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Harnessing liposome technology to deliver a *Rubus ulmifolius* fruit extract to hyperglycemic rats

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ABSTRACT

In this study, liposome technology was proposed to enhance the antihyperglycemic activity of an extract from *Rubus ulmifolius* edible fruits. Liposomes coated with the enteric polymer Eudragit®L100 were developed. The *R. ulmifolius* Eudragit-liposomes were mainly unilamellar, with a mean diameter of 87 ± 7.9 nm. They exhibited a high entrapment efficiency (>70%) of key phenolic compounds: anthocyanins, ellagitannins and flavonols. The protection provided by the vesicle structure and the polymer coating was confirmed by a stability assessment in simulated gastrointestinal fluids. The antihyperglycemic activity of *R. ulmifolius* extract administered orally to hyperglycemic rats was examined. A 20 mg/kg dose of the nanoformulated extract was more effective than a 100 mg/kg dose of the free extract and produced an antihyperglycemic effect similar to that of antidiabetic acarbose.

The results corroborate the hypothesis that the bioactivity of the *R. ulmifolius* fruit extract could be enhanced by harnessing Eudragit-coated liposome technology with the aim of finding new options to manage hyperglycemia.

1. Introduction

Persistent hyperglycemia is a key factor in the development of diabetes, a metabolic disorder that affects millions of people worldwide [1,2]. In diabetes, hyperglycemia is caused by insulin deficiency stemming from β-cell dysfunction, insulin resistance, or both [3]. Diabetes can reduce quality of life due to its daily management requirements and the risk of severe complications [4], such as retinopathy, skin ulcers, cardiovascular diseases, and stroke [5]. Conventional diabetes therapies —including insulin, biguanides, sulfonylureas, and thiazolidinediones — effectively lower blood glucose but often carry unwanted side effects.

Numerous plants have been reported to possess antihyperglycemic properties that are effective in managing diabetes [6,7]. Various parts of plants within the Rosaceae family have demonstrated varying degrees of efficacy for the treatment of diabetes [8]. *Rubus* is the largest genus of the Rosaceae family [9]. Various *Rubus* species have high contents of tannins, flavonoids, anthocyanins, and phenolic acids [9,10]. One particular species, *Rubus ulmifolius* Schott, is used in Chilean folk medicine for its hypoglycemic activity [11]. Considerable research has been performed *in vivo* to assess the antidiabetic effects of various plants [12,13]. One such study tested the antidiabetic activity of extracts from *R. ulmifolius* aerial parts and found that the extracts showed effectiveness in *vivo* as an antidiabetic and in normalizing the liver and kidney profiles of streptozotocin-induced hyperglycemic albino mice [14]. The extracts were administered orally as aqueous suspensions.

Given the poor aqueous solubility of most extracts, which results in reduced bioavailability, the present study developed and assessed the delivery of an extract from *R. ulmifolius* edible fruits (wild blackberries) in liposomes designed for oral administration. The liposomes are expected to increase the solubility and bioavailability of the extract's bioactive compounds while being an aqueous-based formulation that can be safely administered orally. The proposed nanoformulation

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consisted of liposomes coated with an enteric polymer – Eudragit® L100 – to resist gastrointestinal degradation [15]. In a previous study, Eudragit® L100, an anionic polymer that is insoluble at gastric pH and dissolves above pH 6.0, was used to coat liposomes loading an extract from *Ptilostemon casabonae* leaves. These *P. casabonae* Eudragit-coated liposomes reduced glucose levels in sucrose-overloaded rats with one-fourth of the dose required when the extract was administered alone [16].

Capitalizing on the results of our previous research which demonstrated the dose-sparing antihyperglycemic effect of a nanoformulated extract, the current study explored how Eudragit-coated liposomes incorporate a *R. ulmifolius* fruit extract in high amounts, withstand gastrointestinal conditions and potentiate the extract's antihyperglycemic activity. Firstly, we assessed the protection provided by the vesicle structure and the polymer coating in simulated gastrointestinal fluids; secondly, emphasis was placed on enhancing the *in vivo* antihyperglycemic activity of *R. ulmifolius* extract by administering its nanoformulation orally to hyperglycemic rats. The role of an extract from widely available edible fruits and its delivery system, Eudragit-coated liposomes, within emerging therapeutic or nutraceutical options for managing hyperglycemia is discussed, highlighting the ability of the proposed nanocarrier to increase the value of natural products.

2. Materials and methods

2.1. Materials

Soy phosphatidylcholine (≥94%; Phospholipon 90G; P90G) was from Lipoid GmbH (Ludwigshafen, Germany). Phosphate-buffered saline (PBS), methanol, *o*-phosphoric acid 85% *w/w*, acetonitrile, formic acid and stearylamine (SA) were from Merck (Milan, Italy). Eudragit® L100 (Methacrylic Acid - Methyl Methacrylate Copolymer (1:1), approx. Mw 125,000 g/mol) was from Evonik Industries AG (Essen, Germany). Standards of cyanidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside, ellagic acid, quercetin-3-*O*-glucoside and kaempferol-3-*O*-glucoside were from Extrasynthese (Genay, France) and TransMIT GmbH (Gieβen, Germany).

