Nanoformulation prototype based on a natural extract for the potential treatment of type 2 diabetes mellitus.

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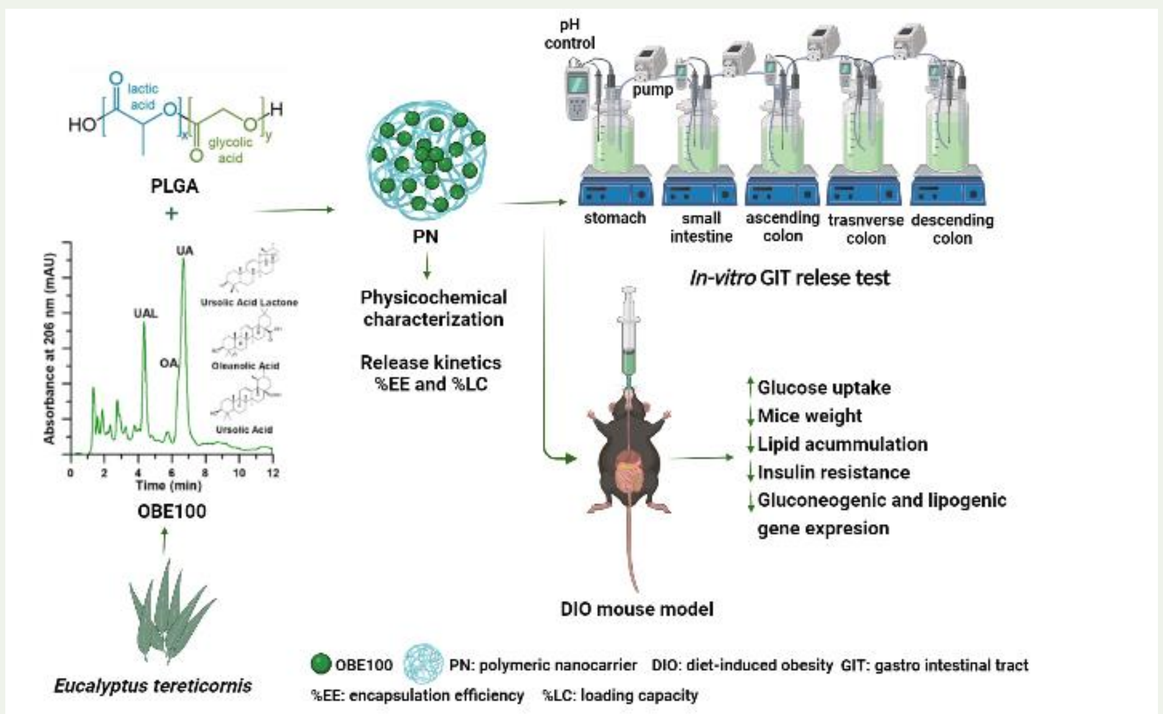
INTRODUCTION

Triterpene-enriched fractions (OBE100) from Eucalyptus tereticornis demonstrated efficiency when administered intraperitoneally to diet-induced obesity (DIO) mouse model to treat obesity-related type 2 diabetes mellitus (T2DM)[1]; however, its oral administration has not yet led to satisfactory results. We aimed to develop an oral nano formulation that enhanced the promising effect of the OBE 100 fraction, guaranteeing higher stability and bioavailability of the pharmacologically active components present in the natural extract through the oral route.

METHODS

Triterpenes were encapsulated in a polymeric nanocarrier (PNs) self-assembled from the poly lactic-co-glycolic acid polymer by a solvent evaporation method. We characterized the NPs size, Z-potential (ZP), polydispersity index (Đ), encapsulation efficiency (EE), loading capacity (LC), and cargo release kinetics. Fifteen doses of 214 mg/kg encapsulated OBE100 were administered using an oral gavage, assessed metabolic biomarkers in-vivo in a DIO mouse model, and finally evaluated NPs in an in-vitro human gastrointestinal model (H-GIM).



RESULTS

We developed PN with a media size of 165 nm (P < 0.05), -35,1 ZP, 0,18 Đ, 98% EE, and 3,5% LC. The release kinetics showed 80% of cargo released in the first 6 hours and up to 85% after 72 hours. The nano formulation decreases the body and liver weight, reduces glucose intolerance, insulin resistance, blood glucose, dyslipidaemia, neutral lipid accumulation and the expression levels of gluconeogenic and lipogenic genes. Additionally, an in-vitro simulator of the human intestinal tract showed a triterpene released up to 91%, suggesting that this nano formulation may have potential use in future human trials.

