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RESEARCH PAPER

Metabolic Activity of Anthocyanin Extracts Loaded into Non-ionic Niosomes in Diet-Induced Obese Mice

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ABSTRACT

Purpose Anthocyanins (ACNs) are polyphenols that might reduce pathological processes associated with type 2 diabetes mellitus and other chronic diseases, but their bioavailability is still controversial. In this study, the metabolic activity of oral delivery of ACN-loaded niosomes was investigated and evaluated in a diet-induced obesity (DIO) mice model.

Methods ACNs extracted from *Vaccinium Meridionale* by the supercritical fluid extraction method were loaded in niosomes. The niosomal formulation was physically characterized and further administrated in drinking water to obese, insulin resistant mouse. We evaluated the effect of ACN loaded niosomes on hyperglycemia, glucose and insulin intolerance and insulin blood levels in C57BL/6 J mice fed with a high-fat diet.

Results The ACN-loaded particles were moderately monodisperse, showed a negative surface charge and 57% encapsulation efficiency. The ACN-loaded niosomes ameliorated the insulin resistance and glucose intolerance in the DIO mice model. Additionally, they reduced animal weight and plasma insulin, glucose, leptin and total cholesterol levels in obese mice.

Conclusion ACN-loaded niosomes administration, as a functional drink, had a beneficial effect on the reversal of metabolic abnormalities associated with obesity.

KEY WORDS anthocyanins · functional drink · niosomes, obesity · type 2 diabetes mellitus

ABBREVIATIONS

T2DM	Type 2 diabetes mellitus
ACNs	Anthocyanins
SCF	Supercritical fluid
DLS	Dynamic Light Scattering
ELS	Electrophoretic Light Scattering
TEM	Transmission Electron Microscopy
Cy-3-Gly	Cyanidin-3-O-glycoside
HFD	High-fat diet
Metf	Metformin
Nios	Niosomes
ACN/Nios	Anthocyanins loaded in niosomes
TC	Total cholesterol
TAG	Triacylglycerides
HDL	High-density lipoprotein
LDL	Low-density lipoprotein cholesterol
IPGTT	Intraperitoneal glucose tolerance test
ITT	Insulin tolerance test

INTRODUCTION

Obesity is a chronic disease that contributes to the development of fatal diseases, including type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD) and cancer (1).

Diana Colorado and Maritza Fernandez contributed equally to this work.

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According to the World Health Organization (WHO), approximately 30% of the world's population suffers from obesity-related disorders (2). Within diet-induced obesity, adipocytes become hypertrophied due to an increase in stored triacylglycerol. As hypertrophy increases and is maintained over time, the endocrine function of adipocytes is altered, establishing a microenvironment that induces oxidative stress, inflammation and release of non-esterified free fatty acids. All these alterations are involved in the generation of insulin resistance (3) and several studies have shown that it precedes the development of hyperglycemia in individuals who eventually develop T2DM (4).

The discovery of new targets that regulate immunometabolic alterations within obesity could lead to the identification of new therapeutic strategies that prevent insulin resistance and its associated complications (5). Under this approach, natural products can be a valuable source in the development of new treatments, with the advantage that information is available concerning their safety and therapeutic effects *in vivo* (6).

Functional foods contain a myriad of bioactive components that are consumed in the daily diet, and in addition to providing nutrients, they may provide potential therapeutic effects (7). Bioactive compounds exert pharmacological effects that modulate therapeutic bodily functions that are beneficial for health. For example, many types of bioactive molecules found in foods have been shown to exert antidiabetic, cardiovascular and neuroprotective effects and are related to the prevention of some types of cancer. Among such bioactive compounds, flavonoids, tannins, and anthocyanins (ACNs) are regarded as the main dietary phenolic compounds (8). The fruits of the genus *Vaccinium Meridionale* Sw (Agraz) possess high ACNs content, which is associated with high antioxidant activity and anti-inflammatory effects, and their consumption might significantly reduce the onset and progress of pathological processes such as T2DM, CVD, chronic neurological deterioration and gradual increase in weight (9–12). The potential health benefits of anthocyanins are often not realized due to their poor water solubility, chemical instability, adverse taste profile, and low oral bioavailability (13).

Nanotechnology is an emerging field recognized to have the potential to make impacts upon the prevention, detection and treatment of different diseases, where the physical, chemical, and biological properties of nanoscale materials are significantly different from those of bulk materials and single atoms or molecules. The application of nanotechnology within the design of drug delivery systems primarily allows for a more effective release of therapeutics to overcome some common pharmaceutical issues (14). Advantages include the achievement of enhanced therapeutic effects with lower drug doses, and the possibility to site-specific deliver hydrophobic–hydrophilic molecules while ensuring the protection of the compounds from degradation (15). Nanoemulsions, stable lipid nanoparticles, polymeric nanoparticles, nanosuspensions, nanogels, liposomes, and niosomes are some of the most often nanosystems utilized in the pharmaceutical industry because they have optimal characteristics for drug delivery (16) Niosomes are one of the vesicular systems that have received the most attention, due to their increased chemical stability, economic advantage, and practicality as well as straightforward methods of preparation without the requirement of pharmaceutically unaccepted solvents (17,18).

The present study aims to evaluate the metabolic activity of ACNs encapsulated in niosomes in a diet-induced murine obesity model, with the intent to increase the oral bioavailability of these unstable compounds to the physical, chemical and biological conditions of the digestive tract. The ultimate goal would be to develop a functional drink prototype for treatment or prevention of obesity and its associated metabolic complications.

MATERIAL AND METHODS

Anthocyanins Extraction from Vaccinium Meridionale Sw

Fruits of *Vaccinium Meridionale* Sw (Agraz) were collected in the area of San Vicente in Antioquia, Colombia (6°17′00.6"N 75°19′54.4"W). Fruits were washed and dried using a fluidized bed (Actum SLF-50 K) at 60°C with an airflow of 3000 rpm for six hours. ACNs were extracted using a Speed ™ SFE-2 supercritical fluid (SCF) system (Applied Separations, Inc. Allentown, PA, USA) with the following conditions: 2900 psi, 45°C, 2 h continued extraction using CO_2 as a solvent.

ACN analysis was performed by chromatographic separation on a reverse-phase using a Zorbax SB-C18 column (50 × 4.6 mm 5 μm) and an HPLC-DAD Agilent 1100 (Agilent Technologies, Santa Clara, CA, USA). Tentative identification of the ACN peaks was made by comparing retention times with delphinidin chloride and cyanidin used as primary standards (Sigma-Aldrich, MO, USA) and by HPLC-MS/API-ES analysis (Agilent Technologies, Santa Clara, CA, USA) (Supplementary information).

Determination of Antioxidant Activity of Extract

The antioxidant capacity of the ACNs extracted by the conventional method with ethanol (19) and by SCF was evaluated *in vitro*. Total phenolic analyses (Folin Ciocalteau analysis) (20), FRAPS (Ferric reducing/antioxidant power) (21), TEAC (Trolox Equivalent Antioxidant Capacity) (22) and ORAC (Oxygen Radical Absorbance Capacity) (23) were performed.

