

Supplementary Table S1. Chronic unpredictable mild stress (CUMS) stressor schedule.

No.	Stressor	Duration	Stress Type
(a)	Continuous light stimulation	24 h	Disruption of circadian rhythm
(b)	Food and water deprivation	24 h	Nutritional and hydration stress
(c)	Damp bedding	24 h	Environmental discomfort
(d)	Behavioural restraint	> 6 h	Physical immobilisation stress
(e)	Cold water immersion (4 °C)	3 min	Acute physical stress
(f)	Empty cage (no bedding)	24 h	Environmental deprivation
(g)	Tail pinching	2 min	Mild pain stimulus
(h)	Inclined cage housing (30°)	24 h	Postural discomfort

Stressors were applied randomly over 6 weeks (1–2 stressors per day), with no *consecutive repetition of the same stressor within 3 days to maintain unpredictability.*

Supplementary Table S2 The effects of aloe-emodin on CUMS-induced depressive behaviour in mice

Category	Behavioural Testing	SPT		TST	FST
		Sucrose preference rate(%)		immobilization time (s)	immobilization time (s)
		0day	46days		
Control		83.91±7.90	88.75±6.60	25.65±6.45	29.29±6.26
CUMS		81.27±4.42	49.94±15.1***	85.06±4.74***	78.74±4.58***
CUMS + Flu		80.75±10.33	76.07±21.29###	37.90±9.89###	34.49±8.28###
CUMS + AE-D		84.92±7.53	67.66±4.12#	51.50±11.96###	45.05±10.28##
CUMS + AE-G		83.24±4.50	82.99±6.19###	31.44±10.91###	34.16±8.02##
p		0.683	0.001	0.001	0.001

n = 6, Statistical significance was determined using one-way ANOVA and Tukey's post hoc test, where **p* < 0.05, ***p* < 0.01, ****p* < 0.001 compared to the control group; #*p* < 0.05, ##*p* < 0.01, ###*p* < 0.001 compared to the CUMS group.

Supplementary Table S3 The effect of aloe-emodin on the number of hippocampal neurons in CUMS mice

Category	Number of neurons
Control	305.67 ± 2.08
CUMS	275.66 ± 11.67***
CUMS + Flu	293.33 ± 5.68##
CUMS + AE-D	302.00 ± 2.64###
CUMS + AE-G	309.00 ± 2.00###
p	0.001

n = 3, Statistical significance determined by one-way ANOVA and Tukey's post hoc test, where **p*<0.05, ***p*<0.01 compared to the control group. #*p*<0.05, ##*p*<0.01 compared to the CUMS group.

Supplementary Table S4 ELISA kits used in this study.

Cytokine	Species	Cat. No.	Method	Supplier	Sample Type
TNF- α	Mouse	E-EL-M3063	Sandwich	Elabscience	Serum, Hippocampus, BV2
IL-6	Mouse	E-EL-M0044	Sandwich	Elabscience	Serum, Hippocampus, BV2
IL-1 β	Mouse	E-EL-M0037	Sandwich	Elabscience	Serum, Hippocampus
IL-10	Mouse	E-HSEL-M0004	Sandwich	Elabscience	BV2 supernatant

All kits were purchased from Elabscience Biotechnology Co., Ltd. (Wuhan, China). BV2, BV2 microglial cell line.

Supplementary Table S5 The effects of aloe-emodin on CUMS-induced pro-inflammatory factors in the serum of mice

Category	IL-6	IL-1 β	TNF- α
Control	36.849 \pm 4.946	10.40 \pm 4.50	13.53 \pm 1.393
CUMS	95.54 \pm 13.89***	26.02 \pm 6.71***	42.71 \pm 7.38***
CUMS + Flu	45.00 \pm 5.62###	13.76 \pm 3.74###	16.62 \pm 2.85###
CUMS + AE-D	65.38 \pm 11.48###	23.72 \pm 2.71#	17.78 \pm 2.83###
CUMS + AE-G	39.97 \pm 3.71###	15.42 \pm 2.68###	16.041 \pm 4.97###
p	0.000	0.000	0.000

n = 3, Statistical analysis was performed using one-way analysis of variance (ANOVA), where **p* < 0.05, ***p* < 0.01, ****p* < 0.01 compared with the control group; #*p* < 0.05, ##*p* < 0.01, ###*p* < 0.01 compared with the CUMS group.

Supplementary Table S6 The effects of aloe-emodin on pro-inflammatory factors in the hippocampus of mice induced by CUMS

Category	IL-6	IL-1 β	TNF- α
Control	21.95 \pm 3.80	35.70 \pm 5.51	114.97 \pm 17.50
CUMS	505.80 \pm 14.78***	227.64 \pm 6.55***	481.52 \pm 22.33***
CUMS + Flu	282.77 \pm 7.98###	168.12 \pm 13.73###	278.81 \pm 10.05##
CUMS + AE-D	302.90 \pm 8.88###	109.92 \pm 41.61##	286.99 \pm 15.73###
CUMS + AE-G	242.82 \pm 11.10###	95.52 \pm 13.61###	257.85 \pm 8.46###
p	0.000	0.000	0.000

n = 3, Statistical analysis was performed using one-way analysis of variance (ANOVA), where **p* < 0.05, ***p* < 0.01, ****p* < 0.01 compared with the control group; #*p* < 0.05, ##*p* < 0.01, ###*p* < 0.01 compared with the CUMS group.