2.2. R. ulmifolius fruit collection and extraction

R. ulmifolius fruits were collected in Jerzu (Sardinia, Italy) in August 2021 and immediately lyophilized. 50 g of dried fruits were ground and extracted (three times for 24 h each) with 80% ethanol in an ultrasonic bath. Ethanol was first removed under vacuum at 40 °C, and water was then removed by freeze-drying. The extraction yield was 25.4% *w/w*.

2.3. Preparation of the liposomes

Uncoated liposomes were prepared by dispersing the phospholipid P90G and the positive charge inducer SA in PBS, with or without the *R. ulmifolius* fruit extract (Table 1) and sonicating this dispersion for 50 alternating cycles, 30 of which at 5 s on and 20 at 3 s on, using an ultrasonic disintegrator. These liposomes were coated with the enteric polymer by dropwise addition to an equal volume of 0.1% *w/v* Eudragit® L100 in PBS [16,17].

Table 1
Composition of empty and *R. ulmifolius* uncoated and Eudragit-coated liposomes.

	P90G (mg)	*R. ulmifolius* extract (mg)	SA (mg)	PBS (ml)	Eudragit solution (ml)
Empty uncoated liposomes	100	-	4	1	-
R. ulmifolius uncoated liposomes	100	4	4	1	-
Empty Eudragit-liposomes	100	-	4	1	1
R. ulmifolius Eudragit-liposomes	100	4	4	1	1

2.4. Characterization of the liposomes

For the characterization of *R. ulmifolius* Eudragit-coated liposomes, three key parameters - average diameter, polydispersity index and zeta potential - were measured via light scattering (Zetasizer nano-ZS; Malvern Panalytical, Worcestershire, UK). *R. ulmifolius* uncoated liposomes, empty uncoated liposomes and empty Eudragit-coated liposomes were studied as well.

The *R. ulmifolius* Eudragit-coated liposomes were dialyzed against water to remove the non-incorporated extract's compounds. Non-dialyzed and dialyzed samples were diluted with methanol (1:50 *v/v*) and injected (10 µl) into a 1260 Infinity II HPLC system equipped with a G4212B photodiode array detector (Agilent Technologies, Cernusco sul Naviglio, Italy) for the detection and quantification of key extract's phenolic compounds: anthocyanins at 520 nm, flavonols and ellagitannins at 360 nm [18]. A 0.22 M phosphoric acid and acetonitrile as mobile phase was delivered to a Luna C18 separation column (150 × 4.60 mm, 2.6 µm; Phenomenex, Castel Maggiore, Italy) in gradient elution at a 0.8 ml/min flow rate. Chromatograms and spectra were processed and analyzed using Agilent Technologies' OpenLab CDS *v.* 2.51 data system. A qualitative evaluation of the extract's phenolic compounds was carried out simultaneously by LC-ESI-QToF-MS-MS [19]. Cyanidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside, ellagic acid pentoside, ellagic acid glucuronide, quercetin-HMG-glucoside, and a kaempferol derivative were quantified in non-dialyzed and dialyzed vesicle dispersions and the entrapment efficiency was calculated using the following formula:

$$\textit{Entrapment efficiency} = \left(\frac{\textit{amount of extract's compound in dialyzed vesicles}}{\textit{amount of extract's compound in non} - \textit{dialized vesicles}}\right) x\ 100$$

The morphology of *R. ulmifolius* Eudragit-coated liposomes was studied via cryogenic-Transmission Electron Microscopy. *R. ulmifolius* uncoated liposomes were also processed to uncover possible structural modifications induced by the coating process. Each sample (3 µl) was deposited onto a glow-discharged grid, which was blotted and vitrified by freezing into ethane using an EM GP plunge freezer (Leica Microsystems Inc., Deerfield, IL, US). Each sample was observed using a 200 kV electron microscope (Tecnai G2 T20 Sphera, FEI Company/ThermoFisher Scientific, Waltham, MA, USA). Micrographs were taken at a magnification of 25,000× under low electron dose conditions using a 4 k × 4 k TemCam-XF416 camera (TVIPS GmbH, Gilching, Germany) in binning mode 1.

The physical stability of *R. ulmifolius* Eudragit-coated liposomes was investigated in simulated gastrointestinal environment. *R. ulmifolius* uncoated liposomes were studied alongside *R. ulmifolius* Eudragit-coated liposomes to evaluate the protection offered by the vesicle structure and the polymer coating.

