

Artículo Científico

Inhibitory effects of *Buddleja scordioides* (salvilla) leaves on digestive enzymes and carbohydrate absorption *in vivo*

Efectos inhibidores de las hojas de *Buddleja scordioides* (salvilla) sobre las enzimas digestivas y la absorción de carbohidratos *in vivo*

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Abstract

The effects of *Buddleja scordioides* (BsLI) leaf infusions on digestive enzymes and carbohydrate absorption were evaluated. The BsLI yield was 21.64 %. In addition, a chemical characterization was carried out identifying hydroxybenzoic acids, hydroxycinnamic acids, flavonols, flavanones and flavones. *In vitro* studies were performed to determine the inhibitory action of BsLI on lipase, α -amylase, and α -glucosidase. Then, in rats, oral starch tolerance tests (OSTT) were carried out using BsLI at a dose of 9.5 mg/kg body weight. Results showed moderate inhibition of lipase and α -glucosidase, but greater inhibition of α -amylase compared to positive controls. During the OSTT trial, the group receiving BsLI showed a significant reduction in glucose levels compared to the negative control group. Bioactive compounds, such as naringenin, luteolin, quercetin, and coumaric acid, were identified after BsLI administration. Furthermore, the consumption of BsLI was safe and showed antioxidant activity like Trolox. In conclusion, BsLI may have an enhanced effect on glucose metabolism by inhibiting carbohydrate absorption.

Keywords: medicinal plant, carbohydrate absorption, antioxidant, *Buddleja scordioides*

Resumen

Se evaluaron los efectos de las infusiones de hojas de *Buddleja scordioides* (BsLI) sobre enzimas digestivas y la absorción de carbohidratos. El rendimiento de BsLI fue del 21.64 %. Además, se caracterizaron componentes químicos: ácidos hidroxibenzoicos, ácidos hidroxicinámicos, flavonoles, flavanonas y flavonas. En estudios *in vitro*, se examinó cómo BsLI inhibe la lipasa, α -amilasa y α -glucosidasa. Luego, en ratas, se probó su efecto en la tolerancia oral al almidón (OSTT) a dosis de 9.5 mg/kg de peso corporal. Resultados indicaron moderada inhibición de lipasa y α -glucosidasa, y mayor inhibición de α -amilasa comparado con controles. Durante la OSTT, el grupo con BsLI tuvo menor glucosa que el control negativo. Tras administrar BsLI, se detectaron compuestos bioactivos: naringenina, luteolina, quercetina y ácido cumárico. Además, BsLI fue seguro, con actividad antioxidante similar al Trolox. En conclusión, BsLI puede tener un efecto beneficioso sobre el metabolismo de la glucosa al inhibir la absorción de carbohidratos.

Palabras clave: planta medicinal, absorción de carbohidratos, antioxidante, *Buddleja scordioides*

1. Introduction

The western lifestyle is characterized by a lack of physical activity and hypercaloric diets. These are the main contributing factors to the development of metabolic diseases such as obesity and type 2 diabetes (Kopp, 2019). A therapeutic target of these diseases is the inhibition of digestive enzymes such as pancreatic lipase, α -amylase, and α -glucosidase (Patil *et al.*, 2015; Irondi *et al.*, 2018).

These digestive enzymes break down dietary lipids and carbohydrates to produce absorbable molecules such as free fatty acids and monosaccharides. Thus, the inhibition of these enzymes reduces the amounts of calories absorbed into the body and postprandial glucose (Awosika *et al.*, 2019). Pharmacological drugs such as acarbose and orlistat inhibit digestive enzymes involved in glucose and lipid metabolism. However, they often exhibit side effects ranging from diarrhea to hepatotoxicity, which limit their use in the clinical setting (Lunagariya *et al.*, 2014). In this regard, previous studies reported that different bioactive compounds (e.g. polyphenols) present in medicinal plants inhibit one or more digestive enzymes with lesser adverse effects than the drugs (Ardeshirlarijani *et al.*, 2019; Gutiérrez-Grijalva *et al.*, 2019).

Several species of *Buddleja* spp. are recognized to treat ills related to inflammatory processes (Estrada-Zúñiga *et al.*, 2019). Additionally, Hwang *et al.*, (2009) found that *B. officinalis* improves hyperglycemia, endothelium-dependent vascular relaxation, and inflammation in a diabetic atherosclerotic mouse model. Previous experimental studies have revealed the diverse biological activities of *Buddleja scordioides*, commonly known as salvilla. In a study published in 2002, Vanderjagt *et al.* demonstrated the anti-inflammatory potential of salvilla. Their findings indicated that the plant extract could effectively inhibit the production of inflammatory cytokines in rats, suggesting its potential for reducing inflammation in conditions like arthritis, asthma, and inflammatory bowel disease. Rocha-Guzmán *et al.* conducted research in 2018 to explore the gastroprotective effects of salvilla. Their study revealed that the extract provided protective benefits to the stomach lining. Furthermore, a study by Villegas-Novoa *et al.* in 2020 reported the antioxidant

effects of *Buddleja scordioides* extract in rats. The research demonstrated that the plant's extract exhibited antioxidant properties, protecting cells from damage caused by free radicals. These observed benefits have been associated with the bioactive compounds found of this herbal product, which include iridoids, phenylpropanoids, sesquiterpenes, saponins, verbascosides, and flavonoids. These compounds have shown a variety of beneficial effects, including antioxidant and anti-inflammatory properties, as reported by Santos-Cruz *et al.* (2012) and Gutiérrez-Rebolledo *et al.* (2019).

However, to the best of our knowledge, there is no evidence investigating the impact of *B. scordioides* on digestive enzymes and carbohydrate absorption *in vivo*. Therefore, the aim of this study was to evaluate the *in vitro* inhibitory effects of BsLI on digestive enzymes and carbohydrate absorption *in vivo*. In addition, the oral BsLI-bioactive-compounds absorption (OBCA), antioxidant activity, and safety of this medicinal plant were investigated.

2. Materials and methods

2.1 Materials

Samples of *Buddleja scordioides* Kunth (salvilla) were collected at Ignacio Ramirez road in Durango, México (coordinates 24°30'17.41" N, length 104°4'51.23" W, elevation 2030 m average). The botanist Dr. Socorro González-Elizondo taxonomically identified leaves and the voucher specimens (47538) were deposited at the Herbarium of the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional del Instituto Politécnico Nacional, Unidad Durango, México.

The leaves were air dried in the shade at 25 °C followed by milling to a particle size of 0.7-1.0 mm.

2.2 Preparation of *Buddleja scordioides* leaves infusions (BsLI)

The infusion concentration was commonly used by the general population (1 % m/v). The dried leaves sample (2 g) was added to 200 mL boiling water and kept stirring for 10 min. Infusions were obtained by subsequent filtration and lyophilized (FreeZone 18 Liter Console Freeze Dry System, Kansas USA). Samples were stored in amber vessels until use.

