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Original Research Article

Synergistic hypolipidemic and hypoglycemic effects of mixtures of *Lactobacillus nagelii*/betanin in a mouse model

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Abstract

Purpose: *To determine the nutraceutical effect of mixtures containing Lactobacillus nagelii/betanin on the carbohydrate and lipid metabolism of mice.*
Methods: *Lactobacillus nagelii and betanin were isolated from the raw materials. These components were subsequently assessed for their anti-hyperglycemic and hypolipidemic activities, and administered individually or combined in normoglycemic and diabetic mice. These animals were subjected to a standard OGTT and given an atherogenic diet.*
Results: *The combination of L. nagelii (2.0 × 10^9 CFU/mL) and betanin (30 mg/kg body weight) exerted a significant ($p < 0.01$) and prolonged reduction of postprandial blood glucose (30 - 120 min). Interestingly, a mixture of L. nagelii (1 ×10^7 CFU/mouse/day) with betanin (10 mg/kg body weight) administered for 30 days, produced favourable effects ($p < 0.01$) in the lipid profile of mice previously treated with an atherogenic diet. These mixtures significantly ameliorated ($p < 0.01$) hypoinsulinemia, hyperleptinemia and increased the levels of adiponectin.*
Conclusion: *The simultaneous administration of L. nagelii and betanin in mice produced a beneficial change in blood glucose and lipids in mice, indicating a synergistic nutraceutical effect. However, there is a need to develop this therapy further for potential application in humans*

Keywords: *Lactobacillus nagelii, Betanin, Anti-hyperglycemic, Hypolipidemic*

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INTRODUCTION

For centuries, traditional beverages and colored berries have been used as natural sources to treat diverse illnesses [1]. The “Pulque” from *Agave* sp. and the “coyol wine” from the fermented sap of *Acrocomia mexicana* are typical fermented juices produced in provinces of Mexico. Despite the low nutritional properties of these beverages [2,3], they are considered as therapeutic agents because of their microbiological and antioxidant content [4,5]. It

has been demonstrated that the combination of probiotics and prebiotics with antioxidant activity produce substantial beneficial effects in human health [6].

Common probiotics (i.e., *Lactobacillus* sp. and *Bifidobacterium* sp.) and fructooligosaccharides from diverse plants promote the synthesis of inhibitory substances such as H_2O_2, bacteriocins and organic acids. These components regulate the growth of pathogenic bacteria because of their antimicrobial properties [6]. On the other hand, betalains are considered as nutraceuticals due to their capacity to improve lipid profile in humans [7,8]. Some medicinal plants such as those of the *Phytolacca* genus are used as traditional medicines and their berries are frequently consumed to treat diverse health problems including type 2 diabetes mellitus (DM2) [7-9]. The berries of these plants accumulate high amounts of betalains with antioxidant activity. In addition, the leaves and berries of some plants belonging to the *Phytolacca* genus have been tested in murine models in order to explore their anti-obesity activities [9].

These reports suggest that some bioactive compounds from the *Phytolacca* genus should be involved in the beneficial changes observed in animal models [9]. Currently, the effect of mixtures containing untested *Lactobacillus* sp. and betanin on the metabolism of lipids and carbohydrates has not been investigated. Thus, the aim of this study was to determine the effect of *L. nagelii*, isolated from the medicinal "coyol wine", and betanin isolated from the medicinal plant *Phytolacca thyrsiflora*, on blood glucose and lipid profile of mice.

EXPERIMENTAL

Plant source

Berries of *Phytolacca thyrsiflora* Fenzl ex J.A. Schmidt were collected in Yaonáhuac Puebla, México (19°52'23.80"N, 97°27'49.47"O) from November 2016 to March 2017. *P. thyrsiflora* was identified by Ramiro Cruz-Durán and a holotype (162543) was kept at FCME-UNAM herbarium. Crude sap from *Acrocomia mexicana* (syn. *A. aculeata*) was collected between November 2016 and April 2017 in San Miguel del Puerto, Pochutla, México (15°55'8.27"N, 96°10'25.44"O) and transported in ice to avoid spontaneous fermentation. A reference voucher 155384 of *A. mexicana* was certified and deposited in the same institution.