Niosomes Preparation

Nanoparticles (NPs) were assembled from a commercial pro-nanosome precursor (Nio-N de NANOVEX Biotechnologies S.L, Asturias, España) Lot 190,308, following a protocol modified from the manufacturer's recommendations. In brief, a 100 mg pro-nanosome vial was added to 1 mL of the ACN aqueous extract and 2 mL of absolute ethanol. The mixture was incubated for one hour at 45°C until complete dissolution. Five mL of deionized water was added and the mixture incubated for an additional one hour at 45°C. The solution was then dispersed with an ultrasound probe with a 1/8 (3 mm) replaceable tip (Branson Sonifier 250, Danbury, USA). The probe was introduced into the middle of the solution, with the instrument set at 40% for three min. The resulting emulsion was then homogenized at 10,000 rpm (L5M Silverson Silverson Machines mixer, Chesham, United Kingdom) for three min. Excess of unbound material was removed by size exclusion chromatography using Sephadex G-25 (Sigma-Aldrich, MO, USA) with water as eluent.

Formulations for animals were elaborated as follows: Aqueous extract of ACNs (containing 514 mg cyanidin-3-O-glycoside) was added to a 1.600 mg pro-nanosome ethanolic solution (16 vials) and treated as described above. Purification process was performed twice by ultracentrifugation at 10.000 rpm for 1 h at 4°C. 256 mg of ACN were recovered and resuspended in 1,6 L of water (160 µg/mL). This solution was prepared once a week and administered to animals. Solutions administered in drinking water were niosomes alone, ACNs alone and ACN-loaded niosomes at concentrations of 100, 280 and 160 µg/mL, respectively.

Our starting point was to administer the amount of ACN recommended by OECD guidelines (24) for the evaluation of chemicals in animal models. By supplying mice with a solution of 280 µg/mL ACN alone, we assume that we would be giving each animal 300 mg of ACN/Kg of body weight. The amount of ACN (160 µg/mL) in the ACN-loaded niosomes (ACN/Nios) solution is equivalent to the amount of ACN encapsulated in the niosomes, after removing non-encapsulated ACN.

Several studies report that niosome-encapsulation of ACN considerably reduces the administered dose required to obtain desired biological effects (25, 26). We tested the hypothesis that ACNs loaded in niosomes induce desired effects at lower doses than ACNs alone and in a shorter time. The solution with niosomes alone (100 µg/mL), was prepared as described in material and methods, and adjusted to the same concentration of niosomes used for encapsulation of ACN.

Nanoparticle Characterization

Particle size and surface charge were estimated by Dynamic Light Scattering (DLS) and Electrophoretic Light Scattering (ELS), respectively, using a Malvern particle size analyzer (Master Sizer X-100, Malvern Instruments). Samples for DLS were kept in polystyrene cuvettes and measurements were made at 25.0 ± 0.1°C after dilution of the nanoparticles in purified water (1: 100). Results were expressed as the mean particle size and polydispersity index (PDI). Samples for ELS were diluted adequately with purified water and placed in a specific cuvette and a potential of +150 mV was applied. All assays were performed in triplicate.

Morphology of the nanoparticles was examined with Transmission Electron Microscopy (TEM). Briefly, 5 µl of each sample was adhered to glow discharged carbon-coated grids for 60 s. Samples were visualized with a Tecnai F20 super Twin TMP microscope (FEI Company, Hillsboro, OR, USA), operating at an accelerating voltage of 200 keV in a bright-field image mode. Digital images were acquired with a GATAN US 1000XP-P and an EDX Oxford Instruments XMAX detector. Image analysis used Image J software. (27)

Encapsulation Efficiency

Nanoparticles were centrifuged at 10,000 rpm for 60 min. The amount of free extract (non-incorporated into the niosomes) was measured by differential pH method using a VarioScan™ LUX multimode microplate reader (ThermoFisher Scientific, Waltham, MA, USA) (28). In brief, samples were mixed with two buffers, potassium chloride (pH 1.0) and sodium acetate (pH 4.5), for measuring the absorbance at 520 and 700 nm, respectively. The concentration of free ACNs (mg/L) was evaluated as cyanidin-3-O-glycoside equivalent according to the following expression $A = [(A_{520} - A_{700})\ pH\ 1.0 - (A_{520} - A_{700})\ pH\ 4.5]$. Total anthocyanin content was calculated using the equation:

$$(\mathrm{Cy{-}3{-}Gly})\ \mathrm{mg/L}\Big) = (A \times MW \times DF)/(\times 1\ \mathrm{cm}) \quad (1)$$

Cyanidin-3-O-glycoside (Cy-3-Gly) was the standard compound; whose molar extinction coefficient (ε) is 26,900 ($L\ cm^{-1}\ mol^{-1}$); DF is the dilution factor; A is the absorbance and MW is the molecular weight (449.2 g/mol). The results were expressed as equivalent milligrams of cyanidin-3-glycoside per 100 L of fresh fruit. This quantification method was standardized according to the International Conference on Harmonization (ICH), International Theme Q2 (R1) guideline (CPMP / ICH / 381/95). The encapsulation efficiency (EE) was determined from the following equation (Eq):

$$(EE\%) = \frac{\text{Total anthocyanins} - \text{Free anthocyanins}}{\text{Total initial anthocyanins}}\ X\ 100 \quad (2)$$

In-Vitro Release Study

Five mg of nanoparticles were suspended in 1.0 mL of PBS (pH = 7.4) for *in vitro* ACN release studies. Samples were incubated at 37°C with intense shaking. At predetermined time intervals (0.5, 1, 2, 4, 6, 8, and 24 h); tubes were centrifuged at 10,000 rpm for 60 min. Concentration of released ACNs were measured using differential pH with the VarioScan (ThermoFisher Scientific, USA). (28)

Animal Model

All animal experiments were performed in compliance with the guidelines for care and use of research animals established by the Institutional Animal Care and Use Committee of The University of Antioquia (Protocol # 65). C57BL/6 mice were obtained from Charles Rivers Laboratories (Wilmington, MA, USA). Male mice over 3 weeks old were used in this study.

Glucose and Insulin Tolerance Test in the Mouse Model of Insulin Resistance

Mice were housed at 22 ± 2°C with a 12:12 h light dark cycle with free access to food and water. Mice were randomly divided into two groups for this study. A control group (n = 10) was fed a standard diet (Chow, 14% fat/54% carbohydrates/32% protein). An HFD group (n = 60) was fed a high-fat diet (HFD, 42% fat/42% carbohydrates/15% proteins) and after 12 weeks under HFD, was divided into 5 different groups. HFD+ (Metf.) group received 10 oral doses of metformin 200 µg/mL; HFD + (Nios) group received a niosomal solution 100 µg/mL; HFD + (ACN) group received an ACNs solution 280 µg/mL, and HFD + (ACN/Nios) group received a solution with 160 µg/ml ACN-loaded niosomes. All treatments were administered over a four-week period. Animals were divided into cages with four mice each. Different treatments were prepared in 200 mL of drinking water, provided to mice ad libitum and changed every two days.

Before and after different treatments, an intraperitoneal glucose tolerance test (IPGTT) and insulin tolerance test (ITT) were performed after 6 h fast. Initial blood glucose levels were determined, followed by administering a glucose load of 2,0 g/kg body weight or by injection of human insulin 0.75 U/kg (Humulin, Eli Lilly, Indianapolis, IN, USA). Blood glucose levels were measured via tail vein blood at 0, 30, 60 and 120 min after the injection using a GlucoQuick Glucometer (Procaps, Barranquilla, Colombia). The zero time was measured just before glucose injection. After glucose metabolism studies, mice were sacrificed and blood, liver and adipose tissue were collected to further analysis.