2.3 Yields of extractable solids

Infusion yield was determined as follows:

$$\text{Yield (\%)} = \frac{\text{Lyophilized infusion (g)}}{\text{Dried plant material (g)}} \times 100 \quad \text{Eq (1)}$$

Results are reported as means of two independent infusion preparation.

2.4 Chemical characterization of BsL

Sample analysis was carried out with an Acquity UPLC system (Waters Corp., Milford) coupled with a tandem Xevo TQ-S triple quadrupole mass spectrometer (Waters Corp., Wexford). The LC system consisted of a sample manager (5 °C) and a quaternary solvent manager. The column used to determine the phenolic profile was an Acquity UPLC BEH C8, 1.7 µm, 2.1 mm x 100 mm (Waters Corp., Wexford), operated at 30 °C. The mobile phase included two solvents: acidified Milli Q water with 7.5 mM formic acid (Solvent A) and acetonitrile LC-MS (Solvent B): Initial – 5 % B, 0.8 min isocratic to 5 % B, 1.2 min gradient to 10 % B; 1.9 min isocratic 10 % B; 2.4 min gradient to 15 % B; 3.7 min isocratic 15 % B; 4.0 min gradient to 21 % B; 5.2 min isocratic 21 % B; 5.7 min gradient to reach 27 % B; 8.0 min gradient to reach 50 % B; 9.0 (linear gradient) for column washing 100 % B; subsequent at 11.5 min linear gradient 5 % B since 13.5 min for column stabilization at a flow rate of 250 µL/min. Electron spray ionization (ESI) in negative mode was as follows: capillary voltage 2.5 kV, desolvation temperature 300 °C, source temperature 150 °C, desolvation gas flow 500 L/h, and cone gas flow 150 L/h, collision gas flow was 0.14 mL/min, MS mode collision energy 5.0 and MS/MS mode collision energy 20.0. For identification and quantification of the phenolic profile, a mixture of standards (20 ng/µL) was used for monitoring retention times, *m/z* values, and MS/MS transitions. Samples and standards were monitoring at multiple modes. The UPLC and Tandem Xevo TQ-S triple quadrupole mass spectrometer control and data processing was using MassLynx v. 4.1 Software (Waters Corp.).

2.5 Inhibitory effects of BsLI on digestive enzymes

2.5.1 Pancreatic lipase inhibition assay

The assay was performed following the methodology reported by McDougall *et al.* (2009), with some modifications. In brief, 150 µL of a solution (10 mg/mL) of porcine pancreatic lipase type II (Sigma- Aldrich, Cat. No. L3126) was mixed with BsLI (at 10, 50, 100, 200, 400, 600, 800, and 1000 µg/µL), 400 µL of Tris buffer (100 mM, pH 8.2), 400 µL of *p*-nitrophenyl laurate (SIGMA Co., St. Louis, USA), and substrate solution (0.08 % w/v dissolved in 5 mM sodium acetate at pH 5.0 containing 1 % Triton X-100). Samples were incubated at 37 °C for 2 h and centrifuged at 16,000 RPM, for 3 min. Finally, the supernatant was read at 400 nm using a microplate reader (MultiScan Go, Thermo Scientific, USA). The positive control was Orlistat (Redustat® Laboratorios Liomont S.A. DE C.V.).

The lipase inhibitory activity was expressed as a percentage of inhibition:

$$\text{Inhibition \%} = 100[S_0 - S_1]/S_0 \quad \text{Eq (2)}$$

Where S_0 is the absorbance of the blank and S_1 is the absorbance of BsLI.

Additionally, BsLI concentration that provided 50 % inhibition (IC_{50}) was calculated by plotting the inhibition percentage versus the log concentration curve (Coruh *et al.*, 2007).

2.5.2 α -Amylase inhibition assay

The assay was performed following the methodology reported by Tamil *et al.* (2010) with several modifications. Substrate solution was prepared using starch (4 %), CaCl₂ 0.01 M, and distilled water (5 mL) for 5 min at 96 °C. The BsLI (at 10, 50, 100, 200, 400, 600, 800, and 1000 $\mu\text{g}/\mu\text{L}$) were incubated for 1 h at 37 °C with 100 μL of porcine pancreas α -amylase solution (2.5 mU/ μL in sodium phosphate buffer (0.02 M, pH 6.9) and sodium chloride (6 mM)) (SIGMA Co., St. Louis, USA). Afterward, to stop the reaction, NaOH (4 mL, 0.1 M) was added, and a centrifugation step was performed (700 xg for 5 min). Glucose concentration in the supernatant was determined using an enzymatic kit (Biosystems Instruments Reagents, Barcelona, Spain). Acarbose (Laboratorios Alparma, CDMX, México) was used as a positive control. Results were expressed as inhibition percentage (equation 2) and IC₅₀.

2.5.3 α -Glycosidase inhibition assessment

The assay was performed following the methodology reported by Apostolidis *et al.* (2007). Sample of BsLI (25 μL at 20, 40, 60, 80, and 100 $\mu\text{g}/\mu\text{L}$), 100 μL of glucosidase enzyme (SIGMA Co., St. Louis, USA) (0.19 mU/ μL), and 50 μL of phosphate buffer (0.1 M, pH 6.9) were mixed and incubated for 10 min at 37 °C. Then, 25 μL of *p*-nitrophenyl- α -D-glucopyranoside solution (5 mM/L prepared in a 0.1 M/L of citrate-phosphate buffer, pH 7) (SIGMA Co., St. Louis, USA) was added and incubated 30 min at 37 °C. The reaction was stopped by adding 1 mL of a 0.05 mol/L NaOH solution. Samples were read at 410 nm with the Synergy HT Microplate Reader (Biotek Instruments, Winooski, Vermont, USA.). Acarbose (Laboratorios Alparma, CDMX, México) was used as a positive control. Results were expressed as inhibition percentage (equation 2) and IC₅₀.

2.6 Antioxidant activity of BsLI *in vitro*.

2.6.1 Determination of radical scavenging activity using the ABTS assay

The ABTS assay was performed following the methodology reported by Re *et al.* (1999). In brief, potassium persulfate (2.5 mM) was mixed and incubated in dark for 16 h at 25 \pm 1 °C with ABTS (7 mM) to produce the radical cations. The BsLI (2.5 μL at 10, 50, 150, 200, 250, 300, 350, and 400 $\mu\text{g}/\text{mL}$) were added to the ABTS solution (200 μL). Trolox was the positive control. Afterward, the absorbance was recorded, and the results were expressed as the inhibition percentage (equation 2) and as IC₅₀.

2.6.2 Radical scavenging assay: Diphenylpicrylhydrazyl (DPPH)

The DPPH assay was performed following the methodology reported by Brand-Williams *et al.* (1995). One mL of the stable free radical DPPH solution (20 mg/L) was mixed with 200 μL of BsLI (at concentration of 10, 50, 100, 200, 400, 500, 600, 700 and 800 $\mu\text{g}/\text{mL}$). Samples were incubated for 30 min in dark at room temperature (26 °C). The absorbance was registered at 515 nm. Trolox was the positive control. The results were expressed as the inhibition percentage (equation 2) and as IC₅₀.

