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Full Length Research Paper

Inhibition of hormone sensitive lipase and pancreatic lipase by *Rosmarinus officinalis* extract and selected phenolic constituents

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Rosmarinus officinalis **L. (Rosemary) has been long claimed to have hypogylcemic-hypolipidemic dual effects in folkloric medicine. In an effort to explain rosemary's claimed benefits, numerous published studies have investigated an array of pharmacologic activities of the plant including anti-inflammatory, anticarcinogenic and metabolic effects. The question remained, however, as how rosemary would target both plasma lipids and glucose levels simultaneously. A newer mechanism has been suggested, in which targeting the hormone sensitive lipase (HSL) would be the common link between the two metabolic effects. In fact, HSL has been extensively studied for its effects on the metabolic switch between glucose and free fatty acids (FFAs) as an energy source. 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. Our results demonstrated *in vitro* inhibitory effects of *R. officnalis* on both HSL and PL in a dose dependent manner. Interestingly, the rosemary extract had an IC_{50} for PL that was several fold lower than the IC_{50} for HSL, indicating a higher affinity to the former enzyme (13.8 and 95.2 µg/mL for PL and HSL, respectively). In addition, we have compared the inhibitory activities of purified constituents found in rosemary to the parent plant [rosmarinic acid (RA), chlorogenic acid (CA), caffeic acid (CaA) and gallic acid (GA)]. Our results showed that all the tested compounds (RA, CA, CaA, and GA) were able to inhibit the PL and HSL activities in a dose dependent manner, but with different potencies. PL and HSL IC_{50} values were calculated for each compound and GA was found to be the most potent (IC_{50} 10.1 and 14.5 for PL and HSL, respectively). Further work is necessary to determine whether our *in vitro* findings would correlate with the *in vivo* effects. Nonetheless, our results are a first step in fully understanding the long claimed hypoglycemic-hypolipidemic dual effects of rosemary. Simultaneous targeting of both HSL and PL is likely to open the door for a new era in our continuous battle against DM type 2 and its cardiovascular complications. Currently we are working on identifying the most active constituents of the plant to evaluate a structure-activity relationship which would pave the road for future therapeutic use.**

Key words: *Rosmarinus officinalis,* Rosemary, obesity, diabetes mellitus, pancreatic lipase, hormone sensitive lipase, phenolic compounds, rosmarinic acid.

INTRODUCTION

Herbal remedies have been used for medical treatment since the dawn of civilization. In recent years, “Non-traditional” or “alternative” treatments using plant extracts and herbal supplements have become extremely popular worldwide fueled by a relentless effort to improve the wellbeing and combat chronic conditions resistant to conventional pharmacologic treatments such as obesity

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and diabetes mellitus (Cicero et al., 2004; Wheatley, 2004) .

Indeed, obesity is one of the most frustrating health burdens in developed countries. It has been ranked as a major risk factor for the development of a plethora of debilitating diseases, including diabetes, chronic kidney disease, cardiovascular disease, and cancer (Erion and Shulman, 2010; Fazio et al., 2010; Nawroth et al., 2010; Roberts et al., 2010).

