

Full Length Research Paper

## Hormone sensitive lipase inhibition by selected medicinal plants

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In the present study, 29 medicinal plants belonging to 20 families were evaluated for their hormone sensitive lipase (HSL) inhibitory potential. The methanolic extracts prepared from plant parts used, were tested *in vitro* at 200 µg/ml concentrations for their inhibitory potential (expressed as % inhibition of XO activity). Twelve plants were found most active (% inhibition more than 30%) and their inhibition profiles (dose-dependent) were further evaluated by estimating the IC<sub>50</sub> values of their corresponding extracts. These active plants are *Malva nicaeensis* All. (IC<sub>50</sub> = 51.07 µg/ml), *Haplophyllum buxbaumii* (Poir.) G. Don. (101.3 µg/ml), *Anchusa italica* Retz. (132.8 µg/ml), *Cleome africana* Botsch. (168.9 µg/ml), *Salvia fruticosa* Mill. (276.7 µg/ml), *Glaucium aleppicum* Boiss. and Hausskn. ex Boiss. (289.7 µg/ml), *Reseda alba* L. (396.6 µg/ml), *Mentha spicata* L. (421.3 µg/ml), *Ononis natrix* L. (422.8 µg/ml), *Chrysanthemum coronarium* L. (463.7 µg/ml), *Anagallis arvensis* L. (751.9 µg/ml), and *Hypericum triquetrifolium* Turra (828.6 µg/ml). The extracts of the majority of other tested plants showed, however, moderate to weak activities. The results of the present screening provide good herbal choices to combat insulin resistance in human, thus playing a role in developing successful herbal remedies for diabetes and related disorders.

**Key words:** Diabetes mellitus, obesity, hormone sensitive lipase, anti-lipase activity, medicinal plants, plant extracts.

### INTRODUCTION

Herbs have been used for medical treatment since the earliest days of mankind. Various Chinese, Egyptian, Greek and Arab scientists enriched our today's knowledge with their expertise in herbal medicine. In the highly competitive environment of contemporary pharmaceutical research, natural products provide a unique element of molecular diversity and biological functionality which is indispensable for drug discovery. The emergence of strategies to deliver drug leads from natural products within the same time frame as synthetic chemical screening has eliminated a major limitation of the past (Dev, 2010; Newman and Cragg, 2007; Qi et al., 2010). Natural products prepared from traditional

medicinal plants and microbial sources have always presented an exciting opportunity for the development of new therapeutic agents. About half of all compounds that were successful in clinical trials in the last twenty-five years were, at least, been derived from natural origin (Dev, 2010; Newman and Cragg, 2007; Qi et al., 2010). Moreover, WHO stated that a majority of the world's population in developing countries still rely on herbal medicine to meet their health needs (WHO, 1999). In recent years, interest has grown in the area of lipid metabolism and its effect on the regulation of glucose control and, in turn, diabetes (Hughes, 2009; Pfeiffer, 2007). The pathogenesis of type 2 diabetes is complex, involving progressive development of insulin resistance and a relative deficiency in insulin secretion, leading to overt hyperglycemia (Ceriello, 2005; Lin and Sun, 2010; Wagman and Nuss, 2001). The disease is largely associated with obesity, which contributes substantially to

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insulin resistance. Importantly, insulin resistance is also implicated in the pathogenesis of other major diseases including atherosclerotic cardiovascular disease, dyslipidemias, and polycystic ovarian syndrome (Saltiel, 2001; Taube et al., 2009).

Moreover, type 2 diabetes mellitus is associated with increased levels of fatty acids and triglycerides. Elevated plasma levels of free fatty acids (FFAs), however, are thought to play a major role in the pathogenesis of insulin resistance and type 2 diabetes by inhibiting glucose uptake and utilization by muscles and causing increased glucose output by the liver (Bergman and Ader, 2000; Pfeiffer, 2007). The increases in plasma FFAs levels are the result of increased mobilization from adipose tissue. Hormone-sensitive lipase (HSL), thought to be the rate limiting enzyme in adipose tissue lipolysis, hydrolyzes the stored triglycerides into monoglycerides and FFAs (Frayn, 2002; Kim et al., 2001; Large et al., 1998; Miles and Nelson, 2007).

Hormone-sensitive lipase (HSL), a neutral lipase, is a vital enzyme in lipid metabolism and general energy homeostasis in mammals. It has broad substrate specificity; it catalyzes the hydrolysis of triacylglycerol, diacylglycerol, monoacylglycerol, and cholesteryl esters as well as retinyl esters (Anthonen et al., 1999; Osterlund, 2001). For that reason, HSL is not only present in adipose tissues but is also found in tissues in which cholesterol esters are stored, for example, adrenal cortex, ovaries, testis, and heart (Kraemer and Shen, 2006).

