

# Supplementary Materials

Article

## Protective Effect of Betulin on Streptozotocin–Nicotinamide-Induced Diabetes in Female Rats

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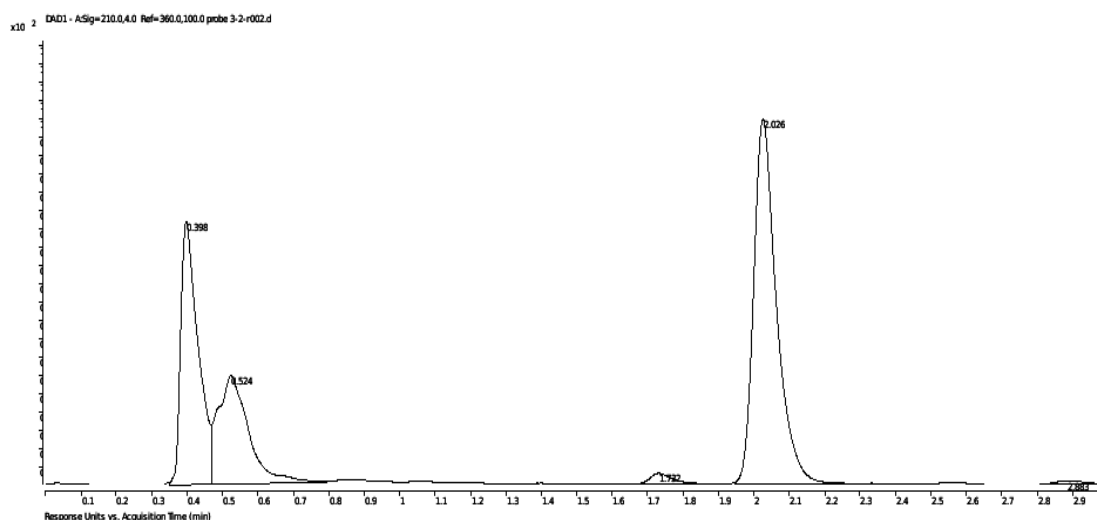
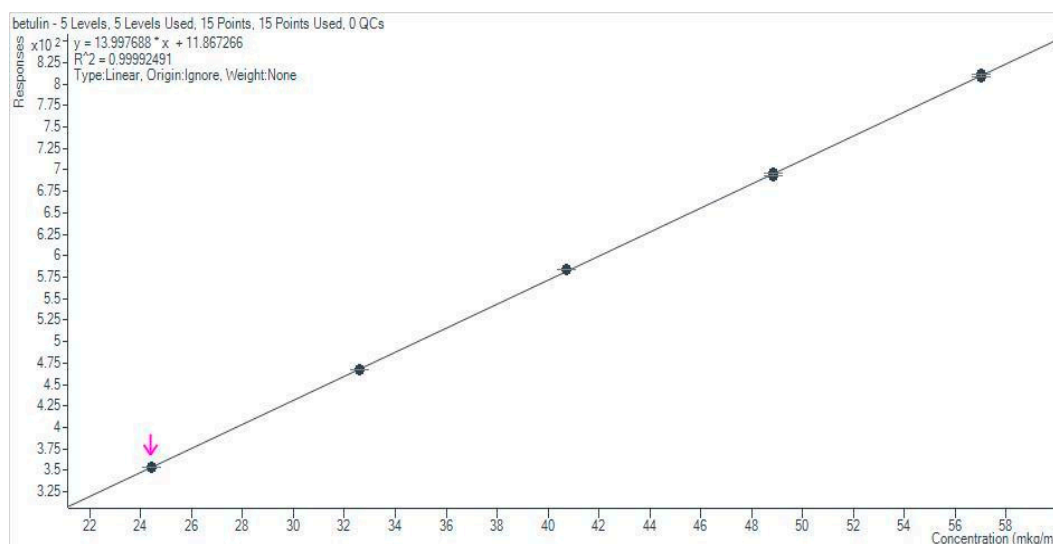


Figure S1. HPLC chromatogram of betulin at a retention time of 2.026 minutes.



**Figure S2.** Calibration curve of betulin standard at different concentrations.

**Table S1.** Concentration of betulin emulsion at different time points before, during, and after the stability tests.

Samples	Exposure time at 55 °C, in hours	Mass concentration of betulin in emulsion, g/dm <sup>3</sup>	Emulsion layering, yes/no
Emulsion	0	4.12 ± 0.11	no
Vial 1	180	4.06 ± 0.04	no
Vial 2	360	4.07 ± 0.07	no
Vial 3	540	4.09 ± 0.11	no
Vial 4	720	4.17 ± 0.08	no

**Table S2.** Effect of betulin intervention on oxidative stress parameters. Data are presented as mean ± standard error of mean (SEM); INT—healthy rats without any treatment, NC—healthy rats administered water, NC + BE50—healthy rats administered betulin (50 mg/kg), T2D—diabetic rats administered water, T2D + BE20—Diabetic rats administered betulin (20 mg/kg), T2D + BE50—diabetic rats administered betulin (50 mg/kg).