Unfortunately, despite significant improvements in public education and pharmacologic management in the last two decades, obesity rates have continued to be alarmingly high. Advances in obesity associated research have shown new biochemical pathways and molecular targets for pharmacologic intervention that will likely pave the road to new treatment strategies in the future. It should be emphasized, however, that these new treatments will only have a clinical impact if significant behavioral and lifestyle changes are strictly followed in order to maintain weight loss and prevent the recurrence of dyslipidemia. Nonetheless, a better understanding of the pathophysiology of obesity and its complications is pivotal to our ability to manage the disease. It is well established that an elevation in plasma triglyceride levels is a key contributor to the development of obesity. Current pharmacologic therapies mainly target the systemic absorption of lipids and their *de novo* biosynthesis. In addition, a handful of the hypolipidemic agents on the market also target the clearance of lipids from the circulation. Dietary triglycerides are not systemically absorbed per se. They have to be hydrolyzed first to free fatty acids and 2-monoacyle glycerol by triacylglycerol lipases (Verger, 1997). Hence, inhibition of the digestion of dietary lipids and therefore limiting their intestinal absorption is a plausible means of therapeutic intervention with minimal systemic adverse reactions (Dipiro, 2007). Indeed, several pharmacologic agents act through inhibiting the absorption of cholesterol and dietary lipids. Among the latter, the only clinically approved pharmacologic agent as pancreatic lipase (PL) inhibitor is Orlistat (Xenical®) (Lean and Campbell, 2004). It is currently utilized as an effective aid in the management of human obesity (Sjostrom et al., 1998). Orlistat, however, possesses several unpleasant adverse reactions that have been shown to compromise the patient compliance. These adverse reactions triggered a wealth of studies that searched for natural inhibitors of the PL with comparable efficacy to orlistat, but void of its side effects. In this current study, we investigated the potential use of rosemary as a natural inhibitor of PL.

Rosemary, *Rosmarinus officinalis* L. (Lamiaceae) is an evergreen perennial shrub cultivated in many parts of the world and is widely used as a spice in a variety of culinary schools (Al-Sereiti et al., 1999; Bai et al., 2010; Harach et al., 2010). In addition, rosemary has been widely investigated for potential therapeutic benefits in curing or managing a wide range of diseases (Sanjust et al., 2008). Traditionally, rosemary has been used in renal colic as an antispasmodic, to relieve symptoms of dysmenorrheal (Takaki et al., 2008), to relieve respiratory disorders, and to stimulate growth of hair (Al-Sereiti et al., 1999; Fabio et al., 2007). Preparations of *R. officinalis* are also indicated for oral hygiene and for the common cold. In addition, a wealth of studies demonstrated antioxidant, cholagogue, choleretic, diuretic, hypoglycemic, anti-inflammatory, antimicrobial, anticarcinogenic, hepatotonic and hypolipidimic activities of rosemary (Dearlove et al., 2008; Erenmemisoglu et al., 1997; Gutierrrez et al., 2010; Harach et al., 2010; Koga et al., 2006; Nabekura et al., 2010; Sotelo-Felix et al., 2002). These biological activities have been attributed to different constituents of rosemary such as monoterpenes, diterpenes and the phenolics -mainly caffeic acid derivative- acids like rosmarinic acid (RA) (Figure 1A), which is an ester of caffeic acid and α-hydroxydihydrocaffeic acid, chlorogenic acid (CA) (Figure 1B), and their hydrolyzed metabolites. Other bioactive phytochemicals identified in rosemary include caffeic acid (CaA) (Figure 1C) and some simple phenolic compounds like gallic acid (GA) (Figure 1D) (El Deeb, 1993; Herrero et al., 2010; Rababah et al., 2004; Wang et al., 2004). Among these bioactive constituents, the pharmacologic properties have been suggested to be highly attributed to rosmarinic acid, the predominant secondary metabolite in rosemary. This suggestion was supported by several *in vitro* studies where rosmarinic acid has been shown to exhibit anti-diabetic, antimutagenic, anti-tumorigenic, anti-HIV, anti-proliferative, and anti-cyclooxygenase activities. Furthermore, rosmarinic acid has also been suggested to have anti-atherogenic activity through preventing the oxidation of low density lipoprotein. The antioxidant properties of the plant have been also demonstrated to be linked to the presence of rosmarinic acid (Dubois et al., 2008; Furtado et al., 2010; Hadafi et al., 1998; Moon et al., 2010; Park et al., 2008; Shetty, 2007; Vanithadevi and Anuradha, 2008).

