Full Length Research Paper

A potential role of *Lavandula angustifolia* in the management of diabetic dyslipidemia

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Diabetic dyslipidemia is a key contributor to insulin resistance and a risk factor for cardiovascular complications in patients with DM type 2. *Lavandula angustifolia* (lavender) is native of the northern region of Jordan and has been long used in Jordanian folkloric medicine in the management of diabetes. To our knowledge, this is the first manuscript to study the potential role of the methanolic extract of the plant in the management of diabetic dyslipidemia. Our results demonstrated *in vitro* inhibitory effects of *L. angustifolia* on both hormone sensitive lipase (HSL) and pancreatic lipase (PL) activities. The results indicated that lavender extract inhibited HSL activity in a dose dependent manner with an IC_{50} of 175.5 µg/ml. Likewise, it inhibited the PL activity in a dose dependent manner with an IC50 of 56.5 µg/ml. Such inhibitory activities could be attributed, but is not exclusive, to the presence of rosmarinic acid (IC_{50} of 125.2 and 51.5 µg/ml for PL and HSL, respectively) and gallic acid (IC_{50} of 10.1 and 14.5 µg/ml for PL and HSL, respectively) in the extract. Interestingly, the inhibitory pattern of the lavender on the enzymatic activities of HSL and PL matched the inhibitory pattern of orlistat.

Key words: Lavender, hormone sensitive lipase, pancreatic lipase, obesity, diabetes mellitus, medicinal plants.

INTRODUCTION

The study of plant secondary metabolites has significantly contributed to drug discovery and unveiled a rich source of structurally novel bioactive molecules with vast pharmacologic actions. Plant-originated drugs such as cyclosporine, sitosterols, the vinca alkaloids, aspirin and digoxin; to name a few, have been globally utilized for a wide range of therapeutic applications. In recent years, "Non-traditional" or "alternative" treatments using plant extracts and herbal supplements have become extremely popular worldwide driven by efforts to improve the wellbeing and conquer the battle against chronic conditions resistant to conventional pharmacologic treatments such as obesity and diabetes mellitus.

Indeed, obesity has emerged to be one of the most intractable health burdens in developed countries. Obesity has been established to be a major risk factor and key contributor to the pathogenesis of a plethora of devastating illnesses such as diabetes, cardiovascular diseases, strokes, renal failure, inflammatory diseases and cancer (Fazio et al., 2010; Hughes, 2009; Roberts et al., 2010).

Unfortunately, despite significant improvements in public education and pharmacologic management in the last two decades, obesity prevalence has continued to escalate into alarmingly high rates. Advances in obesity research have shown new biochemical pathways and molecular targets for pharmacologic intervention that will likely establish a nucleus to new treatment strategies in the future (Cooke and Bloom, 2006; Das and Chakrabarti, 2006; Ioannides-Demos et al., 2006).

It should be emphasized, however, that these new treatments will only have a significant clinical impact if behavioral and lifestyle changes are strictly followed in order to maintain weight loss and reduce the risk of recurrence of dyslipidemia once the target goals are achieved (Gurevich-Panigrahi et al., 2009). Nonetheless, a better understanding of the pathophysiology of obesity and its complications is pivotal to our ability to manage the disease (Fazio et al., 2010). An example is the fact that an elevation in plasma triglyceride levels is a key contributor to the development of diabetic dyslipidemia

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and insulin resistance (Erion and Shulman, 2010; Saltiel, 2001). Hence, a plausible treatment strategy should be able to address hypertriglyceridemia in diabetic patients (Taube et al., 2009). Current pharmacologic therapies, however, mainly target the systemic absorption of lipids and the de novo biosynthesis of cholesterol. In addition, few hypolipidemic agents have been suggested to directly target the clearance of lipids from the circulation (Das and Chakrabarti, 2006). Dietary triglycerides are not systemically absorbed per se. They have to be hydrolyzed first into free fatty acids and 2-monoacyle glycerol by triacylglycerol lipases (Verger, 1997). Hence, inhibition of the digestion of dietary lipids and consequently limiting their intestinal absorption is a reasonable means of therapeutic intervention with minimal systemic adverse effects (Klein, 2004). Unfortunately, despite the fact that several pharmacologic agents act through inhibiting the absorption of cholesterol, very few agents specifically inhibit the absorption of dietary triglycerides. Among the latter, the only clinically approved pharmacologic agent is the pancreatic lipase (PL) inhibitor Orlistat (Xenical®)(Lean and Campbell, 2004). Orlistat is a hydrogenated derivative of lipstatin obtained from *Streptomyces toxitricini*. The drug has been reported to be a potent inhibitor of gastric, pancreatic and carboxylester lipases (Hadvary et al., 1988), and has been widely marketed as a valuable aid in the management of human obesity (Hauptman et al., 1992; Sjostrom et al., 1998). Orlistat, however, causes several unpleasant gastrointestinal adverse effects that may compromise the patient compliance and the acceptability of the drug; including diarrhea, flatulence, abdominal cramps and oily discharge (Lean and Campbell, 2004). These adverse reactions triggered a wealth of studies to identify effective natural inhibitors of the PL with minimal GI side effects. In this current study, we investigated lavender methanolic extract as a potential natural inhibitor of PL.