2.7 Effect of BsLI on carbohydrate absorption *in vivo*.

2.7.1 Animals/ethics approval

Animal experiments were carried out in accordance with the Mexican guidelines (NOM-062-ZOO-1999) and the National Institutes of Health (2002) recommendations for research with animals.

Animals used in the experiments were male Wistar rats (ten weeks old, $n = 8$, 180 ± 20 g of body weight) and male CD-1 mice (eight weeks old, $n=28$, 25 ± 5 g of body weight), as well as non-pregnant female CD-1 mice (eight weeks old, $n=6$, 25 ± 5 g of body weight). All animals were obtained from the same source, Universidad Nacional Autónoma de México (UNAM), campus Juriquilla, Querétaro, México.

They were housed in a controlled environment with a 12–12 h light–dark cycle and maintained at a temperature of 27 ± 1 °C. Water and food (Rodent Lab Chow 5001, Purina®, Québec, Canada) were provided *ad libitum* for all the animals during the experiments.

2.7.2 Oral starch tolerance test (OSTT)

Effects of BsLI on carbohydrate absorption were assessed with an oral starch tolerance test (OSTT). Starch was the negative control, whereas the positive control was acarbose.

In fasting conditions, male Wistar rats (ten weeks old, $n = 8$, 180 ± 20 g of body weight) were given a starch load at a dose of 3 g/kg of body weight (equivalent to 519.26 mg contained in an infusion cup of 240 mL consumed by an adult of 70 kg by a meal). Additionally, 5.12 mg/Kg of body weight of acarbose was used as a positive control (equivalent to 50 mg by a meal in humans). Blood glucose levels were determined in blood samples collected from the tail vein using a glucometer (Stat Strip® Glucose, Nova Biomedical, Waltham, MA, US) at 0, 30, 60, and 120 min. The variations in serum glucose onset time, peak, and AUC were considered to the determination of the relative rate of carbohydrate digestion and absorption.

The human dose extrapolation (HED) to the animal was estimated according to the following formula:

$$\text{HED} = \text{animal dose (mg/kg)} [\text{animal weight (kg/human weight in kg)}]^{0.33}$$

Reagan-Shaw *et al.* (2008)

Eq (3)

2.8 Oral absorption assay of BsLI bioactive compounds (OABC)

The oral absorption assay was conducted on male CD-1 mice (eight weeks old, $n=28$, 25 ± 5 g of body weight). The mice were placed in metabolic cages and fasted for 12 hours before the experiment. Treatments ($n=4$ per time point) were administered through gavage at a dose of 2500 mg/kg of body weight to the intervention groups at various time intervals (0, 0.5, 2, 4, 8, 12, and 24 hours). The negative control group received water as the vehicle. After each treatment, the mice were anesthetized and euthanized by cardiac puncture. Serum was separated from the blood and immediately frozen at -80 °C for later analysis.

The analysis of the serum samples involved adding 100 μL of serum to a mixture containing 37 μL of water with 200 $\mu\text{g}/\mu\text{L}$ of ascorbic acid and 1 $\mu\text{g}/\mu\text{L}$ of EDTA. The mixture was then centrifuged at 14000 g for 10 minutes, and 250 μL of ethyl acetate was added for liquid-liquid micro-extraction. The suspension was mixed using a vortex for 1 minute. The collected volumes were dried using a vacuum centrifugal console, reconstituted in methanol (200 μL), and filtered through 0.45 μm syringe filters. The identification and quantification of BsLI bioactive compounds present in mouse serum were performed following the methodology previously described in section 2.4 on "the chemical characterization of BsLI".

2.9 Toxicity assessment of BsLI

2.9.1 Acute toxicity

The acute toxicity assay was carried out on non-pregnant female CD-1 mice (eight weeks old, $n=6$, 25 ± 5 g of body weight) (Universidad Nacional Autónoma de México, campus Juriquilla, Querétaro, México). The guidelines of the Organization for Economic Cooperation and Development for testing of chemicals 420 (OECD, 2001) were followed. The Mice were divided into two groups of three animals each. A single dose of BsLI (5000 mg/kg of body weight) was administered via gavage (in distilled water). Mice were observed for signs of possible toxicity (convulsions, tremors, lethargy, salivation, diarrhea, sleepiness, and coma) every hour for the first four hours, and then the animals were fed. Thereafter, mice were monitored for any signs of toxicity (changes in the eyes, skin, fur, mucous membranes, and the respiratory, circulatory, and autonomic nervous and central nervous systems) and mortality for 14 days. Measurements of body weight and food and water intake were performed daily. On the last day, animals were sacrificed under deep ether anesthesia and the median lethal dose (LD50) values were estimated. Vital organs such as the heart, kidney, liver, spleen, and lung were isolated and weighted to assess histopathological changes. The organs were fixed in 10 % buffered neutral formalin, embedded in paraffin wax, cut (5 μm) on glass slides, and stained with hematoxylin and eosin. The slides were examined under a light microscope.

2.9.2 Sub-chronic toxicity

For the sub-chronic toxicity assessment, both female and male mice aged eight weeks and weighing 25 ± 5 g of body weight (UNAM, Campus Juriquilla, Querétaro, México) were used. The assay was performed according to the methodology described by the Organization for Economic Co-operation and Development-407 (OECD, 2008). Animals were divided into four groups of 10 each (five males and five females). The control group received distilled water (vehicle), while the three intervention groups received BsLI by gavage at doses of 250, 750, and 2500 mg/kg of body weight, respectively, for 28 days. Mice were observed for signs of abnormalities during the treatment period. Measurements of body weight and food and water intake were performed daily.

Before sacrifice, urine was collected and analyzed using urinalysis strips (Bio-Uridiag-A10) for the measurement of leucocytes, nitrite, urobilinogen, protein, blood, specific gravity, ketone, bilirubin, and glucose. At the end of the treatment period, mice were sacrificed, and blood samples were obtained via cardiac puncture into non-heparinized and ethylenediaminetetraacetic acid (EDTA) containing tubes for biochemical and hematological analyses.

Heart, kidney, liver, spleen, and lung were isolated to assess histopathological changes. These organs were excised, weighed (Scout PRO Ohaus, Mississauga, Ontario, Canada), examined macroscopically, and fixed in 10 % buffered neutral formalin. Fixed organs were processed for paraffin embedding and cuts of 5 mm thick were obtained by microtome and then processed using an alcohol xylene series for being later stained with hematoxylin and eosin.

Hematological parameters such as red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and white blood cell (WBC) were analyzed using an automatic hematology analyzer (Hemat Technology, Newton, Massachusetts, USA).

