

Supplementary Materials: Formulation of Sodium Valproate Nanospanlastics as a Promising Approach for Drug Repurposing in the Treatment of Androgenic Alopecia

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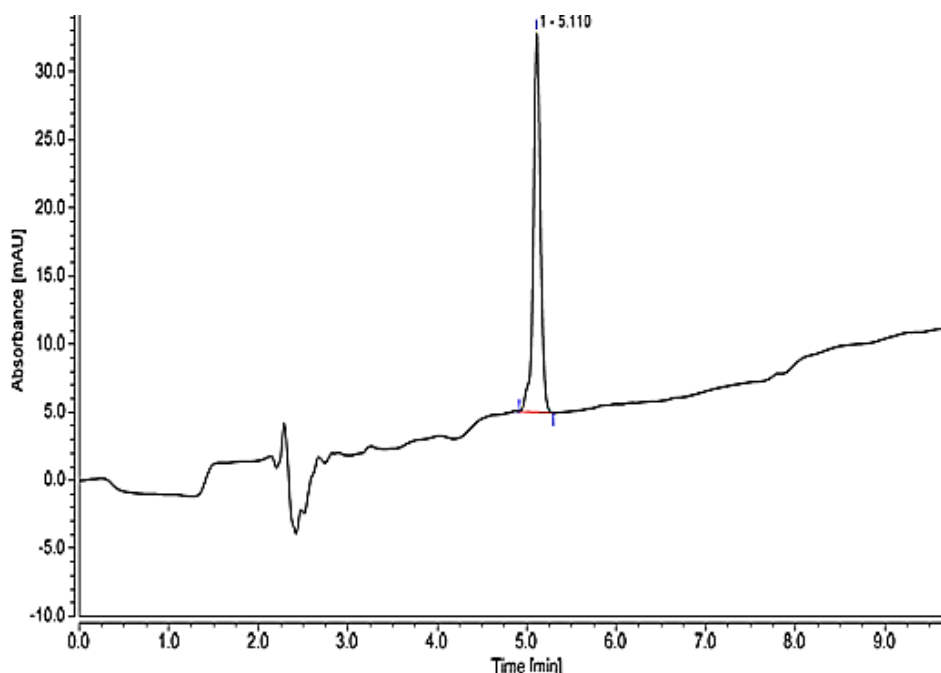


Figure S1. HPLC chromatogram of SV. Abbreviations: SV, sodium valproate.

Table S1. Prescreening study for formulation of SV-loaded SNVs.

Formula	Span 60 to EA ratio	Type of EA	Rotation speed (rpm)	EE %
P1	50:50	Cremophor RH	500	70.33±1.20
P2	60:40	Cremophor RH	500	82.30±0.98
P3	80:20	Cremophor RH	500	97.68±1.93
P4	50:50	Tween 80	500	67.22±1.41
P5	60:40	Tween 80	500	79.44±1.15
P6	80:20	Tween 80	500	95.53±1.46
P7	50:50	Brij 35	500	59.22±1.61
P8	60:40	Brij 35	500	69.23±1.25
P9	80:20	Brij 35	500	86.13±1.33
P10	50:50	Cremophor RH	1000	64.60±1.24
P11	60:40	Cremophor RH	1000	79.48±1.33
P12	80:20	Cremophor RH	1000	93.34±0.92
P13	50:50	Tween 80	1000	60.50±1.27
P14	60:40	Tween 80	1000	70.18±0.62
P15	80:20	Tween 80	1000	90.32±2.18
P16	50:50	Brij 35	1000	55.63±1.33

P17	60:40	Brij 35	1000	65.39±1.22
P18	80:20	Brij 35	1000	80.12±1.18
P19	50:50	Cremophor RH	1500	57.22±1.13
P20	60:40	Cremophor RH	1500	70.18±1.44
P21	80:20	Cremophor RH	1500	90.08±2.11
P22	50:50	Tween 80	1500	52.33±1.14
P23	60:40	Tween 80	1500	64.22±1.28
P24	80:20	Tween 80	1500	87.14±1.65
P25	50:50	Brij 35	1500	47.18±1.14
P26	60:40	Brij 35	1500	60.07±1.38
P27	80:20	Brij 35	1500	76.19±1.29

Notes: All formulations contained 8.3% SV; different values are expressed as mean \pm SD ($n = 3$); 50:50 ratio involves using 50 mg Span 60 and 50 mg EA; 60:40 ratio involves using 60 mg Span 60 and 40 mg EA; 80:20 ratio involves using 80 mg Span 60 and 40 mg EA. Abbreviations: EE, entrapment efficiency; SV-loaded SNVs, Sodium valproate-loaded spanlastic nano-vesicles.