Another key factor in the development of obesity is the hormone-sensitive lipase (HSL). It is a neutral lipase which is essential for lipid metabolism and general energy homeostasis in mammals. It catalyzes the hydrolysis of triacylglycerol, diacylglycerol, monoacylglycerol, and cholesteryl esters into their corresponding components (Anthonsen et al., 1999; Osterlund, 2001). It actions lead to an influx of free fatty acids (FFAs) from the abdominal adipose tissue into the circulation. Indeed, HSL is currently recognized as a component of the metabolic switch between glucose and FFAs as the energy sources in the liver and different peripheral tissues. Adipose HSL activity is normally inhibited by insulin. However, HSL remains active in type 2 diabetes, despite elevated insulin levels, presumably through insulin resistance, leading to significant weakening of the insulin's inhibitory effect on lipolysis. The resulting fatty acid flux stimulates inappropriate hepatic gluconeogenesis, triggers the release of VLDL particles from the liver and results in hypertriglyceridemia.

Furthermore, several adipokines are released due to the FFA mobilization and further augment the insulin resistance. These metabolic changes help sustain a vicious cycle of pathophysiologic events that would ultimately end in what is known as the metabolic syndrome (Bergman and Ader, 2000; Pfeiffer, 2007).

Not surprisingly, the pivotal role of elevated plasma FFA in the development of insulin resistance and type 2 diabetes has raised the interest in HSL as a potential therapeutic target for the simultaneous management of insulin resistance in DM type 2 and hypertriglyceridemia (diabetic dyslipidemia). In fact, many synthetic HSL inhibitors have been recently synthesized (De Jong et al., 2004; Ebdrup et al., 2007; Taha et al., 2008). Safety concerns, however, have precluded the clinical approval of these agents.

In contrast, the identification natural phytochemicals with HSL and or PL inhibitory activities is still a poorly investigated area of research. Effective natural inhibitors of the HSL and PL would be excellent candidates for further development into clinical products. The screening and optimization of safe and effective phytochemicals that inhibit both HSL and PL would provide an excellent new strategy in combating obesity, insulin resistance and diabetic complications. Our current manuscript summarizes a significant amount of work that was undertaken to screen plant species native to Jordan to investigate their potential use as natural HSL and PL inhibitors. After an initial screening of a pool of 30 native plants belonging to six plant families (Bustanji et al., 2010), we proceeded to further investigate the more promising herbal extracts. Lavender was among the top ten. Hence, the HSL and PL *in vitro* inhibitory activities of the methanolic extract of *Lavandula angustifolia* L. (Solanaceae) aerial parts, collected from northern region of Jordan, was further investigated. Lavander (*L. angustifolia*) is rich in phenolic derivatives; mainly gallic acid and rosmarinic acid (Figure 1) and globally known for its aromatic and calmative scent. In addition, lavender has been widely investigated for its mydriatic, antispasmodic, anticholinergic, analgesic and sedative properties. Traditionally, lavender has been also used in asthma, gastric ulcers and Parkinson's disease (Mateus et al., 1998). In the current study, we investigated the inhibitory effects of *L. angustifolia* methanolic extract and two of its phenolic compounds; gallic and rosmarinic acids, on HSL and PL activities. Our study is a first step in an ongoing effort in our lab to investigate new treatment options for DM type II. It establishes a basis for future *in vivo* work in order to determine the full therapeutic potential of the tested plants in the treatment of a plethora of metabolic disorders.

