Screening of Some Medicinal Plants for their Pancreatic Lipase Inhibitory Potential

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ABSTRACT

Obesity is a main risk factor for cardiovascular, metabolic and endocrine disorders. Despite significant improvements in public education and pharmacologic management in the last two decades, obesity rates have continued to be alarmingly high. The need for a combination of multiple therapy approaches to overcome obesity has become widely accepted by the majority of health care systems and guidelines. The rich potential of nature to combat obesity has not been fully explored yet. Several phytochemicals have been investigated for their potential as lipid lowering agents. We have investigated a total of 23 medicinal plants, belonging to 15 different families and compared their pancreatic lipase inhibitory effects. Inhibition of the pancreatic lipase was chosen as the criteria for therapeutic efficacy since such inhibition would serve two functions. It would provide an adjunctive therapy to the pharmacological agents and would minimize systemic adverse reactions by acting topically in the GI tract. Thirteen plants were found to show *in vitro* inhibitory activities. The nine most active plants showed an IC_{50} range of 107.7-342.7 µg/mL. The plants are Anthemis *palaestina* Boiss., *Salvia spinosa* L. *Ononis natrix* L, *Fagonia arabica* L., *Origanum syriaca* L. (Syn. *Majorana syriaca* (L.) Rafin.), *Hypericum triquetrifolium* Turra, *Malva nicaeensis* All., *Chrysanthemum coronarium* L., *Paronychia argentea* Lam. Further isolation, identification and characterization of phytoactive compounds responsible for anti-lipase action is required to evaluate the full therapeutic potentials of these plants.

Keywords: Obesity; Hyperlipidaemia; Pancreatic Lipase; Anti-lipase Activity; Medicinal Plants; Plant Extracts.

INTRODUCTION

Obesity has become an epidemic increasing at an alarming rate. Although it is widely regarded as a problem limited to the developed world, obesity is spreading through the developing nations as well [1]. Obesity is a chronic haemostatic disorder characterized by excessive weight gain[2]. A compelling body of evidence has placed obesity as one of the greatest threats to global health in this millennium, with more than 1 billion overweight adults worldwide and at least 300 million classified as clinically obese with increased risk of morbidity and mortality [3, 4]. Obesity is a major risk factor for cardiovascular disease, diabetes mellitus type 2, dyslipidemia (increased levels of total cholesterol, low-density lipoprotein and triglycerides with decreased levels of high-density lipoprotein) and gallbladder disease [3, 4].

The control of energy homeostasis in metabolic disorders has become one of the most rapidly growing topics in biomedical research. Studies in the molecular

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mechanisms of regulating body weight have also provided potential new targets for therapeutic interventions and resulted in the development of novel drugs [5, 6]. The market for lipid-lowering drugs is potentially huge, as it accounts for about 2–6% of the total health care costs in several developed countries. The need for these drugs is predicted to continue to rise in the next 10 years because of the obesity's growing worldwide prevalence [7].

Despite the significant advances in understanding obesity and the development of effective pharmacologic treatments, obesity is still quite a challenge for many health care systems especially in the developed countries [8]. It is widely accepted that multiple therapeutic modalities are required to achieve the target weight and plasma lipids in obese patients. In fact, a number of pharmacological agents are currently used in combination with dietary and/or lifestyle changes in order to reduce body weight and control the level of plasma lipids [9]. Unfortunately, it is not uncommon to miss the therapeutic goals in obese patients even with pharmacologic treatments and lifestyle changes. [10] The need for an extra tool in combating obesity has led to an extensive research in complementary and alternative medicine.

A main component contributing to the development of obesity and its complications is an elevation in plasma triglyceride levels. However, the dietary triglycerides are not systemically absorbed as such until hydrolyzed to free fatty acids and 2-monoacyle glycerol by triacylglycerol lipases (triacylgycerol acyl hydrolase) [11]. Thus, inhibition of the digestion of dietary lipids and therefore limiting intestinal fat absorption, is a plausible means of therapeutic intervention since it does not involve a systemic or a central mechanism of action [12].

