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

Shereen Arabiyat¹, Ashraf Al-Rabi’ee², Hiba Zalloum³, Mohammad Hudaib², Mohammad Mohammad² and Yasser Bustanji²*

¹Salt College, Balqa Applied University, Salt, ²Faculty of Pharmacy, The University of Jordan, ³Hamdi Mango Research Center for Scientific Research, The University of Jordan, Amman 11942, Jordan

*For correspondence: Email: bustanji@ju.edu.jo; Tel: +962 6 5355000 ext. 23359

Received: 23 June 2015 Revised accepted: 5 March 2016

**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 (IC₅₀) of 0.114 ± 0.004 µg/mL, sage inhibited PL activity in vitro in a dose-dependent manner IC₅₀ 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

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 tetrahydrodrolipstatin (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 °C 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 (IC50).

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 °C 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.
Arabiyyat et al

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](image1.png)

**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)
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
ACKNOWLEDGEMENT

The authors are grateful to Scientific Research Fund of Ministry of Higher Education (no. MPH/1/05/2014) and the Deanship of Academic Research at the University of Jordan (nos. 1630 and 1745) for funding this work. Mrs Jeannine Abusheikh is gratefully acknowledged for proofreading the manuscript.

REFERENCES

1. World Health Organization. Obesity and overweight. Fact sheet N 311; http://www.who.int/mediacentre/factsheets/fs311/en/. Accessed on Jan 2015
2. Kazemipoor M, Radzi CWJWM, Cordell GA, Yazi I. Potential of traditional medicinal plants for treating obesity: a review. International Conference on Nutrition and Food Sciences. IACSIT Press, Singapore. IPCBEE 2012; 39(2012): 1-6.
3. Al-El-Sawi DMH. Flora of Jordan Checklist; revised Edition 1. 2013: The University of Jordan Press, Amman, Jordan.
4. Afifi FU, Abu-Imraileh B. Herbal medicine in Jordan with special emphasis on less commonly used medicinal herbs. J Ethnopharmacol 2000; 72: 101-110.
5. Kasabri V, Afifi FU, Abu Dahab F, et al. In vitro modulation of metabolic syndrome enzymes and proliferation of obesity related-colorectal cancer cell line panel by Salvia species from Jordan. Rev Roum Chim 2014; 59: 693-705.
6. Thomson ABR, Schoeller C, Keelan M, Smith L, Clandinin MT. Lipid absorptions passing through the unstirred layers, brush-border membrane, and beyond. Can J Physiol Pharmacol 1993; 71(8): 531-555.
7. Hill JO, Hauptman J, Anderson JW, Fujioka K, O'Neil PM, Smith DK, Zavoral JH. Orlistat, a lipase inhibitor, for weight maintenance after conventional dieting: a 1-y study. Am J Clin Nutr 1999; 69(6): 1108-1116.
8. Al-Hallaq EK, Kasabri V, Abdalla SS, Bustanji YK, Afifi FU. Anti-obesity and antihyperglycemic effects of Crataegus aronia extracts: In vitro and in vivo evaluations. Food Nutr Sci. 2013; 4(2): 972-983.
9. Al-Hallaq EK, Litescu SC, Kasabri V, et al. Hypocholesterolemic effects of Adiantum capillus veneris L. aqueous extract in high cholesterol diet-fed rats and HPLC-MS determination of its polyphenolics. Rev Roum Chim 2015; 60: 357-365.
10. Mohammad M, Al-masri IM, Issa A, Khdair A, Bustanji Y. Inhibition of pancreatic lipase by berberine and dihydroberberine: an investigation by docking simulation and experimental validation. Med Chem Res 2013; 22(5): 2273-2278.
11. Mohammad M, Kasabri V, Zalloum H, Tayyem R, Aburish E, Al-Hiari Y, Bustanji Y. Antilipolytic property of curcumin: molecular docking and kinetic assessment. Rev Roum Chim 2015, in Press.
12. Kim J, Jang DS, Kim H, Kim JS. Anti-lipase and lipolytic activities of ursolic acid isolated from the roots of Actinidia arguta. Arch Pharmacal Res 2009; 32(7): 983-987.
13. Elouendi CB, Kuaté D, Ngondi JL, Ogen J. Anti-amylose, anti-lipase and antioxidant effects of aqueous extracts of some Cameroonian spices. J Nat Prod 2010; 3(2010): 165-171.
14. Gurbuz I, Ustun O, Yesilada E, Sezik E, Kutsal O. Anti-ulcerogenic activity of some plants used as folk remedy in Turkey. J Ethnopharmacol. 2003; 88(1): 93-97.
15. Bustanji Y, AlMasri I, Mohammad M, et al. Pancreatic lipase inhibition activities of trilactone terpenes of Ginkgo biloba. J Enzyme Inhib Med Chem 2011; 26: 453-459.
16. Guide for the Care and Use of Laboratory Animals 8th edition published by the US National Research Council of National Institute of Health. (https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-use-of-laboratory-animals.pdf)
17. Faranjeh M, Mohammad M, Bustanji Y, Alkhatab H, Abdalla S. Evaluation of immunosuppression induced by metronidazole in Balb/c mice and human peripheral blood lymphocytes. Int Immunopharmacol 2008; 8(2): 341-350.
18. Srinivasan K, Platel K, Rao MVL. Hypotriglyceremic effect of dietary vanillin in experimental rats. Eur J Food Res Technol 2008; 229(1): 103-108
19. Barete M. Targets for medical therapy in obesity. Dig Dis 2012; 30(2): 168-172
20. Mansi K, Abushofia AM, Disi A, et al. Hypolipidemic effects of seed extract of celery (Apium graveolens) in rats. Pharmccog Mag 2009; 5: 301-305.
21. Marrelli M, Loizzo MR, Nicoletti M, et al. In vitro investigation of the potential health benefits of wild Mediterranean dietary plants as anti-obesity agents with α-amylose and pancreatic lipase inhibitory activities. J Sci Food Agric 2014; 94: 2217-2224.
22. BrahmaNaidu P, Nemani H, Meriga B, Merkar SK, Potana S, Ramgopalrao S. Mitigating efficacy of piperine in the physiological derangements of high fat diet induced obesity in Sprague Dawley rats. Chem Biol Interact 2014; 221:42-51.
23. Bansal P, Paul P, Shankar G, et al. Flavonoid rich fraction of Pilea microphylla (L.) attenuates metabolic abnormalities and improves pancreatic function in C57BL/KsJ-db/db mice. Biomed Prev Nutr 2011; 1: 268-272.