MATERIALS AND METHODS

Plant materials

Botanic materials were collected from several lavender plants,
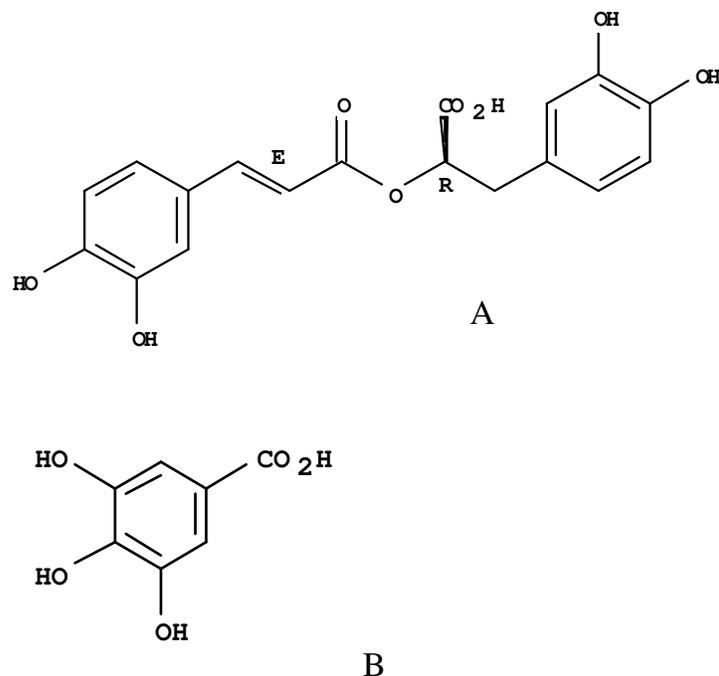
Figure 1. Chemical structure of rosmarinic acid (A) and gallic acid (B).

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, Jordan University), and voucher specimens were deposited at the Herbarium Museum of the Faculty of Pharmacy, Jordan University of Science and Technology. The aerial parts of the lavender 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 et al., 2011a).

Methanolic extraction

Methanolic extraction of lavender was carried out using a protocol that has been validated and optimized in our lab. Briefly, a 500 mg sample of ground plant material was mixed with 10 mls of methanol (80%) then incubated at 37°C for 3 h using a shaker water bath. At the end of the incubation period, the samples were cooled to room temperature then were centrifuged at 1500 (g) for 10 min. The supernatant was carefully recovered then the solvent was evapo-rated under vacuum conditions at 40°C using a rotary evaporator. The solid residues were collected and stored in a cool and dry condition until the time of subsequent activity assays as described previously (Bustanji et al., 2010, 2011b; Mohammad et al., 2010).

Preparation of lavender extract and phenolic compounds stock solutions

The collected solid residues were initially dissolved in DMSO to give five different extract stock solutions with a concentration range of 0.62 to 10.0 mg/ml. Subsequently, 20 µl aliquot of each stock solution was used in the reaction mixture to give a final concentration range of 12.5 to 200 µg/ml. For the HSL assay, a higher concentration was needed and plant extracts were diluted to give a final concentration range of 12.5 to 400 µg/ml in the reaction mixtures. Rosmarinic acid and gallic acid were purchased from Sigma and 5 concentrations (10, 20, 50, 100 and 200 µg/ml) were prepared by dissolving the pure compound in ultra pure water just before the IC_{50} determination assays as described below.

Pancreatic lipase preparation

The enzyme solutions were freshly prepared immediately prior to 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 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 et al., 1989).