Each sample was diluted (1:100 *v:v*) with a simulated gastric fluid (SGF; 0.1 M hydrochloric acid, pH 1.2) or a simulated intestinal fluid (SIF; disodium hydrogen phosphate buffer, pH 7; Carlo Erba reagents Srl, Cornaredo, Milan, Italy), both adjusted with 0.3 M sodium chloride to increase ionic strength. The diluted samples were incubated at 37 °C and analyzed by measuring vesicle mean diameter, polydispersity index and zeta potential after 2 h in SGF or 6 h in SIF, respectively [16].

2.5. Animals

Male Sprague-Dawley rats (Charles River, Milan, Italy), each weighing between 200 and 250 g, were used in the study. The rats were housed in cages (four per cage) under controlled environmental conditions of 22 ± 2 °C, 50-60% humidity, and 12-h light/dark cycle. The rats had unlimited access to both drinking water and a standard laboratory rat food pellet (Stefano Morini, S. Polo D'Enza, Italy). The experiments were conducted in accordance with the ARRIVE 2.0 guidelines, the European Union's Directive (2010/63/UE) and the Italian legislation (DL No. 26, March 2014), which regulate the use of animals in scientific research. Every effort was made to minimize distress and reduce the number of animals used. Ethics approval number: 186/2023-PR.

2.6. Evaluation of antihyperglycemic activity

The rats ($n = 56$) were randomly assigned to control and treatment groups (at least eight rats per group) without any pre-determined criteria for inclusion or exclusion. The rats were fasted overnight. Food was withheld during the study. At the beginning of the experiment, blood glucose level was 86 ± 1.4 mg/dL. Blood glucose level was measured by withdrawing ~5 μL of blood from rat's caudal vein with a OneTouch Verio Reflect glucometer (LIFESCAN ITALY Srl, Milan, Italy; reported result range: 20–600 mg/dL). Immediately after basal glycemia was measured, the rats received an intragastric administration of the test samples at time 0: *R. ulmifolius* fruit extract dissolved in water with 1% Tween 80 was administered at doses 10, 40, and 100 mg/kg (in a fixed volume of 3 ml/kg); acarbose, reference compound and positive control, was administered at a dose of 30 mg/kg (2 ml/kg) [20]; the negative control group rats received an equal volume of vehicle (1% Tween 80 in water; 2 ml/kg); Eudragit-coated liposomes loaded with *R. ulmifolius* fruit extract (2 mg/ml) were administered at doses 10 and 20 mg/kg (in a fixed volume of 3 ml/kg). Acarbose was administered at a volume of 2 ml/kg because its concentration allowed accurate dosing within a lower administration volume, thereby minimizing gastric distension and reducing potential stress associated with oral gavage. The extract samples were administered at 3 ml/kg to achieve the required doses. Both volumes fall within the accepted range for oral administration in rats and were selected to optimize dosing accuracy and tolerability.

Thirty min after the intragastric administration of the test samples, the rats were intragastrically administered a 40% sucrose solution (3 g/kg) immediately after the second measurement of blood glucose. This was measured again after 30, 60, 90, and 120 min from sucrose administration.

Results are presented as the percent change from basal glucose level and as the area under the curve (AUC) and as the percentage of decrease of hematic glucose from control animals.

2.7. Statistical analysis

Results are presented as mean values ± standard deviations (SD). A Student's t-test was used to compare pairs of data, and a p-value less than 0.05 was considered significant. I*n vivo* results are presented as mean values ± standard error (SEM) and represent the percentage change from basal glycemia values (before sucrose administration) and the percentage of decrease from sucrose-induced value. *In vivo* data were analyzed by two- or one-way ANOVA with treatment and time and treatment as factors, respectively, followed by Dunnet's post hoc test with the significance level set at $p < 0.05$, using GraphPad Prism *v.* 10.0.

3. Results and discussion

3.1. Characterization of the liposomes

R. ulmifolius Eudragit-coated liposomes were characterized in terms of size, homogeneity and charge (Table 2). They were then compared with *R. ulmifolius* uncoated liposomes and empty uncoated/Eudragit-coated liposomes to uncover possible modifications of the vesicles' characteristics induced by the extract and/or by the polymer coating. Both empty and *R. ulmifolius* uncoated liposomes exhibited a mean diameter of around 70 nm, a polydispersity index around 0.27 and a zeta potential around +37 mV (Table 2). These data indicate that small, homogeneous cationic liposomes were produced and remained as such when the *R. ulmifolius* extract was incorporated. The coating with Eudragit led to statistically significant increases in vesicles' mean diameter (approximately 85 nm) and polydispersity index (around 0.43) and a decrease in zeta potential (around +24 mV) compared to uncoated liposomes, regardless of the presence of the extract (Table 2). Overall, these results suggest that the effect of the extract was negligible and that the enlarged vesicles and less positive charge indicate that the anionic polymer surrounded the cationic liposomes through electrostatic interaction.