Purification of betanin from *Phytolacca thyrsiflora*

Berries of *Phytolacca thyrsiflora* (500 g) were homogenized in a mortar for immediate extraction with 1 L of acidified acetone (0.01% HCl) in the dark for 12 h at 4°C. The extract was filtered with Whatman filter paper 1 and then partitioned with 250 mL of chloroform. The aqueous phase was recovered and concentrated to a minimal volume (about 1 mL). The extract was subsequently separated by reversed-phase high-performance liquid chromatography (RP-HPLC). This experimental procedure was done using a Dionex Ultimate 3000 coupled to a diode array detector DAD-3000, equipped with a Varian Cromospher C_{18} column (250 - 4.5 mm ID, 5 µm particle size). A Hewlett Packard 1050 system coupled to a HP G1306A diode array detector was used to scale the purification of betanin. The mobile phase, flow rate, temperature and wavelengths were those reported by Luczkiewicz and Cisowsky [10].

Authentic betanin (Sigma-Aldrich Co.) was used to determine the retention time of this compound in RP-HPLC experiments. Betanin from *P. thyrsiflora* was collected manually according to the elution profile in multiple runs and finally lyophilized for further use. A calibration curve using authentic betanin was made to quantify this compound in *P. thyrsiflora* berries (mg 100 g fresh weight^{-1}).

Assessment of betanin on enzymes involved in carbohydrate hydrolysis

In vitro inhibitory assays using alpha-amylase and alpha-glucosidase (Sigma-Aldrich Co.) were done under the conditions previously reported [11]. IC_{50} were estimated from the dose response curves (0.005-0.5 mg mL^{-1}) using betanin isolated from *P. thyrsiflora*. The data were processed using GraphPad Prism 7.02.

Isolation and identification of *L. nagelii* from "coyol wine"

The crude sap from *A. mexicana* (20 mL) was deposited in a sterile glass flask and thawed on ice for subsequent incubation and lightly shaken (60 rpm) for 24 h at 30°C. One milliliter of the fermented sap was centrifuged at 10,000 *g* for 2 min and the precipitate was recovered in 100 µL of 15% glycerol. Different volumes (10 - 50 µL) were inoculated in Petri dishes containing MRS culture medium [12]. The Petri dishes were incubated under microaerophilic conditions using an anaerobic jar (Oxoid™) at 30°C for 2 days in the dark. The identity of the species was initially

corroborated with biochemical characterization [12] and by molecular tests performed on the basis of 16s rRNA gene sequence analyses [4,13]. Amplicons were cloned in TOPO-TA cloning vector (Invitrogen™) and sequenced in an ABI PRISM 3700 apparatus. The genic sequences were contrasted with the information available at NCBI database.

For further experiments, *Lactobacillus nagelii* was routinely grown in liquid MRS for 24 h under microaerophilic conditions. The CFU/mL of *L. nagelii* administered to the mice were estimated by measuring the optical density at 600 nm and also, by direct colony plate counting as reported for *Lactobacillus* sp. [14]. The OD_{600} values were correlated with the number of CFU in accordance with the same report [14]. Two concentrations of *L. nagelii* were adjusted to 2×10^9 and 1×10^7 CFU/mL based on OD_{600} readings. The CFU administered to the mice were selected based on the bioactive concentrations of *Lactobacillus* sp. which produced a noticeable immunomodulatory effect in mouse model [14-16]. Each stock of *L. nagelii* was centrifuged, pelleted and then resuspended in 50 µL of sterile phosphate buffered saline before administration.

