

Icaritin Promotes Myelination by Simultaneously Enhancing the Proliferation and Differentiation of Oligodendrocyte Precursor Cells

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Material and Methods (catalog no for antibodies)

Primary antibodies: mouse monoclonal anti-Glial Fibrillary Acidic Protein (GFAP, Millipore (Burlington, MA, USA), cat. no. G6171), rabbit polyclonal anti-Microtubule Associated Protein 2 (MAP2, Millipore, cat. no. AB2290A4), Anti-Galc (Millipore, cat. no. MAB342), mouse monoclonal anti-CNPase (Millipore, cat. no. c5922), Anti-A2B5 (Millipore, cat. no. MAB312), mouse monoclonal anti-O4 (Millipore, cat. no. O7139), mouse monoclonal anti-Myelin Basic Protein (anti-MBP, Abcam (Cambridge, UK), cat. no. ab62631), PDGFR α (Abcam, cat. no. ab203491), Anti-Olig2 (R&D (Minneapolis, MN, USA), cat. no. AF2418) and Anti-MOG (Santa Cruz (Dallas, TX, USA), cat. no. sc-376138). Secondary antibody: Alexa Fluor 488 Goat anti-mouse IgG (Thermo Fisher Scientific, Waltham, MA, USA. cat. no. A-11001), Alexa Fluor 568 Goat anti-mouse IgM (Thermo Fisher Scientific, USA. cat. no. A-21043), Alexa Fluor 488 Goat anti-rabbit IgG (Thermo Fisher Scientific, USA, cat. no. A-11008), and Alexa Fluor 555 Goat anti-rabbit IgG (Thermo Fisher Scientific, USA. cat. no. A-21428).

Supplementary Figures

Figure S1 Non-toxic dose screening of herbal monomers through MTT method. PPD (A), PPT (B), 1226 (C), Geniposide (D), Curcumin (E), Catalpol (F), B4(G), Genipin (H), Emodin (I), Loganin (J), Icaritin (K), Icariin (L), Quercetin (M), Apigenin (N), Morroniside (O), Resveratrol (P), Longistyline A (Q), Cajanine (R).

Figure S2 PDGFR α ⁺ positive OPCs (human). Scale bars represent 25 μ m.

Figure S3. Expression of hOLs markers: (A) GFAP⁺ astrocytes and MAP2⁺ neurons, scale bar 25 μ m; (B) O4⁺ and Olig2⁺ OL, scale bar 50 μ m; (C) Galc⁺ and MOG⁺ OL, scale bar 25 μ m; (D) CNPase⁺ and GFAP⁻ OL, scale bar 25 μ m; (E) MBP⁺ and Olig2⁺ OL, scale bar 50 μ m.