Trop J Pharm Res, April 2016; 15(4): 727
24. el-Sayed NH, Khalifa TI, Ibrahim MT, Mabry TJ. Constituents from Salvia triloba. Fitoterapia 2001; 72(7): 850-853.
25. Haas C, Hengelhaupt KC, Kummritz S, Bley T, Pavlov A, Steingroewer J. Salvia suspension cultures as production systems for oleanolic and ursolic acid. Acta Physiol Plant 2014; 36(8): 2137-2147.
26. Wozniak L, Skaska S, Marszalek K. Ursolic acid - A pentacyclic triterpenoid with a wide spectrum of pharmacological activities. A review. Molecules 2015; 20: 20614-20641.
27. Chicco AG, D'Alessandro ME, Hein GJ, Oliva ME, Lombardo YB. Dietary chia seed (Salvia hispanica L.) rich in alpha-linolenic acid improves adiposity and normalises hypertriacylglycerolaemia and insulin resistance in dyslipaemic rats. Br J Nutr. 2009; 101(1): 41-50.
28. Ninomiya K, Matsuda H, Shimoda H, Nishida N, Kasajima N, Yoshino T, Morikawa T, Yoshikawa M. Carnosic acid, a new class of lipid absorption inhibitor from sage. Bioorg Med Chem Lett. 2004; 14(8): 1943-1946.
29. Kasabri V, Afifi FU, Abu-Dahab R, Mhaidat N, Bustanji YK, Abaza IF, Mashallah S. In vitro modulation of metabolic syndrome enzymes and proliferation of obesity related-colorectal cancer cell line panel by Salvia species from Jordan. Rev Roum Chim 2014; 60(10):1-10.
30. Wierzbicki AS, Hardman TC, Viljoen A. New lipid-lowering drugs: an update. Int J Clin Pract 2012; 66: 270-280.
31. Mnafgui K, Hamden K, Ben SH, et al. Inhibitory activities of Zygophyllum album: a natural weight-lowering plant on key enzymes in high-fat diet fed rats. Evid Based Complement Altern Med 2012; Article ID 620384, 9 pp.
32. Jeong SM, Kang MJ, Choi HN, et al. Quercetin ameliorates hyperglycemia and dislipidemia and improves antioxidant status in type 2 diabetic db/db mice. Nutr Res Prac 2012; 6: 201-207.