The ability of a hypolipidemic agent to lower blood glucose levels is a significant advantage in the management of diabetes mellitus (DM). DM is a group of metabolic disorders that share the common feature of hyperglycemia. Diabetes affects approximately 6.4% of the world's population with the highest prevalence in North America and Caribbean (10.2%) followed by middle East and North Africa (9.3%) (International Diabetes Federation, 2010).

Hormone-sensitive lipase (HSL), a neutral lipase, is a component of the metabolic switch between glucose and free fatty acids (FFAs) as an energy source. HSL catalyzes the hydrolysis of triglycerides leading to a flux of free fatty acids from the adipocytes (Anthonsen et al., 1999; Osterlund, 2001). Adipose HSL activity is normally inhibited by insulin. However, the regulatory inhibition of HSL activity in type 2 diabetes is significantly compromised by the development of insulin resistance.

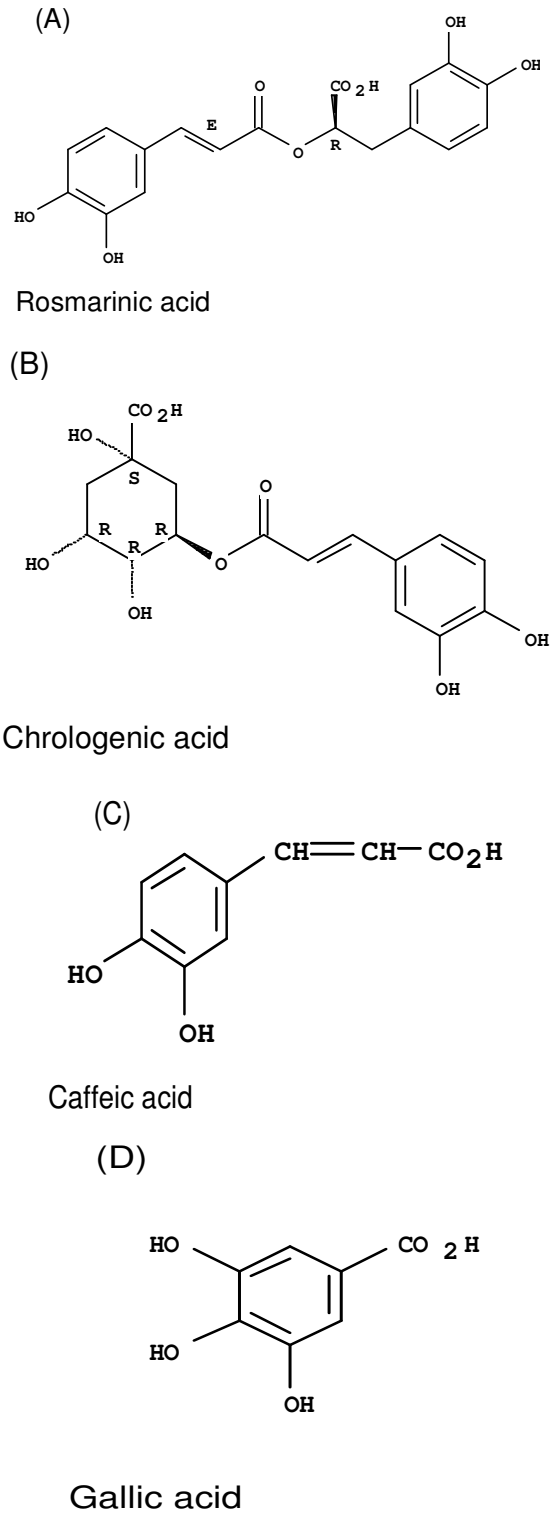

Figure 1. Chemical structure of (A) rosmarinic acid, (B) chrologenic acid, (C) caffeic acid and (D) gallic acid.

