Evaluation of hypocholesterolemic activity of extracts of *Bidens odorata* and *Brickellia eupatorioides*

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Abstract: We sought to evaluate the hypolipidemic activity of extracts of *Bidens odorata* and *Brickellia eupatorioides* using a model of hyperlipidemia induced in rats by Triton WR-1339 (300mg/kg intraperitoneally). The rats were divided into 5 groups of 3 rats each: normal control group, hyperlipidemic control group, hyperlipidemic with 20 mg/kg atorvastatin, hyperlipidemic with 300 mg/kg *B. odorata* extract, and hyperlipidemic with 300mg/kg *B. eupatorioides* extract, respectively. After 10 d of treatment by intragastric administration, the extract of *B. odorata* caused a significant decrease of serum total cholesterol and triglyceride levels without altering the liver enzymes aspartate transaminase and alanine aminotransferase. In addition, the extract had antioxidant potential as shown by the 2,2′-diphenyl-1-picrylhydrazyl technique. These findings indicate that *B. odorata* has potential as a hypolipidemic agent and might be beneficial in treatment of hyperlipidemia and atherosclerosis.

Keywords: Cholesterol, hypercholesterolemia, plant extracts, triglycerides.

INTRODUCTION

Obesity has reached proportions of being considered a pandemic (Kopelman 2000). In 2014, 1.9 billion people worldwide were estimated as being overweight and 600 million obese (Chestnov *et al*., 2014).

Obesity is associated with the appearance of other diseases, including cardiovascular diseases. At present, these diseases, according to reports of the World Health Organization (WHO), are the main cause of mortality in the world.

Hyperlipidemia is the main cause of atherosclerosis, a disease that begins with the deposition of low density lipids (LDLs) on the wall of the arteries. These lipids are attacked by reactive oxygen species (ROS) causing the release of chemokines and inflammatory cells, finally resulting in the formation of an atherosclerotic plaque (Weber, 2011).

There are several drugs for the treatment of hyperlipidemia such as fibrates, niacin, bile acid sequestrants, and inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) called statins, the latter being the most commonly used (Tiwari and Khokhar, 2014).

Despite the existence of various alternatives for the treatment of high blood lipid levels, a large percentage of patients with this disease do not receive proper treatment for the control of the levels of lipids in the blood, thus increasing the risk of cardiovascular problems such myocardial infarction and stroke (WHO, 2011).

In the search for new hypolipidemic agents, various plants have been evaluated. In particular, some plants in the family Asteraceae have demonstrated effects on reducing serum lipids (Wider *et al*., 2002; Bahar, 2016; Hong *et al*., 2012). In addition, various plants of this family have an important antioxidant activity (Kenny *et al*., 2014; Teugwa *et al*., 2013; Dewan *et al*., 2013), and other biological activities such as hypoglycemic (Palacios *et al*., 2008; Abdullahi *et al*., 2015) and hepatoprotective effects (Achika *et al*., 2014; Syed *et al*., 2014).

In Northeast Mexico there is a great diversity of plants in the family Asteraceae (Villaseñor, 2004). These include *Bidens odorata* and *Brickellia eupatorioides.*

B. odorata, popularly known as aceitilla, is used in Mexican folk medicine to treat gastrointestinal and kidney disorders, and for the treatment of diabetes (Astudillo-Vázquez *et al*., 2015). An aqueous extract has been assessed for diuretic activity (Camargo *et al*., 2004), and a chloroformic extract for antidiarrheal activity, where various fatty acids (oleic, palmitic, linoleic, and stearic acids) in the active fraction of the extract were identified. Kaempferol, quercetin, and flavonoids quercetin and luteolin have been isolated from *B. eupatorioides*, popularly known as the false eupatorium (Wollenweber *et al*., 1996). However, there are no reports of any pharmacological activity. Other species of the same genus of this plant, *Brickellia cavanillesii* and *Brickellia veronicaefolia*, have been shown to possess hypoglycemic activity (Escandon *et al*., 2012; Pérez *et al*., 2000).

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This study aimed to evaluate antihyperlipidemic activity and antioxidant capacity of *B. odorata* and *B. eupatorioides* in an animal model of hyperlipidemia in Wistar rats.