Table 2
Mean diameter (MD), polydispersity index (PI), and zeta potential (ZP) of empty and *R. ulmifolius* uncoated and Eudragit-coated liposomes. Values are the means ± standard deviation (SD; $n > 10$). *$p < 0.001$ *vs.* empty Eudragit-liposomes; °$p < 0.001$ *vs. R. ulmifolius* Eudragit-liposomes.

	MD (nm ± SD)	PI ± SD	ZP (mV ± SD)
Empty uncoated liposomes	*71 ± 2.2	*0.28 ± 0.01	*+36 ± 5.4
R. ulmifolius uncoated liposomes	°70 ± 3.2	°0.27 ± 0.01	°+38 ± 3.8
Empty Eudragit-liposomes	83 ± 4.2	0.44 ± 0.05	+25 ± 2.9
R. ulmifolius Eudragit-liposomes	87 ± 7.9	0.42 ± 0.05	+24 ± 7.3

Six phenolic compounds – two anthocyanins, two ellagitannins and two flavonols –characteristic of the *R. ulmifolius* fruit extract were quantified in non-dialyzed and dialyzed liposomes, both uncoated and Eudragit-coated, to determine the entrapment efficiency (Table 3). Entrapment efficiency was high (>70%) for all six compounds in Eudragit-coated liposomes and good for ellagitannins and flavonols in uncoated liposomes. Marked differences in entrapment efficiency were observed for the anthocyanins cyanidin-3-*O*-glucoside and pelargonidin-3-*O*-glucoside, which showed low values in uncoated liposomes (17% and 35%, respectively) and high values in Eudragit-coated liposomes (71% and 81%, respectively). The distinct chemical and structural properties of the anthocyanins might influence the interactions with the phospholipid, preventing their efficient incorporation during liposome formation. The addition of the Eudragit coating might retain some of the unentrapped anthocyanins, possibly adsorbed on the liposomes' surface, leading to higher entrapment efficiency. Karim et al. [21] also found that the entrapment efficiency of anthocyanin pelargonidin-3-*O*-glucoside was low in uncoated liposomes (28%) and increased in liposomes coated with chitosan (53%) or with chitosan and pectin (61%). This difference was attributed to the polymer coating that could reduce the

Table 3
Entrapment efficiency (E %) of *R. ulmifolius* uncoated and Eudragit-coated liposomes. Values are the means ± standard deviation (SD; $n = 4$). Mean values within a row with different letters are significantly different at $p \leq 0.05$.

Compound	E% ± SD	E% ± SD
	uncoated liposomes	Eudragit-liposomes
cyanidin-3-*O*-glucoside	17 ± 0.6[a]	71 ± 6.5[b]
pelargonidin-3-*O*-glucoside	35 ± 2.6[a]	81 ± 6.0[b]
ellagic acid pentoside [a]	72 ± 3.5[a]	71 ± 2.8[a]
ellagic acid glucuronide [a]	75 ± 7.5[a]	72 ± 6.8[a]
quercetin-HMG-glucoside [b]	74 ± 8.3[a]	80 ± 5.8[a]
kaempferol derivative [c]	68 ± 1.6[a]	76 ± 4.5[b]

[a] Expressed as ellagic acid equivalents.
[b] Expressed as quercetin-3-*O*-glucoside equivalents.
[c] Expressed as kaempferol-3-*O*-glucoside equivalents.

fluidity of the liposomal bilayer making it less permeable, thus retaining a greater amount of the anthocyanin and resulting in higher entrapment efficiency for the polymer-coated liposomes.

The morphological assessment performed by cryo-TEM indicated that *R. ulmifolius* uncoated liposomes were uni- and bilamellar, smaller than 100 nm, and in spherical shape (Fig. 1A). Some flattened vesicles were observed, likely caused by surface tension compression during the vitrification process and/or high vesicle concentration. *R. ulmifolius* Eudragit-coated liposomes were larger and densely packed, consisting of slightly inhomogeneous, spherical unilamellar vesicles, with a fraction of oligolamellar vesicles (Fig. 1B). During the coating process, Eudragit approaches the liposome surface and interacts via electrostatic attraction. Some segments of the anionic Eudragit can intercalate into the outer phospholipid bilayer, but its chains primarily wrap around the positively charged liposomes. This typically results in increased overall vesicle size. During Eudragit deposition, the liposome phospholipids undergo thermodynamic readjustments to compensate for the lateral pressure stress exerted by the polymer and preserve structural integrity. The deposition of the polymer is often non-uniform across all vesicles. This variation leads to a broader size distribution and a higher polydispersity index. The size and uniformity data observed in the micrographs are in line with those obtained from dynamic light scattering measurements (Table 2).