	Glutathione in blood plasma, μmol/L	MDA in blood plasma μmol/L	SOD units/ GmHb	CAT mmol/GmHb
INT	20.5 ± 5.3	3.19 ± 0.23	112.5 ± 5.0	50.2 ± 2.0
NC	88.2 ± 8.4	3.75 ± 0.38	132.4 ± 3.5	48.5 ± 2.8
NC + BE50	47.2 ± 3.4	2.60 ± 0.31	130.8 ± 6.6	37.0 ± 2.6
T2D	30.9 ± 4.8	6.27 ± 0.25	132.8 ± 8.7	48.9 ± 1.9
T2D + BE20	77.7 ± 7.9	5.73 ± 0.43	132.2 ± 4.6	43.3 ± 1.9
T2D + BE50	46.1 ± 5.3	4.46 ± 0.19	110.9 ± 13.7	45.3 ± 1.2

**Table S3.** Effect of betulin on oral glucose tolerance (OGTT) and Area under the curve (AUC) in rats. Data are presented as mean ± standard error of mean (SEM); INT—healthy rats without any treatment, T2D—diabetic rats administered water, T2D + BE20—Diabetic rats administered betulin (20 mg/kg), T2D + BE50—diabetic rats administered betulin (50 mg/kg).

	Glucose level, mmol/L				AUC, mmol/L·min
	Before load	30 minutes	60 minutes	120 minutes	
INT	5.9 ± 0.3	8.8 ± 0.3	6.5 ± 0.8	5.3 ± 0.8	802 ± 35
T2D	10.3 ± 0.4	15.7 ± 1.9	14.2 ± 0.7	12.4 ± 0.8	1636 ± 113
T2D + BE20	6.3 ± 0.6	8.1 ± 1.0	6.4 ± 0.5	4.6 ± 0.4	721 ± 53
T2D + BE50	7.2 ± 0.5	10.7 ± 1.9	9.9 ± 1.7	6.6 ± 0.6	1061 ± 136

**Table S4.** Effect of betulin on serum insulin concentration and HOMA-IR. Data are presented as mean ± standard error of mean (SEM); INT—healthy rats without any treatment, NC + BE50—healthy rats administered betulin (50 mg/kg), T2D—diabetic rats administered water, T2D + BE20—Diabetic rats administered betulin (20 mg/kg), T2D + BE50—diabetic rats administered betulin (50 mg/kg).

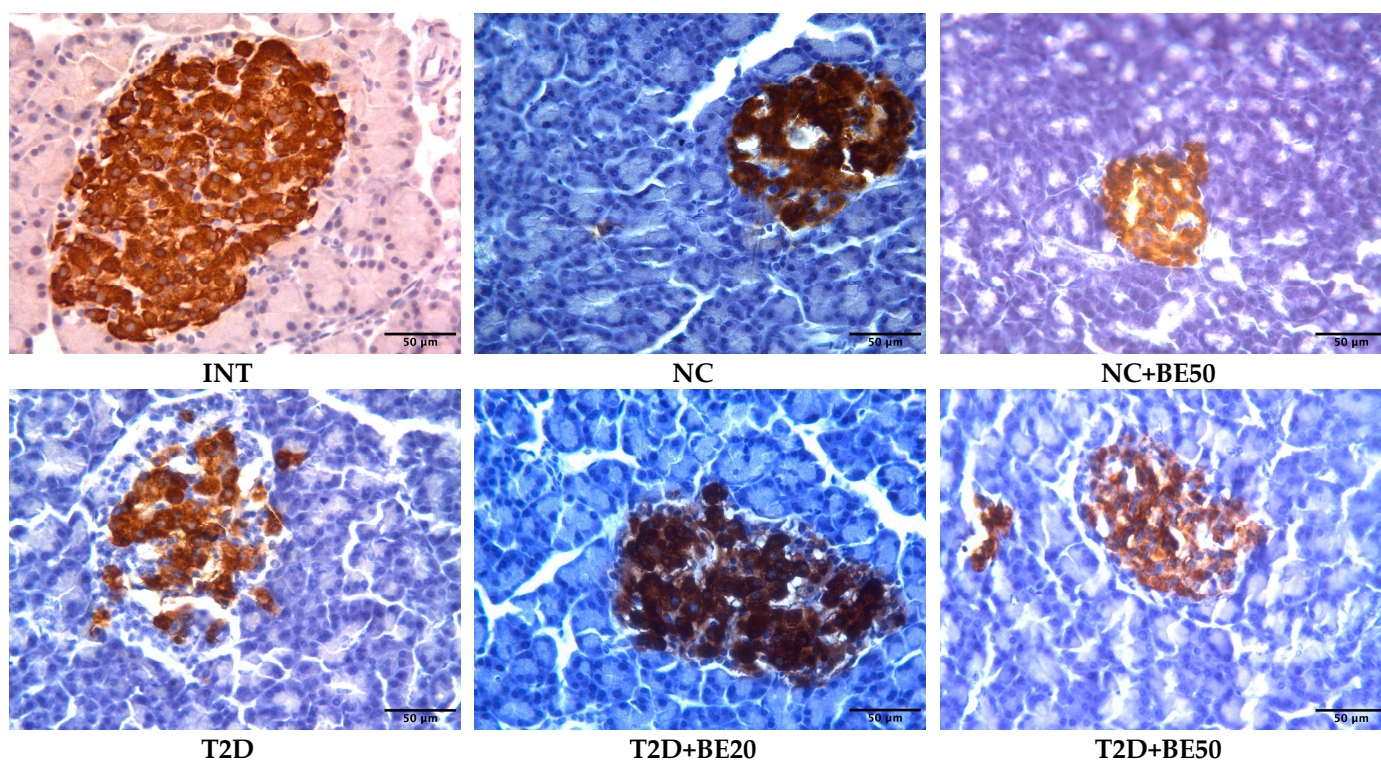
	Insulin μIU/mL	HOMA-IR
INT	1.9 ± 0.25	0.5 ± 0.09
NC + BE50	2.12 ± 0.72	0.81 ± 0.28
T2D	1.98 ± 0.05	0.91 ± 0.06
T2D + BE20	1.93 ± 0.48	0.55 ± 0.19
T2D + BE50	2.22 ± 0.26	0.72 ± 0.13