Serum biochemical parameters such as glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-c), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total protein, and total bilirubin were analyzed using Biosystem commercial test kits with an automated A15 spectrophotometer (Biosystems Instruments Reagents, Barcelona, Spain). The concentrations of low-density lipoprotein cholesterol (LDL-c) were estimated using the formula by Friedewald *et al.*, (1972):

$$\text{LDL-c (mg/dL)} = [\text{total cholesterol (TC) (mg/dL)} - \text{HDL (mg/dL)} - (\text{triglycerides (TG) (mg/dL)/5})] \quad \text{Eq (4)}$$

2.10 Statistical analysis

Data were expressed as mean values \pm standard error (SE). Statistical significance was determined by one-way variance analysis (ANOVA) ($p < 0.05$) followed by Tukey's test, Statistical analysis was conducted using the Sigma Plot software version 13.0 (Systat Software, Inc., San Jose, CA, USA).

3. Results and discussion

3.1 Yield and chemical characterization

The yield of BsLI was 21.64 %. Regarding chemical characterization results, hydroxybenzoic acids, hydroxycinnamic acids, flavonols, flavanones, and flavones were identified and quantified. The protocatechuic acid, salicylic acid, vanillic acid, and quinic acid were the phenolic acids with the highest concentration; whereas, quercetin, quercetin 3-O-glucoside, and luteolin were the main flavonol compounds detected (Table 1).

Table 1. Chemical characterization of *Buddleja scordioides* leaves infusion (BsLI)(1 %).**Tabla 1.** Caracterización química de la infusión de hojas de *Buddleja scordioides* (BsLI)(1 %).

No.	Compound	Rt (min)	[M-H] ⁻ m/z	Transitions	Content [ng/mg of crude extract]
<i>Hydroxybenzoic acids</i>					
1.	Gallic acid	1.64	169	125, 79	8.529 ± 0.73
2.	Protocatechuic acid	3.13	153	109, 91	258.914 ± 9.73
3.	2,5-dihydroxybenzoic acid	4.21	153	109, 81	183.945 ± 0.27
4.	4-hydroxybenzoic acid	4.41	137	93, 65	106.790 ± 2.60
5.	Vanillic acid	4.85	167	152, 123	138.336 ± 1.13
6.	Tri-hydroxybenzaldehyde	6.21	153	83	16.584 ± 1.33
7.	Salicylic acid	7.70	137	93, 65	401.480 ± 43.62
<i>Hydroxycinnamic acids</i>					
8.	Quinic acid	1.01	191	93, 85	210.486 ± 24.05
9.	Chlorogenic acid	4.32	353	191, 85	27.11 ± 2.95
10.	4-O-caffeoylquinic acid	4.51	353	353, 179	13.717 ± 0.48
11.	Caffeic acid	4.85	179	135	93.348 ± 6.94
12.	Coumaric acid	6.19	163	119, 98	47.224 ± 10.48
13.	Ferulic acid	6.54	193	178, 134	51.178 ± 1.08
14.	4,5-dicaffeoylquinic acid	7.14	515	353, 179	3.446 ± 0.47
15.	Rosmarinic acid	7.73	359	197, 161	36.189 ± 0.02
<i>Flavonols</i>					
16.	Rutin	6.25	609	300, 271	69.162 ± 1.15
17.	Quercetin 3-O-glucoside	6.48	463	300, 271	433.894 ± 1.48
18.	Quercetin 3-O-β-glucuronide	6.54	477	301, 151	164.200 ± 9.09
19.	Quercetin	8.41	301	179, 151	466.554 ± 2.06
<i>Flavanones</i>					
20.	Neohesperidin	7.77	609	301, 164	48.328 ± 1.00
21.	Eriodictyol	8.42	287	151, 135	5.225 ± 0.90
<i>Flavones</i>					
22.	Luteolin	8.40	285	151, 133	231.853 ± 4.55
23.	Apigenin	8.80	269	148, 117	13.022 ± 1.36
24.		9.80	283	268, 211	21.021 ± 1.30

Values are means of duplicate determinations ± standard error.

3.2 Inhibition of digestive enzymes

3.2.1 Lipase inhibition

Results of lipase assessment are depicted in Fig. 1. The BsLI showed a lower percentage of lipase inhibition (~44%) than orlistat (~92%). Additionally, the IC₅₀ values were significantly higher as compared with the positive control (16-fold) (Table 2).

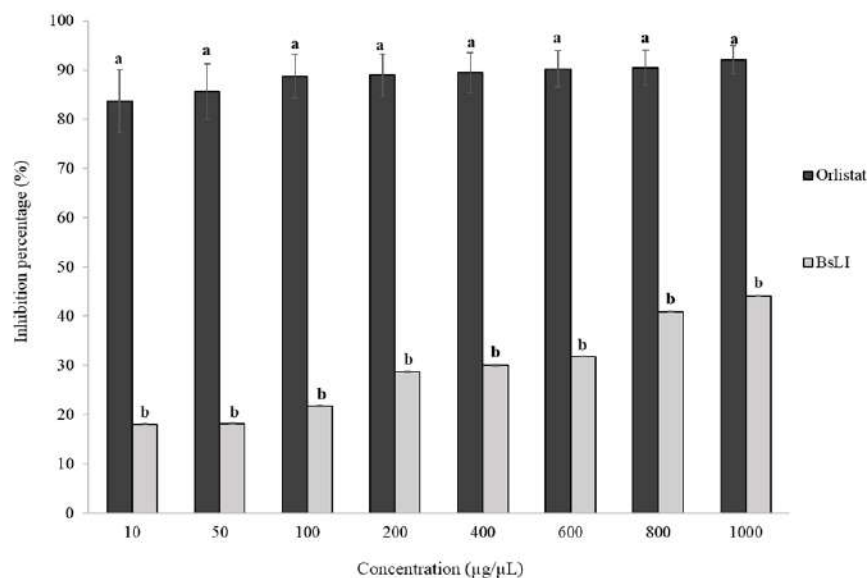


Figure 1. Lipase inhibition of *Buddleja scordioides* leaves infusion (BsLI). Orlistat was the positive control. Different letters in the graph indicate significant differences in the percentage of lipase inhibition. Values are means of duplicate determinations \pm standard error. ($p < 0.05$) by Tukey's test.

Figura 1. Inhibición de la lipasa de la infusión de hojas de *Buddleja scordioides* (BsLI). Orlistat fue el control positivo. Las letras diferentes en el gráfico indican diferencias significativas en el porcentaje de inhibición de la lipasa. Los valores son medias de determinaciones duplicadas \pm error estándar. ($p < 0.05$) mediante la prueba de Tukey.

3.2.2 α -amylase inhibition

The amylase results are shown in Fig. 2. The BsLI had a higher percentage of α -amylase inhibition than acarbose, achieving a ~96% while acarbose had a ~78% inhibition. On other hand, no differences were observed for the IC₅₀ values between BsLI and positive control (acarbose) (Table 2).