Figure S1

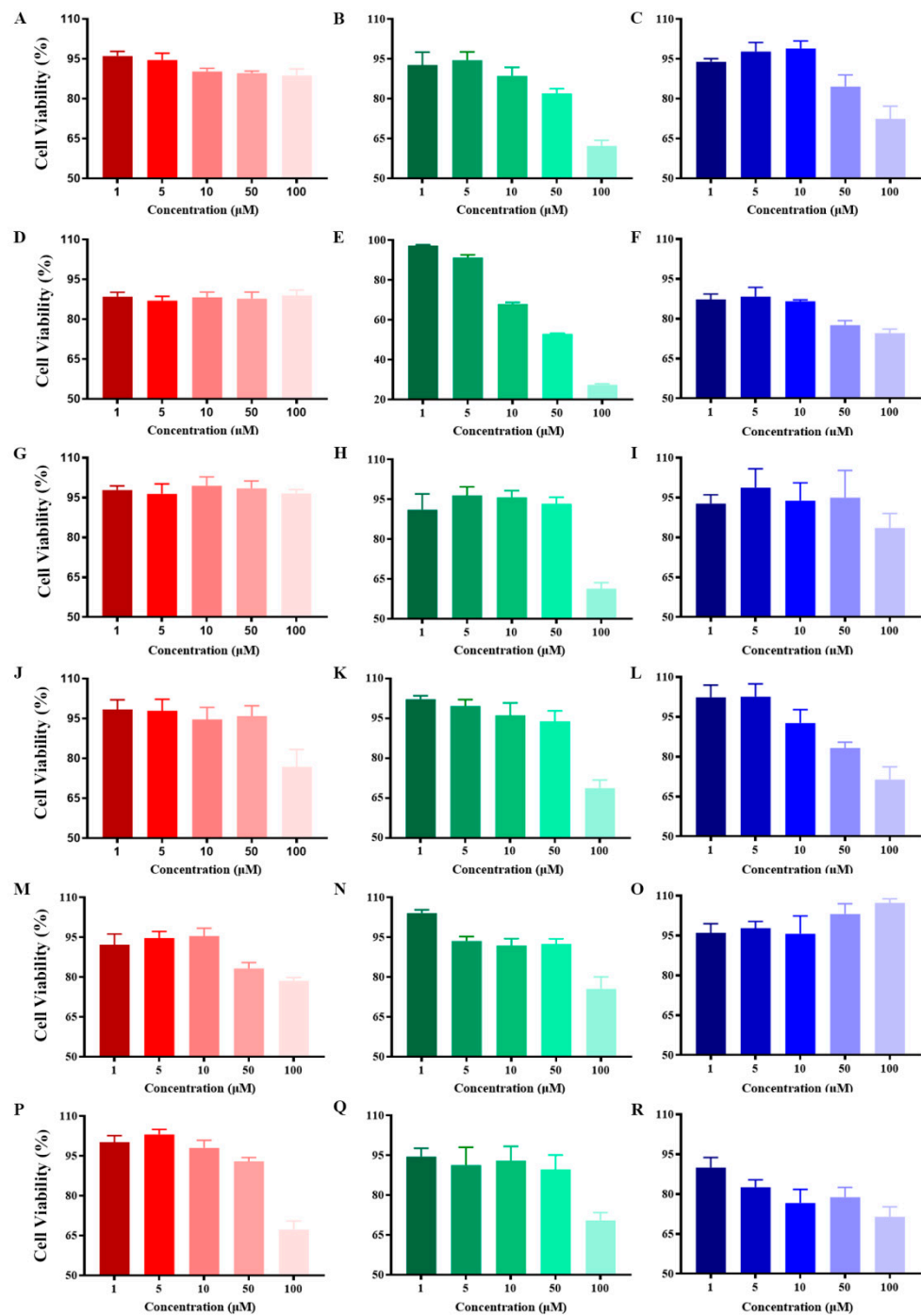


Figure S2.

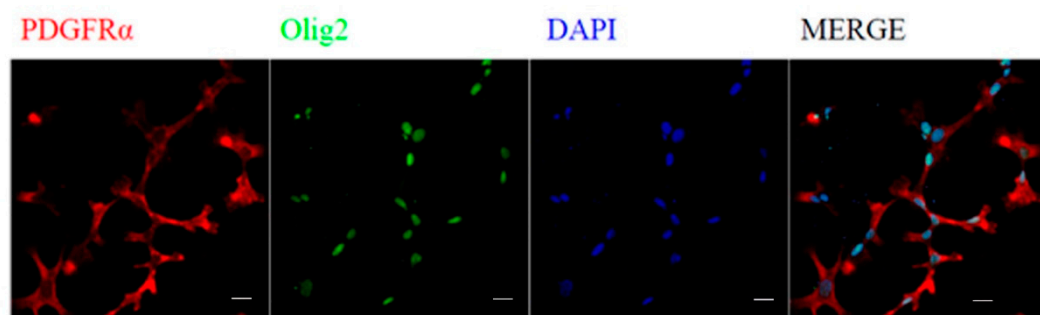
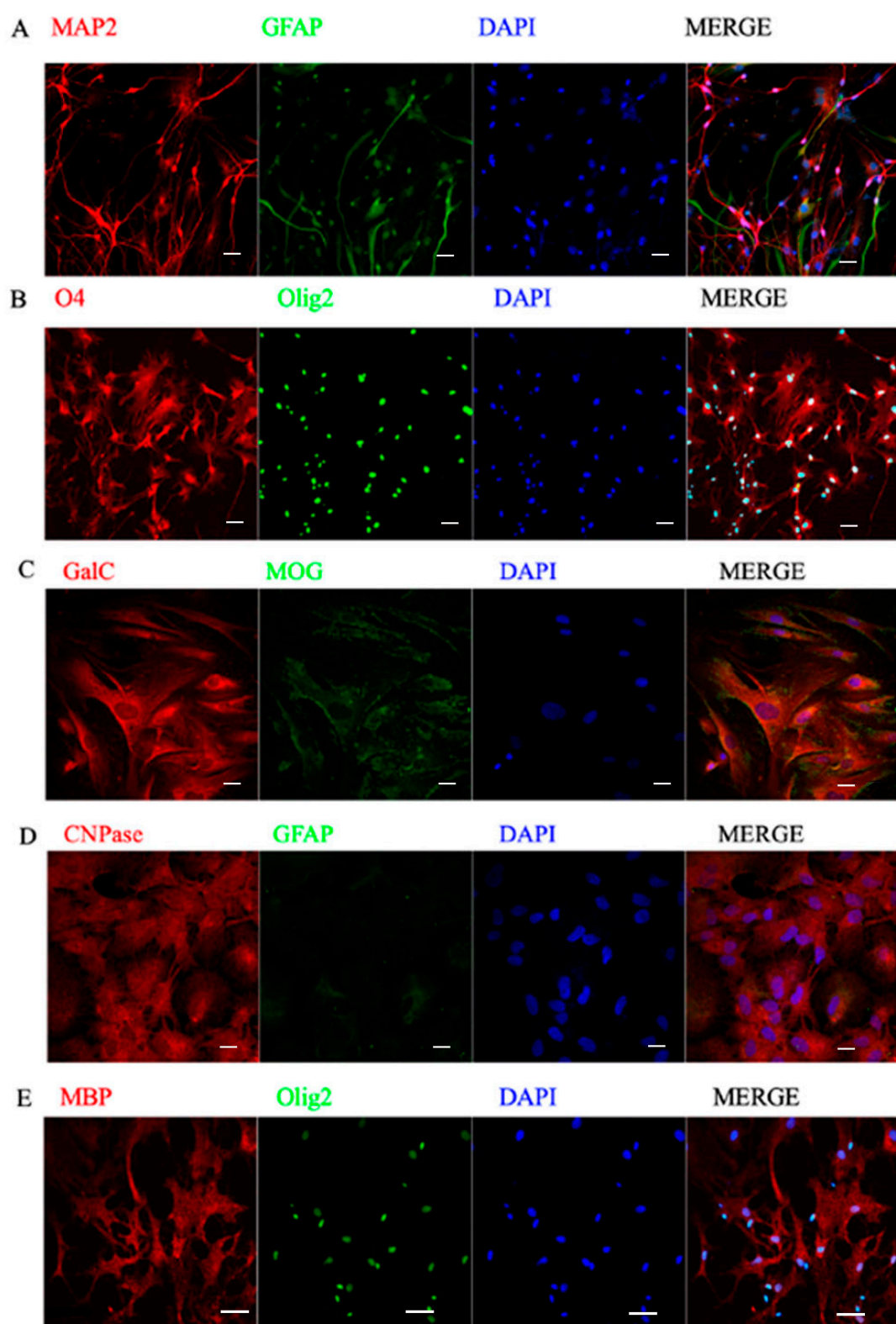