Animals

Institute of Cancer Research (ICR) male mice were kept at 25 °C and 50 % relative humidity under a cycle of 12 h light and 12h dark [24]. Water was administered as previously reported [24]. The animal experiments were approved by the institutional ethical committee (approval no. R1627). These procedures were conducted in accordance with the Mexican norm NOM-062-ZOO-1999 and with International Standards on Animal Welfare [25].

Administration of *L. nagelii* and betanin in glucose tolerance test

Institute of Cancer Research (ICR) male mice (8-week old weighing approximately 30 g) were separated into 8 groups of 6 animals and fed by oral gavage (2 mL/kg). The first experimental group was fed a soluble glucose solution (Glucox^{MR}, 1.5 g/kg) and betanin (30 mg/kg). The second experimental group was fed with a mixture of a soluble glucose solution (Glucox^{MR}, 1.5 g/kg) and fresh *L. nagelii* cells (2×10^9 CFU/mL). The third experimental group was administered with a soluble glucose solution (Glucox^{MR}, 1.5 g/kg) mixed with fresh *L. nagelii* cells (2×10^9 CFU/mL) and betanin (30 mg/kg body weight). Diabetic mice previously treated with streptozotocin (groups 4 - 6), were evaluated using the same conditions described for the groups 1 - 3 [17]. Animals with basal glucose levels higher than 200 mg/dL after 2 h of an OGTT were considered as diabetics. The control groups (normoglycemic and diabetic mice) were only fed with a soluble glucose solution (Glucox^{MR} 1.5 g/kg body weight). Blood samples were obtained from the tail vein at 0, 15, 30, 60 and 120 min. Blood plasma was obtained by centrifugation at 3000 g for 10 min. Blood glucose was measured in accordance with the SPINREACT® protocol whereas the levels of plasma insulin were determined with commercial ELISA kits (Sigma-Aldrich Co.).

Administration of *L. nagelii* and betanin in mice subjected to high fat diet

Four groups of six mice were fed by oral gavage twice a day for 30 days with an atherogenic diet (2 mL/kg body weight) previously reported [9]. The diet of the first experimental group was mixed with 10 mg betanin/kg body weight. The diet of the second experimental group was mixed with 1×10^7 of *L. nagelii* CFU//mouse/day. The diet of the third experimental group was combined with 1×10^7 CFU/mouse/day and betanin (10 mg/kg body weight). Two other control groups (4-5) were fed with the atherogenic diet/water (2 mL/kg body weight) and with a standard laboratory diet (LabDiet®)/water (2 mL/kg body weight). The body weights of the mice were measured every 5 days. At the end of the experimental procedure, the contents of cholesterol and triglycerides in mice were determined in accordance with a previous report [24] whereas blood glucose was determined using SPINREACT® reagent. Insulin leptin, and adiponectin, were quantified using commercial ELISA kits (Sigma-Aldrich Co).

Statistical analysis

ANOVA coupled with a Dunnett´s test ($p < 0.05$ and $p < 0.01$) were conducted to determine statistically significant differences in the biochemical profile among the animal groups assayed. The data were processed using GraphPad Prism 7.02 software.

RESULTS

Betanin content and its inhibitory activity on hydrolytic enzymes

The chemical profile of the berries from *P. thyrsiflora* revealed betanin as the most abundant compound (about 97 %) in the pigment fraction (Figure 1). According to the results, the content of betanin was 276 mg 100 g fresh weight^{-1}. Enzymatic assays revealed that betanin

produced a moderate inhibition on alpha-amylase (IC_{50}, 117.3 ± 1.6 µg/mL) and alpha glucosidase (IC_{50}, 146.6 ± 2.5 µg/mL).