The only clinically approved pharmacologic agent as Pancreatic Lipase (PL) inhibitor is Orlistat (Xenical®)[13, 14], a hydrogenated derivative of lipstatin obtained from *Streptomyces toxitricini*. Orlistat has been reported to be a potent inhibitor of gastric, pancreatic and carboxylester lipases [14], and has been shown to be an effective tool for the treatment of human obesity [15-17]. Although it is one of the best-selling drugs worldwide, it's use is compromised by unpleasant gastrointestinal adverse reactions like oily stools, oily spotting and flatulence [15-17]. The success of orlistat has prompted research for the identification of newer PL inhibitors that lack some of these adverse reactions.

The use of botanical materials is gaining renewed interest as potential source of new drugs or lead compounds in the search for new medicaments for the treatment of different diseases [18]. Natural products prepared from traditional medicinal plants and microbial sources have always presented an exciting opportunity for the development of new therapeutic agents. An analysis of the origin of the drugs launched in the last twenty-five years showed about half of all compounds that were successful in clinical trials have, at least, they have been derived from a natural origin [19]. The structural diversity of natural products combined with the fact that they were elaborated within the living systems render them often more tolerable than totally synthetic molecules [20].

Natural products provide a vast pool of PL inhibitors that can possibly be developed into clinical products. At present, the potential of developing successful natural products for the management of obesity is still largely unexplored. The screening and optimization of safe and effective lipid lowering phytochemicals would provide an excellent new strategy in combating obesity and its complications [21].

Jordan’s geographic and climatic characteristics allowed the growth of a quite diverse group of natural flora. Indeed, a total of 485 species of medicinal plants, belonging to 99 families have been identified as natural remedies from the flora of Jordan [22].

In the current study, we have optimized a protocol to screen the methanolic extracts of various medicinal plants, collected from the several areas of Jordan, for their anti-pancreatic lipase activity. A total of 23 traditional medicinal plants belonging to 15 families, regardless of their claimed ethnopharmacological uses, were tested using a simple, fast, efficient and reliable spectrophotometeric method, in an attempt to find new compounds with potential PL inhibitory activities.

MATERIALS AND METHODS

Plant Materials

Plant materials were collected from selected plants, growing wild or cultivated in different locations of Jordan, during the flowering periods of these plants. The collected plants were identified taxonomically, by Dr. Khaled Tawaha (Faculty of Pharmacy, University of Jordan), and voucher specimens were deposited at the Herbarium Museum of the Faculty of Pharmacy, Jordan University of Science and Technology. The plant materials were cleaned of residual soil and air-dried at room temperature. Plants were ground to a fine powder using a laboratory mill and passed through a 24 mesh sieve to generate a homogeneous powder, stored at room temperature (22–23 °C), and protected from light until extraction.

Plant Extraction

Methanolic extractions were conducted using 250 mg sample of each ground plant material, of the used parts (see Table (1), in 10 mL methanol (80%), at 37 °C for 3 h, in a shaking water bath. After cooling, the extract was centrifuged at 1500 g for 10 min, and the supernatant was recovered. The solvent was evaporated under vacuum at 40°C using a rotary evaporator. The solid residues were collected and stored in dry condition until analysis [23].

Preparation of Extract for *In Vitro* Assay

The tested extracts were initially dissolved in DMSO to give five different stock solutions with a concentration range of 0.625-10.0 mg/mL (0.625, 1.25, 2.5, 5.0 and 10mg/mL). Subsequently, 20 µL aliquot of each stock solution was used in the reaction mixture to give a final concentration range of 12.5- 200 µg/mL (12.5, 25, 25, 50 and 200 µg/mL).

Enzyme Preparation

The enzyme solutions were prepared immediately before use. Crude porcine pancreatic lipase type II (Sigma, USA, EC 3.1.1.3) was suspended in tris-HCl buffer (2.5 mmol, pH 7.4 with 2.5 mmol NaCl) to give a concentration of 200 unit/ml.