Figure S3



Supplementary Tables:

Table S1. Effects of TCM constituents on Glu-induced reduction viability of mNPCs. (Data are presented as mean \pm SD, n=6), Glu: glutamate.

Table S2. Effect of TCM constituents on CORT-induced reduction viability of mNPCs. (Data are presented as mean \pm SD, n=6), CORT: corticosterone.

Table S1 Effects of TCM constituents on mNPCs under Glu-induced pathological condition (Data are presented as mean \pm SD, n=6)

Group	Concentration	Cell viability (%)
CTRL	/	100.23 \pm 1.82
Glu	500 μ M	81.56 \pm 2.23
DNPZ	20 μ M	75.82 \pm 1.37
	100 μ M	48.67 \pm 3.49
	2 μ M	82.59 \pm 4.92
PPT	10 μ M	78.45 \pm 3.27
	50 μ M	70.33 \pm 3.96
	2 μ M	80.98 \pm 4.25
PPD	10 μ M	75.32 \pm 3.97
	50 μ M	61.23 \pm 3.44
	2 μ g/mL	72.49 \pm 3.75
1226	10 μ g/mL	68.43 \pm 2.66
	50 μ g/mL	55.43 \pm 4.94
	2 μ M	82.25 \pm 3.76
Catalpol (CAT)	10 μ M	83.51 \pm 4.75
	50 μ M	88.66 \pm 1.53
	2 μ M	82.73 \pm 3.24
Geniposide	10 μ M	81.52 \pm 2.24
	50 μ M	75.43 \pm 4.97
	2 μ M	77.33 \pm 5.96
Emodin	10 μ M	65.72 \pm 4.28
	50 μ M	40.42 \pm 2.15
	2 μ M	77.25 \pm 4.36
Cajanine	10 μ M	49.51 \pm 3.27
	50 μ M	36.95 \pm 1.48
	2 μ M	91.32 \pm 1.76
Longistyline A (LoA)	10 μ M	79.34 \pm 5.28
	50 μ M	52.38 \pm 2.40
	2 μ g/mL	89.77 \pm 2.37
B4	10 μ g/mL	94.35 \pm 1.92
	50 μ g/mL	76.92 \pm 2.51