Quantification of baseline 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 (Bustanji et al., 2010; Mohammad et al., 2010; Sharma et al., 2005; Taha et al., 2008), with minor modification. Here, *p*-nitrophenyl butyrate (PNPB), dissolved in acetonitrile, was used in the enzymatic assays as the PL substrate at 100 µM concentration instead of 5 mM. An aliquot (0.10 ml) of the 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 5 time points (1 to 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
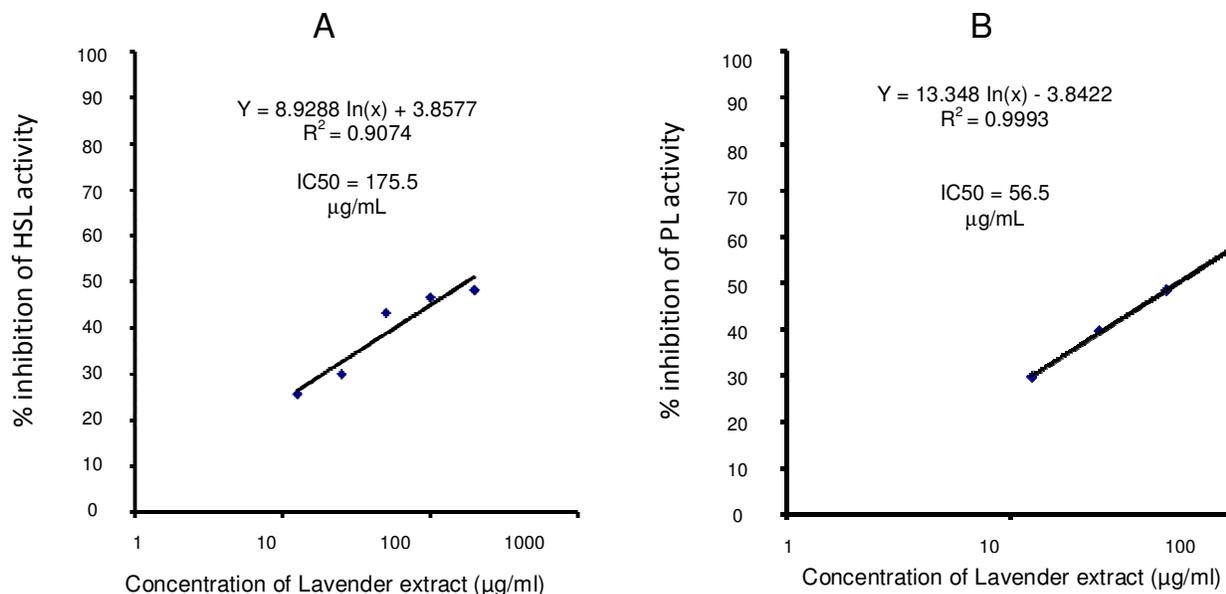
Figure 2. The inhibitory effects of lavender extract concentrations on the activity of extract plotted on a log scale (A) hormone sensitive lipase and (B) pancreatic lipase.

spectrophotometer, against a blank that contained the same reaction mixture, but denatured enzyme. Enzyme activity was indirectly measured as an increase in the absorbance per minute. The pancreatic lipase activity was defined as the increase in the rate of p-nitrophenol release which can be calculated from the slope of the linear segment of (absorbance vs time) profiles. The procedure was repeated three times and the average of the three slopes was designated as the uninhibited PL activity.

PL inhibition by lavender extracts - Determination of IC$_{50}$

The inhibition of pancreatic lipase activity by the prepared lavender extracts was measured using the spectrophotometric assay described above. PL was pre-incubated 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 the extract to the calculated uninhibited activity as determined above. The concentration required to give 50% inhibition (IC$_{50}$) was calculated for the lavender extracts using concentrations ranging from (12.5 to 200 µg/ml) (Figure 2). All assays were in triplicates and the calculated inhibition percentages were the mean of 3 measurements. Orlistat, a known inhibitor of PL, was used as a positive control in the assay mixture.

$$\% \text{ of inhbition} = (1 - \frac{\text{Test Inclination}}{\text{Blank Inclination}})$$

Extraction of the HSL enzyme

Isolated fat cells were extracted from rat epididymal adipose tissues as described earlier (Bustanji et al., 2010; Rodbell, 1964). Brief ly, Wistar male rats were sacrificed by cervical dislocation, and their epididymal fat pads were removed quickly and rinsed several times in ice-cold normal saline. The tissue was weighed and minced into small pieces and placed in a flask. The resulting mass was treated as follows: for each 1.0 g of tissue, 3 mls of KRB (pH 7.4) supplemented with 4% BSA were added, followed by 10 mg of collagenase. The mixture was incubated and agitated in a metabolic shaker (Shaking Incubator, Daiki Scientific Corporation) over a 2 h period at 37°C. Subsequently, fat cells were liberated from the tissue fragments by gentle stirring with a rod.