Table 2. Inhibitory digestive enzymes and antioxidant assessment of *Buddleja scordioides* leaves infusion (BsLI) IC₅₀ values.**Tabla 2.** Inhibición de enzimas digestivas y evaluación antioxidante de la infusión de hojas de *Buddleja scordioides* (BsLI) Valores IC₅₀.

	Orlistat ($\mu\text{g}/\mu\text{L}$)	Acarbose ($\mu\text{g}/\mu\text{L}$)	Trolox ($\mu\text{g}/\mu\text{L}$)	BsLI ($\mu\text{g}/\mu\text{L}$)
Lipase	0.34 ± 0.01^a	---	---	5.50 ± 0.01^b
α -amylase	---	1.69 ± 0.81^a	---	1.83 ± 0.01^a
α -glucosidase	---	1.64 ± 0.04^a	---	7.60 ± 0.10^b
ABTS	---	---	1.93 ± 0.01^a	1.48 ± 0.01^a
DPPH	---	---	2.11 ± 0.01^a	2.05 ± 0.01^a

Results are expressed as inhibitory median concentration (IC₅₀) values. Values are means of duplicate determinations \pm standard error. a-c Different letters indicate statistical differences ($p < 0.05$) by Tukey's test.

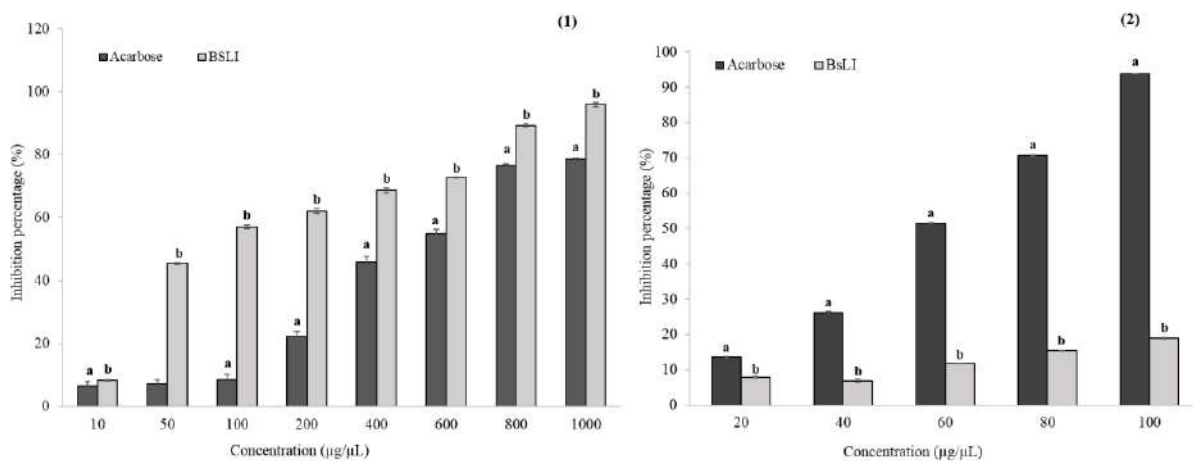
**Figure 2.** α -amylase inhibition (1) and α -glucosidase inhibition (2) of *Buddleja scordioides* leaves infusion (BsLI). Acarbose was the positive control. Values are means of duplicate determinations \pm standard error. a,b Different letters indicate statistical differences between BsLI and positive control ($p < 0.05$) by Tukey's test.

Figura 2. Inhibición de α -amilasa (1) e inhibición de α -glucosidasa (2) de la infusión de hojas de *Buddleja scordioides* (BsLI). La acarbose fue el control positivo. Los valores son medias de determinaciones duplicadas \pm error estándar. a,b Las letras distintas indican diferencias estadísticas entre BsLI y el control positivo ($p < 0.05$) mediante la prueba de Tukey.

3.2.3 α -glucosidase inhibition

Fig. 2 shows the results of α -glucosidase inhibition. The BsLI had a lower percentage of α -glucosidase inhibition (~19 %) than acarbose (~94 %). Regarding IC_{50} values, BsLI was 4-fold higher than the positive control (Table 2).

3.4 Oral starch tolerance test (OSTT)

Variations in serum-TG onset time and area-under-time-curve (AUC) are depicted in Fig. 3. Both groups treated with acarbose and BsLI showed significantly reduced glucose levels at 60 (above 12 %) and 120 min (above 14 %) in comparison with the control group.

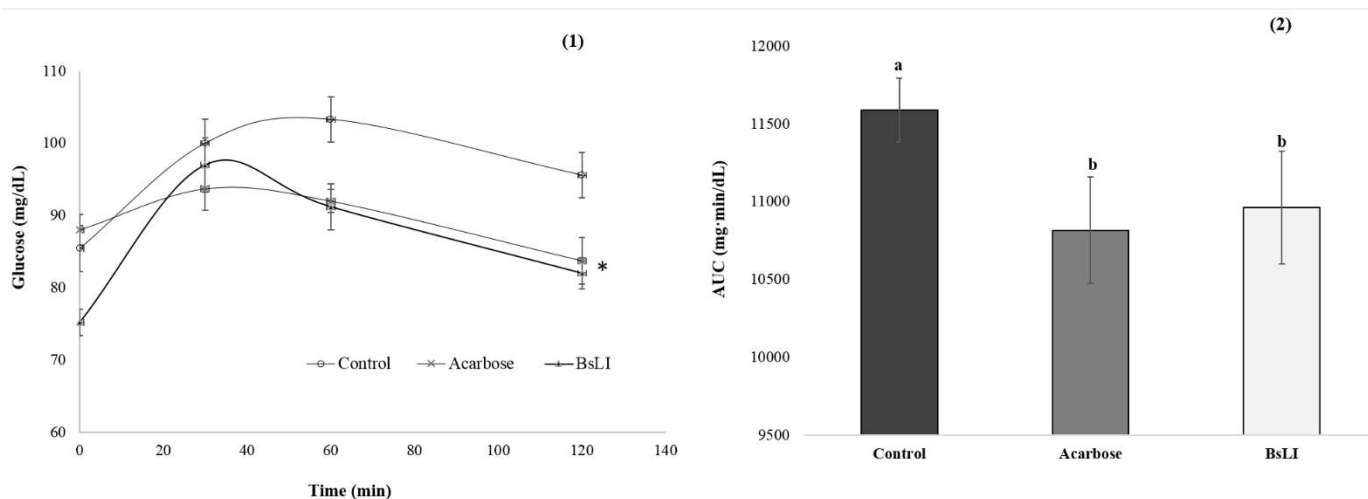


Figure 3. Oral starch tolerance test (OSTT) (1) and Area-under-time-curve (AUC) (2) in rats treated with *Buddleja scordioides* leaves infusion (BsLI). Acarbose was used as a positive control. Values are means of duplicate determinations \pm standard error. (*) indicates statistical differences between study groups, whereas for AUC a,b different letters indicate statistical differences between groups ($p < 0.05$) by Tukey's test.