Serum Biochemical Parameters

The following commercial kits were used to determine serum biochemical parameters. Total cholesterol (TC, 11505), triacylglycerides (TAG, 11528), high-density lipoprotein (HDL, 11557), low-density lipoprotein cholesterol (LDL, 11585), alkaline phosphatase (21590), alanine aminotransferase (11832), aspartate aminotransferase (23531) (BioSystems S.A, Barcelona, España), leptin (ab100718) and adiponectin (ab108785) (Abcam, Cambridge, UK). Insulin in mouse serum was quantified using an ultrasensitive enzyme immunoassay (10–1247-01 Mercodia, Uppsala, Sweden). All determinations were done following the manufacture's recommendation, and the absorbance of each sample was measured using a Varioskan™ LUX multimode micro plate reader (ThermoFisher Scientific, USA).

Statistical Analysis

Results are expressed as means ± sem. Comparisons between groups were analyzed using a one-way analysis of variance (ANOVA) followed by a Dunnett post hoc test. Student's t-tests were used to compute individual pairwise comparisons of least square means. The trapezoidal rule was used to determine the area under the curve (AUC). Differences were considered to be significant at $P < 0.05$. All analyses were performed with Prism 8 (GraphPad Software, Inc., La Jolla, CA, USA) statistical software.

RESULTS

ACN Characterization

ACN extracts obtained with the SCF system had less of cyanidin-3-glycoside (Cy-3-Gly) content but higher amounts of total phenols and antioxidant capacity compared with ethanolic extraction (Table I)(Fig. 1a). SCF extracts also contained other ACNs, such as delphinidin-3-glycoside, malvidin-3-glycoside and some other unidentified compounds (Supplementary information).

Characterization, Encapsulation Efficiency and Release Profile of Nanoparticles

Characterization of ACN encapsulated particles showed a mean diameter of 219.7 ± 3.1 nm with a PDI of 0.25 ± 0.1, slightly higher than empty particles (200 ± 5.1 nm; 0.073 ± 0.1 PDI)(Table II). The intensity-diameter profile indicated that all particles were moderately monodisperse (Fig. 1b). ζ-potential showed a negative surface charge for empty and loaded nanoparticles, suggesting moderate stability due to

Table I *In Vitro* Evaluation of Antioxidant Activity of an Anthocyanin-Rich Extract from *Vaccinium meridionale*

Sample	Total Phenolics (μg GAE/g extract)	ORAC (uM TE/g extract)	FRAP (μg aa/g extract)	TEAC (uM TE/g extract)	Cyd 3 glu (mg/100 g extract)
SFE	22,089.38	1629.59	161,225.30	291.07	0.79
Ethanol extraction	10,032.09	442.42	62,577.08	157.25	1.90

SFE, supercritical fluid extraction; *Cyd 3 glu*, Cyanidin 3-glucoside; *GAE*, Gallic acid equivalents; *ORAC*, Oxygen Radical Absorbance Capacity; *FRAP*, Ferric Reducing Antioxidant Power; *TEAC*, Trolox-Equivalent Antioxidant Capacity

electrostatic repulsion forces that prevent vesicle aggregation (Table II).

Encapsulation efficiency was estimated to be 57%. This apparent low efficiency may be attributed to the complexity of the extract and quantification methodology. Only Cy-3-Gly was assessed in ACN-loaded niosomes. TEM showed that niosomes were quasi-spherical with a diameter size of 200 ± 0.7 nm ($n = 30$), consistent with vesicular nature (Fig. 1c). Cyanidin-3-glycoside determinations show a sustained release profile in the first hours of the experiment, reaching 81% after 8 h and attaining a plateau that persisted to hour 24 (Fig. 1d).

Anthocyanin-Loaded Niosomes Improve Carbohydrate Metabolism in Obese, Insulin-Resistant Mice

Results obtained from glucose and insulin tolerance tests were analyzed as a total area under the curve (AUC) between 0 and 120 min for IPGTT and between 0 and 60 min. For ITT. After four weeks of treatment, HFD-fed mice treated with metformin (HFD+ (Metf.) and ACN-loaded niosomes (HFD + (ACN/Nios) showed improved glucose tolerance and insulin sensitivity as compared to HFD-fed mice group with no treatment (Fig. 2a-d). Standard diet-fed mice exhibited a 36% reduction in glucose level 15 min after insulin administration. However, the glucose level of HFD-fed mice was increased by

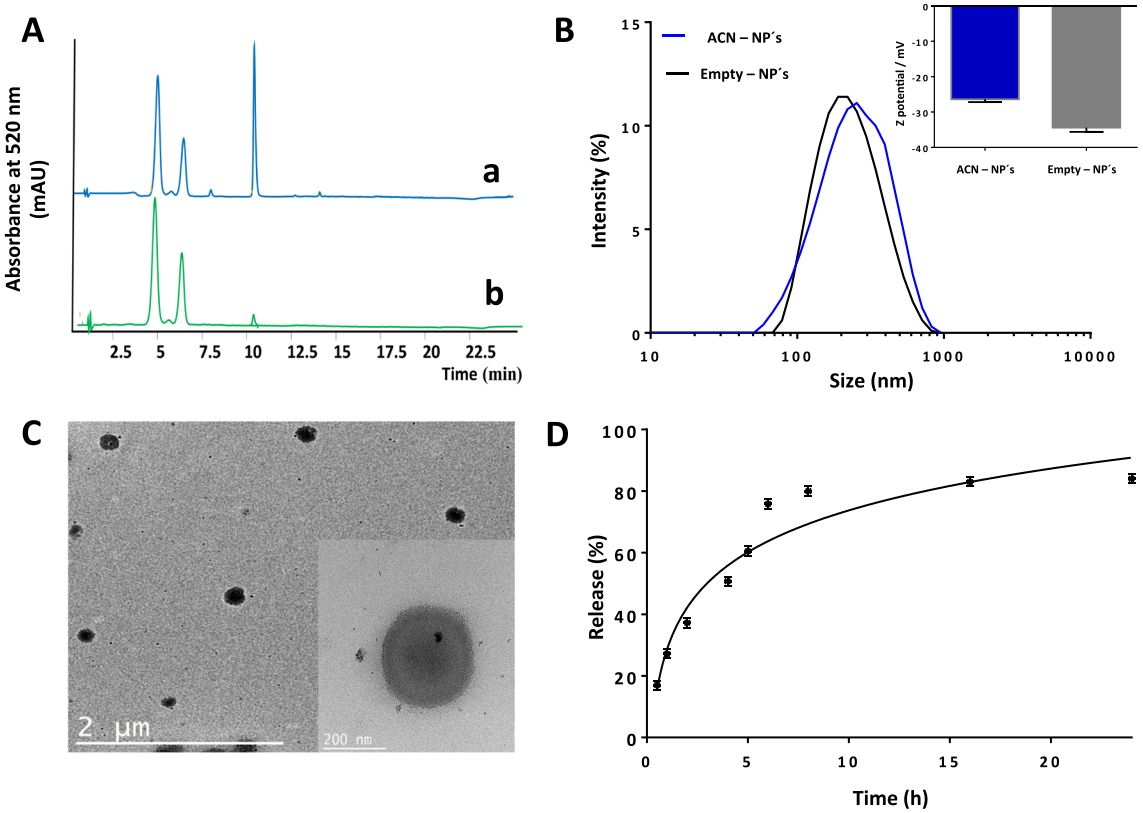

Fig. 1 Chromatograms are comparing the peaks present in the extraction of ACN from Agraz by supercritical fluids and ethanolic extraction (**a**) and the ACN-loaded niosomes (**b-d**). Particle size (**b**) and surface charge (inset) of loaded and empty nanoparticles. Transmission Electron Microscopy images of loaded (**c**) and empty nanoparticles (inset); and *in vitro* release profile of agraz from the niosomes under simulated conditions, with buffer pH 7.4 as a release medium. Data are expressed as mean ± standard deviation of the data ($n = 3$).

