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Comparative evaluation of *Baccharis trimera, Pimpinella anisum* and statin on the biochemical profile of Wistar rats

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Abstract

Many are the plants for therapeutic purposes. *Baccharis trimera* is known to treat rheumatism, diabetes, and liver disorders. *Pimpinella asinum* is known to control colds, cough, bronchitis, fever, cramps and inflammation. The aim of this study was to evaluate the use of *B. trimera* and *P. anisum* and compare with statin effects on plasma lipids of Wistar rats. Sixty Animals were divided in control group (CG) and G2 (treated with *anise*), G3 (*B. trimera*) and G4 (statin). Plants and statin were administrated by intra-gastric route twice a day for 30 days. No modifications in glycaemia were observed in the experimental groups. Reductions were observed in cholesterol levels in treated groups. For LDL-c levels, significant differences were observed in G2 and G4. G3 showed significant reduction in the triglycerides levels and no significant differences were observed in the glycaemia in the studied groups. Increased levels of HDL-c were presented by the groups treated with the plants. The group treated with *B. trimera* showed significant reduction in triglycerides when compared to the control group. Our results suggest that the plants used in this work have similar effects in the lipid profile of Wistar rats when compared to the use of statin.

Keywords: *Pimpinella* anisum, *Baccharis trimera,* estatin, cholesterol, LDL-c, HDL-c

Introduction

The use of medicinal plants for therapeutic purposes is one of the mankind oldest forms of medical practice and many studies have been designed to show the effects of these plants in many pathologies. As chronic degenerative diseases such as cardiovascular disease, diabetes and obesity are among the leading causes of death in the modern world, they are used as models to investigate the effect of plants on the genesis and treatment of these diseases [1-2].

Baccharis trimera (popularly known in Brazil as "carqueja") is well known for its various medicinal properties and it is used to treat liver and kidney diseases, rheumatism, diabetes, and digestive disorders. Some studies have shown antioxidant, anti-inflammatory and antimutagenic properties, as well as, analgesic effects [3-8]. It presents apigenin, genkwanin, cirsimaritin, eupatorin, hispidulin, 7,4′-di-O-methyl-apigenin, luteolin, nepetin, quercetin, 3-O-methylquercetin and rutin as major flavonoid constituents, essential oils, saponins and diterpenoid [9-10].

Pimpinella anisum L. is another well-known plant much used by the population. Also known as anise, it has a strong sweet taste and aroma and is commonly used as a medication for the control of colds, coughs, bronchitis, fever, cramps, inflammation, bad digestion, and loss of appetite as well as a condiment [11]. Many biological effects of *Pimpinella anisum* have been identified. For example, it can display antiepileptic effects, antispasmodic and gastric protection [12-14]. It can also promote hypoglycemic effects and can reduce the effects of oxidative stress by reducing the action of free radicals [15].

Because of the wide range of applications of the above plants, this paper aims to evaluate their effects on the biochemical profile of Wistar rats and compare with those promoted by simvastatin, which is a synthetic lipid lowering drug and belongs to a class of drugs widely used in combating the deviations in lipid profile.

Materials and Methods

Experimental animals

Sixty male Wistar rats weighing approximately 250g were used. They were kept in the vivarium at UNIMAR (University of Marília) under a dark/light cycle of 12 hours, room temperature of 22 ± 2 C, and relative air humidity of 60 ± 5%. After a period of seven days of acclimation to the laboratory, the animals were divided randomly into 4 groups (n=15) treated for 30 days, as follows: G1 received water (Control Group); G2 received *P. anisum* by gavage route; G3 received *B. trimera* by gavage route and G4 that received statin also by intra-gastric route. Animals of all groups received water and food *ad libitum* during the treatment period.

This work was approved by the Animal Research Ethics Committee of the University of Marilia (UNIMAR) with registration number 2500000764/2007-47 de 18.01.2007. The

animals were treated according to the "*Guide for the Care and Use of Experimental Animals*" (that follows principles for the care of laboratory animals).