Given that *R. ulmifolius* Eudragit-liposomes were developed for oral administration, their physical stability was assessed in fluids that simulate physiological gastrointestinal conditions. The behavior of *R. ulmifolius* uncoated liposomes was also studied to discriminate between the protection provided by the vesicle structure and that of the Eudragit coating. After 2 h in SGF, *R. ulmifolius* uncoated liposomes exhibited a moderate increase in polydispersity index (from 0.27 to 0.31; Table 2 *vs.* Table 4) while maintaining the same mean diameter (around 70 nm) and having a lower zeta potential (from +38 to +26 mV; Table 2 *vs.* Table 4), which can be attributed to the anions in SGF (Cl^-). On the contrary, the *R. ulmifolius* Eudragit-liposomes showed a decrease in size (from 87 to 74 nm; Table 2 *vs.* Table 4) accompanied by an improved homogeneity (polydispersity index from 0.42 to 0.29; Table 2 *vs.* Table 4), which could be explained by adjacent vesicles being at a greater distance due to sustained surface charge (+27 mV; Table 4).

After 6 h in SIF, *R. ulmifolius* uncoated liposomes increased in size (from 70 to 88 nm; Table 2 *vs.* Table 4) and polydispersity (from 0.27 to 0.42; Table 2 *vs.* Table 4), while the *R. ulmifolius* Eudragit-liposomes were basically unchanged (93 nm with a polydispersity index of 0.42; Table 4). Both formulations showed a reversed zeta potential to weakly negative values (−2 and −0.1 mV; Table 4) due to the ionic species in SIF (Cl^- and HPO_4^{2-}). Zeta potential is a measure of the electrostatic charge at the slipping plane of a vesicle and is heavily dependent on its surrounding ionic environment. Variables such as ionic strength, ion type, and pH dictate the electrical double layer of a vesicle. The changes in zeta potential values observed upon incubation of the vesicle formulations with the two simulated gastrointestinal fluids indeed reflect the interactions between uncoated and Eudragit-coated vesicles and the ionic species present in the fluids.

Table 4
Mean diameter (MD), polydispersity index (PI), and zeta potential (ZP) of *R. ulmifolius* uncoated and Eudragit-coated liposomes in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) determined after incubation for 2 h (t_2) and 6 h (t_6). Values are the means ± standard deviation (SD; $n = 4$).

	Simulated fluid	MD (nm ± SD)	PI ± SD	ZP (mV ± SD)
R. ulmifolius uncoated liposomes t_2	SGF	73 ± 1.7	0.31 ± 0.04	+26 ± 6.5
R. ulmifolius Eudragit-liposomes t_2		74 ± 4.7	0.29 ± 0.05	+27 ± 7.9
R. ulmifolius uncoated liposomes t_6	SIF	88 ± 6.0	0.42 ± 0.05	−2 ± 2.4
R. ulmifolius Eudragit-liposomes t_6		93 ± 7.0	0.42 ± 0.06	−0.1 ± 1.1

Overall, the data from the stability assessment in simulated gastrointestinal fluids indicate that there was no relevant vesicle degradation after incubation of both formulations in the two fluids, but the dual protection provided by the Eudragit-liposomes was more effective, which confirms the potential of this delivery system for oral administration.

3.2. Antihyperglycemic activity of *R. ulmifolius* fruit extract in rats

Fig. 2 shows the effects of the intragastrical administration of *R. ulmifolius* fruit extract on its own or formulated in Eudragit-coated

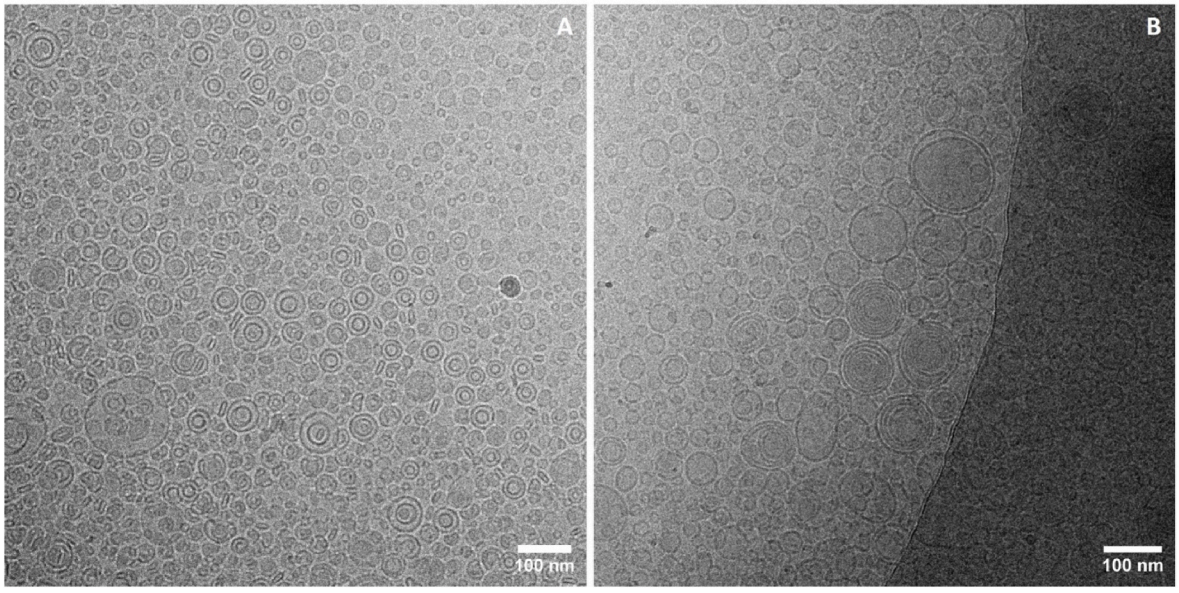

Fig. 1. Cryo-TEM micrographs of *R. ulmifolius* uncoated (A) and Eudragit-coated liposomes (B).