Table II Characterization of Anthocyanins (ACN) - Encapsulated Niosomes

Formulation	Size (nm)	PDI	PZ (mv)	EE (%)
ACN nanoparticle	219.7 ± 3.1	0.25 ± 0.1	−26.4 ± 0.8	57.4 ± 1.2
Empthy nanoparticle	200 ± 5.1	0.073 ± 0.1	−34.4 ± 1.2	NA

Data represent the average ± SD (n = 3)

PDI, polydispersity index; *PZ*, ζ-potential; *NA*, Not apply

4%. Glucose reduction, 15 min after insulin challenge, was 18.6, 5.7, 17.7 and 21.9% for those treated with metformin, niosomes, ACN and ACN/Nios, respectively (Fig. 2c).

Animals treated with Metf (23.2%), ACN (22.4%) and ACN/Nios (30.7%), displayed significantly reduced fasting glucose levels after treatments, reaching glucose levels observed in animals fed with a standard diet (Fig. 2e). AUCs derived from glucose tolerance test were only significantly reduced by Metf and ACN/Nios treatments (Fig. 2f).

Anthocyanin-Loaded Niosomes Reverse Metabolic Parameters in Obese, Insulin-Resistant Mice

Bodyweight measurements over time showed that ACN-loaded niosomes only caused a gradual decreased in weight starting after week two of treatment (Fig. 3a). A significant difference ($p < 0.05$) was observed as compared to the HFD-fed mice at the end of 4 weeks of treatment. The reduction in body weight is partially explained by a significant reduction in visceral fat and liver weight (Fig. 3b).

Mice treated with Metf and ACN/Nios showed a significant reduction in blood insulin concentrations compared with HFD group animals (Table III). Further, blood insulin levels in animals treated with ACN/Nios were significantly lower than levels in mice treated with ACNs alone. Fasting glucose levels decreased significantly in animals treated with metformin, ACN and ACN/Nios compared with levels in mice fed HFD.

Levels of triacylglycerol (TAG), high-density lipoproteins (HDL) and low-density lipoproteins (LDL) did not change significantly among treatment groups. In contrast, total cholesterol levels (CT) decreased significantly in mice treated with ACN/Nios. Treatments did not alter serum adiponectin levels compared to levels in mice fed HFD. However, concentrations of leptin decreased significantly in mice treated with ACN/Nios. Administration of ACN/Nios reversed some of the metabolic abnormalities associated with obesity. Additionally, ACNs loaded into niosomes reduce the expression of pro-inflammatory cytokines in the adipose tissue of obese mice. We could not identify a specific antioxidant activity, either in the animal model or in a macrophage cell line, J774. (Supplementary information).

Levels of serum alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase were measured to evaluated liver function. Animals administered Nios, ACN or ACN/Nios did not show altered levels of these enzymes when compared with mice fed either a standard diet (chow) or HFD. Niosomes and ACN thus do not show obvious hepatotoxicity when added to drinking water.

Table III Serum Parameters for the Male C57BL/6 Mice Fed with Chow, HFD, HFD Plus Niosomes or ACN or ACN/NIOS

	Chow	*p	HFD	HFD + Metf	*p	HFD + Nios.	*p	HFD + ACN	*p	HFD + ACN/Nios.	*p
Insulin (μg/mL)	1,0 ± 0,1	d	5,4 ± 0,6	2,9 ± 0,4	b	3,9 ± 0,5	n.s	5,2 ± 0,6	n.s	2,8 ± 0,5	b
Glucose (mg/dL) #	150[91,159]	c	194[180,240]	153[130,184]	a	168[146,213]	n.s	160[135,166]	a	141[105,165]	c
TC (mg/dL) #	60,8[31,91]	d	247,6[135,311]	179,5[130,204]	n.s	179,2[169,261]	n.s	183,3[150,242]	n.s	156,6[86,191]	a
TAG (mg/dL) #	71,2[17,114]	n.s	81,2[13,141]	39,2[12,157]	n.s	33,2[18,235]	n.s	75,4[56,99]	n.s	71,4[17,162]	n.s
LDL (mg/dL) #	54,6[32,68]	n.s	57,3[53,76]	57,2[45,77]	n.s	54,0[51,61]	n.s	53,3[47,68]	n.s	58,8[46,67]	n.s
HDL (mg/dL) #	70,1[62,85]	n.s	78,6[65,92]	69,6[55,113]	n.s	68,4[63,106]	n.s	78,3[68,81]	n.s	79,9[73,108]	n.s
Leptin (pg/mL)	6263 ± 1877	d	84,818 ± 6032	58,016 ± 6533	n.s	78,008 ± 10,632	n.s	56,455 ± 10,632	n.s	36,714 ± 12,963	c
Adiponectin (ng/mL)	4111 ± 79,3	a	3420 ± 141,3	3261 ± 141,1	n.s	3752 ± 180,6	n.s	3850 ± 230,6	n.s	3631 ± 232,8	n.s
AP (U/L)	39,2 ± 5,2	a	41,3 ± 4,7	21,4 ± 4,6	a	34,1 ± 2,7	n.s	32,4 ± 2,4	n.s	35,0 ± 5,2	n.s
AST (U/L)	61,1[44,119]	n.s	86,2[49,135]	85,7[74,135]	n.s	66,3[50,88]	n.s	59,5[52,154]	n.s	100,0[60,206]	n.s
ALT (U/L)	37,2 ± 7,1	n.s	55,0 ± 6,5	50,1 ± 8,4	n.s	31,3 ± 5,4	n.s	30,6 ± 6,3	n.s	41,6 ± 7,3	n.s

TC, Total Cholesterol; *TAG*, triacylglycerol; *LDL*, Low density lipoprotein; *HDL*, High density lipoprotein; *AP*, alkaline phosphatase; *AST*, Aspartate transaminase; *ALT*, alanine transaminase; *HFD*, High fat die; *Metf*, Metformin; *Nios*, Niosomes; *ACN*, Anthocyanins; *ACN/NIOS*, Anthocyanins loaded into niosomes. N = 6–9 mice/group. The values represent the means ± SE, ANOVA with Tukey post-test of multiple comparisons and as medians ± interquartile range, #: Kruskal-Wallis.* a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.001$, d: $p < 0.0001$ n.s: non-significant. All Groups *vs* HFD Group

