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Original Research Article

Antilipolytic and hypotriglyceridemic effects of dietary *Salvia triloba* Lf (Lamiaceae) in experimental rats

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Abstract

Purpose: *Pancreatic triacylglycerol lipase (PL) is a noteworthy pharmacological target for the management of dyslipidemia, and diabetes and obesity. This study was aimed to evaluate the modulatory effects of Salvia triloba L.f. (Lamiaceae) leaves methanol extract (ME) on a high fat diet (HFD)-induced hypertriglyceridemia in rats, with complementary in vitro evaluation of sage PL-inhibitory potential.*

Methods: *Pre-induction of HFD hypertriglyceridemia sage leaves ME (750 mg/kg) was orally supplemented (via gastric intubation) to overnight fasting rats (n = 5). Potential plant modulation of PL was also quantified in vitro by a colorimetric assay (n = 3). For comparison, the effect of Orlistat was similarly evaluated as reference standard.*

Results: *Compared to Orlistat, supplementation of S. triloba at a dose of 750 mg/kg b.wt significantly reversed the HFD-induced postprandial hypertriglyceridemia in experimental overnight fasting rats ($p < 0.001$ vs. HFD rats). Dietary sage caused 66.4 % reduction in plasma triglycerides. Compared to Orlistat which exerted antilipolytic activity, with half-maximal inhibitory concentration (IC_{50}) of 0.114 ± 0.004 µg/mL), sage inhibited PL activity in vitro in a dose-dependent manner IC_{50} of 100.80 ± 9.07 µg/mL)*

Conclusion: *Sage has dual hypotriglyceridemic and antilipolytic properties which indicate that it can potentially be used to suppress body weight gain.*

Keywords: *Pancreatic lipase, Salvia triloba, Sage, Methanol extract, Hypertriglyceridemia, Orlistat*

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INTRODUCTION

Obesity and its cardiovascular comorbidities have increasingly become an alarming public health concern despite the remarkable progress in the discovery and development of novel obesity-diabetes therapeutics. For many years, plant-derived compounds have been at the forefront as important sources of phytotherapeutic and/or preventive agents due to plants' availability and relatively high safety [1,2]. Salvia as one of the largest genera in the family Lamiaceae is represented by more than 900 species. Several Salvia species are closely linked to Jordanian traditional medicine in the treatment of multiple ailments [3,4]. Sage (*Salvia triloba* L.f.) specifically is a medicinal plant widely used in Jordan both as a folk remedy and in the food and beverage industry [5].

Pancreatic lipase (PL) is a key enzyme for lipid breakdown that is necessary for the absorption of

dietary lipids [6]. The success of tetrahydrolipstatin (orlistat) which is a specific pancreatic lipase inhibitor has prompted research to identify new pancreatic lipase inhibitors derived from natural sources [7-9]. Natural compounds, like berberine, dihydroberberine and curcumin have substantially been proven as PL-inhibitors [10-11]. Phytochemically, the pentacyclic triterpenes, oleanolic acid and ursolic acid in *S. triloba* are established as attractive constituents with PL modulatory activities [12,13]. As a continuation of our interest in the phytoprinciples-based PL inhibitors with hypotriglyceridemic efficacies, the present study was designed to determine the in vivo postprandial plasma triglycerides lowering properties of *S. triloba* crude methanol extracts in high fat fed animals and the complementary investigation of its *in vitro* PL-inhibition capacities. For comparison, the effect of orlistat was similarly evaluated.

EXPERIMENTAL

Chemicals and instruments

Unless otherwise stated, all reagents and chemicals of analytical grade were procured from Sigma® (Dorset, UK). In the UV determinations, a UV-VIS spectrophotometer from SpectroScan® 80D (UK) was used. Reference drugs were purchased from local suppliers.

Plant collection and extraction

Aerial parts of *Salvia triloba* (sage) were procured from Amman, Jordan in the summer of 2014. Taxonomic identification of the plant was carried out by Professor Khaled Tawaha (Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan) and a voucher specimen deposited in the herbarium. Sage leaves were dried for three days and then ground into a fine powder. In a Soxhlet apparatus (Sigma-Aldrich, USA), the powder was subsequently extracted with absolute methanol for 72 h. The solvent was afterwards filtered and then evaporated using Rotavapor (Laborota 4000-efficient, Heidolph, Germany). The extract obtained was further left at 24 ± 0.5 ºC for 24 h in order to allow for complete drying and then stored in a refrigerator until used.