The resulting fatty acid flux stimulates inappropriate hepatic gluconeogenesis. Furthermore, high FFAs levels are suspected to augment insulin resistance and contribute to the inhibition of glucose uptake and utilization by muscles and the increase in gluconeo-genesis (Bergman and Ader, 2000; Erion and Shulman, 2010; Pfeiffer, 2007). In this current study we investigated the *in vitro* effects of rosemary on HSL activity. The goal of the study was to explain a molecular basis for the traditional use of rosemary in the management of both diabetes and hyperlipidemia by evaluating its inhibitory potential on PL and HSL activities.

The current manuscript summarizes a significant amount of work that was undertaken to identify plant species native to Jordan with potential HSL and PL inhibitory activities. After an initial screening of a pool of 30 native plants belonging to twenty plant families (Bustanji et al., 2010a, 2010b) we proceeded to further investigate the more promising herbal extracts. Hence, rosemary was selected. We then measured the HSL and PL *in vitro* inhibitory activities of the methanolic extract of *R. officinalis* aerial parts and compared that to the purified rosmarinic acid, chlorogenic acid, caffeic acid and gallic acid. 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

Materials

Crude porcine pancreatic lipase type II (Sigma, USA, EC 3.1.1.3); *p*-nitrophenyl butyrate (Sigma, USA); orlistat (kindly donated from Jordan Sweden Medical Company (JOSWE), Amman, Jordan; BSA (Sigma, USA); collagenase, type II (Cabiochem Germany); protease inhibitor tablet (Sigma, USA); rosmarinic acid (Sigma, USA); chlorogenic acid (Acros Organics, Belgium); caffeic acid (Acros Organics, Belgium); and gallic acid (Sigma, USA).

Plant materials

Plant materials were collected from Northern region of Jordan. The collected plants were identified taxonomically, by Dr. Khaled Tawaha (Faculty of Pharmacy, Jordan University), and voucher specimens were deposited at the department of pharmaceutical sciences / Jordan University. 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

The methanolic extraction of rosemary and other plants has been optimized in our lab to produce a maximum yield (Mohammad et al., 2010a). Briefly, methanolic extractions of rosemary were conducted using 500 mg sample of ground plant material in 10 ml methanol (80%), at 37°C for 3 h, in a shaking water bath. After cooling to room temp, 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 as described previously (Mohammad et al., 2010a).

Preparation of extract and compounds for *in vitro* assay

The tested material was initially dissolved in DMSO to give six different stock solutions with a concentration range of 0.31 - 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 6.3 - 200 µg/ml for both the extracts and pure compounds.

Pancreatic lipase preparation

The enzyme solutions were prepared immediately before use. For the *in vitro* assays, crude porcine pancreatic lipase type II 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. Porcine PL was selected due to its high homology to the human enzyme (85% homology) and similar enzyme kinetics and behavior (Mark et al., 1989).

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 (Bustanji et al., 2010a; Lee et al., 1993; Mohammad et al., 2010b), 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 ultraviolet (UV) spectrophotometer, against a blank that contain the same mixture but denaturated enzyme. Enzyme activity was detected as an increase of the absorbance per minute. The pancreatic lipase activity is defined as an increase of the rate of *p*-nitrophenol release which can be calculated from the slope of the linear segment of (absorbance *vs* time) profiles.

Pancreatic lipase inhibition by test extract

The inhibition of pancreatic lipase activity by the prepared test was measured using the spectrophotometric assay described above. PL was pre-incubated with each concentration of the tested material for at least 1 min at 37°C before adding the substrate. The final concentration of DMSO was fixed and did not exceed 2.0%; in order to rule out any potential biological activity by DMSO itself. Further, DMSO was at equal concentrations in both the test samples and controls. 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 (IC_{50}) was determined for the tested extract. PL was pre-incubated with escalating six concentrations (see above) of the test materials and the percentage of residual activity of PL data was 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.