Quantification of Pancreatic Lipase Activity by a Spectrophotometric Assay

The lipase activity of PL was quantified by a colorimetric assay that measures the release of *p*-nitrophenol as previously described [24-27], however, with minor modification. Here, *p*-nitrophenyl butyrate (PNPB), dissolved in acetonitrile, was employed, in the enzymatic assays, as PL substrate at 100 µM concentration instead of 5 mM. Aliquot (0.10 mL) of PL solution was added to the reaction mixtures. The volume was completed to 1 mL using the tris-HCl buffer before measuring the solution absorbances spectrophotometrically, at 410 nm, at a minimum of 5 time points (1-5 min). The reaction, maintained at 37 °C, was started by adding the substrate to the reaction mixture. The release of *p*-nitrophenol was measured as the increase in absorbance measured at 410 nm, by a UV spectrophotometer, against blank using denaturated enzyme. Enzyme activity was defined as an increase of an absorbance per minute.

The pancreatic lipase activity is defined as an increase of the rate of *p*-nitrophenol release which can be estimated from the slope of the linear segment of (absorbance *vs* time) profiles.

PL Inhibition by Test Extract

The inhibition of pancreatic lipase activity by the prepared plant extracts was measured using the spectrophotometric assay described above. PL was preincubated with each particular extract for at least 10 min at 37 °C before adding the substrate. 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 (IC_{50}) was determined for each tested extract. PL was preincubated with different concentrations (12.5-200 µg/mL) of each extract and the percentages of residual activity of PL data were used to evaluate the IC_{50} values. All assays were triplicated and the calculated inhibition percentages were the mean of 3 observations. Orlistat, a known inhibitor of PL, was used as a positive control in the assay mixture.

RESULTS AND DISCUSSION

Pancreatic Lipase (PL), the principal lipolytic enzyme synthesized and secreted by the pancreas, plays a key role

in the efficient digestion of triglycerides. PL is responsible for the hydrolysis of 50–70% of total dietary fats [28]. PL inhibition is one of the most widely studied mechanisms for the determination of the potential efficacy of natural products as antiobesity 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. The need for a combination of multiple therapy approaches to overcome obesity has become widely accepted by the majority of health care systems and guidelines.

Phytochemicals identified from traditional medicinal plants present an exciting opportunity for the development of newer therapeutics [21]. Previous reports have screened for biologically active agents derived from natural herbal sources for their anti-PL activity [26, 29].

In this study, we have developed a protocol to screen twenty four different plant extracts for their potential as PL inhibitors. The results are summarized in Table (1). Lipase inhibition is expressed by the concentration of extract residues that inhibits 50% of the enzymatic activity (IC_{50} value). Thirteen of these plant extracts were able to inhibit the PL in a dose-dependent manner, with an IC_{50} range between 108-938 µg/mL, and these are *Anthemis palaestina* Boiss. (107.7 µg/mL), *Salvia spinosa* L. (156.2 µg/mL) *Ononis natrix* L.(167 µg/mL), *Fagonia arabica* L. (204.1 µg/mL), *Origanum syriaca* (L.) Kostel "Syn. Majorana syriaca (L.) Rafin. (234 µg/mL), *Hypericum triquetrifolium* Turra (236.2 µg/mL), *Malva nicaeensis* All. (260.7 µg/mL), *Chrysanthemum coronarium* L. (286.1 µg/mL), *Paronychia argentea* Lam. (342.7 µg/mL), *Convolvulus althaoieds* L. (664.5 µg/mL), *Reseda alba* L. (738 µg/mL), and *Adonis palaestina* Boiss (937.5 µg/mL). Figure 1 shows the inhibitory profiles of the two most potent plant extracts; *A. palaestina* and *S. spinosa*. Both extracts exhibited a dose-dependent PL inhibitory effect using a concentration range of 12.5-200 µg/mL. The extracts exhibited an inhibition rate ranging from 20 to 56% and 5-55% for *A. palaestina* and *S. spinosa*, respectively. The percent inhibition was plotted against the logarithmic transformation of the corresponding extract concentrations for determining the IC_{50} value from the regression equations. However, IC_{50} values were about 107.7 µg/mL and 156.2 µg/mL for *A. palaestina and S. spinosa*, respectively. Orlistat, a pancreatic lipase inhibitor, showed an IC_{50} value of 0.65 µg/mL.