The resulting suspension was centrifuged for 1 min at 400 (g) at 20°C. Fat cells floated to the surface while stromal-vascular cells settled at the bottom. Stromal-vascular cells were removed by aspiration. Fat cells were decanted and washed by suspending them in 10 mls of warm (37°C) KRB-BSA solution followed by centrifugation (for 1 min at 400 g at 20°C) and a second round of removal of stromal-vascular cells by aspiration. The washing procedure was repeated three times. HSL was extracted from epididymal fat cells as described earlier (Morimoto et al., 1999). Briefly, 1 ml of suspended fat cells (in KRB-BSA solution) was further diluted by 2.5 mL KRB-BSA and incubated at 37 °C for 30 min. Subsequently, the suspension was centrifuged at 100 (g) for 1 min to separate the infranatant from the fat cells. For each 1.0 ml of suspended fat cells, a 1.125 ml homogenization buffer (each 100 ml prepared from 50 mM Tris-HCl, pH 7.0, 250 mM sucrose, and 1 crushed protease inhibitor tablet) was added and the mixture was manually agitated 20 times. The homogenate was centrifuged at 4540 g and 4°C over 10 min. Subsequently, 250 µl of diethyl ether was added to the homogenate and abruptly shaken and centrifuged at 1200 g over 5 min at 4°C. The upper ether layer was aspirated. The subsequent supernatant was used as HSL extract. HSL extract aliquots (0.5 ml) were stored at -80°C for later use. The rat HSL (1068 aa) is almost identical to human HSL (1072 aa) and has been shown to have almost identical physicochemical and kinetic properties (Holm et al., 1994).

Quantification of hormone sensitive lipase activity by a spectrophotometric assay

The lipase activity of HSL was quantified by a colorimetric assay that measures the release of p-nitrophenol as previously described

(Bustanji et al., 2010, 2011b; Petry et al., 2005), and using the same substrate and procedures described above for the quantification of PL. Orlistat was also used as a standard inhibitor for the hormone sensitive lipase (Fex and Mulder, 2008; Smith et al., 1996).

HSL inhibition by lavender extract - Determination of IC$_{50}$

The inhibition of HSL activity by the prepared lavender 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 at concentration of 200 µg/ml. The final concentration of DMSO was fixed and did not exceed 2.0%. The percentage of residual activity of HSL was determined for each extract by comparing the lipase activity of HSL with and without the extract. Inhibition of HSL by tested extract was calculated from the residual activity of the uninhibited HSL control using the following formula.

$$\% \text{ of inhbition} = (1 - \frac{\text{Test Inclination}}{\text{Blank Inclination}})$$

where test inclination is the linear change in absorbance per minute of test material, and blank inclination is the linear change in absorbance per minute of blank (uninhibited reaction). HSL was pre-incubated with different concentrations (12.5-400 µg/ml) of the lavander extracts and the percentages of HSL inhibition data were used to evaluate the IC$_{50}$ value. The percent inhibition was plotted against the logarithmic transformation of the corresponding test extract concentrations for determining the IC50 value (Figure 2). All assays were done in triplicates and the calculated inhibition percentages were the mean of 3 observations.

RESULTS AND DISCUSSION

Phytochemicals identified from traditional medicinal plants present an exciting opportunity for the development of new therapeutic agents (Dev, 2010; Newman and Cragg, 2007). Jordan lies in the heart of the Middle East with a special dynamic typography and climate that allow Jordan to encompass a unique and rich habitat for a wide variety of plant species. Indeed, more than 2500 wild plant species from 700 genera are found in Jordan (Anonymous, 2001; Oran and Al-Eisawi, 1998). *L. angustifolia* (Lavender) is commonly cultivated in the northern region of Jordan and utilized for a wide range of folkloric medical and cosmetic indications. Interestingly, lavender has been traditionally claimed to have therapeutic benefits in the management of DM type 2. In fact, people in the northern parts of Jordan have been using antidiabetic remedies containing lavender extracts for ages.

The chemical constituents of extracts and essential oils from various Lavandula species have been previously investigated and several classes of phytochemicals have been identified, including phenolics (Areias et al., 2000) and terpenes (Aburjai et al., 2005; Salido et al., 2004). In addition, Tiliacos et al. (2008) determined that a substantial percentage of *Lavandula intermedia* was made up of two compounds; coumarin and herniarin(7-methoxycoumarin). Further, Angioni et al. (2006)