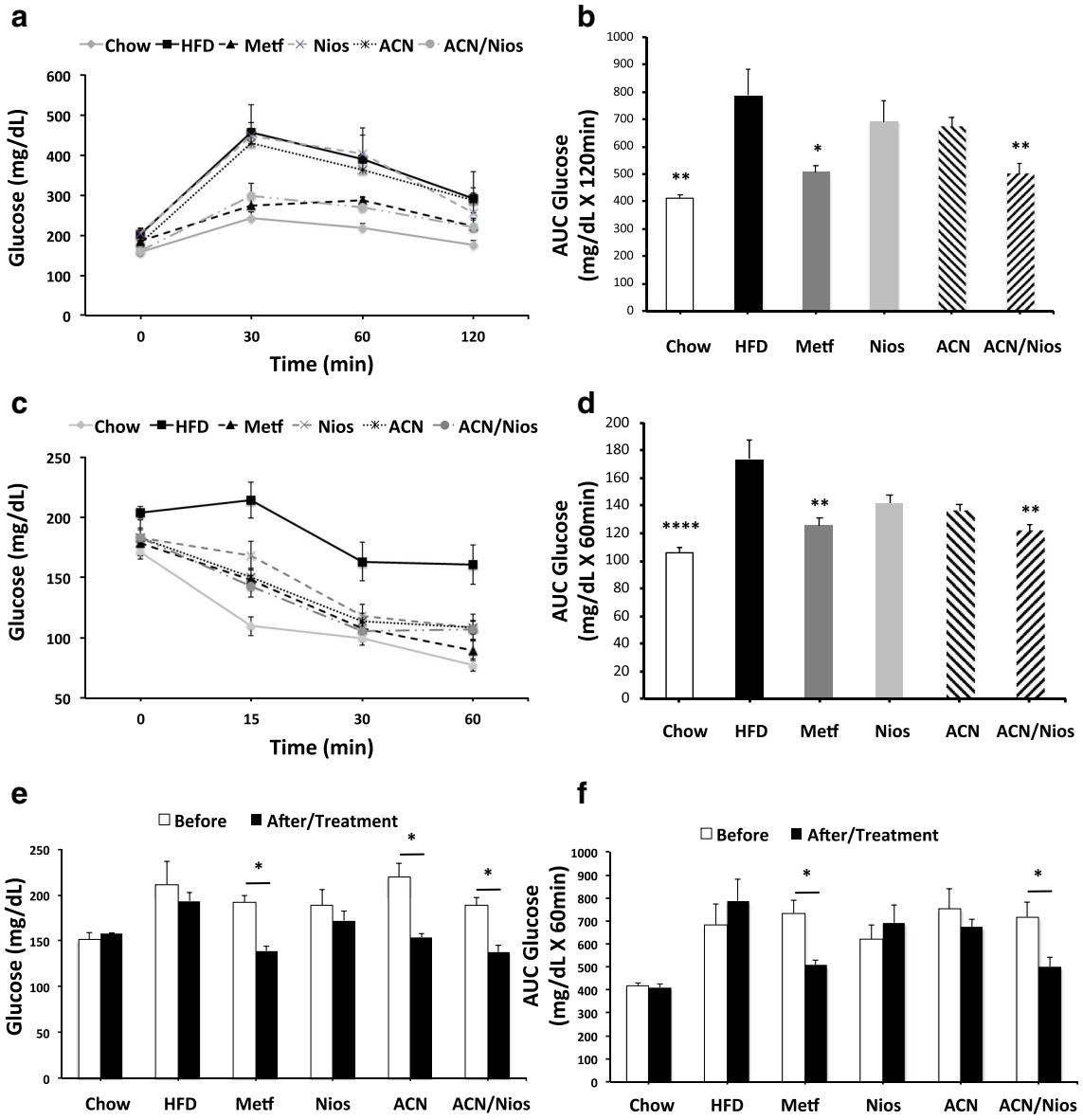

Fig. 2 Glucose (**a** and **b**) and insulin (**c** and **d**) tolerance test in obese animals treated with metformin (Metf.), Niosomes (Nios), anthocyanins (ACN) and ACN-loaded niosomes (ACN/Nios). All tests were performed after 4 weeks of treatment or the administration of 10 doses in the case of Metf. (**b**, **d**) the area under the curve (AUC), calculated from the data obtained in A and C. Comparison of fasting glucose levels (**e**) and areas under the curve (AUC) of glucose tolerance tests (**f**), before and after treatments. HFD: High Fat Diet. $N = 10$ *: $p < 0.05$, **: $p < 0.01$. ***: $p < 0.0001$, ANOVA with Tukey post-test of multiple comparisons (**a**, **b**,**c** and **d**). E and F, a t-Student test was performed. Values were expressed as mean ± standard error.

DISCUSSION

In the present work, the metabolic activity of an ACNs extract obtained from *Vaccinium Meridionale* Sw (Agraz) was packaged into non-ionic niosomes and evaluated in a diet-induced obesity mouse model.

The supercritical fluid (SCF) extraction method used here revealed the presence of four anthocyanins: cyanidin-3-glucoside, cyanidin-3-arabinoside, cyanidin-3-galactoside, and delphinidin-3-hexoside. These data correspond to those reported by Garzón *et al.* (2010) (19), for anthocyanins identified in agraz fruits grown in native habitats of Colombia. In the previous study, ACNs extraction was performed using methanol as a solvent, with resultant yields of 329 mg cyanidin-3 glycoside and total phenol content of 758.6 mg GAE per 100 g of extract. This method results in isolation of higher amounts of cyanidin 3-glucoside, but less total phenols, when compared to the ethanol extraction method used in the present study, with the difference being more profound when compared with SCF extraction. Extraction with SCF incurs a beneficial advantage in that it is a clean technology that does not generate contaminant by-products. On the other hand, a higher