MATERIALS AND METHODS

Reagents

Triton WR-1339 (Tyloxapol), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and quercetin (Sigma). Atorvastatin (Lipitor, Pfizer). The biochemical parameters were determined using commercial kits for laboratory IL Test Instrumentation Laboratory (Italy).

Plant material

Plants were collected in 2014. *B. eupatorioides* in Galeana, Nuevo Leon (Mexico) (25°39′08.23″N, 100°42′40.14″W, 1169m) and *B. odorata* in Saltillo, Coahuila (Mexico) (25°39′08.23″N, 100°42′40.14″W, 1169 m) were identified in the Department of Botany at the School of Biological Sciences at the University Autónoma de Nuevo Leon and assigned voucher numbers: 26846 (*B. eupatorioides*) and: 16378 (*B. odorata*).

Plants were washed with distilled water and dried in the shade at room temperature for 7d. They were triturated in a manual mill and the extracts were obtained using a Soxhlet apparatus with ethanol as the solvent system.

Phytochemical screening of plant extracts

Ethanolic extracts of plants under study underwent a preliminary phytochemical screening to determine the presence of carboxyl groups (sodium bicarbonate), phenolic hydroxyls (ferric chloide), saponins (foam formation), flavonoids (Shinoda), carbohydrates (Antrona), unsaturated hydrocarbons (potassium permanganate), sterols and triterpenes (Liebermann-Burchard), coumarins (Ehrlich), alkaloids (Dragendorff), sesquiterpene lactone (Baljet), quinones (Bornträger), and carbonyls (2, 4-dinitrophenylhidrazine). (Verde *et al*., 2016).

Antiradical activity assay using 2,2-diphenyl-2-picrylhydrazyl (DPPH)

DPPH was prepared at a concentration of 125µM and stored in darkness. Solutions of each extract at 1mg/mL in ethanol were prepared as serial 1:2 dilutions. To each dilution was added 0.5mL 125µM DPPH for a total reaction volume of 1mL, allowed to stand for 30 min in darkness and the optical density at 517 nm was measured in a spectrophotometer (Jenway 320d). As a positive control we used quercetin 1mg/mL and ethanol was used as a negative control. With the data obtained, we calculated the reduction ratio for each of the different dilutions using the following equation.

$$\%\text{Reduction} = \frac{(\text{absorbance negative control}) - (\text{absorbance of sample})}{(\text{Absorbance negative control})} \times 100$$

The reduction rates, plotted versus the concentration. The equation of the line was calculated from the graph and the necessary concentration was obtained for a 50% reduction (IC_{50}).

Experimental animals

This study was conducted after approval from the ethics committee of the Faculty of Medicine of the Universidad Autónoma de Nuevo Leon (Docket No. HI14-003) following the provisions of the Official Mexican Standard NOM-062-ZOO-1999 technical specifications for the production, care, and use of laboratory animals. The rats were housed in polycarbonate cages in conditions of appropriate temperature and humidity and under cycles of 12 h/light, 12 h/dark and with free access to water and standard food for rodents (Prolab diet 2500).

Animal model of hyperlipidemia

Male Wistar rats 250-200g were divided randomly into 5 groups of 3 rats. Group 1: normal control; Group 2: Triton WR-1339 treatment, Group 3: atorvastatin treatment, Group 4: *B. odorata* extract treatment, and Group 5: *B. eupatorioides* extract treatment. Rats in groups 1 and 2 were administered distilled water intragastrically for 10 d, atorvastatin group 3 (20mg/kg), the group 4 BO extract (300mg/kg) and group 5 BE extract (300mg/kg) (Moreno-Peña *et al.,* 2016). On the 10th day, rats in group 1 were treated with saline intraperitoneally and groups 2, 3, 4 and 5 with Triton WR-1339 300mg/kg. Tail blood was obtained 24 h after administration of Triton WR-1339 and saline solution to the respective groups. Blood samples were centrifuged at 3300rpm for 10min to separate the serum, in which the concentrations of cholesterol, triglycerides and liver enzymes aspartate transaminase (AST) and alanine aminotransferase (ALT) were determined.