Curcumin (CUR)	2 μ M	92.84 \pm 1.86
	10 μ M	98.72 \pm 1.40
	50 μ M	64.59 \pm 3.82
Resveratrol	2 μ M	80.32 \pm 4.58
	10 μ M	79.40 \pm 2.14
	50 μ M	72.83 \pm 4.59
Genipin (GEN)	2 μ M	83.10 \pm 2.59
	10 μ M	90.26 \pm 0.84
	50 μ M	75.25 \pm 3.83
Icaritin (ICT)	2 μ M	84.59 \pm 2.40
	10 μ M	115.56 \pm 1.79
	50 μ M	123.02 \pm 1.64
Icariin	2 μ M	72.39 \pm 4.30
	10 μ M	74.66 \pm 2.29
	50 μ M	63.14 \pm 3.71
Quercetin	2 μ M	82.42 \pm 3.72
	10 μ M	72.03 \pm 0.71
	50 μ M	55.23 \pm 3.93
Apigenin	2 μ M	84.38 \pm 3.56
	10 μ M	66.95 \pm 3.25
	50 μ M	66.38 \pm 4.58
Loganin	2 μ M	82.92 \pm 2.96
	10 μ M	80.50 \pm 3.83
	50 μ M	79.31 \pm 2.12
Morroniside	2 μ M	85.49 \pm 2.25
	10 μ M	79.56 \pm 3.01
	50 μ M	79.31 \pm 2.29

Table S2 Effects of TCM on mNPCs constituents under CORT-induced pathological condition(Data are presented as mean \pm SD, n=6)

Group	Concentration	Cell viability (%)
CTRL	/	98.29 \pm 2.52
CORT	100 μ M	67.01 \pm 1.86
DNPZ	20 μ M	65.82 \pm 1.73
	100 μ M	63.59 \pm 2.78
	2 μ M	68.77 \pm 3.21
PPT	10 μ M	70.24 \pm 3.25
	50 μ M	55.78 \pm 3.52
	2 μ M	59.32 \pm 4.51
PPD	10 μ M	64.59 \pm 2.18
	50 μ M	53.47 \pm 3.86
	2 μ g/mL	68.34 \pm 2.71
1226	10 μ g/mL	57.59 \pm 3.50
	50 μ g/mL	53.24 \pm 4.67
	2 μ M	69.16 \pm 3.82
Catalpol (CAT)	10 μ M	71.23 \pm 2.56
	50 μ M	76.90 \pm 1.54
	2 μ M	70.12 \pm 3.25
Geniposide	10 μ M	64.87 \pm 2.14
	50 μ M	60.74 \pm 3.89
	2 μ M	63.27 \pm 1.82
Emodin	10 μ M	61.90 \pm 2.78
	50 μ M	52.34 \pm 3.25
	2 μ M	62.18 \pm 3.42
Cajanine	10 μ M	59.63 \pm 1.39
	50 μ M	38.72 \pm 2.91
	2 μ M	63.58 \pm 3.46
Longistyline A	10 μ M	59.10 \pm 2.17
	50 μ M	36.54 \pm 1.35
	2 μ g/mL	69.36 \pm 2.47
B4	10 μ g/mL	68.12 \pm 1.79
	50 μ g/mL	68.45 \pm 2.36

Group	Concentration	Cell viability (%)
Curcumin (CUR)	2 μ M	73.67 \pm 1.71
	10 μ M	75.82 \pm 1.55
	50 μ M	42.35 \pm 3.68
Resveratrol	2 μ M	66.38 \pm 2.37
	10 μ M	59.48 \pm 3.32
	50 μ M	52.31 \pm 2.90
Genipin (GEN)	2 μ M	71.2 \pm 3.84
	10 μ M	75.21 \pm 1.74
	50 μ M	78.32 \pm 1.99
Icaritin (ICT)	2 μ M	70.45 \pm 1.95
	10 μ M	92.43 \pm 3.62
	50 μ M	100.30 \pm 2.15
Icariin (ICA)	2 μ M	71.04 \pm 3.91
	10 μ M	69.85 \pm 2.76
	50 μ M	62.31 \pm 2.32
Quercetin	2 μ M	71.03 \pm 3.63
	10 μ M	42.99 \pm 2.64
	50 μ M	38.78 \pm 3.20
Apigenin	2 μ M	72.24 \pm 3.10
	10 μ M	69.72 \pm 3.09
	50 μ M	44.98 \pm 2.20
Loganin	2 μ M	71.41 \pm 2.53
	10 μ M	72.03 \pm 2.11
	50 μ M	71.85 \pm 2.11
Morroniside	2 μ M	70.37 \pm 2.25
	10 μ M	72.07 \pm 2.70
	50 μ M	71.51 \pm 3.81