**Table S5.** Effect of betulin intervention on hepatic and renal functions. Data are presented as mean  $\pm$  standard error of mean (SEM); INT—healthy rats without any treatment, NC—healthy rats administered water, NC + BE50—healthy rats administered betulin (50 mg/kg), T2D—diabetic rats administered water, T2D + BE20—Diabetic rats administered betulin (20 mg/kg), T2D + BE50—diabetic rats administered betulin (50 mg/kg).

	AST $\mu\text{mol}/\text{min}\cdot\text{L}$	ALT $\mu\text{mol}/\text{min}\cdot\text{L}$	ALP $\mu\text{mol}/\text{min}\cdot\text{L}$	Total protein g/L	Urea mmol/L	Creatinine $\mu\text{mol}/\text{L}$
INT	16.1 $\pm$ 0.6	12.6 $\pm$ 0.6	51.5 $\pm$ 3.3	70.5 $\pm$ 1.3	5.2 $\pm$ 0.2	62.3 $\pm$ 1.5
NC	18.5 $\pm$ 0.8	12.8 $\pm$ 0.7	48.5 $\pm$ 4.3	67.3 $\pm$ 2.2	5.9 $\pm$ 0.3	60.2 $\pm$ 1.7
NC + BE50	17.4 $\pm$ 1.7	13.1 $\pm$ 0.8	32.0 $\pm$ 2.4	67.6 $\pm$ 1.3	5.2 $\pm$ 0.4	64.3 $\pm$ 1.6
T2D	24.7 $\pm$ 1.7	18.6 $\pm$ 1.2	40.8 $\pm$ 4.0	64.3 $\pm$ 2.1	7.9 $\pm$ 0.4	67.7 $\pm$ 0.7
T2D + BE20	21.7 $\pm$ 1.4	13.6 $\pm$ 0.8	30.4 $\pm$ 1.4	62.1 $\pm$ 1.6	7.0 $\pm$ 0.3	68.0 $\pm$ 3.3
T2D + BE20	20.2 $\pm$ 2.6	11.3 $\pm$ 1.4	27.6 $\pm$ 1.7	68.0 $\pm$ 1.5	5.8 $\pm$ 0.1	64.3 $\pm$ 1.9

**Table S6.** Effect of betulin on blood glucose, glycosylated haemoglobin,  $\alpha$ -amylase, and glucose tolerance. Data are presented as mean  $\pm$  standard error of mean (SEM); INT—healthy rats without any treatment, NC—healthy rats administered water, NC + BE50—healthy rats administered betulin (50 mg/kg), T2D—diabetic rats administered water, T2D + BE20—Diabetic rats administered betulin (20 mg/kg), T2D + BE50—diabetic rats administered betulin (50 mg/kg).

	Glucose mmol/L	HbA1c %	$\alpha$ -amylase mg/s·L
INT	5.9 $\pm$ 0.3	4.5 $\pm$ 0.2	30.9 $\pm$ 1.8
NC	7.7 $\pm$ 0.3	4.4 $\pm$ 0.3	34.7 $\pm$ 0.7
NC + BE50	8.6 $\pm$ 0.1	4.8 $\pm$ 0.4	45.0 $\pm$ 2.3
T2D	10.3 $\pm$ 0.4	6.1 $\pm$ 0.4	43.7 $\pm$ 1.6
T2D + BE20	6.3 $\pm$ 0.6	5.5 $\pm$ 0.7	38.1 $\pm$ 1.2
T2D + BE20	7.2 $\pm$ 0.5	4.9 $\pm$ 0.2	35.1 $\pm$ 1.6



**Figure S3.** Pancreatic sections with immunohistochemical stain for insulin antibodies (brown) at  $\times 400$  magnification. INT—healthy rats without any treatment, NC—healthy rats administered water, NC + BE50—healthy rats administered betulin (50 mg/kg), T2D—diabetic rats administered water, T2D + BE20—Diabetic rats administered betulin (20 mg/kg), T2D + BE50—diabetic rats administered betulin (50 mg/kg).