HSL is a component of the metabolic switch between glucose and FFAs as energy sources. The hormonal and neuronal regulation of HSL activity is mediated through reversible cyclic adenosine monophosphate-dependent serine phosphorylations mediated by protein kinase A. However, the activity of HSL is acutely activated via cAMP and protein kinase A-mediated phosphorylation (Anthonen et al., 1998; Kraemer Fredric and Shen, 2006) in response to epinephrine / norepinephrine (Plee-Gautier et al., 1996). Insulin stimulation of adipocytes prevents HSL activation, leading to a decrease in the release of FFA and glycerol (Langin et al., 1996).

The pivotal role of elevated plasma FFAs in the development of insulin resistance and type 2 diabetes have rendered HSL as a potential therapeutic target for this disease; lowering plasma FA levels and, thereby, reducing insulin resistance. However, many synthetic HSL inhibitors have recently been identified (Claus et al., 2005; De Jong et al., 2004; Lowe et al., 2004; Taha et al., 2008; Ebdrup et al., 2005, 2007)

Natural products can provide a vast pool of HSL inhibitors that can possibly be developed into clinical products (Bustanji et al., 2011a, b; Mohammad et al., 2010; Rout et al., 2009). At present, the potential of developing successful natural products for the management of diabetes and obesity is still largely unexplored. The screening and optimization of safe and

effective phytochemicals that inhibit HSL would provide an excellent new strategy in combating insulin resistance and its complications. Herein, we have optimized a protocol to screen the methanolic extracts of various medicinal plants, collected from the several areas of Jordan, for their HSL inhibitory potential. A total of 29 traditional medicinal plants belonging to 16 families, regardless of their claimed ethnopharmacological uses, were tested using a simple, fast, efficient and reliable method, in an attempt to find new extracts with potential HSL inhibitory activities.

## MATERIALS AND METHODS

### Plant materials

Plant materials were collected from 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 plant materials were cleaned of residual soil and air-dried at room temperature, 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 to 23°C) and protected from light until extraction.

### Plant extraction

Methanolic extractions were conducted using 500 mg sample of each ground plant material of the used parts (Table 1), in 20 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 (Cai et al., 2004).

### Extraction of the HSL enzyme

Isolated fat cells were extracted from rat epididymal adipose tissues as described by Claus et al. (2005), Rodbell (1964) and 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 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; Taha et al., 2008). 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,

**Table 1.** Hormone sensitive lipase (HSL) inhibitory activity of the methanolic extracts of plants of Jordan.

Plant name	Family	% of inhibition at 200 µg/ml	IC <sub>50</sub> (µg/ml)
<i>Achillea biebersteinii</i> Afanasiev	Asteraceae	-9.08	-
<i>Adonis palaestina</i> Boiss	Ranunculaceae	16.77	-
<i>Anagallis arvensis</i> L.	Primulaceae	42.3	751.9
<i>Anchusa italica</i> Retz.	Boraginaceae	57.41	132.8
<i>Anthemis palestina</i> Reut. ex Boiss.	Asteraceae	3.81	-
<i>Calendula arvensis</i> L.	Asteraceae	-10.06	-
<i>Chrysanthemum coronarium</i> L.	Asteraceae	49.7	463.7
<i>Cinnamomum verum</i> J.Presl	Lauraceae	25.8	-
<i>Cleome africana</i> Botsch.	Capparaceae	53.8	168.9
<i>Convolvulus althaeoides</i> L.	Convolvulaceae	22.55	-
<i>Eryngium creticum</i> Lam.	Apiaceae	18.68	-
<i>Fagonia arabica</i> L.	Zygophyllaceae	0.65	-
<i>Glaucium aleppicum</i> Boiss. & Hausskn. ex Boiss.	Papaveraceae	41	289.7
<i>Haplophyllum buxbaumii</i> (Poir.) G. Don.	Rutaceae	60.1	101.3
<i>Helianthemum ledifolium</i> Mill.	Cistaceae	1.2	-
<i>Hypecoum dimidiatum</i> Delile	Papaveraceae	25.03	-
<i>Hypericum triquetrifolium</i> Turra	Clusiaceae	31.92	828.6
<i>Linum pubescens</i> Banks & Sol.	Linaceae	25.5	-
<i>Majorana syriaca</i> (L.) Kostel.	Lamiaceae	17.42	-
<i>Malva nicaeensis</i> All.	Malvaceae	55.8	51.07
<i>Mentha spicata</i> L.	Lamiaceae	37.33	421.3
<i>Ononis natrix</i> L.	Fabaceae	37.58	422.8
<i>Onosma giganteum</i> Lam.	Boraginaceae	14.16	-
<i>Paronychia argentea</i> Lam.	Illecebraceae	19.96	-
<i>Reseda alba</i> L.	Resedaceae	39.5	396.6
<i>Reseda lutea</i> L.	Resedaceae	12.36	-
<i>Salvia fruticosa</i> Mill.	Lamiaceae	51.4	276.7
<i>Silene aegyptiaca</i> L.f.	Caryophyllaceae	4.79	-
<i>Varthemia iphionoides</i> Boiss. & Blanche	Asteraceae	23.62	-

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.