Preparation of aqueous extracts of *P. anisum* and *B. trimera*

The extracts of *P. anisum* seed and *B. trimera* leaf were prepared by infusion at a concentration of 5 g / mL. The extract was stored in amber glass vials of 30 mL and kept in the freezer for later use.

Preparation of Statin

Simvastatin were obtained in local pharmacy and the tablets were ground and mixed in water immediately before administration (5 g / mL).

Administration of the plants and statin

The administration of the plant extracts and statin (simvastatin) was done twice a day: in the early morning and late afternoon (according to the weight of animals) and the treatment lasted for 30 days. The administered doses were 200 μl of saline solution for GC and 0.3 mg / kg of aqueous extract of the plants (G2 and G3) and statin (G4).

Collection of blood samples and determination of the biochemical profile and Atherogenic Index (AI)

After 30 days of treatment, the animals were anesthetized with Thiopental (sodium pentobarbital) until complete sedation, after which blood samples were drawn to determine their biochemical profile: total cholesterol, LDL-c, HDL-c, triglycerides and glycaemia. The glucose and lipid levels were measured in mg/dL.

The exams were performed at the Clinical Analysis Lab of the University Hospital of UNIMAR (São Francisco Labortory) and the results were interpreted according to the ADA [16].

Atherogenic Index (AI) was calculated after Schulpis, Karikas [17] and also used by Munshi, Joshi, Rane [18]: AI = (Total cholesterol – HDL-c)/HDL-c.

Statistical analysis

Data analyses were performed using Analysis of Variance (ANOVA) and complemented by the Tukey test at significance level of 5%.

Results and Discussion

Table 1 shows the statistical results from the biochemical parameters in the studied groups (G1-G4). Groups treated with plants and statin showed significant reduction on the cholesterol levels. For HDL-c levels, no significant differences between the control group (G1) and the group treated with statin (G4) were observed, but there was a significant increase of this parameter in animals treated with *P. anisum* (G2) and *B. trimera* (G3). For LDL-c levels, significant differences were observed in G2 and G4 when compared to control group. G3 showed significant reduction in the triglycerides levels and no significant differences were observed in the glycaemia in the studied groups.

Table 1- Results of the biochemical profile (mg/mL) of G1, G2, G3 and G4. Control group (G1), group treated with *Pimpinella anisum* (G2), *Baccharis trimera* (G3) and statin (G4).

	G1	G2	G3	G4
Cholesterol p = 0,0059	89,18 ± 4,09A(1)	78,25 ± 4,81B	76,31± 6,51B	78,59 ± 5,20B
HDL-c p = 0,0000	21,00 ± 1,61A	30,33 ± 1,50C	24,31± 1,49B	21,83 ± 2,79A
LDL-cp = 0,0000	34,93 ± 4,61B	21,37 ± 7,11A	33,77± 6,63B	25,57± 6,29A
Triglycerides p = 0,0014	118,00 ± 14,62BC	122,50 ± 22,76BC	91,15± 18,33A	105,17± 21,20AB
Glucose p = 0,1303	75,18 ± 4,09A	78,25 ± 4,81A	76,31± 6,51A	85,00 ± 11,20A

(1)Means followed by at least one same letter do not differ statistically
(Analysis of Variance complemented by the Tukey test at significance level of 5%).

