41.
8. Cynthia L. Ogden, Margaret D. Carroll, Brian K. Kit and Katherine M. Flegal. Prevalence of Obesity in the United States, 2009–2010. (http://www.cdc.gov/nchs/data/databriefs/db82.pdf)
9. Yun JW. Possible anti-obesity therapeutics from nature – A review. Phytochem 2011; 71: 1625-41.
10. Mukherjee M. Human digestive and metabolic lipases-abrief review. J. Mol. Catal. B: Enzym. 2003; 22:369-376.
11. Martins F., Noso T.M., Porto V.B., Curiel A., Gambero A., Bastos D.H.M., Ribeiro M.L. and Carvalho P.O. (2009) *Obesity*, 18(1), 42-47.
12. Birari R.B. and Bhutani K.K. (2007) *Drug Discovery Today*, 12(19-20), 879-889.
13. Sharma, N., V.K. Sharma and S.Y. Seo, 2005. Screening of some medicinal plants for anti-lipase activity. J. Ethnopharmacol., 97: 453-456.
14. Xie, W., W. Wang and H. Su, 2007. Hypolopidemic mechanisms of *Ananas comosus* L. leaves in mice: Different from fibrates but similar to statins. J. Pharmacol. Sci., 103: 264-274.
15. Kumar, A.S., A. Mazumder and V.S. Saravanan, 2008. Antihyperlipidemic activity of camellia sinensis leaves in triton wr-1339 induced albino rats. Phcog. Mag., 4: 60-64.
16. Won SR, Kim SK, Kim YM, Lee PH, Ryu JH, Kim JW, Rhee HI. Licochalcone A: a lipase inhibitor from the roots of *Glycyrrhiza uralensis*. *Food Res Intern* 2007; 40: 1046–50.
17. Zhao HL, Kim YS. Determination of the kinetic properties of platycodin D for the inhibition of pancreatic lipase using a 1, 2- diglyceride-based colorimetric assay. *Arch Pharm Res* 2004; 27: 1048–52.
18. Udaya Prakash NK et al., (2014) Int. J. Res. Phar. Scie. 5(2) 147-152.
19. Kwon CS, Sohn HY, Kim SH, Kim JH, Son KH, Lee JS, Lim JK, et al. Anti-obesity effects of *Dioscorea nipponica* Makino with lipase inhibitory activity in rodents. *Biosci Biotechnol Biochem* 2003; 67:1451–6.
20. Ono Y, Hattori E, Fukaya Y, Imai S, Ohizumi Y. Anti-obesity effect of *Nelumbo ucifera* leaves extract in mice and rats. *J Ethnopharmacol* 2006; 106:238–44.
21. Bustanji Y, Hudaib M, Tawaha K, Mohammad M, Almasri I, Hamed S, Oran S (2011a). In Vitro Xanthine Oxidase Inhibition by Selected Jordanian Medicinal Plants. J. Pharm. Sci., 1: 1-8.

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