#### Preparation of extracts for *in vitro* assay

The tested extracts were initially dissolved in DMSO to give five different stock solutions with a concentration range of 1.25 to 20.0 mg/ml. Subsequently, 20 µl aliquot of each stock solution was used in the reaction mixture to give a final concentration range of 25 to 400 µg/ml.

#### Quantification of HSL 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 (Petry et al., 2005; Taha et al., 2008), however, with minor modification. Here, *p*-nitrophenyl butyrate (PNPB), dissolved in acetonitrile, was employed, in the enzymatic assays, as HSL substrate at 200 µM concentration. Aliquot (0.10 ml) of HSL solution was added to the reaction mixtures. The volume was completed to 1 ml using the tris-HCl buffer before measuring the solution absorbances at 410 nm, by a UV spectrophotometer, at a minimum of 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, against blank using denaturated enzyme. The HSL 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.

#### HSL inhibition by test extract

The inhibition of HSL activity by the prepared plant extracts was measured using the spectrophotometric assay previously described. Pancreatic lipase (PL) was pre-incubated 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:

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

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 concentration required to give 50% inhibition ( $IC_{50}$ ) was determined for the twelve extracts having the best inhibitory activities at 200  $\mu\text{g/ml}$  concentration (above 30%). HSL was pre-incubated with five different concentrations (25 to 400  $\mu\text{g/ml}$ ) of these extracts and the percentages of HSL inhibition data were used to evaluate the  $IC_{50}$  values.

The percent inhibition was plotted against the logarithmic transformation of the corresponding test extract concentrations for determining the  $IC_{50}$  value. All assays were triplicated and the calculated inhibition percentages were the mean of 3 observations.

## RESULTS AND DISCUSSION

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.

Phytochemicals identified from traditional medicinal plants present an exciting opportunity for the development of newer therapeutics (Birari and Bhutani, 2007). In the present study, as a part of the continuing search for biologically active agents for management of diabetes mellitus and dyslipidemia from natural herbal sources, twenty nine different plant extracts, belonging to sixteen different families, were investigated as potential HSL inhibitors. Although several previous studies have screened the extracts of various medicinal plants as pancreatic lipase (PL) inhibitors for hyperlipidemia management, this is the first study that screens the extracts of selected medicinal plants, as potential HSL inhibitors. The degree of HSL inhibition was evaluated for all extracts at concentration of 200  $\mu\text{g/ml}$ . While the  $IC_{50}$  values (concentration of extract that inhibits 50% of the enzymatic activity) were determined only for the plants ( $n = 12$ ) that showed inhibitory activity of more than 30% when compared to uninhibited enzymatic reaction.

Evaluating the obtained results, it was found that 12 of these plant extracts (Table 1) were able to inhibit the HSL in a dose-dependent manner, with an  $IC_{50}$  range of 51.0 to 828.6  $\mu\text{g/ml}$  and these are *Malva nicaeensis* All. ( $IC_{50} = 51.07 \mu\text{g/ml}$ ), *Haplophyllum buxbaumii* (Poir.) G. Don. (101.3  $\mu\text{g/ml}$ ), *Anchusa italica* Retz. (132.8  $\mu\text{g/ml}$ ), *Cleome africana* Botsch. (168.9  $\mu\text{g/ml}$ ), *Salvia fruticosa* Mill. (276.7  $\mu\text{g/ml}$ ), *Glaucium aleppicum* Boiss. and Hausskn. ex Boiss. (289.7  $\mu\text{g/ml}$ ), *Reseda alba* L. (396.6  $\mu\text{g/ml}$ ), *Mentha spicata* L. (421.3  $\mu\text{g/ml}$ ), *Ononis natrix* L.

(422.8  $\mu\text{g/ml}$ ), *Chrysanthemum coronarium* L. (463.7  $\mu\text{g/ml}$ ), *Anagallis arvensis* L. (751.9  $\mu\text{g/ml}$ ), and *Hypericum triquetrifolium* Turra (828.6  $\mu\text{g/ml}$ ).

Figure 1 shows the inhibitory profiles of the three most potent plant extracts, namely *M. nicaeensis*, *H. buxbaumii* and *A. italica*. The extracts of these plants exhibited a dose-dependent HSL inhibitory effect using a concentration range of 25 to 400  $\mu\text{g/ml}$ . The  $IC_{50}$  values were estimated from the best fit regression of the % of HSL inhibition vs extract concentration profiles. The  $IC_{50}$  values were about 51.1, 101.3 and 132.8  $\mu\text{g/ml}$  for *M. nicaeensis*, *H. buxbaumii* and *A. italica*, respectively.