Preparation of *S. triloba* ME and orlistat for *in vitro* PL activity assay

Sage methanol extracts were initially dissolved in a Tris-HCl buffer (2.5 mM [Promega®, USA], pH 7.4 with 2.5 mM NaCl) to give five initial stock solutions with a concentration range of 0.3125 – 10.0 mg/mL. Subsequently, a 20 µL aliquot of each stock solution was used in the reaction mixture to give a final concentration range of 6.25 – 200 µg/mL. Extracts were prepared according to traditional use, so DMSO or any other organic solvent, even in a minimum concentration, was avoided [14].

Finally, orlistat, the reference drug (Hayat Pharmaceutical Industries Co. PLC, Jordan; 1 mg/mL DMSO), was prepared into six different stock solutions with a concentration range of 0.625 - 20 µg/ mL. Thereafter, a 20 µL aliquot of each stock solution was used in the reaction mixture to give a final concentration range of 0.0125 – 0.4 µg/mL.

Spectrophotometric quantification of PL inhibition by test extracts and orlistat

Pancreatic lipase activity was determined by measuring the rate of release of p-Nitrophenol (p-NP) from p-Nitrophenol butyrate (p-NPB). Enzyme assay was conducted by the spectrophotometric method as per the protocol from Al-Hallaq *et al* [8-9] and Bustanji *et al* [15]. Subsequent determinations were undertaken for the tested extracts and orlistat (n = 3) to calculate the concentration required for the PL 50 % inhibition (IC_{50}).

Animals

All experiments related to diet-induced lipid profile derangements (hypertriglyceridemia) were conducted with male Albino rats at the experimental animal laboratory at the University of Jordan. All animal experiments comply with the Guide for the Care and Use of Laboratory Animals 8th edition published by the US National Institute of Health [16,17].

The ethical approval for conducting animal studies was obtained from the ethical and scientific research council at the faculty of Pharmacy/ the University of Jordan (Ref No. 27/2/2010/3). Before the investigations, rats initially weighing 375 - 425 g (average 400 g) were housed in groups of five per cage with free access to tap water and proper pellet chow *ad libitum* at 25 ± 0.5 ºC with a relative humidity 50 – 60 % and a 12 h light/dark cycle [16].

As the acute oral toxicity studies were performed in overnight fasting animals, no abnormal behavior or mortality was evidenced at the sage dose range of 500 – 1000 mg/kg b.wt. Hence, the dose 750 mg/kg b.wt was selected as the therapeutic/preventive dose in the present study.

Experimental protocol

Overnight fasting rats were randomly divided into 4 groups with 5 animals each, assigned to:

Group I – Normal diet control (standard rat chow)

Group II – High fat (30%) diet (HFD) control [18]

Group III – Orlistat (5 mg/kg b.wt) + HFD

Group IV – Sage ME (750 mg/kg b.wt) + HFD

Except for group I, overnight fasting male Albino rats were fed a HFD following an initial supplementation with 1 mL of vehicle (20 % ethanol + 80 % water; group II), orlistat (5 mg/kg b.wt; group III), or sage ME (750 mg/kg b.wt; group IV) as per assigned to treatment groups via gastric intubation. Tail vein blood samples were collected under ether anesthesia and subsequently centrifuged for 5 min at 4000 rpm (round per minute).

Using an enzymatically commercial kit (Joaquim Costa®, Barcelona, Spain), postprandial HFD induced changes in plasma triglycerides were determined 2 h-post HFD administration.

Statistical analysis

Data are represented as mean ± standard deviation (SD, n = 3 – 5). Statistical differences between the control and different treatment groups were determined with Graphpad Prism® (version 3.02 for Windows; GraphPad Software, San Diego, CA, USA) using one way analysis of variance (ANOVA) followed by Newman Keuls' post test whenever appropriate. Values were considered significantly different if $p < 0.05$, and highly significantly different if $p < 0.01$ and $p < 0.001$.