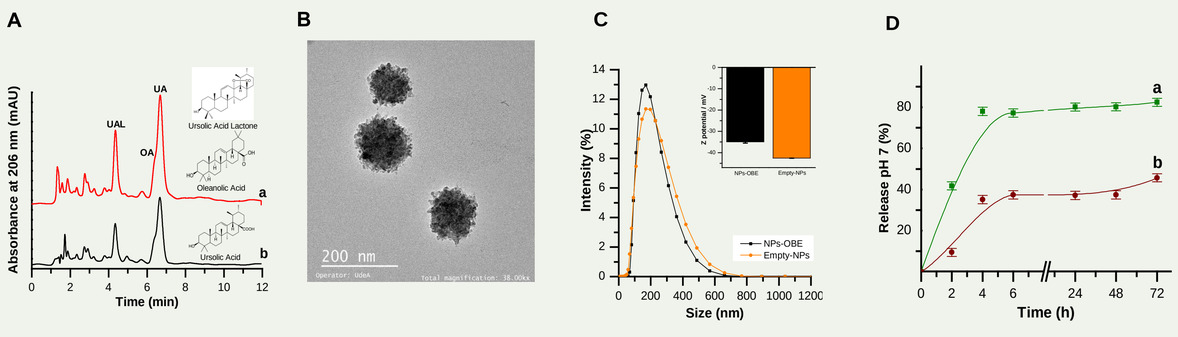


Figure 1. Physicochemical characterization and release profile for polymeric nanoformulation (PN). (A) chromatographic profiles, OBE100 and their main triterpenes (B) Morphologic characterization by TEM. (C) Hydrodynamic size and ζ-potential of OBE100-loaded NP (black line and bar) and empty NP (Orange line and bar). (D) the release profile for OBE100-loaded NP; main triterpenes LAU (a) and OA+UO mix (b) in sink conditions pH 7 .

RESULTS

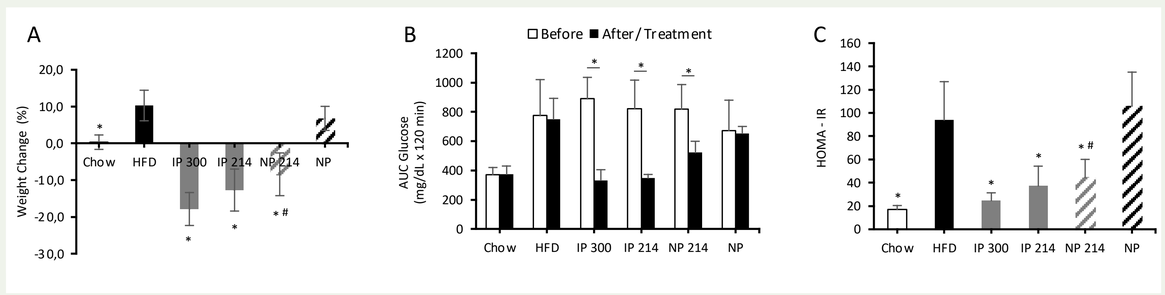


Figure 2. Nanoformulation effect in the assessment of animal weight and carbohydrate metabolism. (A) weight change in prediabetic mice treated intraperitoneally with doses of 300mg/kg (IP300) and 214mg/kg (IP 214) of OBE100; 214 mg/kg of OBE100-loaded NP (NP214) and empty NP (NP) orally. (B) AUC glucose intolerance test before/after treatments. (C) Homeostasis model assessment of insulin resistance (HOMA-IR) in prediabetic animals with the different treatments. HFD: High Fat Diet. (n=6-8 mice/group). Values were expressed as mean ± standard error; the statistical significance was considered with p <0.05. ANOVA with Dunnet's post hoc of multiple comparisons was performed. *: p<0.05, all groups vs HFD group. #: p<0.05, NP214 vs NP, ns: non-significant.