determined through gas chromatography/ mass spectrometry (GC/MS) that the main ingredient of the essential oils fraction of *Lavandula stoechas* was fenchone. The latter is a monoterpene that comprised about 52.6% of the leaf/stem and 66.2% of flowers oily extracts. Interestingly, the essential oil fraction of the lavender species has been suggested to contribute to the antioxidant and antibacterial activities of the plant (Lu et al., 2010). The pharmacologic properties of the more polar methanolic extracts of the aerial parts, however, have not been elucidated. In one study, rosmarinic acid, a previously identified component in the lavender methanolic extract, has been suggested to prevent lipid peroxidation with possible implications in the management of dyslipidemia (Georgiev et al., 2009). The current manuscript summarizes a significant amount of work that was undertaken to identify plant species native to Jordan with potential HSL and pancreatic lipase (PL) inhibitory activities. The central role of HSL in regulating fatty acid metabolism makes it an interesting pharma-cological target for the treatment of insulin resistance and dyslipidemia where a decrease in the release of free fatty acids into the circulation is highly desirable. Our preliminary screening of more than 30 plants led to the selection of lavender as one of the stronger candidates for possible inhibitory activities of HSL and PL. Our results demonstrated *in vitro* inhibitory effects of *Lavandula angustifolia* on both HSL and PL activities. The results indicated that lavender extract was able to inhibit HSL in a dose dependent manner with an IC$_{50}$ of 175.5 µg/ml. Likewise, it was able to inhibit the PL activity in a dose dependent manner with an IC50 of 56.5 µg/ml. Such inhibitory activity could be attributed at least in part, but is not exclusive to the presence of rosmarinic acid (IC$_{50}$ of 125.2 and 51.5 µg/ml for PL and HSL, respectively) and gallic acid (IC$_{50}$ of 10.1 and 14.5 µg/mL for PL and HSL, respectively) (Table 1) in the extract. The deviation of the IC$_{50}$ values of the methanolic extract from those of either rosmarinic acid or gallic acid was anticipated. The extract contains a number of additional phenolic compounds that would likely contribute to or even dictate the net inhibitory effects of the extract. The current study is a first step in identifying compounds in lavender that may explain its folkloric use in the management of diabetes and dyslipidemia. Interestingly, the IC$_{50}$ of the lavender extract for the PL was about one third the value for its IC$_{50}$ for HSL; indicating an approximately 3-fold higher potency against PL activity. The same pattern of preferential inhibition was evident with orlistat, the only commercially available FDA-approved PL inhibitor. The higher HSL and PL IC$_{50}$ values for lavender extract compared to orlistat would necessitate the intake of larger amounts of the plant in order to achieve comparable effects on lipid metabolism. The latter would be feasible due to the safety profile of lavender. In fact, quantities in the range of double and triple digits of milligrams, have been deemed "generally recognized as safe, GRAS" by a number of scientific and

Table 1. IC$_{50}$ values (µg/ml) of extracts and pure compounds for both hormone sensitive lipase (HSL) and pancreatic lipase (PL).

Tested extract / compound	Pancreatic lipase (µg/ml)	HSL (µg/ml)
Lavander methanolic extract	56.5	175.5
Rosmarinic acid	125.2	51.5
Gallic Acid	10.1	14.5
Orlistat	0.65	2.1

regulatory panels in the US (Anonymous, 2010). Oral extracts of lavender have been widely used in the management of insomnia at doses that far exceed the microgram range used in this study (Anonymous, 2010). Interestingly, when comparing the monograph of lavender to that of orlistat, the GI adverse effects of lavender are milder and less frequent than those of orlistat. In addition, the number of listed drug and food interactions of lavender is considerably fewer than the listed interactions of orlistat.

Hence, the future use of lavender as an aid in the management of diabetic dyslipidemia does not seem to be farfetched. On the other hand, the lower potency of lavender extract compared to orlistat suggests that the extract per se should be only used as a complementary rather than alternative therapy to hyopilipidemic drugs. Further structure activity relationship (SAR) assessment is required to enhance the potencies of the active constituents in the lavender extract. In addition, the lower potency of the rosemary extract against PL and HSL activities compared to orlistat could explain why lavender traditionally required longer times to exert a noticeable hyoplipidemic effect.

Conclusion

To our knowledge, this is the first study in which the effects of *L. angustifolia* and rosmarinic/gallic acids on HSL and PL activities are directly compared. Most of the published literature on lavender had focused on the volatile oil fraction and its uses as a sleep aid, calmative, fragrant, insect repellant and flavoring agent (Natural Medicines Comprehensive Database, 2010). In contrast, the main focus of this work was to investigate a possible rationale for the folkloric use of lavender in the management of DM type 2 in Jordan. Our results demonstrated *in vitro* inhibitory effects of the lavender methanolic extract on both HSL and PL in a dose dependent manner.

Interestingly, the extract had an IC$_{50}$ for PL that was about 3-fold lower than the IC$_{50}$ for HSL, indicating a higher affinity to the former enzyme.

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