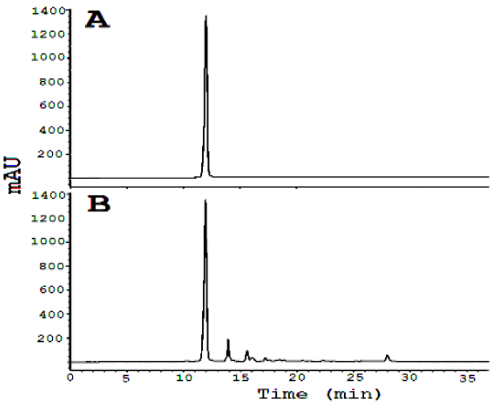

Figure 1: Detection of betanin by Reversed-Phased High-Performance Liquid Chromatography**.** A, authentic standard of betanin. B, betanin isolated from berries of *Phytolacca thyrsiflora*

Identity of *Lactobacillus* sp.

The *Lactobacillus* species isolated from "coyol wine" showed similar biochemical parameters such as those described by Edwards *et al*. [12]. Blast-N and ClustalW alignments of the 16S ribosomal gene sequence revealed a high similarity (>98%) between the isolated sequence and those reported for *L. nagelii* in the gen bank of the NCBI (Figure 2).

TCATGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGAAGCTGTTTTAACTGGTG
CTTGCACCGACTTAAACAACTGAGTGGCGAACGGGTGAGTAACACGTGGGTAACCTGCCCCAAAGCGGGG
GATAACATTTGGAAACAGATGCTAATACCGCATAACCACAGAAACCACATGGTTTCTGTGTCAAAGATGG
TTTCGGCTATCACTTTGGGATGGACCCGCGGCGTATTAGCTAGTTGGTAAGGTAATGGCTTACCAAGGCA
ATCATACGTAGCCGAACTGAGAGGTTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGG
AGGCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCCCGTGAGTGAAGAAGGT
TTTCGGATCGTAAAACTCTGTTGTCAGAGAAGAACGTGTGCGAGAGGAACTGCTCGTGCAGTGACGGTAT
CTGGCCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGG
ATTTATTGGGCGTAAAGGGAACGCAGGCGGTCTTTTAAGTTTGATGTGAAAGCCTTCGGCTTAACCGAAG
TCGTGCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAGAGTGGAACTCCATGTGTAGCGGTGAAATG
CGTAGATATATGGAAGAACACCAGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGTTCGAAA
GCGTGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATGAATGGTAAGTGTTGGAN
GGTTACCGCCCTTCCGTGCCGCATCAAACGCATTAAGCATTCCGCCTGGGGAGTACGACCGCAAGGTTGA
AACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCACCGCGAAAA
CCTTACCAGGTCTTGACATCTTCTGATAGCCTAAGAAATTAGGTGTTCCCTTGGGGACAGAATGACAGGT
GGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATT
GTCAGTTGCCAGCATTAAGTTGGGCACTCTGGCAAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATG
ACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGATACAACGAGTCGCT
AGACCGCGAGGTTAAGCTAATCTCTGAAAGTCGTTCTCAGTTCAGATTGCAGGCTGCAACTTCGCCTGCA
TGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACAC
CGCCCGTCACACCATGAGAGTTTGTAACACCCAAAGCCGGTGGGGGAACCAATAGGAGCCAGCCGTCTAA
GGTGGGACAGATGATTGGGGTGAAGTCGTAACAAGGTAGCCGTAGGAGAACCTGCGGTTGA

Figure 2: 16S ribosomal gene sequence from the *Lactobacillus* sp. isolated form the fermented sap of *Acrocomia mexicana*

Anti-hyperglycemic activity

Oral administration of betanin in normoglycemic mice produced a significant decrease ($p < 0.05$) in the glucose levels at 30 min. However, after 30 min, this effect was not significant (Figure 3 A). Interestingly, in this experimental group, the levels of insulin at 15 - 30 min increased when compared with those of the untreated group (Table 1). The single administration of *L. nagelii* exerted a more sustained anti-hyperglycemic effect than that of pure betanin (Figure 3 A).