Our results exhibit that animals treated with *B. trimera* and *P. anisum* showed no change in blood glucose values. Tirapelli et al [13] evaluated the effects of *B. trimera* on glycaemia in diabetic and non-diabetic rats and their results suggested that this plant has anti-diabetic activity. Similar effects were found by Oliveira et al [5], Dickel *et al* [19] and Trojan-Rodrigues *et al*[6]. In the literature no studies about the effect of *P. anisum* aqueous extract in relation to glycaemia were found, although Kreydiyyeh et al [20] found that aniseed oil increases glucose absorption by increasing the activity of the Na+-K+ ATPase and consequently the sodium gradient needed for the sugar transport in rats. Saddala et al [21] studied the aqueous extract of *P. tirupatiensis* tuberous root on cardiac oxidative stress and lipid peroxidation in non-diabetic and streptozotocin-induced diabetic rats and found that this extract promoted normalization of glutathione and xanthine oxidase activity in diabetic animals and possible protects heart against oxidative damages promoted by hyperglycaemia. Lee et al [15] studied the effects of ethanol

extract of *P. brachycarpa* in diabetic mice and concluded that this plant significantly reduces levels of thiobarbituric acid reactive substances. It also increases superoxide dismutase, catalase and glutathione peroxidase activity what can be useful to control glucose levels and oxidative stress what is related to heart diseases.

Our results also show that the lipid profile can present similar or better results when animals are treated with the studied plants when compared to those treated with statin in the experimental period of this work. These effects can be explained by the presence of antioxidants and flavonoids, which are also recognized by their effects on the lipid profile. The main antioxidants from *P. anisum* are terpenoids such as linalool, terpinene-4-ol, -terpineol, p-anisaldehyde, foeniculin, anethole, trans-anethole, trans-pseudoisoeugenila, 2-metillbutirate, para-anisaldehyde, estragol and metilcavicol [11, 22-23]. *B. trimera* also possesses bioactive compounds that may be related to the effects observed in the lipid profile of the studied animals. These compounds include flavones, flavonols, saponins and diterpenes, and phenolics such as apigenin, cirsimaritina, eupatorina, genkwanina, hispidulin, isoquercitina, luteolin, nepetin, quercetin and rutin compounds. Among terpenoid compouds it contains mainly saponins. The presence of these compounds also gives to *B. trimera* antioxidant, anti-inflammatory, analgesic and muscle relaxing effects and liver protection [9, 23-26]. Muneera, Majeed, Naveed [27] compared the effects of using simvastatin and *Nigella sativa* to treat hyperlipidemia and found that both promoted significant and comparable improvement in the lipid profile of rats after treatment.

Low HDL-c levels contrasted with elevated plasma concentrations of triglycerides and LDL-c are related to the metabolic syndrome and increased risk of cardiovascular events and death [28-29]. In order to improve the lipid profile, doctors commonly make prescription of statins to their patients. These drugs are structural inhibitors of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA) that is a limiting enzyme in hepatic cholesterol biosynthesis, resulting in upper regulation of LDL-c receptors which lead to a decrease in LDL-c, total cholesterol and triglycerides levels [16]. However, the use of these drugs may be associated to adverse effects, such as liver damage, development of collateral circulation, stenosis, activation of nitric oxide synthase and increased incidence of diabetes. Moreover, these drugs are not recommended in patients with acute or chronic liver disease what leads patients to seek alternative treatments [30-34].

Table 2 brings the results to the calculation of the Atherogenic Index that is associated with the deposition of foam cells, plaque, fatty infiltration or lipids in the heart, coronaries, aorta or in the liver. It may be also related to the size of the pro and anti-atherogenic lipoprotein particles. When AI increases, there is also an increase in the risk of oxidative stress damages and it is an indication of cardiovascular risk [18]. Our results show that the plants exhibit lower AI than control and the group treated with the simvastatin showing they may have protective effects for cardiovascular diseases probably due to the presence of bioactive compounds described above.

Table 2- Atherogenic Index (AI) in groups G1, G2, G3 and G4. Control group (G1), group treated with *Pimpinella anisum* (G2), *Baccharis trimera* (G3) and statin (G4).