The animals in group 4 and group 5 were given BO extract and BE extract at the dose of 300mg/kg. It is recommended that antihyperlipidemic effects of *B. odorata* extract and *B. eupatorioides* extract should also be tested at different doses.

Cholesterol analysis in hepatic tissue

Liver tissue of rats from the different groups was extracted 24h after the administration of Triton WR-1339, and preserved at -80°C until their analysis. The total cholesterol was measured by following the instructions in the total cholesterol assay kit (Cell Biolabs).

STATISTICAL ANALYSIS

ANOVA was performed to determine significant differences in reduction of serum lipids in rats from different groups using MiniTab version 17 software. A $p< 0.05$ was considered significant.

Table 1: Phytochemical screening of plant extracts

Chemical type	*B. odorata*	*B. eupatorioides*
Carboxyl group	+	–
Phenolic hydroxyls	+	+
Saponins	–	–
Flavonoids	+	+
Carbohydrates	+	+
Unsaturated hydrocarbons	+	+
Sterols and triterpenes	+	+
Coumarins	+	+
Alkaloids	+	+
Sesquiterpene lactones	–	+
Quinones	–	+

RESULTS

Phytochemical profile of the plants

The Phytochemical screening of plant extracts it is presented in the table 1.

Table 2: Antiradical activity by DPPH. Results expressed ± DS. Quercetin was used as positive control

Treatment	IC_{50}µg/mL
B. odorata	55.92±3.5
B. eupatorioides	46.49±1.8
Quercetin	3.29±2.1

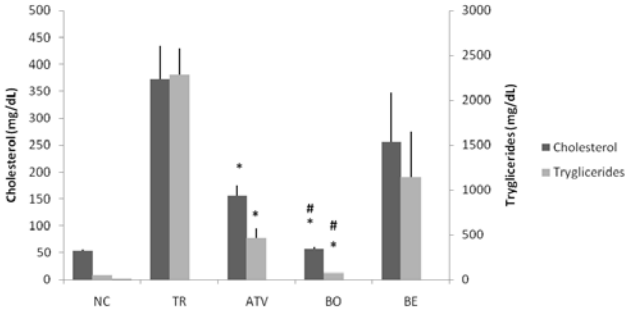

Fig. 1: Comparison of serum lipids in the experimental groups after 24h of administration of Triton WR-1339. Results expressed as mean ± SD. **p*<0.05 vs Triton WR-1339 group. #*p*<0.05 vs atorvastatin. NC: Normal control, TR: Triton WR-1339 group, ATV: Atorvastatin group, BO: *B. odorata* group, BE: *B. eupatorioides* group.

Antiradical activity by DPPH

Plants under study showed antiradical capacity with an IC_{50} of 46.49±1.84 and 55.92±3.51µg/mL for *B. eupatorioides* and *B. odorata* respectively. Quercetin was used as a positive control (table 2).

Antihyperlipidemic activity

The rats in the group treated with Triton WR-1339 had significantly increased serum lipid levels (cholesterol 372.3±61.6mg/dL and triglycerides 2289.0±284.9mg/dL) compared with normal control (cholesterol 53.7±2.1 mg/dL and triglycerides 51.3±4.7mg/dL), AST only was significantly increased, compared with rats in the normal control group. Rats in the group treated with atorvastatin had significantly reduced cholesterol (155.7± 20.5 mg/dL) and triglycerides (467.0±102.4mg/dL) compared with rats from the group treated with Triton WR-1339, while AST and ALT were increased more than they were in rats in the group treated with Triton WR-1339 and the healthy control group. *B. odorata* extract reduced cholesterol (57.7±3.0mg/dL) and triglycerides (73.7±1.5mg/dL) compared with the Triton WR-1339 group. However, no increase in liver enzymes was observed in rats from the group treated with atorvastatin. Rats in the group treated with *B. eupatorioides* extract did not have significantly reduced levels of serum lipids compared with rats from the group treated with Triton WR-1339 (figs. 1 and 2).