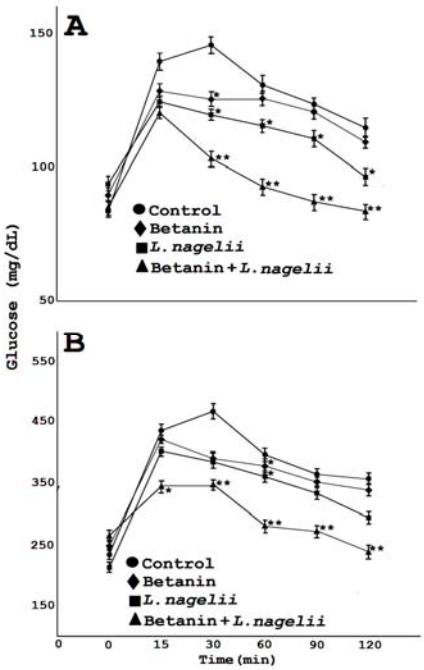

Figure 3: Anti-hyperglycemic activity of betanin isolated from *Phytolacca thyrsiflora* and *Lactobacillus nagelii* isolated from "coyol wine" in mice. A, effect of the oral administration of betanin and *L. nagelii* in normoglycemic ICR male mice. B, effect of the oral administration of betanin and *L. nagelii* in diabetic mice. Bars represent the SD (n=6). *Statistically significant differences compared with control at $p < 0.05$; **$p < 0.01$ compared with control

Nevertheless, no significant changes in the levels of insulin were observed (Table 1). Simultaneous administration of both components resulted in a significant decrease of blood glucose ($p < 0.01$) which stayed low during the test (Figure 3 A).

Remarkably, the combination of betanin and *L. nagelii* produced a substantial reduction of blood glucose in treated mice (about 35 %).

A significant anti-hyperglycemic effect of betanin ($p < 0.05$) was observed in diabetic mice between 30 and 60 min, which suggested a more prolonged effect than in normoglycemic mice (Figure 3B). In comparison with the hyperglycemic control, mice treated with betanin showed a significant and prolonged insulinogenic effect after 15 min (Table 2).

Table 1: Plasma insulin (mU L^{-1}) of normoglycemic mice following different treatments+

Component	0 min	15 min	30 min	60 min	90 min	120 min
Control group	5.5±0.15	8.7±0.32	17.7±0.26	16.2±0.18	8.2±0.37	3.3±0.32
Betanin	4.9±0.18	12.3±0.24*	25.5±0.32*	15.9±0.32	8.5±0.31	3.8±0.28
L. nagelii	6.1±0.35	9.1±0.41	18.0±0.27	17.7±0.14	8.3±0.25	3.6±0.14
Betanin + *L. nagelii*	5.2±0.32	17.2±0.81*	27.3±0.63*	16.2±0.39	7.9±0.65	4.9±0.43

+The results are presented as the mean ± SEM of samples from six mice (n=6). *p < 0.01 in comparison with control group

Table 2: Plasma insulin (mU L^{-1}) of diabetic mice under different treatments+

Component	0 min	15 min	30 min	60 min	90 min	120 min
Control group	2.6±0.09	3.1±0.05	2.4±0.09	2.0±0.08	3.1±0.02	3.3±0.32
Betanin	2.3±0.03	2.8±0.06	5.8±0.07*	5.3±0.04*	5.9±0.05*	4.7±0.04*
L. nagelii	3.1±0.09	3.5±0.07	3.7±0.06*	2.8±0.09	2.2±0.08	2.9±0.02
Betanin+*L. nagelii*	2.8±0.01	5.9±0.02*	7.2±0.05*	6.4±0.03*	6.3±0.06*	5.2±0.05*

+The results are presented as the mean ± SEM of samples from six mice (n=6). *p < 0.01 in comparison with control group

The administration of *L. nagelii* cells produced a statistically significant reduction (p < 0.05) between 30 - 60 min, however, such effect was not observed after 60. This consequence was not apparently related to the production of insulin (Table 2). Interestingly, the anti-hyperglycemic activity was significantly increased (p < 0.01) and sustained during the test after the simultaneous administration of betanin and *L. nagelii* cells (Figure 3B).