	G1	G2	G3	G4
AI	3,25	1,58	2,14	2,60

This work shows important results in the lipid profile after using *P. anisum* and *B. trimera*. This combination of decreasing LDL-c and triglycerides levels and increase HDL-c levels is the desired goal for any treatment for dyslipidemia and metabolic syndrome. Furthermore, the use of plants and its association with the presence of flavonoids and other antioxidants can bring numerous benefits to the body, such as reducing or inhibiting lipid peroxidation, which is a factor implicated in many diseases, including cardiovascular diseases. Padua et al [35] showed that the use of *B. trimera* can act by increasing the antioxidant defense system and decreasing the effects of reactive species. *P. anisum* also exert antioxidant effects because of its potential in reducing nitric oxide radicals and hydrogen and may show metal chelating activities, what are related to the prevention of oxidative damages and their correlated diseases such as diabetes, metabolic syndrome and cardiovascular diseases. Paiva et al [3] found that *B. trimera* has anti-oxidant and neuro-protective effects.

Conclusions

Our findings suggest that *B.* trimera and P. anisum have potential to be used to control lipid levels. Despite the prospect of the use of medicinal plants is benefic for the population, clinical and laboratory studies in humans are needed to analyze the positive and possible side effects.

Authors' contributions

SMB, ACQ and ELG carried out the conception and design of the study, treated the animals and drafted the manuscript.
PCSB performed the statistical analysis.
MSSS prepared the extracts of the plants and treated the animals.
CGM performed the laboratorial analysis.
All authors read and approved the final manuscript.

References

[1]. Lee CY, Lee MJ, Kim JM, Choi SM, Kang KK, Kim OJ, Lee BM. Oryza sativa L. extracts inhibit nitric oxide production and inducible nitric oxide synthase expression in murine macrophage cells and lungs of antigen-challenged allergic mice. Int Journalof Phytomedicine 2015; 7 (1) 01-07.

[2]. Ghosh S, Ahire M, Patil S, Jabgunde A, Bhat Dusane M, Joshi BN, Pardesi K, Jachak S, Dhavale DD, Chopade BA. Antidiabetic Activity of Gnidia glauca and Dioscorea bulbifera: Potent Amylase and Glucosidase Inhibitors. Evid Based Complement Alternat Med 2012;2012:929051. doi: 10.1155/2012/929051.

[3]. Paiva AF, Bonomo LF Boasquivis PF, Paula ITBR, Guerra JF, Leal WM, Silva ME, Pedrosa ML, Oliveira RP. Carqueja (Baccharis trimera) Protects against Oxidative Stress and β-Amyloid-Induced Toxicity in Caenorhabditis elegans. Oxid Med Cell Longev 2015; 2015:740162. doi: 10.1155/2015/740162.

[4]. Pádua B C, Rossoni Júnior JV, Magalhães CL, Chaves MM, Silva ME, Pedrosa ML, de Souza GH, Brandão GC, Rodrigues IV, Lima WG, Costa DC. Protective effect of Baccharis trimera extract on acute hepatic injury in a model of inflammation induced by acetaminophen. Mediators Inflamm 2014; 2014:196598. doi: 10.1155/2014/196598.

[5]. Oliveira CB, Comunello LN, Lunardelli A, Amaral RH, Pires MG, da Silva GL, Manfredini V, Vargas CR, Gnoatto SC, de Oliveira JR, Gosmann G. Phenolic enriched extract of Baccharis trimera presents anti-inflammatory and antioxidant activities. Molecules 2012; 17(1):1113-23.

[6]. Trojan-Rodrigues M, Alves TL, Soares GL, Ritter MR. Plants used as antidiabetics in popular medicine in Rio Grande do Sul, southern Brazil. J Ethnopharmacol 2012;139 (1):155-63. Review.

[7]. Mendes FR, Tabach R, Carlini EA. Evaluation of Baccharis trimera and Davilla rugosa in tests for adaptogen activity. Phytother Res 2007, 21 (6): 517-522.

[8]. Borella JC, Duarte DP, Novaretti AAG, Menezes Jr A, França SC, Camila B. Rufato CB, Santos PAS, Rodrigo C.S. Veneziani RCS, Lopes NP. Seasonal variability in the content of saponins from Baccharis trimera (Less.) DC (Carqueja) and isolation of flavone. Rev Bras Farmacogn 2006; 16(4): 557-561.