Extraction of the HSL enzyme

Isolated fat cells were extracted from rat epididymal adipose tissues as described earlier (Bustanji et al., 2010b; Rodbell, 1964; Taha et al., 2008). Briefly, 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 ml 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 2 h 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 ml of warm (37°C) KRB-BSA solution followed by centrifugation (for 1 min at 400 g at 20°C) and a second round of removing stromal-vascular cells by aspiration. This washing procedure was repeated three times. HSL was extracted from epididymal fat cells as reported 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., 2010b; Petry et al., 2005; Taha et al., 2008), 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 test extract

The inhibition of HSL activity by the prepared plant extracts and the pure compounds was measured using the spectrophotometric assay described above. HSL was preincubated with each particular inhibitor for at least 3 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 HSL was determined for each test material by comparing the lipase activity of HSL with and without the extract. Inhibition of HSL by the test material was calculated from the residual activity of the uninhibited HSL control using the following formula:

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

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

	Pancreatic lipase (µg/mL)	HSL (µg/ml)
Rosemary extract	13.8	95.2
Rosmarinic acid	125.2	51.5
Chlorogenic acid	96.5	21.3
Caffeic acid	32.6	40.1
Gallic Acid	10.1	14.5
Orlistat	0.65	2.1

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

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). The percent inhibition was plotted against the logarithmic transformation of the corresponding test extract concentrations for determining the IC_{50} values (concentration required to give 50% inhibition (IC_{50}). All assays were triplicated 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 strategies (Birari and Bhutani, 2007). Noteworthy, there are many successful stories in drugs discovery which introduced vital drugs from natural sources like cyclosporine, mevinolin, the vinca alkaloids, taxol and others.

Natural products can provide a vast pool of enzyme inhibitors with the potential to be developed into clinical products. The possibility of finding effective and safe PL and HSL dual natural inhibitors would provide an excellent new strategy in combating both obesity and insulin resistance and their complications. In this study, the effects of methanolic extract of *R. officinalis*, on PL and HSL activities were investigated. The plant extract exhibited a significant inhibitory activity on both enzymes, PL and HSL, as calculated from the residual activity of the uninhibited enzyme controls (Figure 2). Interestingly, the rosemary extract was more effective against PL than that of HSL (Table 1). This differential inhibition could be explained, at least partly by differences in the catalytic sites and the sizes of both enzymes (465 aa Vs 1072 aa for human PL and HSL, respectively). In addition, our results showed that the inhibitory activity of the extract on both enzymes is dose dependent (Figure 2). At concentrations of 6.3 - 200 µg/ml, the percentage of enzyme inhibition ranged from 36.8 to 95.1% and 2.4 to 64.9% for the PL and HSL enzymes, respectively (Figure 2). The IC_{50} values of the plant extract were calculated from the least regression fit to be 13.8 and 95.2 µg/ml for PL and HSL respectively.

RA, the major phenolic constituent of *R. officinalis* has been previously identified in the methanolic extracts of the plant (Bassani et al., 1990; Scarpati and Oriente, 1958). Moreover, RA has been frequently reported as the bioactive phenolic constituent to which most of the plant activities are attributed (Shetty, 2007). Therefore the effect of rosmarinic acid was also investigated on both PL and HSL. In addition, other common phenolic constituents of the plant including CA, CaA, and GA, which are also extractable by methanol, were investigated. Results showed that all the tested compounds (RA, CA, CaA, and GA) were able to inhibit the PL and HSL activities in a dose dependent manner, but with different potencies. PL and HSL IC_{50} values were calculated for each compound as shown in Table 1.