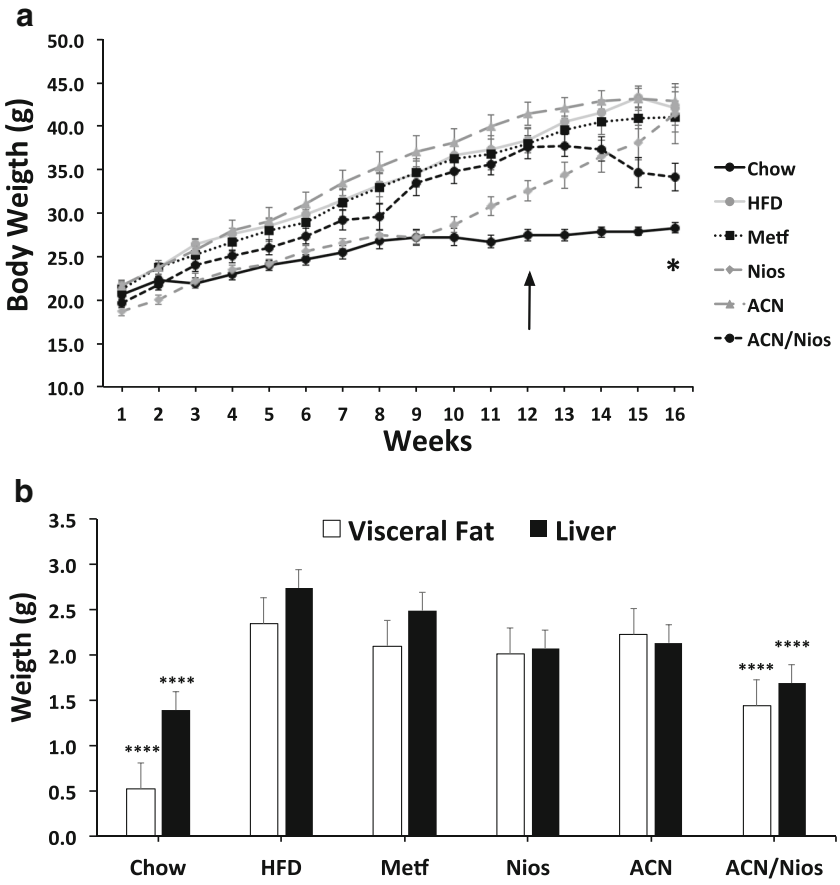

Fig. 3 Evaluation of body weight (**a**) and visceral fat and liver (**b**) of the experimental mice. The treatments with metformin (Metf), Niosomes (Nios), anthocyanins (ACN) and ACN-loaded niosomes (ACN/Nios), was given for 4 weeks and started at week 12. HFD: High Fat Diet. $N = 10$. (**a**) *: $p < 0.05$, ***: $p < 0.001$, ****: $p < 0.0001$, ANOVA with Tukey post-test of multiple comparisons. (**b**) A t-Student test was performed. Values were expressed as mean ± standard error. All groups were compared to the group fed with HFD.

amount of other phenolic compounds is obtained, which include flavonoids such as quercetin that have been shown to exhibit high anti-inflammatory, cardioprotective, neuroprotective and anti-carcinogenic activities (9,29).

Several studies demonstrate the role of ACNs in the prevention and treatment of metabolic syndrome and type 2 diabetes. ACNs have been reported to reverse insulin resistance and improve glucose tolerance, dyslipidemia, glycemia and protect β-cell functionality through different mechanisms. ACNs regulate GLUT4 expression and translocation and increase PPARγ activation in adipose tissue and muscle cells. They increase expression of AMP-activated protein kinase, the secretion of leptin and adiponectin and reduce the expression of retinol-binding protein 4 (RBP4). Further, they inhibit intestinal α-glucosidase and pancreatic α-amylase, reducing the amount of carbohydrate absorbed and mimicking caloric restriction. Likewise, the anti-inflammatory activity of ACN is associated with reduced expression of TNF-α, MCP-1 and IL-6 in adipose tissue, probably via modulating the AMPK pathway (30,31).

Several studies have demonstrated the role of ACNs in the prevention and treatment of T2DM through different mechanisms (11,32). However, the use of ACN-rich extracts as a functional food or medical treatment is limited due to the low stability of this type of molecule in various environmental conditions during processing and storage, such as heat, the presence of oxygen, light and pH (33). These compounds are also susceptible to interaction with other foods and additives that result in both reduced bioavailability and bioactivity (13,34). The beneficial effect(s) upon obese individuals might be reached following regular consumption, for which quantities and duration remain undefined.

The vesicular systems, also known as niosomes, have been comparatively better accepted within the pharmaceutical industry as compared to other systems due to the presence of non-ionic surfactants that improves their safety profile (14). Furthermore, they have the potential to be custom-made and encapsulate both hydrophobic and hydrophilic compounds. Herein, an ACNs aqueous extract was encapsulated into niosomes by an in-house modified production process with ethanol as a solvent, sonication and a homogenization set-up. The resultant slightly increased size of the ACN-loaded nanosomes concerning the empty ones, maybe explained by the cargo that having a high phenolic content coming from the SCF extraction process may have more interaction with the components of the formulation, thereby increasing the nanoparticle size (35). Remarkably, modifications of this methodology produced smaller nanoparticles with much lower PDI than those reported by the manufacturer and other authors (18,36), which is highly beneficial for our purpose.

The ζ-potential of the loaded nanoparticles was probably affected by the ACNs, which have a positive charge at the oxygen atom of the C-ring of the flavonoid structure that may affect the chemical nanoparticle environment, thus decreasing the resultant ζ-potential.

The encapsulation efficiency was similar to those reported in other studies (37,38), and the resultant size of the ACN-loaded niosomes is ideal for oral administration. It is known that particles <5 μm will pass through lymphatic vessels and M cells of Peyer's patches and those with sizes <500 nm can pass through the epithelial cell membrane via endocytosis (18).

The cyanidin-3-glycoside showed a sustained release profile attaining a plateau at 24 h of the assay. We speculate that the remaining ACNs were released entirely after 24 h. However, since the solution underwent a colour change, which may indicate instability of the pH-dependent anthocyanins (34), we expect that such colour change impacted the quantification of the amount of released compound following 24 h, so that these data were not taken into account. This characteristic force us to supplement the loaded nanoparticles every 24 h to ensure a continuous supply of ACNs to the test animals. Moreover, the release profile of these niosomes could be improved by modulating the amount of cholesterol and surfactant of the formulation, by tuning membrane stiffness, and thus decreasing the outflow (39).

When studying the ACN release kinetics, data fit well with a Korsmeyer-Peppas model, which implies that erosion and dilution of the matrix may be taking place. The *n* value was slightly lower than 0.5, indicating the porosity of the material and the agraz components are coming out from the niosomes by a combined mechanism, i.e. partial diffusion through both, the swelled matrix and the water-filled pores. This kind of release mechanism had been reported for other niosomes (40,41), (see Supplementary information).

In the present study, ACNs were dissolved in water and packaged in niosomes (i.e. ACN/Nios) and further administered in the animals' drinking water at concentrations of 280 and 160 μg/ml, respectively for four weeks. Although it is difficult to compare these doses and administration times with other studies, the results of the present work highlight two important aspects. The administration of the ACN/Nios formulation as a functional drink and the biological effect of such on the employed animal model is achieved following 4 weeks of administration, while the administration of dissolved ACNs does not have a marked effect upon the reversal of the metabolic abnormalities associated with obesity.

Studies carried out by Chen *et al.* (2018) (42) showed that the administration of 200 and 400 mg/kg per day for 28 days of a soybean extract enriched with anthocyanins slightly reduces hyperglycemia and hyperlipidemia in a T2DM mouse model. This study has been validated in various animal models, in which the beneficial effect of anthocyanin administration within the reduction of immunometabolic markers associated with obesity and diabetes has been determined. These studies have evaluated the administration of raw extracts in food amounts ranging from 0.2% (wt/wt) of cyanidin-3 glycoside, to mixtures of 10% (wt/wt), depending on the plant source from which the ACNs were obtained. However, administration times vary widely, from 3 to 8 weeks, depending on the animal model used and the source of anthocyanins administered. (43–46).

In the animal model used here, the biological activity of ACNs is evident when they are packaged in niosomes. These nanoparticles appear to increase the bioavailability of ACNs, as only the animal group treated with ACN/Nios reduced fasting glucose and insulin levels, as well as intolerance to glucose and insulin. These results suggest that treatment with the ACN/Nios formulation significantly affects the reversal of the systemic insulin resistance established within the animal model. This reversion was not identified in animals having received only ACNs, even though a higher concentration was administrated. Most likely, the administration of the treatment based on ACNs alone for an extended time can equal the effects identified in the animals treated with ACN/Nios.

Niosomes serve as an alternative for the oral administration of aqueous extracts (47). Here, we observed improvement in the bioavailability profile of the metabolites present in agraz, likely due to absorption in the stomach and small intestine of the gastrointestinal tract (18). Besides, others have indicated that phospholipids and lipoid surfactants are not hydrolyzed by gastric lipases suggesting that vesicular systems such as niosomes remain protected in the stomach (48).