Table S2. Determination of the elasticity of SV-loaded SNVs and the corresponding niosomes.

Formula	PS before extrusion (nm)	PS after extrusion(nm)	DI
F8	222.9 \pm 2.39	210.5 \pm 1.88	22.57 \pm 1.5
Niosomes	293.6 \pm 1.33	44.93 \pm 1.55	0.98 \pm 0.03

Notes: F8, the optimized SV-loaded SNVs; the values are expressed as mean \pm SD; $n = 3$. Abbreviations: EA, edge activator; DI, deformability index; PS, particle size.

Table S3. The correlation between the hair follicles number/cm² in the studied groups.

Duration	SV-loaded SNVs ($n = 40$)	Minoxidil ($n = 40$)	p -value
Pretreatment	17.9 \pm 2.1	18.3 \pm 2.75	0.358
1 month after treatment	17.2 \pm 1.28	14.4 \pm 1.98	0.047
2 month after treatment	16.76 \pm 2.07	13.1 \pm 2.51	0.031
3 month after treatment	19.45 \pm 4.03	19.97 \pm 4.18	0.593
4 month after treatment	26.27 \pm 4.97	25.55 \pm 5.12	0.328
5 month after treatment	29.19 \pm 5.28	30.87 \pm 6.11	0.314
6 month after treatment	31.58 \pm 6.49	32.54 \pm 6.39	0.286

Notes: P-value less than 0.05 indicate model terms are significant. Abbreviations: SV, sodium valproate; SNVs, spanlastic nanovesicles; P- value, probability value.

Table S4. The correlation between the hair shaft diameter (μm) in the studied groups.

Duration	SV-loaded SNVs ($n = 40$)	Minoxidil ($n = 40$)	p -value
Pretreatment	51.5 \pm 5.27	50.7 \pm 5.44	0.371
1 month after treatment	42.9 \pm 4.28	43.2 \pm 4.52	0.482
2 month after treatment	43.18 \pm 3.98	43.9 \pm 3.89	0.539
3 month after treatment	46.17 \pm 4.03	47.27 \pm 4.83	0.294
4 month after treatment	57.9 \pm 6.94	56.35 \pm 5.83	0.271
5 month after treatment	59.36 \pm 5.29	60.27 \pm 4.39	0.287
6 month after treatment	62.4 \pm 7.54	63.19 \pm 7.49	0.483

Notes: P-value less than 0.05 indicate model terms are significant. Abbreviations: SV, sodium valproate; SNVs, spanlastic nanovesicles; P- value, probability value.

Table S5. The correlation between the hair density/cm² in the studied groups.

Duration	SV-loaded SNVs ($n = 40$)	Minoxidil	p -value
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	(n = 40)		
Pretreatment	143.2 ± 10.11	146.04 ± 11.23	0.129
1 month after treatment	130.27 ± 12.28	131.94 ± 13.55	0.366
2 month after treatment	144.27 ± 18.31	142.07 ± 18.69	0.157
3 month after treatment	153.77 ± 21.62	155.39 ± 22.65	0.163
4 month after treatment	184.53 ± 20.59	186.28 ± 21.65	0.148
5 month after treatment	190.15 ± 23.77	193.27 ± 22.36	0.116
6 month after treatment	191.26 ± 24.72	197.71 ± 26.23	0.059

Notes: P-value less than 0.05 indicate model terms are significant. Abbreviations: SV, sodium valproate; SNVs, spanlastic nanovesicles; P- value, probability value.

Table S6. Side effects among the studied groups.

Side effects	SV-loaded SNVs (n = 40)	Minoxidil (n = 40)	P-value
Initial shedding	5 (12.5%)	32 (80%)	<0.001
Irritation	4 (10%)	12 (30%)	0.005
Facial hypertrichosis	0 (0%)	3 (7.5%)	0.128
Dandruff	2 (5%)	1 (2.5%)	0.322

Notes: P-value less than 0.05 indicate model terms are significant. Abbreviations: SV, sodium valproate; SNVs, spanlastic nanovesicles; P- value, probability value.