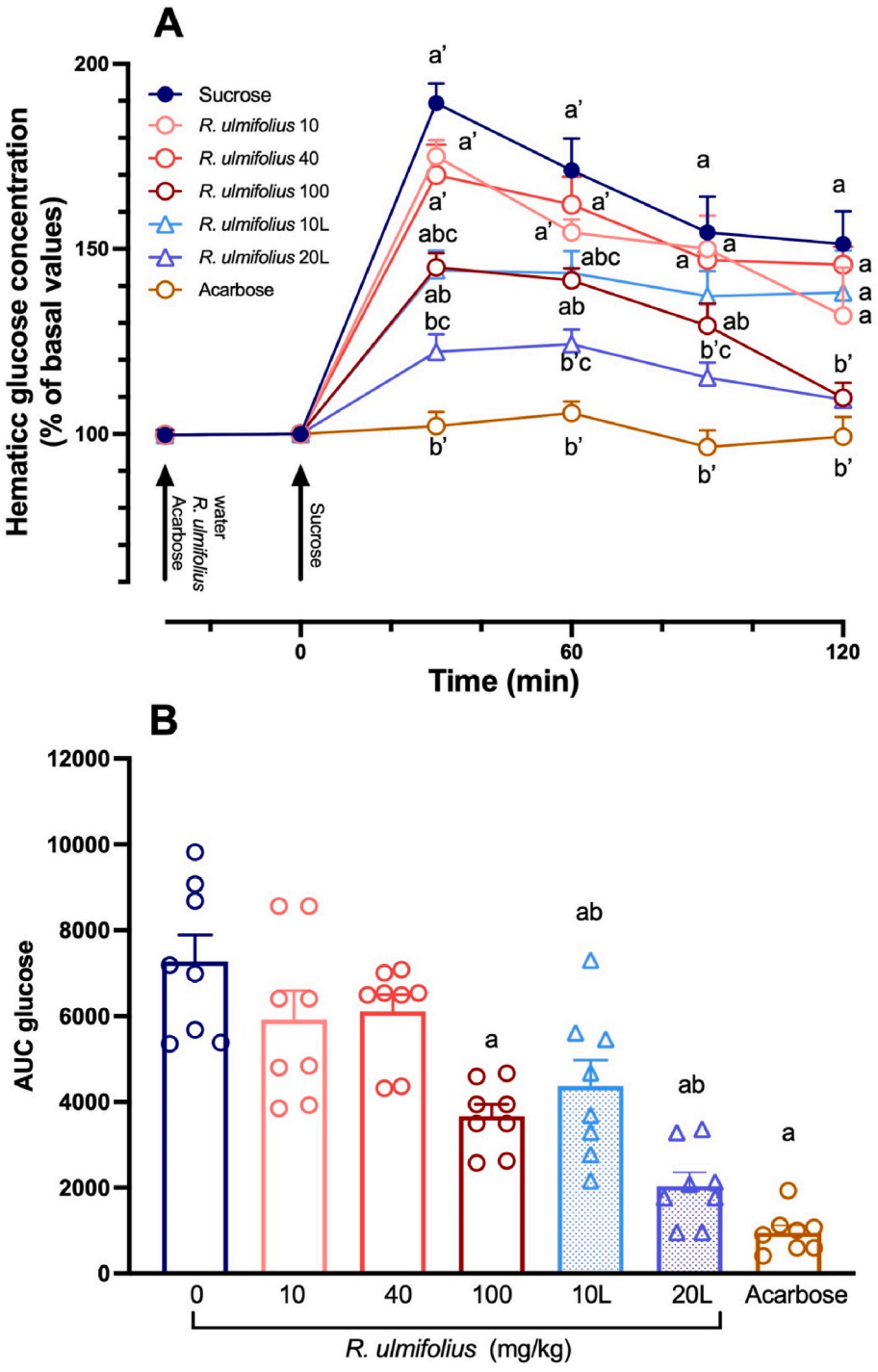

Fig. 2. Antihyperglycemic effect of the intragastric administration of *R. ulmifolius* extract in 1% Tween 80 in water or in Eudragit-liposomes. Panel A shows the dose-dependent effects of *R. ulmifolius* extract on the sucrose-induced increase in glycemia. Panel B shows the curve (AUC) of glycemia after administration of the different test samples. Data are expressed as the mean ± SEM of eight rats per group and represent the percentage increase from the basal glycemia value, which was taken as 100%. [a] $p < 0.05$ and [a'] $p < 0.01$ *vs.* basal value; [b] $p < 0.05$ and [b'] $p < 0.01$ *vs.* the same time point on the sucrose curve; [c] $p < 0.01$ *vs.* the same time point on same dose of extract in water.