Figura 3. Prueba de tolerancia oral al almidón (OSTT) Prueba oral de tolerancia al almidón (OSTT) (1) y área bajo la curva de tiempo (AUC) (2) en ratas tratadas con infusión de hojas de *Buddleja scordioides* (BsLI). La acarbose se utilizó como control positivo. Los valores son medias de determinaciones duplicadas \pm error estándar. (*) indica diferencias estadísticas entre los grupos de estudio, mientras que para AUC a,b letras diferentes indican diferencias estadísticas entre los grupos ($p < 0,05$) por la prueba de Tukey.

3.5 Oral BsLI-bioactive-compounds absorption evaluation (OBAC)

Polyphenols detected in the serum of mice are shown in Fig. 4. Bioactive compounds such as 4-hydroxybenzoic acid, naringenin, eriodictyol, luteolin, quercetin, and coumaric acid were identified in serum mice at different times after BsLI administration. The coumaric acid (13.84 ng/ μ L) and luteolin (16.01 ng/ μ L) exhibited the highest concentration; nevertheless, these levels declined rapidly.

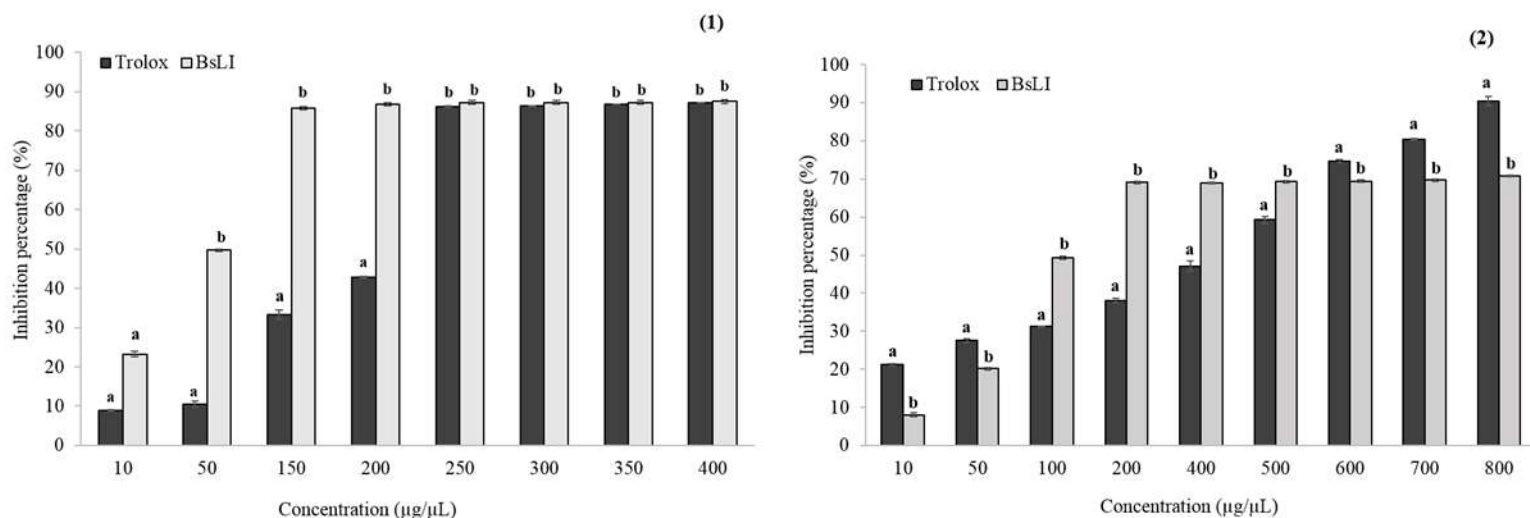


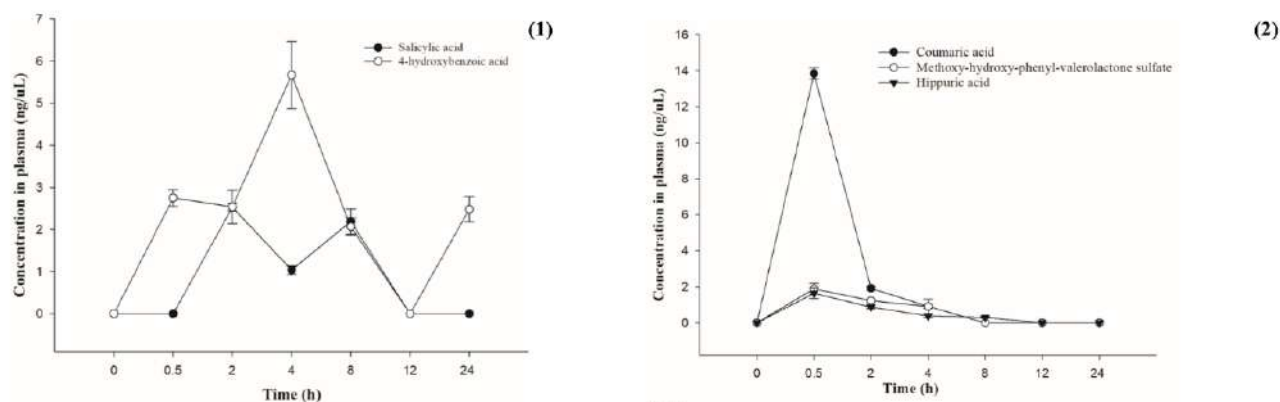
Figure 4. Inhibition percentage of *Buddleja scordioides* leaves infusion (BsLI) on ABTS (1) and DPPH (2) assessment. Trolox was the positive control. Values are means of duplicate determinations \pm standard error. a,b Different letters indicate statistical differences between BsLI and positive control ($p < 0.05$) by Tukey's test.

Figura 4. Porcentaje de inhibición de la infusión de hojas de *Buddleja scordioides* (BsLI) en la evaluación ABTS (1) y DPPH (2). Trolox fue el control positivo. Los valores son medias de determinaciones duplicadas \pm error estándar. a,b Las letras distintas indican diferencias estadísticas entre BsLI y el control positivo ($p < 0.05$) mediante la prueba de Tukey.

3.6 Antioxidant activity of BsLI *in vitro*.

3.6.1 ABTS and DPPH

Antioxidant evaluation of the BsLI by ABTS and DPPH assays is shown in Fig. 5. On one hand, ABTS results, showed that the BsLI had inhibition percentage like Trolox. On other hand, BsLI exhibited a lower inhibition percentage (~70 %) of DPPH radical compared with Trolox (~91 %). There were no significant differences for IC_{50} between the treatments for both, ABTS and DPPH assessment (Table 2).