RESULTS

Inhibitory effect of S. triloba ME on PL activity

The IC_{50} of Orlistat was 114.0 ± 4.0 ng/mL (equivalent to 0.2 ± 0.0 µM, n = 3). Compared to orlistat, a marked concentration-dependent PL inhibition trend was obtained from the *S. triloba* tested extracts. The inhibitory profiles of *S. triloba* leaves ME are shown in Figure 1. The IC_{50} of sage value obtained for a minimum of triple independent determinations was 100.8 + 9.07 µg/mL.

Effect of sage on postprandial plasma triglycerides in HFD rats

A promising hypotriglyceridemic influence of dietary sage at 750 mg/kg is observed in HFD-induced hypertriglyceridemic rats. Figure 2 demonstrates the effect of dietary *S. triloba* ME (750 mg/kg) on plasma triacylglyceride levels. Expectedly, plasma triglycerides (mg/dL) in HFD-fed animals are highly significantly greater than those in the control rats (217.8 ± 81.92 vs. 62.8 ± 14.53 respectively, n = 5 rats/group, $p < 0.001$, Figure 2).

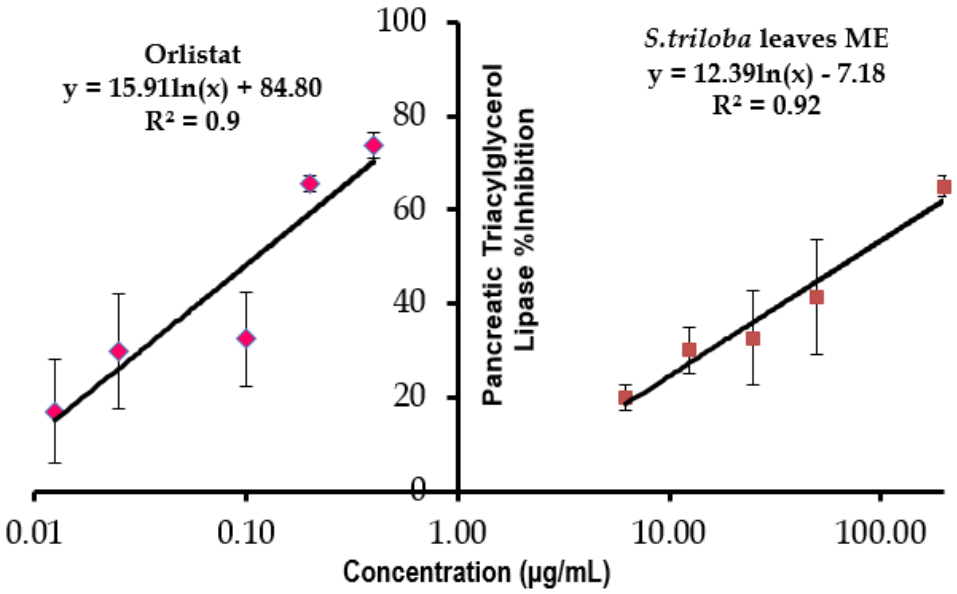

Figure 1: *In vitro* inhibitory effects of ascending concentrations (µg/mL) of *S. triloba* ME and orlistat on pancreatic triacylglycerol lipase activity. Results are mean ± SD (n = 3 independent replicates)

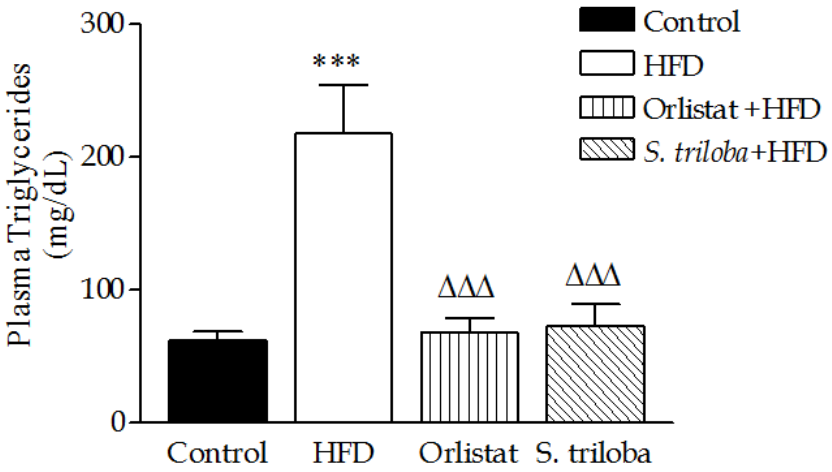

Figure 2: *In vivo* effects of dietary *S. triloba* ME (750 mg/kg b.wt) on postprandial hypertriglyceridemia in HFD-fed animals (n = 5). Results are expressed as mean ± SD of plasma triacylglycerides using ANOVA followed by Newman Keuls' post-test where ***P<0.001 compared to normal diet control group, $^{\Delta\Delta\Delta}$P<0.001 compared to HFD group