liposomes on the increase in blood glucose induced by a sucrose overload. In rats that received sucrose, the blood glucose concentration increased by 90% compared to baseline values, remaining elevated (+50%) for two successive hours. Administration of the reference antihyperglycemic compound acarbose (30 mg/kg, orally) prevented the sucrose-induced increase in glycemia almost completely. The administration of *R. ulmifolius* fruit extract in water with 1% Tween dose-dependently reduced the increase in glycemia. The maximal increases in glycemia were 75 ± 6.5%, 70 ± 9.2% (not significantly different from that observed after sucrose administration) and 45 ± 4.1% ($p < 0.05$) over basal values with doses 10, 40 and 100 mg/kg, respectively. Interestingly, the administration of the *R. ulmifolius* extract in Eudragit-liposomes, at doses 10 and 20 mg/kg, demonstrated a more effective antihyperglycemic effect than the free extract. The 10 mg/kg dose produced an effect similar to that observed at the higher dose (100 mg/kg) of the extract in water (+45 ± 4.1%) and the 20 mg/kg dose was

even more effective (+22 ± 3.9%), with an effect slightly weaker than that observed with acarbose, which *per se* did not significantly change basal glycemia levels.

A two-way ANOVA displayed significant main effects of treatment [$F(6,49) = 9.060$; $p < 0.0001$], a significant main effect of repeated measures [$F(3.199,156.8) = 148.5$; $p < 0.0001$], and a significant interaction between the two factors [$F(30,245) = 7.991$; $p < 0.0001$].

To better evaluate the antihyperglycemic activity of the different samples, Fig. 2 (panel B) shows the area under the curve (AUC) of the glucose concentration in the blood after the administration of each sample. AUC analysis confirms that the administration of *R. ulmifolius* extract in water significantly reduced sucrose-induced hyperglycemia only at the higher dose (100 mg/kg), while *R. ulmifolius* Eudragit-liposomes exhibited greater antihyperglycemic activity with only one-fifth of the dose used of the extract on its own. One-way ANOVA showed a significant main effect of treatment on the AUC [$F(8,63) = 16.52$; $p < 0.0001$].

To better appreciate the antihyperglycemic effect of the different doses of the *R. ulmifolius* extract in 1% Tween 80 in water or in Eudragit-liposomes in comparison with reference acarbose, Table 5 reports the percentages of decrease in glycemia with respect to sucrose-only treated animals. Acarbose lowers blood glucose by acting almost exclusively in the intestinal lumen with minimal systemic signaling effects. Its antihyperglycemic action is limited to delaying carbohydrate digestion [22]. In contrast, *R. ulmifolius* extract's bioactive compounds, such as the identified anthocyanins, ellagitannins, and flavonols, combine local α-glucosidase inhibition with systemic actions on insulin signaling pathways (PI3K/Akt, AMPK, and GLUT4), hepatic glucose production, and adipose and muscle insulin sensitivity [23–28]. Thus, though our results show that the antihyperglycemic action of the extract resembles that of acarbose (around −30% glycemia change; Table 5) in terms of lowering postprandial glucose via α-glucosidase inhibition, the extract with its diverse array of phenolic compounds likely exerts broader, pleiotropic antihyperglycemic effects that extend beyond acarbose's classical mechanism.

The *in vivo* antihyperglycemic/antidiabetic potential of *R. ulmifolius* extract is supported by the findings of Akhtar et al. [14], who demonstrated the antihyperglycemic effect of extracts from the aerial parts of *R. ulmifolius* given orally to streptozotocin-induced diabetic albino mice. The antidiabetic potential of another *Rubus* species, *R. fruticosus*, was correlated with the inhibition of α-glucosidase and α-amylase, key enzymes in carbohydrate digestion (Akyüz [29]. This inhibitory activity was found to be dependent on the maturity stage of *R. fruticosus* fruits and the chemical composition of the extracts with proved antioxidant activity. Additional support to the antidiabetic potential of *Rubus* species is provided by Joshi et al. [30] who reviewed various *in vitro* and *in vivo* studies on *Rubus* species and reported their hypoglycemic properties. The antidiabetic effects included stimulation of insulin secretion, increase in hepatic glycogen synthesis, inhibition of digestive enzymes, enhancement of β-cells functions in pancreas, increase in peripheral glucose uptake, and improvement of antioxidant status. A strong connection between these antidiabetic effects and *Rubus* extracts and isolated bioactive compounds, including flavonoids, phenolic acids, tannins, alkaloids, glycosides, triterpenoids and sterols, has been highlighted. These compounds have been reported to exert antihyperglycemic activity via various mechanisms that primarily include promoting AMPK and Akt phosphorylation, preserving the function of pancreatic β-cells and insulin secretion, enhancing glucose consumption, inhibiting the digestive enzymes involved in glucose digestion, inhibiting apoptosis, promoting the proliferation of β-cells, reducing postprandial blood glucose levels, and increasing the percentage of good gut microbiota [30].