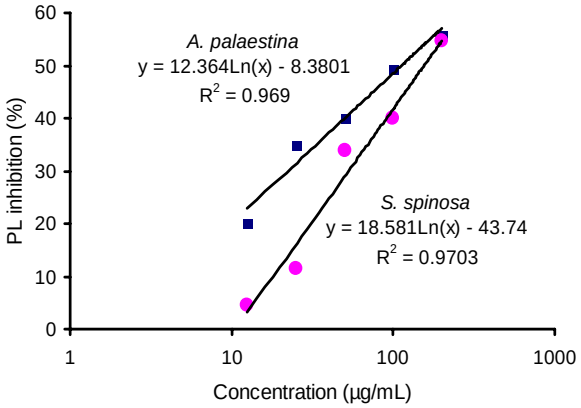

Figure 1: The inhibitory effect of *A. palaestina* (■) *and S. spinosa* (●) extracts concentrations on the activity of pancreatic lipase.

On the other hand, the extracts of 11 plants showed poor lipase activity, with IC_{50} more than 1000 µg/mL, and are considered inactive. These plants are *Calendula arvensis* L., *Cleome africana* Botsch., *Onosma giganteum* Lam., *Achillea biebersteinii* Afan., *Anagallis arvensis* L., *Haplophyllum buxbaumii* (Poir.) G. Don., *Hypecoum dimidiatum* Delile, *Linum pubescens* Banks & Sol., *Reseda lutea* L., *Silene aegyptiaca* (L.) L.f., and *Varthemia iphionoides* Boiss. & Blanche.

To the best of our knowledge, all the plants evaluated in this study have not been screened before for their anti-lipase activity.

Table 1: Pancreatic Lipase (PL) inhibitory activities, expressed as IC_{50} (µg/mL), of the methanolic extracts of 23 plant species that were collected from different locations in Jordan.

Plant Name	Family	Part used	IC_{50} (µg/mL)
Achillea biebersteinii Afanasiev	Asteraceae	Aerial parts	Inactive
Adonis palaestina Boiss	Ranunculaceae	Aerial parts	937.5
Anagallis arvensis L.	Primulaceae	Aerial parts	Inactive
Anthemis palestina Reut. ex Boiss.	Asteraceae	Aerial parts	107.7
Calendula arvensis L.	Asteraceae	Aerial parts	Inactive
Chrysanthemum coronarium L.	Asteraceae	Leaves and flowers	286.1
Cleome africana Botsch.	Capparaceae	Aerial parts	Inactive
Convolvulus althaeoides L.	Convolvulaceae	Aerial parts	664.5
Fagonia arabica L.	Zygophyllaceae	Aerial parts	204.1
Haplophyllum buxbaumii (Poir.) G. Don.	Rutaceae	Aerial parts	Inactive
Hypecoum dimidiatum Delile	Papaveraceae	Aerial parts	Inactive
Hypericum triquetrifolium Turra	Clusiaceae	Aerial parts	236.2
Linum pubescens Banks & Sol.	Linaceae	Aerial parts	Inactive
Origanum syriacum L.	Lamiaceae	Aerial parts	234
Malva nicaeensis All.	Malvaceae	Aerial parts	260.7
Ononis natrix L.	Fabaceae	Aerial parts	167
Onosma giganteum Lam.	Boraginaceae	Aerial parts	Inactive
Paronychia argentea Lam.	Illecebraceae	Aerial parts	342.7
Reseda alba L.	Resedaceae	Aerial parts	738
Reseda lutea L.	Resedaceae	Aerial parts	Inactive
Salvia spinosa L.	Lamiaceae	Aerial parts	156.2
Silene aegyptiaca L.f.	Caryophyllaceae	Aerial parts	Inactive
Varthemia iphionoides Boiss. & Blanche	Asteraceae	Aerial parts	Inactive

Interestingly, our results showed that *A. palaestina* possesses the most potent inhibitory action (IC_{50} = 107.7µg/mL). *A. palaestina* was previously reported to have an antioxidant activity [29], which was suggested to be attributed to its content of phenolic compounds including some identified flavonoid constituents [30]. A lot of these compounds, obtained from various sources, were also reported to exhibit anti-PL activity [21], which accordingly may justify the anti-PL activity of *A. palaestina* observed in the present study.