It is worth mentioning that several previous reports have suggested the potential use of rosemary extracts in reduction of blood glucose and modification of the lipid profile (Harach et al., 2010; Hasani-Ranjbar et al., 2009). Interestingly a vast number of botanical therapeutic preparations which contained constituents from rosemary extract have been used traditionally throughout the years. These alternative therapies have been recommended to reduce blood triglycerides and reduce body weight (Yamamoto et al., 2005). On the other hand, there have been few previous reports suggesting the potential inhibition of pancreatic lipase by rosemary extracts, hence, explain its reported anti-hyperlipidimc effect (Slanc et al., 2009). To our knowledge, this is the first study that compares the PL and HSL IC_{50} values for the methanolic extract and the purified major phenolic constituents of the plant. Interestingly, the most potent compound was gallic acid. It had IC_{50} values of 10.1 and 14.5 (µg/ml) for PL and HSL, respectively. This finding was unexpected since previous reports attributed most of the tested pharmacological activities of rosemary to its content of RA. It is noteworthy, however, that in comparison to RA, gallic acid has smaller molecular size, but RA has higher aqueous solubility (1.5 g/100 ml Vs 2.5 g/100 ml respectively). Therefore, the higher potency could not be explained simply by solubility factors. Instead, we propose that a better interaction between gallic acid and the PL and HSL enzymes could be a function of the smaller size. It would be quite interesting to correlate the *in vivo* behavior of gallic acid with its *in vitro* inhibitory activity. Nonetheless, the current study confirms that rosemary extract was able to inhibit PL and HSL *in vitro*.

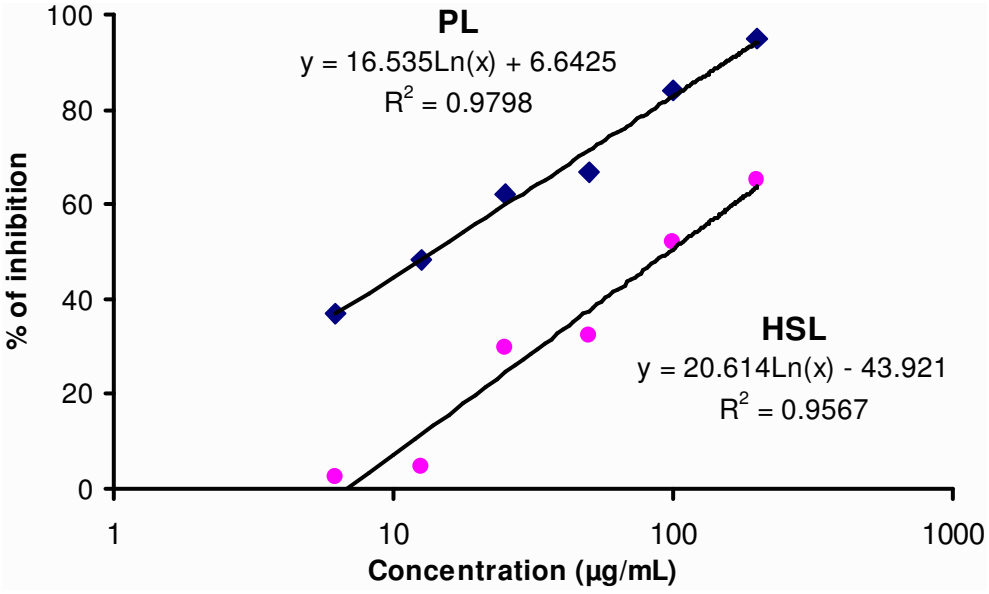

Figure 2. The inhibitory effects of Rosemary extract concentrations on the activity of hormone sensitive lipase (HSL, ●) and on pancreatic lipase (PL, ■).

That inhibition, however, was distinct from the inhibitory activities of purified RA. A possible explanation is the fact that the extract contains a variety of constituents that may each contribute individually and synergistically to the inhibition of PL and HSL. The latter suggestion is supported by our findings that confirm *in vitro* inhibitory activities for RA, CA, CaA, and GA against PL and HSL (Table 1). The differences between the PL and HSL IC_{50}s for the extract and purified phenolics further suggest specific enzymatic interactions with the phenolic compounds. Overall, it seems that the constituents in the extract are more potent against PL, which may explain the lower IC_{50} value compared to the one for HSL.