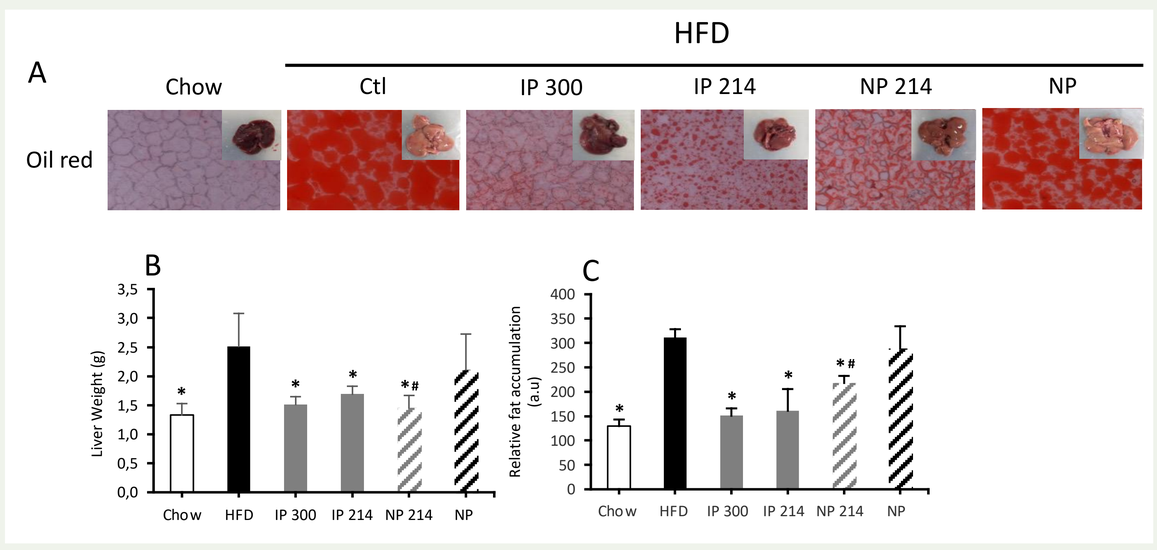


Figure 3. Effect of oral nanoformulation treatment on DIO mouse model liver. (A) Liver oil red staining for fatty acids in prediabetic mice treated intraperitoneally with doses of 300mg/kg (IP300) and 214mg/kg (IP 214) of OBE100; 214 mg/kg of OBE100-loaded NP (NP214) and empty NP (NP) orally. (B) mice liver weight. (C) mice liver tissue area dyed with oil red.

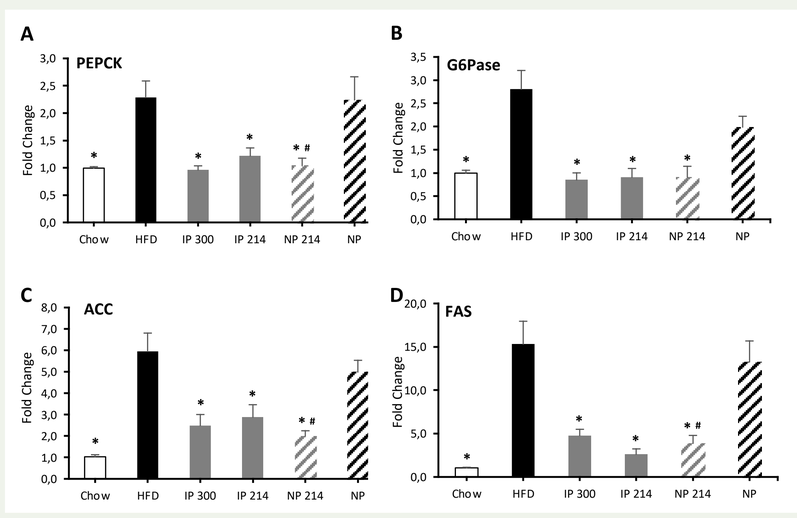


Figure 4. Effect of oral nanoformulation treatment in liver gene expression on DIO mouse model. qRT-PCR and relative expression (fold change) of mRNA transcripts for gluconeogenic genes (A) Phosphoenolpyruvate carboxykinase (PEPCK), (B) Glucose 6-phosphatase (G6Pase). Lipogenic genes, (C) acetyl-CoA carboxylase (ACC), (D) fatty acid synthase (FAS). High Fat Diet. (n=4 mice/group). Values were expressed as mean ± standard error; the statistical significance was considered with p <0.05. ANOVA with Dunnet's post hoc of multiple comparisons was performed. *: p<0.05, all groups vs HFD group. #: p<0.05, NP214 vs NP, ns: non-significant.

Table 1. Digestive conditions and triterpenes released assay using in a modified simulator of the Human gastrointestinal Ecosystem (**SHIME**)

Reactor	Description	pH	Residence time (h)	Accumulated Time (h)	Released UAL x reactor (%)	SD	Accumulated UAL Release (%)	Released OA+UA x reactor (%)	SD	Accumulated OA+UA release (%)
1	Stomach	2	3	3	63	0,26	63	39	0,06	39
2	Small Intestine	6,5-7,0	4	7	11	1,86	74	29	0,23	68
3	Ascending colon	5,0-5,5	20	27	8	0,52	82	17	0,30	85
4	Transverse colon	6,0-6,4	32	59	ND	-	-	6	0,23	91
5	Descending colon	6,4-6,8	24	83	ND	-	-	ND	-	-

UAL. Ursolic acid lactone. OA. Oleanolic acid. UA. Ursolic acid. ND. Non detected. SD. The values represent the mean ± SE. Three independent experiments with three replicas each one.

CONCLUSIONS

We developed an oral polymeric nanocarrier to encapsulate a natural product that showed the potential to decrease the effects of pre-diabetes and obesity in mice and open the way toward developing new strategies of therapeutic agents using natural products combined with nanobiotechnology to control T2DM and obesity. These results show an alternative to being evaluated in future pre-clinical and clinical trials because this nanoparticle releases up to 91% of the main triterpenes of extract in an in-vitro gastrointestinal model.

References: 1.- Acin, S., Muñoz, DL., Guillen, A., Soscue, D., Castaño, A., Echeverri, F., Balcazar, N. 2021. Triterpene-enriched fractions from Eucalyptus tereticornis ameliorate metabolic alterations in a mouse model of diet-induced obesity. *J. Ethnopharmacol*. 265: 113298.

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