While the antihyperglycemic/antidiabetic potential of *Rubus* extracts and isolated bioactive compounds is supported by previous research, their delivery via liposomes was investigated for the first time in this study. Formulating *R. ulmifolius* fruit extract in Eudragit-liposomes enhanced the potency of its antihyperglycemic action compared to its aqueous solution due to the liposomes' physicochemical properties. Small liposomes in the sub-200 nm range have been shown to consistently exhibit superior oral bioavailability compared to larger vesicles. In our study, the size of around 85 nm falls within the optimal sub-200 nm range that is consistently associated with maximal oral liposome uptake and bioavailability [31–33]. Furthermore, the high entrapment efficiency (>70%) aligns with ranges where proportional gains in payload protection, availability for absorption, and systemic exposure occur with increases in entrapment efficiency [32,34,35]. This results in dose sparing at a fixed administered dose. Finally, gastrointestinal protection strategies, such as Eudragit coating, protect liposomes in gastric media and shift release to the intestine. These strategies yield 2-5-fold increases in bioavailability and reduced food effects [36–38]. Together, the combination of small size, high entrapment efficiency, and demonstrated gastrointestinal stability and protection provides a coherent physicochemical explanation for the enhanced *in vivo* efficacy and observed dose sparing shown in Fig. 2.

In light of the promising results of the *in vivo* hyperglycemic model, additional research and a comprehensive examination of an antidiabetic effect in an *in vivo* model of diabetes are necessary to fully validate the health potential of the *R. ulmifolius* fruit extract and confirm the value of the nanotechnology approach.

4. Conclusions

The extract produced from *R. ulmifolius* edible fruits was formulated in a liposomal system with the aim of exploiting this nanotechnology to reduce hyperglycemia in sucrose-overloaded rats upon oral administration. The proposed Eudragit-coated liposomes efficiently incorporated key phenolic compounds (anthocyanins, ellagitannins and flavonols) and provided protection against simulated stress conditions of the gastrointestinal tract. The *R. ulmifolius* Eudragit-coated liposomes were proved to reduce hyperglycemia with only one-fifth of the dose used of the extract on its own.

This study provides a sustainable approach to utilizing widely available *R. ulmifolius* edible fruits and advancing the development of oral liposome-based medicinal or nutraceutical products.

Table 5
Percentage of change in glycemia over time. Data are expressed as the mean of eight rats per group and represent the percentage of change *vs.* the same time point of sucrose-treated animals, which was considered as 100%.

	R. ulmifolius 10 mg/kg	*R. ulmifolius* 40 mg/kg	*R. ulmifolius* 100 mg/kg	*R. ulmifolius* Eudragit-liposomes 10 mg/kg	*R. ulmifolius* Eudragit-liposomes 20 mg/kg	Acarbose 30 mg/kg
Time (min)	(% change)					
0	0	0	0	0	0	0
30	-11.1	-12.4	-21.8[a]	-23.9[a,b]	-36.3[a,b]	-49.5[a*]
60	-9.9	-5.2	-16.6[a]	-18.9[a,b]	-29.7[a,b]	-38.4[a*]
90	-5.7	-7.9	-16.8[a]	-15.8	-29.4[a*,b]	-37.7[a*]
120	-22	-6.2	-31.7[a*]	-14.6	-32.3	-36.6[a*]

[a] $p < 0.05$ and [a*] $p < 0.01$ *vs.* the same time point on the sucrose curve; [b] $p < 0.01$ *vs.* the same time point on same dose of extract in water.

Future research will focus on investigating the antihyperglycemic effect of the *R. ulmifolius* Eudragit-coated liposomes in an animal model of diabetes.

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CRediT authorship contribution statement

Simone Pani: Investigation, Software, Writing – original draft. **Carla Caddeo:** Conceptualization, Investigation, Supervision, Writing – review & editing. **Laura Dazzi:** Investigation, Writing – original draft. **Enrico Sanna:** Investigation, Writing – original draft. **Giuseppe Talani:** Investigation. **Valentina Masala:** Investigation, Software. **Carlo Ignazio Giovanni Tuberoso:** Investigation, Writing – original draft. **Aurélien Dupont:** Investigation. **Cinzia Sanna:** Investigation, Writing – review & editing. **Francesca Pintus:** Conceptualization, Investigation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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