On the other hand, the extract, was less potent than orlistat, the only FDA approved PL inhibitor on the market (Table 1). The lower potency compared to orlistat suggests that rosemary 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 rosemary extract. On the other hand, the lower potency of the rosemary extract against PL and HSL activities compared to orlistat could explain why rosemary traditionally required several weeks to exert a noticeable hyoplipidemic effect. It could also explain why rosemary had a much favorable safety profile compared to orlistat.

The central role of HSL in regulating fatty acid metabolism makes it an interesting pharmacological target for the treatment of insulin resistance and dyslipidemic disorders where a decrease in delivery of fatty acids to the circulation and thereby reducing insulin resistance is desirable. In this study, *R. officinalis* and other pure phenolic constituents (Rosmarinic acid, chlorogenic acid, caffeic acid and gallic acid) were evaluated as potential *in vitro* inhibitors for the HSL. Results indicated that rosemary extract was able to inhibit the HSL in dose dependent manner with an IC_{50} of 95.2. Such inhibitory activity could be attributed at least in part to the presence of RA and other phenolics. Noteworthy, that all investigated phenoilc showed, relatively, potent inhibitory activities on HSL. The most potent was gallic acid with an IC_{50} of 14.5 µg/mL.

It is widely accepted in the traditional medicine to use rosemary in the management of hyperglycemia because of its effect on glucose homeostasis and antioxidant defense capabilities. Despite accumulating literature regarding rosemary, the exact mechanism by which it produces its antihyperglycemic activity is still controversial.

In one study, it was concluded that the remarkable antidiabetogenic effects of *R. officinalis* could be due to its potent antioxidant properties. However, other findings indicate that it might be producing its hypoglycaemic activity by a mechanism independent from insulin secretion e.g Inhibition of protein glycation, and the inhibition of endogenous glucose production (Bakırel et al., 2008). In fact, many plant polyphenols are known to form hydrophobic bonds to proteins, the phenolic components in the rosemary extract would affect the functions of many of proteins or transporters such as intestinal Na+-dependent glucose transport proteins (Koga et al., 2006). Interestingly, some recent reports suggested that rosemary extract might inhibit the intestinal absorption of glucose by inhibition through inhibition of intestinal α-amylase enzyme (McCue and Shetty, 2004) or

α-glucosidase enzyme (Koga et al., 2006).

Another yet possible mechanism of the hypoglycemic action of *R. officinals* was suggested to be through increasing the insulin level as evident from analysis of data in some diabetic animal models (Vanithadevi and Anuradha, 2008). Based on our results, we believe that the potential inhibition of HSL by rosemary extract could be, at least in part, a newly suggested mechanism by which *R. officinalis* and or its constituents produce their reported hypoglycemic activities.

Furthermore, the HSL-inhibiting activity may be promoted by mechanistic synergies among the present phenolic substances like RA and other phenolics (chlorogenic, caffeic and gallic acids).

Conclusion

To our knowledge, this is the first study in which the effects of *R. officinals* and some of its purified constituents on HSL and PL activities are directly compared. Our results demonstrated *in vitro* inhibitory effects of *R. officnalis* on both HSL and PL in a dose dependent manner. Interestingly, the rosemary extract had an IC_{50} for PL that was several fold lower than the IC_{50} for HSL, indicating a higher affinity to the former enzyme. To our surprise, the *in vitro* inhibitory activity did not correlate with the RA activity as evident from the IC_{50} values of the purified constituents.

Further work is necessary to deter-mine whether our *in vitro* findings would correlate with the *in vivo* effects. Nonetheless, our results are a first step in fully understanding the long claimed hypoglycemic-hypolipidemic dual effects of rosemary. Simultaneous targeting of both HSL and PL is likely to open the door for a new era in our continuous battle against DM type 2 and its cardiovascular complications. Our results provide a possible mechanism to explain the long history of the traditional use of rosemary in the management of diabetes. Further work is still necessary to identify the most active constituents of the plant and enhance their efficacies through optimizing their SAR.

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