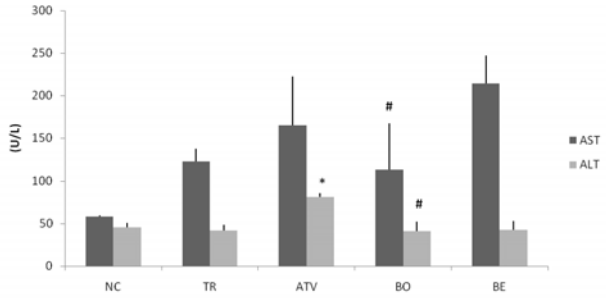

Fig. 2: Comparison of hepatic transaminases in the experimental groups 24h after administration of Triton WR-1339. Results are expressed mean ± SD. **p*<0.05 vs Triton WR-1339 group. #*p*<0.05 vs atorvastatin. NC: Normal control, TR: Triton WR-1339 group, ATV: atorvastatin group, BO: *B. odorata* group, BE: *B. eupatorioides* group.

Cholesterol in the liver tissue

No differences were found in the cholesterol content of the samples from the normal control group (total cholesterol 145.67±16.2, free cholesterol 125.4±21.7), samples of the Triton WR-1339 group (total cholesterol 171.63±44.0, free cholesterol 177.4±84.1) and those treated with the extract of *B. odorata* (total cholesterol 204.44±105.3, free cholesterol 152.1± 87.7)

DISCUSSION

In Mexico, obesity is a public health problem. Studies in the Mexican population indicate a direct relationship between the degree of obesity and level of blood cholesterol (Lara *et al*., 2004). High levels of cholesterol and triglycerides in the blood are considered an important risk factor for myocardial infarction (Unzueta *et al*., 2000) and type II diabetes mellitus, representing more than half of the deaths due to ischemic heart disease in Mexico (Escobedo *et al*., 1994). Despite the existence of established pharmacological therapy to decrease the levels of blood cholesterol, a high percentage of people remain without effective treatment for this condition. A number of medicinal plants have therefore been studied to assess their hypolipidemic activity using various animal models. Triton WR-1339 is a nonionic detergent used to induce a model of acute hyperlipidemia, which has been widely used to evaluate synthetic drugs and plant extracts (Schurr *et al*., 1972). The objective of the present study was to evaluate the antihyperlipidemic activity of *B. odorata* and *B. eupatorioides*, two plants present in the Northeast of Mexico. The results showed that only treatment with the extract of *B. odorata* was associated a marked reduction in the serum lipids in the model, even though a greater reduction was observed compared with rats in the group treated with the statin. The phytochemical profile of *B. odorata* revealed the presence of flavonoids. Extracts that contain these compounds reduce the impact of the hyperlipidemia induced by Triton WR-1339 (Mäkynen *et al*., 2013; Ibrahim *et al*., 2016). Hyperlipidemia is a pathological condition where there is an excessive release of ROS, which produces oxidative stress and accelerates the formation of atherosclerotic plaques (Hannan *et al*., 2016); natural antioxidants reduce lipid peroxidation and decrease the progression of atherosclerotic lesion formation (Sahebkar, 2015). In the present work, the two extracts evaluated showed an important antioxidant capacity, which would protect the vascular wall tissue against oxidative damage caused LDL cholesterol. But only *B. odorata* showed a hypocholesterolemic effect reducing cholesterol and triglycerides to a lower level than atorvastatin, and maybe these effects can be bolstered by its antioxidant potential suggested by the identification of flavonoids and terpenes. By contrast, AST and ALT, used as markers of liver damage, were not significantly increased in rats in the groups treated with the extracts in the present study. However, AST and ALT were found increased in the group treated with atorvastatin, indicating that the extracts did not induce liver damage at the dose evaluated in the animal model.

CONCLUSION

To our knowledge, this is the first report of the antihyperlipidemic activity of *B. odorata*. It is remarkable that an extract reduced the levels of lipids to lower levels than found in rats in the group treated with the statin without altering the enzymes indicative of liver damage. The results show that the extract of *B. odorata* has promise as a hypolipidemic agent, and because it shows an important antioxidant activity, has promise to prevent or treat atherosclerosis. Further studies are needed to identify the bioactive compounds in the extract.

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