Based on the lipid nature of niosomes, drug bioavailability following oral administration is enhanced via a mechanism similar to conventional lipid-based carriers. Surfactants increase the permeability of the intestinal membrane in a concentration-dependent manner, but not all non-ionic surfactants enhance the drug absorption when present at concentrations above their critical micelle concentration. Conversely, toxic effects reported are more pronounced when this critical concentration is surpassed.

A differential biological effect between the animal groups treated with ACN *vs* ACN/Nios was evidenced in other metabolic markers as well. In the latter group, the levels of total cholesterol, leptin, and expression of pro-inflammatory cytokines within adipose tissue were reduced. Likewise, it was observed that the administration of the ACN/Nios formulation significantly reduced animal weight with 4 weeks of treatment. Therefore, this work provides essential information in that encapsulation was shown to protect ACNs from degradation, thereby increasing their bioavailability and bringing us closer to a determination of the amount and duration required for the administration of these compounds.

In animals that received the ACNs treatment alone (dissolved in water), a reduction in fasting glucose could be observed, but no changes in glucose or insulin tolerance were

noted. The decrease in fasting glucose concentration within these animals could be explained by the fact that at the intestinal level, ACNs inhibit the absorption of glucose from the diet into the general circulation (49), and thus its hepatic storage as glycogen is reduced. Thus, the amount of glucose released via glycogenolysis is reduced during fasting.

Encapsulation of ACN mixtures within niosomes both protects the different components of the mixture as well as increases their availability and therapeutic effect. *In vitro* studies have shown that ACNs encapsulation reduces the necessary effective dose when used in formulations for management of mitochondrial dysfunction or cancer (50–52). This system of administration of ACN-loaded niosomes has been successfully used for the treatment of other pathologies in animal models. For example, multiple groups have shown that ACNs loaded into niosomes resulted in a more significant effect in reducing memory impairment in a mouse model of Alzheimer's disease than the administration of ACN alone (53,54). Additionally, Priprem *et al.* (2018) identified a higher anti-inflammatory activity after using this drug delivery form within a model of oral injury in rats (38).

In conclusion, the functional drink prepared with anthocyanins-loaded niosomes was well tolerated by animals and resulted in an increased anti-hyperglycemic/anti-diabetic effect within a diet-induced mice obesity model, without inducing any apparent toxicity.

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AUTHOR CONTRIBUTIONS

Diana Colorado: Methodology, **Sergio Acín**: Methodology, supervision, **Diana Lorena Muñoz:** Methodology, **Maritza Fernandez:** Methodology, formal analysis, **Jahir Orozco**: Conceptualization, formal analysis, writing - reviewing & editing, **Yasmin Lopera**: Methodology, formal analysis, **Norman Balcazar**: Conceptualization, formal analysis, writing - review, editing & supervision.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest The author reports no conflict of interest relevant to this work.

REFERENCES

1. Bluher M. Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol. 2019;15(5):288–98.
2. Organization WH. Obesity and overweight. WHO Web site http://www.hoint/mediacentre/factsheets/fs311/en/ Updated March 2011. 2011.
3. Ahima RS. Digging deeper into obesity. J Clin Invest. 2011;121(6):2076–9.
4. Czech MP. Insulin action and resistance in obesity and type 2 diabetes. Nat Med. 2017;23(7):804–14.
5. Israili ZH. Advances in the treatment of type 2 diabetes mellitus. Am J Ther. 2011;18(2):117–52.
6. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod. 2012;75(3):311–35.
7. Konstantinidi M, Koutelidakis AE. Functional foods and bioactive compounds: a review of its possible role on weight management and obesity's metabolic consequences. Medicines (Basel). 2019;6(3).
8. Blesso CN. Dietary anthocyanins and human health. Nutrients. 2019;11(9).
9. Scalbert A, Manach C, Morand C, Remesy C, Jimenez L. Dietary polyphenols and the prevention of diseases. Crit Rev Food Sci Nutr. 2005;45(4):287–306.
10. Lee YM, Yoon Y, Yoon H, Park HM, Song S, Yeum KJ. Dietary anthocyanins against obesity and inflammation. Nutrients. 2017;9(10).
11. Rozanska D, Regulska-Ilow B. The significance of anthocyanins in the prevention and treatment of type 2 diabetes. Adv Clin Exp Med. 2018;27(1):135–42.
12. Kimble R, Keane KM, Lodge JK, Howatson G. Dietary intake of anthocyanins and risk of cardiovascular disease: a systematic review and meta-analysis of prospective cohort studies. Crit Rev Food Sci Nutr. 2019;59(18):3032–43.
13. Lila MA, Burton-Freeman B, Grace M, Kalt W. Unraveling anthocyanin bioavailability for human health. Annu Rev Food Sci Technol. 2016;7:375–93.
14. Iqbal MA, Md S, Sahni JK, Baboota S, Dang S, Ali J. Nanostructured lipid carriers system: recent advances in drug delivery. J Drug Target. 2012;20(10):813–30.
15. Speranza B, Petruzzi L, Bevilacqua A, Gallo M, Campaniello D, Sinigaglia M, *et al.* Encapsulation of active compounds in fruit and vegetable juice processing: current state and perspectives. J Food Sci. 2017;82(6):1291–301.
16. Mena-Giraldo P, Perez-Buitrago S, Londono-Berrio M, Ortiz-Trujillo IC, Hoyos-Palacio LM, Orozco J. Photosensitive nanocarriers for specific delivery of cargo into cells. Sci Rep. 2020;10(1):2110.
17. Aditya NP, Espinosa YG, Norton IT. Encapsulation systems for the delivery of hydrophilic nutraceuticals: food application. Biotechnol Adv. 2017;35(4):450–7.
18. Fidan-Yardimci M, Akay S, Sharifi F, Sevimli-Gur C, Ongen G, Yesil-Celiktas O. A novel niosome formulation for encapsulation of anthocyanins and modelling intestinal transport. Food Chem. 2019;293:57–65.
19. Garzón G, Narváez CE, Riedl KM, Schwartz SJ. Chemical composition, anthocyanins, non-anthocyanin phenolics and antioxidant activity of wild bilberry (Vaccinium meridionale Swartz) from Colombia. Food Chem. 2010;122:980–6.
20. Swain T, Hillis, W.E. The phenolic constituents of Prunus domestica I—the quantitative analysis of phenolic constituents. Journal of Science of Food and Agriculture 1959;10:63–68.
21. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239(1):70–6.

22. Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 2001;73:239–44.
23. Prior RL, Hoang H, Gu L, Wu X, Bacchiocca M, Howard L, *et al.* Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC(FL))) of plasma and other biological and food samples. J Agric Food Chem. 2003;51(11):3273–9.
24. Organization for Economic Co-operation and Development OECD guidelines for the testing of chemicals Organization for Economic Co-operation and Development, Paris (2002). https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf.
25. Samadder A, Abraham SK, Khuda-Bukhsh AR. Nanopharmaceutical approach using pelargonidin towards enhancement of efficacy for prevention of alloxan-induced DNA damage in L6 cells via activation of PARP and p53. Environ Toxicol Pharmacol. 2016;43:27–37. https://doi.org/10.1016/j.etap.2016.02.010.
26. Samadder A, Tarafdar D, Abraham SK, Ghosh K, Khuda-Bukhsh AR. Nano-pelargonidin protects hyperglycemic-induced L6 cells against mitochondrial dysfunction. Planta Med. 2017;83:468–75. https://doi.org/10.1055/s-0043-100017.
27. Schneider CA, Rasband WS, Eliceiri KW. NIH image to ImageJ: 25 years of image analysis. Nat Methods. 2012;9(7):671–5.
28. Gaviria C, Cifuentes O, Monsalve C, Rojano B. Actividad antioxidante de extractos metanólicos de attalea butyracea. Scientia et Technica Año XIII. 2007;33:297–8.
29. Nayak B, Liu RH, Tang J. Effect of processing on phenolic antioxidants of fruits, vegetables, and grains–a review. Crit Rev Food Sci Nutr. 2015;55(7):887–919.
30. Rozanska, D & Regulska-llow, B. The significance of anthocyanins in the prevention and treatment of type 2 diabetes. Adv Clin Exp Med. 2018; 27(1):135–142. doi: https://doi.org/10.17219/acem/64983.
31. Naseri R, Farzaei F, Haratipour P, Nabavi SF, Habtemariam S, Farzaei MH, Khodarahmi R, Tewari D, Momtaz S. Anthocyanins in the management of metabolic syndrome: a pharmacological and biopharmaceutical review. Front Pharmacol. 2018; 9:1310. Published 2018 Dec 4. doi:https://doi.org/10.3389/fphar.2018.01310.
32. Takikawa M, Inoue S, Horio F, Tsuda T. Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase in diabetic mice. J Nutr. 2010;140(3):527–33.
33. Fang J. Bioavailability of anthocyanins. Drug Metab Rev. 2014;46(4):508–20.
34. Fernandes I, Faria A, Calhau C, de Freitas V, Mateus N. Bioavailability of anthocyanins and derivatives. J Funct Foods. 2014;7:54–66.
35. Lopez-Padilla A, Ruiz-Rodriguez A, Restrepo Florez CE, Rivero Barrios DM, Reglero G, Fornari T. Vaccinium meridionale swartz supercritical co_2 extraction: effect of process conditions and scaling up. Materials (Basel). 2016;9(7).
36. Yeo LK, Chaw CS, Elkordy AA. The effects of hydration parameters and co-surfactants on methylene blue-loaded niosomes prepared by the thin film hydration method. Pharmaceuticals (Basel). 2019;12(2).
37. Un RN, Barlas FB, Yavuz M, Ag Seleci D, Seleci M, Gumus ZP, *et al.* Phyto-Niosomes: in vitro assessment of the novel Nanovesicles containing Marigold extract. Int J Polym Mater Polym Biomater. 2015;64(17):927–37.
38. Priprem A, Damrongrungruang T, Limsitthichaikoon S, Khampaenjiraroch B, Nukulkit C, Thapphasaraphong S, *et al.* Topical Niosome gel containing an anthocyanin complex: a potential Oral wound healing in rats. AAPS PharmSciTech. 2018;19(4):1681–92.
39. Varshosaz J, Pardakhty A, Hajhashemi VI, Najafabadi AR. Development and physical characterization of sorbitan monoester niosomes for insulin oral delivery. Drug Deliv. 2003;10(4):251–62.
40. Kamboj S, Saini V, Bala S. Formulation and characterization of drug loaded nonionic surfactant vesicles (niosomes) for oral bioavailability enhancement. ScientificWorldJournal. 2014;2014:959741.
41. Morakul B, Teeranachaideekul V, Buraphacheep JV. Niosomal delivery of pumpkin seed oil: development, characterisation, and physical stability. J Microencapsul. 2019;36(2):120–9.
42. Chen Z, Wang C, Pan Y, Gao X, Chen H. Hypoglycemic and hypolipidemic effects of anthocyanins extract from black soybean seed coat in high fat diet and streptozotocin-induced diabetic mice. Food Funct. 2018;9(1):426–39.
43. Guo H, Ling W, Wang Q, Liu C, Hu Y, Xia M, *et al.* Effect of anthocyanin-rich extract from black rice (Oryza sativa L. indica) on hyperlipidemia and insulin resistance in fructose-fed rats. Plant Foods Hum Nutr. 2007;62(1):1–6.
44. Jayaprakasam B, Olson LK, Schutzki RE, Tai MH, Nair MG. Amelioration of obesity and glucose intolerance in high-fat-fed C57BL/6 mice by anthocyanins and ursolic acid in cornelian cherry (Cornus mas). J Agric Food Chem. 2006;54(1):243–8.
45. Nizamutdinova IT, Jin YC, Chung JI, Shin SC, Lee SJ, Seo HG, *et al.* The anti-diabetic effect of anthocyanins in streptozotocin-induced diabetic rats through glucose transporter 4 regulation and prevention of insulin resistance and pancreatic apoptosis. Mol Nutr Food Res. 2009;53(11):1419–29.
46. Sun CD, Zhang B, Zhang JK, Xu CJ, Wu YL, Li X, *et al.* Cyanidin-3-glucoside-rich extract from Chinese bayberry fruit protects pancreatic beta cells and ameliorates hyperglycemia in streptozotocin-induced diabetic mice. J Med Food. 2012;15(3):288–98.
47. Chen BH, Stephen Inbaraj B. Nanoemulsion and nanoliposome based strategies for improving anthocyanin stability and bioavailability. Nutrients. 2019;11(5).
48. Tavano L, Muzzalupo R, Picci N, de Cindio B. Co-encapsulation of antioxidants into niosomal carriers: gastrointestinal release studies for nutraceutical applications. Colloids Surf B Biointerfaces. 2014;114:82–8.
49. Adisakwattana S, Yibchok-Anun S, Charoenlertkul P, Wongsasiripat N. Cyanidin-3-rutinoside alleviates postprandial hyperglycemia and its synergism with acarbose by inhibition of intestinal alpha-glucosidase. J Clin Biochem Nutr. 2011;49(1):36–41.
50. Shirode AB, Bharali DJ, Nallanthighal S, Coon JK, Mousa SA, Reliene R. Nanoencapsulation of pomegranate bioactive compounds for breast cancer chemoprevention. Int J Nanomedicine. 2015;10:475–84.
51. Samadder A, Tarafdar D, Abraham SK, Ghosh K, Khuda-Bukhsh AR. Nano-Pelargonidin protects hyperglycemic-induced L6 cells against mitochondrial dysfunction. Planta Med. 2017;83(5):468–75.
52. Lagoa R, Silva J, Rodrigues JR, Bishayee A. Advances in phytochemical delivery systems for improved anticancer activity. Biotechnol Adv. 2020;38:107382.
53. Ali T, Kim MJ, Rehman SU, Ahmad A, Kim MO. Anthocyanin-loaded PEG-gold nanoparticles enhanced the Neuroprotection of Anthocyanins in an Abeta1-42 mouse model of Alzheimer's disease. Mol Neurobiol. 2017;54(8):6490–506.
54. Amin FU, Shah SA, Badshah H, Khan M, Kim MO. Anthocyanins encapsulated by PLGA@PEG nanoparticles potentially improved its free radical scavenging capabilities via p38/JNK pathway against Abeta1-42-induced oxidative stress. J Nanobiotechnology. 2017;15(1):12.