[9]. Oliveira CB, Comunello LN, Maciel ES, Giubel SR, Bruno AN, Chiela EC, Lenz G, Gnoatto SC, Buffon A, Gosmann G. The inhibitory effects of phenolic and terpenoid compounds from Baccharis trimera in Siha cells: differences in their activity and mechanism of action. Molecules 2013;18(9):11022-32.

[10]. Silva, F G, Oliveira CBA, Pinto J E B P; Vivian E. Nascimento V E, Santos S C, Seraphin J C, Pedro H. Ferri P H 2007. Seasonal variability in the essential oils of wild and cultivated Baccharis trimera. J Braz Chem 18 (5): 990-997.

[11]. Koeduka T, Baiga TJ, Noel JP, Pichersky E. Biosynthesis of t-anethole in anise: characterization of t-anol/isoeugenol synthase and an O-methyltransferase specific for a C7-C8 propenyl side chain. Plant Physiol 2009; 149(1): 384-94.

[12]. Janahmadi M, Farajnia S, Vatanparast J, Abbasipour H, Kamalinejad M. The fruit essential oil of Pimpinella anisum L. (Umblliferae) induces neuronal hyperexcitability in snail partly through attenuation of after-hyperpolarization. J Ethnopharmacol 2008; 20(3):360-5.

[13]. Tirapelli CR, de Andrade CR, Cassano AO, De Souza FA, Ambrosio SR, da Costa FB, de Oliveira AM. Antispasmodic and relaxant effects of the hidroalcoholic extract of Pimpinella anisum (Apiaceae) on rat anococcygeus smooth muscle. J Ethnopharmacol 2007;110(1):23-9.

[14]. Al Mofleh I A, Alhaider AA, Mossa JS, Al-Soohaibani MO, Rafatullah S. Aqueous suspension of anise [quot]Pimpinella anisum [quot] protects rats against chemically induced gastric ulcers. World J Gastroenterol 2007; 13(7):1112-8.

[15]. Lee SJ1, Choi HN1, Kang MJ1, Choe E2, Auh JH3, Kim JI1. Chamnamul [Pimpinella brachycarpa (Kom.) Nakai] ameliorates hyperglycemia and improves antioxidant status in mice fed a high-fat, high-sucrose diet. Nutr Res Pract 2013;7(6):446-52.

[16]. American Diabetes Association. Standards of medical care in diabetes--2014. Diabetes Care 2014;37 Suppl 1:S14-80. doi: 10.2337/dc14-S014.

[17]. Schulpis K, Karikas GA. Serum cholesterol and triglyceride distribution in 7767 school-aged Greek children. Pediatrics. 1998 May;101(5):861-4.

[18]. Munshi RP, Joshi SG1, Rane BN. Development of an experimental diet model in rats to study hyperlipidemia a ndinsulin resistance, markers for coron ary heart disease. Indian J Pharmacol. 2014 May-Jun;46(3):270-6. doi: 10.4103/0253-7613.132156.

[19]. Dickel ML1, Rates SM, Ritter MR. Plants popularly used for loosing weight purposes in Porto Alegre, South Brazil. J Ethnopharmacol 2007;109(1):60-71.

[20]. Kreydiyyeh SI, Usta J, Knio K, Markossian S, Dagher S. Aniseed oil increases glucose absorption and reduces urine output in the rat. Life Sci 2003;74(5):663-73.

[21]. Saddala RR, Thopireddy L, Ganapathi N, Kesireddy SR. Regulation of cardiac oxidative stress and lipid peroxidation in streptozotocin-induced diabetic rats

treated with aqueous extract of Pimpinella tirupatiensis tuberous root. Exp Toxicol Pathol 2013 Jan;65(1-2):15-9. doi: 10.1016/j.etp.2011.05.003.