Of the other highly active plants, *S. spinosa* showed also a strong inhibitory action (IC_{50} = 156.2 µg/mL). Although potent inhibitory activities were previously reported for some common salvia species like *S. officinals*, *S. miltiorrhiza* and *S. sclarea* [31-32], this is the first report about *S. spinosa*. Moreover, the reported activity of these plants was found to be mainly attributed to carnosic acid [31], a diterpene, and some dihydroxyphenylcarboxylic acid derivatives which are identified to occur as common constituents of Salvia species. These constituents may, however, explain the results obtained for *S. spinosa* in the present study.

A herbal mixture, based on a Malva species (*M. sylvestris*) as one of its components, was reported to be

used to control body weight and to combat obesity [33]. Based on our results concerning *M. nicaeensis*, which showed a strong activity (IC_{50} = 260.7 µg/mL), we believe that the anti-PL activity would be, at least partly, one of the potential mechanisms of the reported use of this herbal mixture.

Of the remaining active species, three plants, possessing IC_{50} less than 300 µg/mL, have not been previously reported to have any anti-PL activity. These plants are *O. syriacum*, *H. triquetrifolium*, and *C. coronarium.* However, some other species of the same plants genera were reported to have similar activities such as *Origanum majorana* [29], *Hypericum erectum* [34] and *Chrysanthemum morifolium* [32].

On the other hand, the present study reports for the first time the anti-PL activity of seven other active plants (namely *O. natrix*, *G. aleppicum*, *F. arabica*, *P. argentea*, *C. althaoieds*, *A. palaestina*, and *R. alba*) whose various genera's species were not reported before to possess such activity.

Our results suggest that these plants could serve as crude drugs for the treatment of hypertriglyceridemia. However, further studies are needed, using animal models, to verify the inhibitory activities of these plants *in vivo*. 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 therapeutics. Our results suggest that the rich potential of nature to combat obesity has not been fully explored yet and many newer leads may be obtained from natural sources as, out of 23 screened plants, 12 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. 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 antiobesity functional agents.

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مسح و تقييم مجموعة من النباتات الطبية كعوامل مثبطة لإنزيم محلل الدهنيات البنكرياسي (Pancreatic Lipase)

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ملخـص

يعتبر فرط الوزن من أهم العوامل المساعدة والمحفزة لانتشار أمراض القلب بالإضافة إلى ارتباط زيادة الوزن باختلال في عمليات الأيض ووظائف الغدد الصماء.
لذلك أصبحت الحاجة ملحة لإيجاد أدوية للسيطرة على الوزن الزائد لما له من تأثيرات على صحة الفرد و ادائه.
لقد تم في هذا البحث دراسة القدرة التثبيطية لإنزيم محلل الدهنيات البنكرياسي (Pancreatic Lipase) حيث يساعد تثبيط عمل هذا الإنزيم في السيطرة على الوزن الزائد. أشارت نتائج هذه الدراسة الى أن النباتات الطبية يمكن أن تكون مصدراً أساسياً لمثبطات جديدة وفعالة لهذا الإنزيم؛ فمن أصل 23 نبتة تمت دراستها كان هنالك 13 نبتة فعالة و بقوة تثبيطية عالية.
تشير الدراسة إلى أنه لابد من إجراء دراسات تفصيلية لهذه النباتات من أجل معرفة المواد الفعالة فيها المسؤولة عن تثبيط هذا الإنزيم.

الكلمات الدالة: السمنة الزائدة، ارتفاع الدهنيات، إنزيم محلل الدهنيات البنكرياسي، النباتات العشبية، مستخلصات النباتات.

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