In orlistat- treated HFD-fed rats (group III), plasma triglycerides (mg/dL) are substantially less than those in untreated HFD-fed rats (68.1 ± 24.8 vs. 217.8 ± 81.9 respectively, n = 5 rats/group, $p < 0.001$, Figure 2) with a 68.8 % reduction. Also, postprandial hypertriglyceridemia (mg/dL) has been impressively normalized in group III as in the controls' (68.1 ± 24.8 vs. 62.8 ± 14.53 respectively, n = 5 rats/group, $p > 0.05$). Similarly, in the *S. triloba* 750 mg/kg b treated HFD-fed rats (group IV), plasma triglycerides (mg/dL) are highly markedly less than in those in HFD-fed animals (73.2 ± 36.8 vs. 217.8 ± 81.92 respectively, n = 5 rats/group, $p < 0.001$) with a 66.4 % reduction. Postprandial plasma triglycerides in group IV are comparable to controls' (73.2 ± 36.8 vs. 62.8 ± 14.53 respectively, n = 5 rats/group, $p > 0.05$) and orlistat-treated HFD animals' (73.2 ± 36.8 vs. 68.1 ± 24.77, n = 5 rats/group, $p > 0.05$). Taken together, these outcomes are perfectly aligned with *in vitro* PL inhibitory effects of sage.

DISCUSSION

Potential anti-obesity pharmacotherapeutics were intensely scrutinized as obesity was reaching alarming epidemic proportions globally [19]. Pharmacological intervention with natural product-based drugs is an effective and safe alternative for mitigating obesity. Pancreatic triacylglycerol lipase (PL) is an interesting pharmacological target for the management of dyslipidemia, atherosclerosis, and obesity-related dyslipidemia [19-21]. The orlistat PL-IC_{50} value obtained in this current study is comparable to the other reported PL-IC_{50} values [20]. These significant anti-lipase effects of sage may be solidly related to the effect of the major compounds identified in the crude extract [8,20] acting additively or synergistically in optimal ratio [22]. Hydrocarbons, sterols, triterpenes, fatty acids, phenolic acids, and flavonoids have been identified in *S. triloba*. *Salvia* species [23-25]. The presence of pentacyclic triterpenes (oleanolic acid, carnosic acid, and ursolic acid) in *Salvia* species may account for the plant PL inhibitory propensities [26-29]. All in all, pharmacological inhibition of dietary lipid digestion and absorption may induce favorable amelioration of dyslipidemia, atherosclerosis, and obesity. Impressively, pancreatic triacylglycerol lipase natural inhibitors offer the utility for adjuvant or alternative treatment to statins or orlistat as likely synergies can exist between new and established lipid-lowering drugs [30].

In a study performed by Mnafgui *et al* [31], it was found that the inhibitory action of PL leads to a decrease in lipid profiles. Our *in vitro* and *in vivo* findings combined are in agreement with those of Mnafgui *et al* [31]. As the occurrence of obesity is on the rise, various recent studies have been done regarding the treatment of obesity through the suppression of triglyceride accumulation by inhibiting the digestion of dietary lipids. This may minimize intestinal fat absorption with a remarkable body weight reducing influence countering the abdominal fat accumulation [32].

CONCLUSION

Sage extract can inhibit crucial gastrointestinal enzymes involved in lipid digestion and absorption, which indicates that sage is a potential phytotherapeutic/prophylactic strategy to control obesity-associated

hypertriglyceridemia. Future studies would focus on the *in vitro* and *in vivo* testing of therapeutic and/or preventive potentials of sage extract both alone and in combination with established pharmacological and natural agents used for the management of adiposity-related dyslipidemia and insulin resistance.

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