[22]. Jamshidzadeh A, Heidari R, Razmjou M, Karimi F, Moein MR, Farshad O, Akbarizadeh AR, Shayesteh MR. An in vivo and in vitro investigation on hepatoprotective effects of Pimpinella anisumseed essential oil and extracts against carbon tetrachloride-induced toxicity. Iran J Basic Med Sci 2015; 18(2):205-11.

[23]. Shojaii A, Abdollahi Fard M. Review of Pharmacological Properties and Chemical Constituents of Pimpinella anisum. ISRN Pharm 2012; 2012:510795. doi: 10.5402/2012/510795.

[24]. Simões-Pires CA, Queiroz EF, Henriques AT, Hostettmann K. Isolation and on-line identification of antioxidant compounds from three Baccharis species by HPLC-UV-MS/MS with post-column derivatisation. Phytochem Anal 2005; 16(5): 307-14.

[25]. Padua B C, Rossoni Júnior JV, Magalhães CL, Chaves MM, Silva ME, Pedrosa ML, de Souza GH, Brandão GC, Rodrigues IV, Lima WG, Costa DC. Protective effect of Baccharis trimera extract on acute hepatic injury in a model of inflammation induced by acetaminophen. Mediators Inflamm 2014; 2014:196598. doi: 10.1155/2014/196598.

[26]. Soicke H, Leng-Peschlow E. Characterization of flavonoids from Baccharis trimera and their antihepatotoxic properties. Planta Med 1987; 53(1):37-9.

[27]. Muneera KE, Majeed A, Naveed AK. Comparative evaluation of Nigella sativa (Kalonji) and simvastatin for the treatment of hyperlipidemia and in the induction of hepatotoxicity. Pak J Pharm Sci 2015;28(2):493-8.

[28]. Kapourchali FR, Surendiran G, Goulet A, Moghadasian MH. The Role of Dietary Cholesterol in Lipoprotein Metabolism and Related Metabolic Abnormalities: A Mini-review. Crit Rev Food Sci Nutr 2015 Jun 9:0. [Epub ahead of print].

[29]. Baibata D, Ionescu G, Petcov B, Mancas S. Non-High-Density Lipoproteins Cholesterol and Cardio-Metabolic Risk. Maedica (Buchar) 2015; 10(1):33-8.

[30]. Sirtori CR. The pharmacology of statins. Pharmacol Res 2014; 88:3-11. doi: 10.1016/j.phrs.2014.03.002.

[31]. Macedo AF, Taylor FC, Casas JP, Adler A, Prieto-Merino D, Ebrahim S. Unintended effects of statins from observational studies in the general population: systematic review and meta-analysis. BMC Med 2014;12:51. doi: 10.1186/1741-7015-12-51.

[32]. Covelli D, Vannucchi G, Campi I, Currò N, D'Ambrosio R, Maggioni M, Gianelli U, Beck-Peccoz P, Salvi M. Statins may increase the risk of liver dysfunction in patients treated with steroids for active graves' orbitopathy. J Clin Endocrinol Metab 2015; 100(5):1731-7.

[33]. Ovbiagele B, Buck BH, Liebeskind DS, Starkman S, Bang OY, Ali LK, Villablanca JP, Salamon N, Yun SW, Pineda S, Saver JL 2008. Prior antiplatelet use and infarct volume in ischemic stroke. J Neurol Sci 15; 264(1-2): 140-4.

[34]. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002; 346(16): 1221-31.

[35]. Padua BC, Rossoni Junior JV, de Brito Magalhaes CL, Seiberf JB, Araujo CM, Bianco de Souza GH, Chaves MM, Silva ME, Pedrosa ML, Costa DC. Baccharis trimera improves the antioxidant defense system and inhibits iNOS and NADPH oxidase expression in a rat model of inflammation. Curr Pharm Biotechnol 2014; 14(11):975-84.