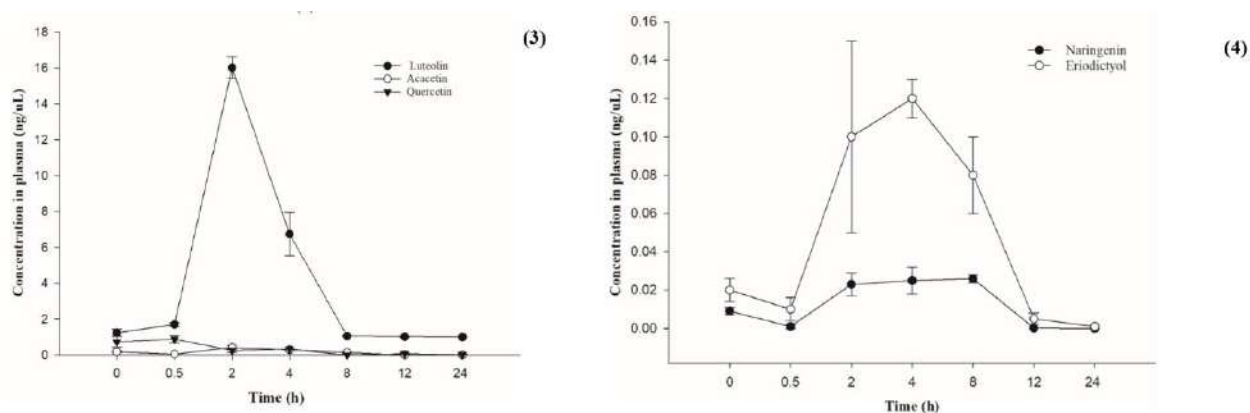


Figure 5. Means of total polyphenols of *Buddleja scordioides* leaves infusion (BsLI) in mice serum: (1) hydroxybenzoic acids, (2) hydroxycinnamic acids, (3) flavones y flavonols, and (4) flavanones. Values are expressed as mean \pm standard error.

Figura 5. Medias de polifenoles totales de la infusión de hojas de *Buddleja scordioides* (BsLI) en suero de ratones: (1) ácidos hidroxibenzoicos, (2) ácidos hidroxicinámicos, (3) flavonas y flavonoles, y (4) flavanonas. Los valores se expresan como media \pm error estándar.

3.7 Acute and sub-chronic toxicity evaluation

A single oral dose of infusion of BsLI (5000 mg/kg of body weight) produced no signs of toxicity (tremors, convulsions, salivation, diarrhea, lethargy, sleepiness, or coma) or mortality after 14 days. No significant difference in body weight gain was observed. No gross pathological abnormalities were observed in both groups. Furthermore, histological analysis of isolated organs showed no abnormal changes (data not shown). Thus, the LD50 value was found to be greater than 5000 mg/kg.

Regarding sub-chronic toxicity evaluation, there were no significant differences between the study groups for body weight, water, and food consumption (data not shown).

Macroscopic analysis of liver, lung, spleen, kidney, and heart exhibited no significant changes in color and texture compared with the control group. Additionally, intervention with BsLI samples at different doses did not affect the weight of organs.

Sub-chronic oral administration BsLI samples at different doses had no significant differences in the urinary biochemical parameters compared with the control group (data not shown). Moreover, treatment with BsLI showed no significant changes in serum concentrations of glucose, liver enzymes, total bilirubin, total protein content, albumin, total cholesterol, HDL-c, LDL-c, and triglycerides in comparison with the controls (Table 3).

Sub-chronic oral administration BsLI samples at different doses exhibited no significant changes on hematological profile compared with the control group (Table 4).

Histological analysis of heart, kidney, liver, spleen, and lung of intervention groups treated with different doses of BsLI (250, 750 y 2500 mg/kg of body weight) revealed no abnormal changes compared with the control group (data not shown).

Our results showed that BsLI decreases carbohydrate absorption *in vivo*, which could be explained by the inhibition of digestive enzymes involved in glucose metabolism. Also, we found an antioxidant activity in these herbal infusions.

Although the BsLI exhibited a lower percentage of lipase inhibition than orlistat, this effect could reduce the absorption of lipids *in vivo*. However further studies to examine the impact of BsLI on lipid metabolism are required.

Regarding carbohydrate digestive enzymes, BsLI showed higher inhibition of α -amylase than acarbose. This effect could be attributed to bioactive compounds present in BsLI such as polyphenols. It has been reported that the hydroxyl groups of these compounds interact with the enzyme active site, allowing the formation of ligands with the catalytic residues of the binding site and, consequently the generation of a conjugated system, resulting in the inactivation of the enzyme (Narita and Inouye, 2009).

On other hand, BsLI had a minimal inhibitory effect on α -glucosidase. This lack of effect could be because we employed *p*-nitrophenyl glucoside as substrate and α -glucosidase from a yeast, *Saccharomyces cerevisiae*. This test only indicates a general glucosidase activity (Williamson, 2013); therefore, further investigation to determine the effect of BsLI on α -glucosidase inhibition using other substrates such as sucrose and enzyme from other sources (e.g. porcine small intestine) is mandatory.

Our results showed that the BsLI decreases the absorption of starch similarly to acarbose. We attribute this effect to the inhibitory activity of BsLI on α -amylase. Given that the hydrolysis of the polysaccharide to produce limited dextrans, maltose, and maltotriose is catalyzed by α -amylase, inhibition of both salivary and pancreatic α -amylase decreases meal-derived carbohydrate absorption (Robyt *et al.*, 2008).

In the present study, we detected phenolic compounds (quercetin, rutin, luteolin, and phenolic acids) with well-established biological activity (Deng *et al.*, 2020; Sok *et al.*, 2021), which could be responsible for the beneficial effects of BsLI. However, it is important to consider that the biological action of polyphenols depends on their absorption and interaction with target tissues (Silberberg *et al.*, 2006). In this regard, we analyzed the absorption of bioactive compounds of BsLI in serum mice. The main metabolites identified in BsLI were phenolic acids and flavonoids. Additionally, the plasma levels of these compounds suggest relatively rapid absorption, which is partly consistent with previous studies (Liu *et al.*, 2002).

Also, it has been reported that the bioactive compounds contained in this herbal infusion have antioxidant effects (Moretti *et al.*, 2012; Tian *et al.*, 2021). In this line, the results of our study demonstrated that BsLI exhibits antioxidant properties by scavenging free radicals (ABTS and DPPH). It has been suggested that an ideal antidiabetic agent should have antioxidant activity (Mai *et al.*, 2007); thus, in addition to the hypoglycemic action, the BsLI could emerge as a therapeutic alternative for diabetes management.