Hypolipidemic activity

Prolonged administration of pure betanin and *L. nagelii* cells generated significant decreases (p < 0.01) in body weight, liver weight, LDL-c, triglycerides as well as a significant increase (p < 0.01) in HDL-c (Figure 4A-F). Surprisingly, slight increase in HDL-c levels was observed for *L. nagelii* treatment compared with betanin treatment.

The oral administration of betanin and *L. nagelii* cells substantially improved the lipid profile and decreased body (~28%) and liver (35%) weights of treated mice. The mixtures of betanin and *L. nagelii* cells produced an evident reduction of blood glucose at the end of the treatment (Table 3). Under prolonged administration (30 days), the levels of insulin and leptin decreased whereas the levels of adiponectin increased in mice treated with the mixture of betanin and *L. nagelii*.

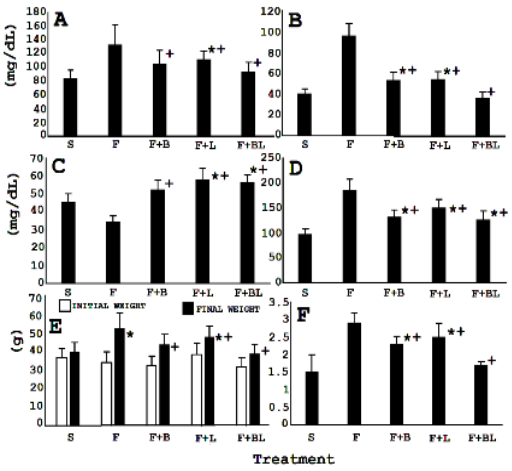

Figure 4: Lipid profile of mice treated with mixtures of *L. nagelii* and bentanin**.** A, total cholesterol content, B, LDL-c content. C, HDL-c content. D, triglyceride content. E, body weight. F, liver weight. S, standard diet; F, fat diet (atherogenic diet); F+B, fat diet plus betanin; F+L, fat diet plus *Lactobacillus nagelii* cells; F+BL, fat diet plus betanin and *L. nagelii* cells. Bars represent the SD (n=6). *p < 0.01 compared with standard diet; + p < 0.01 compared with atherogenic diet.

Table 3: Levels+ of glucose (mg dL^{-1}), insulin (mU L^{-1}), leptin (ng mL^{-1}) and adiponectin (µg mL^{-1}) in mice after the administration of an atherogenic diet (AD) combined with *L. nagelii*/betanin for 30 days

Group	Glucose	Insulin	Leptin	Adiponectin
Standard diet (control)	84.4±1.48*	6.5±1.10*	1.7±0.15*	2.5±0.11*
AD	118.6±2.33	23.5±1.88	7.4±0.17	0.5±0.04
AD + betanin	97.3±0.38*	11.4±0.42*	3.1±0.27*	4.1±0.42*
AD + *L. nagelii*	99.8±3.51*	13.8±0.54*	3.9±0.58*	4.3±0.30*
AD + betanin + *L. nagelii*	95.4±1.39*	9.1±0.83*	2.8±0.33*	4.9±067*

+The results are presented as the mean ± SEM of samples from six mice (n=6). * p < 0.01 in comparison with the group treated with AD

DISCUSSION

The berries of *P. thyrsiflora* are rich in betanin, a common nutraceutical found in beetroot. Interestingly, these plant organs are used to prepare traditional beverages in the northern highlands of Puebla, Mexico. The endogenous levels of betanin in *P. thyrsiflora* were similar to those reported for *Phytolacca americana*, beetroot cultivars and the Malabar spinach [18,19]. Thus, the berries of *P. thyrsiflora* can be considered as a potential source to obtain betanin.