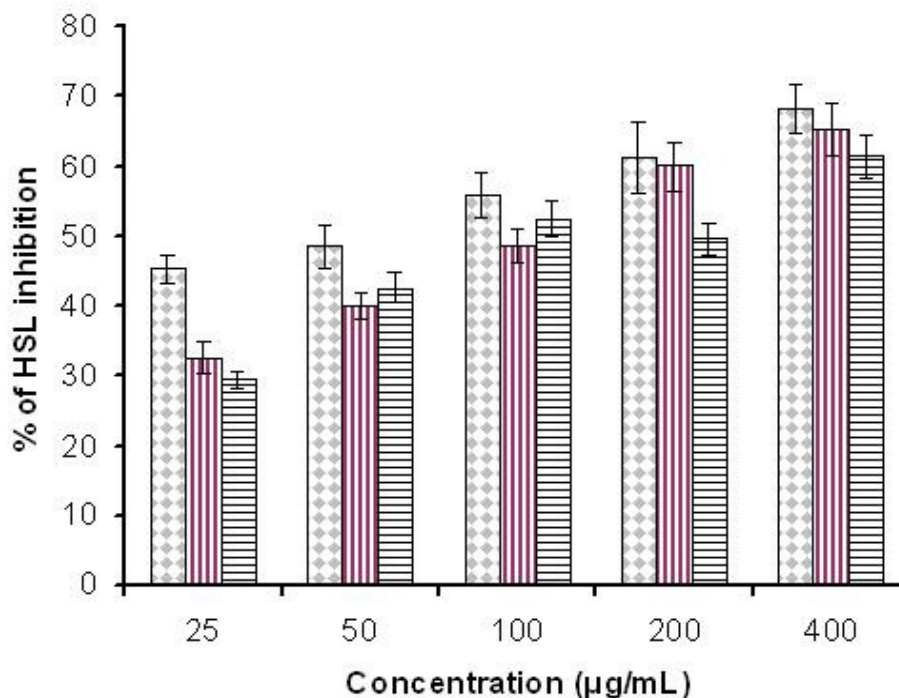
On the other hand, the extracts of 9 plants (Table 1) showed poor anti-lipase activity, with an inhibition range of 15 to 26%, and their  $IC_{50}$  values were not, accordingly, determined. The latter plants are *Cinnamomum verum* J. Presl (25.8% of inhibition), *Linum pubescens* Banks and Sol. (25.5%), *Hypocoum dimidiatum* Delile (25.0%), *Varthemia iphionoides* Boiss. and Blanche (23.6%), *Convolvulus althaoieds* L. (22.6%), *Paronychia argentea* Lam. (20.0%), *Eryngium creticum* Lam. (18.7%), *Majorana syriaca* (L.) Kostel (17.4%), *Adonis palaestina* Boiss (16.77%).

To the best of our knowledge, all the plants evaluated in this study, have not been screened before for their anti-HSL activity. Noteworthy, most of the studied plants whose extracts showed notable anti-HSL activities like *M. nicaeensis*, *A. nchusa*, *S. fruticosa*, *G. aleppicum*, *R. alba*, *M. spicata*, *O. natrix*, *C. coronarium*, and *H. triquetrifolium* have been previously reported to possess PL inhibitory activities (Bustanji et al., 2011). However, further chemical investigations are required for the identification of the potential active constituents responsible for the inhibition of either enzyme. Interestingly, many of the studied plants that showed notable HSL inhibition have been reported to have antidiabetic activities, for example, *A. italica*, *M. nicaeensis* (Liu, 2009), *C. Africana* (Yaniv et al., 1987), and *S. fruticosa* (Takamura et al., 2008). We believe that the inhibition of HSL participated, at least partially, in decreasing insulin resistance, which is responsible of the reported hypoglycemic activities. Further studies, however, are needed to verify these activities and identify the responsible active components.

Our results suggest that the plants, studied here, could serve as crude drugs for the treatment of insulin resistance and its consequence complications. However, further studies are needed, using animal models and / or cell lines, 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 plants.

## Conclusion

Natural products prepared from traditional medicinal plants have always presented an exciting opportunity for



**Figure 1.** The inhibitory effect of *M. nicaeensis* (diamond), *H. buxbaumii* (vertical lines) and *A. italica* (horizontal lines) extracts concentrations on the activity of hormone sensitive lipase.

the development of new types of therapeutics. Our results suggest that the rich potential of nature to combat insulin resistance has not been fully explored yet and many newer leads may be obtained from natural sources as, out of 29 screened plants, 12 of them were found to be potentially active against HSL. 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 anti-HSL functional agents.

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