The toxicological activity of medicinal plants is an important aspect that must be assessed before being recommended as alternative therapies (Nasri, 2013). In this context, we evaluate the acute and chronic toxicological effects of BsLI and our results revealed no signs of toxicity, macroscopic abnormalities, or mortality, suggesting non-toxic effects of these herbal infusions. Usually, the consumption of infusions in Mexican communities is one cup per day at 1 % (m/v) concentration (equivalent to 33 mg/kg of body weight). Interestingly, although we used higher doses (7.5 [250 mg/kg], 22.7 [750 mg/kg] and 75 [2500 mg/kg] times), none of these caused signs of toxicity, damage symptoms or mortality. Additionally, all hematological and biochemical parameters remained within the reference range for CD1 mice (Titlow, 2013) and no significant differences were observed between the study groups. Particularly, it is noteworthy that after treatment with BsLI we found no significant differences in transaminase levels or abnormal changes in liver tissue by histological examination, suggesting that this natural agent does not induce hepatocellular damage. Also, macroscopic and microscopic evaluation of the organs from mice receiving oral administration of BsLI for 28 days at different doses (250, 750, and 2500 mg/kg) revealed normal architecture indicating no detrimental changes or morphological disorders in the sub-chronic toxicity assessment. Hence, the no-observed-adverse-effect level (NOAEL) may be considered for this herbal product. The BsLI could be considered a promising and attractive strategy in the treatment of chronic diseases such as obesity and type II diabetes; however, future clinical studies investigating the effects of these herbal infusions are needed to confirm our findings.

Table 3. Liver function indices of CD-1 mice administrated orally with *Buddleja scordioides* leaves infusion (BsLI) for 28 consecutive days.

Tabla 3. Índices de función hepática de ratones CD-1 administrados por vía oral con infusión de hojas de *Buddleja scordioides* (BsLI) durante 28 días consecutivos.

	Control group	250 (mg/kg)	750 (mg/kg)	2500 (mg/kg)
AST (IU/L)	193.00 ± 6.20 ^a	184.55 ± 11.46 ^a	180.78 ± 13.87 ^a	193.52 ± 19.53 ^a
ALT (IU/L)	65.47 ± 1.85 ^a	59.75 ± 4.17 ^a	49.56 ± 3.16 ^a	49.05 ± 5.70 ^a
Total Protein (g/dL)	4.99 ± 0.06 ^a	5.05 ± 0.06 ^a	5.31 ± 0.05 ^a	5.07 ± 0.10 ^a
Albumin (g/dL)	2.53 ± 0.28 ^a	1.61 ± 0.07 ^a	1.96 ± 0.07 ^a	1.86 ± 0.08 ^a
Total bilirubin (mg/dL)	0.14 ± 0.09 ^a	0.12 ± 0.09 ^a	0.15 ± 0.35 ^a	0.28 ± 0.18 ^a
Total cholesterol (mg/dL)	104.12 ± 1.89 ^a	99.14 ± 3.25 ^a	101.71 ± 2.87 ^a	98.43 ± 1.47 ^a

HDL-c (mg/dL)	58.16 ± 1.64 ^a	62.18 ± 2.23 ^b	59.03 ± 3.29 ^a	58.26 ± 1.53 ^a
LDL-c (mg/dL)	38.85 ± 2.31 ^a	24.22 ± 2.20 ^b	31.46 ± 1.82 ^a	30.73 ± 2.09 ^a
Triglyceride (mg/dL)	59.86 ± 3.13 ^a	69.86 ± 2.68 ^a	68.50 ± 3.83 ^a	77.2 ± 3.23 ^b

ALT: alanine aminotransferase, AST: aspartate aminotransferase, HDL-c: high density lipoprotein, and LDL-c: low density lipoprotein. Values are expressed as mean ± standard error. Different letters indicate statistically significant differences between groups * $p \leq 0.05$ significantly different from the control were determined by using one way ANOVA followed by Tukey's multiple comparison tests.

Table 4. Effect on hematological parameters of oral administration with infusions of *Buddleja scordioides* leaves infusion (BsLI) for 28 consecutive days.

Tabla 4. Efecto sobre los parámetros hematológicos de la administración oral con infusión de hojas de *Buddleja scordioides* (BsLI) durante 28 días consecutivos.

	Control group	250 (mg/kg)	750 (mg/kg)	2500 (mg/kg)
RBC ($\times 10^6/\mu\text{L}$)	9.02 ± 0.19 ^a	8.31 ± 0.26 ^b	9.56 ± 0.14 ^a	8.67 ± 0.17 ^b
Hemoglobin (g/dL)	17.24 ± 0.37 ^b	16.51 ± 3.28 ^a	18.45 ± 0.34 ^a	16.88 ± 0.29 ^a
Hematocrit (%)	46.52 ± 0.98 ^b	44.13 ± 1.26 ^a	49.62 ± 0.90 ^a	44.77 ± 0.79 ^a
MCV (fL)	51.56 ± 0.07 ^a	53.26 ± 0.29 ^a	51.73 ± 0.33 ^a	51.671 ± 0.29 ^a
MCH (pg)	19.10 ± 0.04 ^a	19.93 ± 0.08 ^a	19.24 ± 0.15 ^a	19.50 ± 0.11 ^a
MCHC (g/dL)	37.03 ± 0.06 ^a	37.44 ± 0.13 ^a	37.18 ± 0.08 ^a	37.73 ± 0.13 ^a
Platelets ($\times 10^3/\mu\text{L}$)	1171.66 ± 4.77 ^a	727.85 ± 4.91 ^b	1047.33 ± 3.97 ^a	978.28 ± 4.41 ^a
WBC ($\times 10^3/\mu\text{L}$)	8.26 ± 2.91 ^a	5.68 ± 2.71 ^a	6.26 ± 3.03 ^a	5.20 ± 2.54 ^a
Neutrophils (%)	11.00 ± 0.51 ^b	9.71 ± 0.75 ^b	14.11 ± 0.58 ^a	11.86 ± 0.57 ^a
Eosinophils (%)	0.55 ± 0.11 ^a	0.57 ± 0.16 ^a	0.00 ± 0.00 ^b	0.57 ± 0.16 ^a
Basophils (%)	0.00 ± 0.00 ^c	0.14 ± 0.05 ^a	0.11 ± 0.04 ^b	0.14 ± 0.05 ^a
Lymphocytes (%)	87.22 ± 0.49 ^a	86.71 ± 0.93 ^a	83.56 ± 0.59 ^b	85.71 ± 0.43 ^a
Monocytes (%)	1.22 ± 0.12 ^c	2.86 ± 0.30 ^a	2.22 ± 0.90 ^a	1.71 ± 0.15 ^a

RBC: red blood cell, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, WBC: white blood cell. Values are expressed as mean ± standard

error. Different letters indicate statistically significant differences between groups * $p \leq 0.05$ significantly different from the control were determined by using one way ANOVA followed by Tukey's multiple comparison tests.

4. Conclusion

The study demonstrates that the infusion of *Buddleja scordioides* (BsLI) exerts inhibitory effects on carbohydrate absorption *in vivo* by targeting digestive enzymes involved in glucose metabolism. Moreover, BsLI exhibits antioxidant properties and contains bioactive compounds, including phenolic acids and flavonoids. The infusion proves to be safe, with no indications of toxicity or adverse effects on organ histology. These findings suggest that BsLI holds promise as a potential therapeutic alternative for managing conditions such as obesity and type II diabetes. However, further clinical studies are necessary to validate these results and explore the full potential of BsLI as a treatment option.

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Conflict of Interest statement

The authors declare no conflicts of interest.

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