It is known that steroidal alkaloids are abundant constituents of the leaves and shoots of the *Phytolacca* genus [20]. however, the presence of these anti-nutrients in its berries is undetectable [20]. The inhibitory effect of betanin on alpha amylase and alpha glucosidase suggests that this compound may be participating as a possible modulator of carbohydrate absorption. However, further *in vivo* trials are required to support these enzymatic findings. The results on the administration of pure betanin in normoglycemic mice, strongly suggest that this natural pigment produces a significant anti-hyperglycemic effect in this animal model.

A previous study on the prolonged administration of betanidin (aglycone) demonstrated a significant reduction of glucose levels in BALB/c mice [21]. Nevertheless, the present work suggests the effect of betanin (glucoside form of betanidin) in postprandial glucose regulation. Contrary to the betanin effect, the administration of *L. nagelii* produced a more prolonged anti-hyperglycemic activity with no modifications on insulin levels. This property of *L. nagelii* cells could probably be related to their natural capacity to transform glucose into lactic acid. However, the participation of gut microbiota in the absorption of glucose cannot be discarded.

A similar biochemical tendency was previously reported with oral administration of viable lyophilized cells of *Lactobacillus rhamnosus* and *Lactobacillus delbrueckii* subsp. *vulgaricus* in the same normoglycemic mouse model [22]. These results suggest that *L. nagelii* may be acting as a peripheral regulator of glucose absorption during postprandial situation. Nevertheless, the possible interaction of *L. nagelii* with gut microbiota should be investigated in further works. On the other hand, the present investigation suggests that betanin can exert a sustained anti-hyperglycemic effect in diabetic mice whereas the single administration of *L. nagelii* was apparently less effective in diabetic model than in the normoglycemic mice.

Remarkably, a combination of both components resulted in a significant anti-hyperglycemic effect in the diabetic model. This synergistic effect could probably be based in the capacity of *L. nagelii* for converting glucose into lactic acid plus the moderate anti-alpha amylase/anti-alpha glucosidase and insulinogenic effects of betanin. Beneficial effects such as body weight loss, liver weight loss, decreases of LDL-c and triglycerides as well a substantial increase in HDL-c were observed. This experimental evidence may be related to the strong antioxidant activity of betanin and its capacity to ameliorate LDL-c oxidation [7].

The findings of this investigation suggest that a prolonged administration of *L. nagelii* cells could also contribute in the regulation of cholesterol and triglycerides in mice. Consequently, treated mice showed a significant decrease in body weight (about 28 %) and liver weight (about 35 %). This evidence shows the anti-obesity potential of the mixture of betanin and *L. nagelii*. Similar changes in lipid profile were reported by the continuous administration of *Lactobacillus plantarum* in obese rats [23]. However, the observed effect was lower than that of *L. nagelii*. Furthermore, the present work suggests that betanin could be one of the principal anti-obesity agents in *Phytolacca* berries [9].

The results obtained in this work shows that mixtures of betanin and *L. nagelii* produce favorable changes in the biosynthesis of key hormones involved in the metabolism of lipids and carbohydrates (insulin, leptin, adiponectin and insulin). Thus, the effect of these mixtures could probably ameliorate insulin resistance.

CONCLUSION

Betanin and *Lactobacillus nagelii* have been isolated and identified from *P. thyrsiflora* and the Mexican “coyol wine”, respectively. The results of the single or combined administration of these components in mice show a substantial improvement in the metabolism of carbohydrates and lipids. Simultaneous administration of both components produces a significant enhancement of anti-hyperglycemic and hypolipidemic activities. These properties may be related to the natural capacity of *L. nagelii* to metabolize glucose to lactic acid. The beneficial effect may be linked to a moderate anti-alpha-amylase and anti-alpha-glucosidase activities of betanin as well as its effect on insulin synthesis. The design of new food products based on these constituents should be considered.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this study.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All the authors named in this work participated equally in the design of experimental procedures, collection, analysis of the data and writing.

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