Supplementary Table S7. Top 15 hub genes in the aloë-emodin–depression PPI network ranked by degree

Gene	Degree	Betweenness Centrality	Closeness Centrality	Functional Description
<i>TNF</i>	65	0.06241571	0.78378378	IL-17 signaling pathway; PI3K/AKT pathway; MAPK signaling pathway; TNF signaling pathway
<i>TP53</i>	64	0.15458516	0.79090909	MAPK signaling pathway; ErbB signaling pathway; Relaxin signaling pathway
<i>IL1B</i>	60	0.04624122	0.75	IL-17 signaling pathway; PI3K/AKT pathway; MAPK signaling pathway; TNF signaling pathway
<i>AKT1</i>	60	0.04590488	0.75652174	TNF signaling pathway; Estrogen signaling pathway; MAPK signaling pathway; Relaxin signaling pathway; PI3K-Akt signaling pathway; AGE-RAGE signaling pathway in diabetic complications; Prolactin signaling pathway; EGFR tyrosine kinase inhibitor resistance; ErbB signaling pathway
<i>BCL2</i>	58	0.04684285	0.73728814	Estrogen signaling pathway; PI3K-Akt signaling pathway; Prolactin signaling pathway; ErbB signaling pathway
<i>CASP3</i>	58	0.0295499	0.73728814	IL-17 signaling pathway; PI3K/AKT pathway; MAPK signaling pathway; TNF signaling pathway
<i>EGFR</i>	58	0.0417475	0.73728814	Estrogen signaling pathway; MAPK signaling pathway; Prolactin signaling pathway; EGFR tyrosine kinase inhibitor resistance
<i>MYC</i>	57	0.07226287	0.73728814	MAPK signaling pathway; Relaxin signaling pathway; EGFR tyrosine kinase inhibitor resistance; ErbB signaling pathway

Supplementary Table S7. Top 15 hub genes in the aloe-emodin–depression PPI network ranked by degree (Continued)

Gene	Degree	Betweenness Centrality	Closeness Centrality	Functional Description
<i>ESR1</i>	50	0.01965676	0.68503937	Estrogen signaling pathway; Relaxin signaling pathway; AGE-RAGE signaling pathway in diabetic complications
<i>MAPK3</i>	49	0.01585066	0.6796875	IL-17 signaling pathway; TNF signaling pathway; Estrogen signaling pathway; MAPK signaling pathway; Relaxin signaling pathway; PI3K-Akt signaling pathway; AGE-RAGE signaling pathway in diabetic complications; Prolactin signaling pathway; EGFR tyrosine kinase inhibitor resistance; ErbB signaling pathway
<i>MMP9</i>	47	0.02464199	0.66923077	IL-17 signaling pathway; TNF signaling pathway; Estrogen signaling pathway
<i>GSK3B</i>	47	0.0254368	0.6744186	IL-17 signaling pathway; Relaxin signaling pathway; PI3K-Akt signaling pathway; AGE-RAGE signaling pathway in diabetic complications; Prolactin signaling pathway; ErbB signaling pathway
<i>PTGS2</i>	46	0.01648132	0.66923077	IL-17 signaling pathway; TNF signaling pathway
<i>MAPK1</i>	43	0.01521087	0.64925373	IL-17 signaling pathway; TNF signaling pathway; Estrogen signaling pathway; MAPK signaling pathway; Relaxin signaling pathway; PI3K-Akt signaling pathway; AGE-RAGE signaling pathway in diabetic complications; Prolactin signaling pathway; EGFR tyrosine kinase inhibitor resistance; ErbB signaling pathway
<i>PIK3CA</i>	43	0.009402	0.6	PI3K-Akt signaling pathway

Topological parameters were calculated using the Network Analyzer tool in Cytoscape 3.7.1. Degree indicates the number of direct interactions; betweenness centrality measures the extent to which a node lies on shortest paths; closeness centrality reflects proximity to all other nodes.

Supplementary Table S8. Molecular docking results for aloe-emodin and key molecular targets

Sample	AKT1	GSK3B	PIK3CA	PTGS2	MAPK1	MAPK3	BCL2	Casp3	ESR1	TNF	IL1B	MMP9	MYC	TP53	EGFR
Aloe-emodin	-10.1	-8.6	-8.1	-7.7	-7.6	-7.6	-7.43	-7.13	-7.00	-6.95	-6.99	-6.72	-6.52	-6.2	-6.03

Using AutoDock Vina software, molecular docking results were obtained for aloe-emodin and 15 key depression targets identified through network pharmacology screening. The lower the binding energy, the more stable the binding conformation; when the binding energy is less than $-5.0 \text{ kcal}\cdot\text{mol}^{-1}$, the drug compound can bind tightly to the target.

Supplementary Table S9 Primary antibodies used in western blotting (WB) and immunofluorescence (IF).

Antibody	Host	Cat. No.	Dilution	MW	Supplier	Application
PI3K	Rabbit	4249	1:1000	110 kDa	Cell Signaling Technology	WB
Phospho-PI3K	Rabbit	17366	1:1000	85 kDa	Cell Signaling Technology	WB
AKT	Rabbit	4691	1:1000	60 kDa	Cell Signaling Technology	WB
Phospho-AKT	Rabbit	4060	1:2000	60 kDa	Cell Signaling Technology	WB
NF-κB p65	Rabbit	8242	1:1000	65 kDa	Cell Signaling Technology	WB
Phospho-NF-κB p65	Rabbit	3033	1:1000	65 kDa	Cell Signaling Technology	WB
β-actin	Mouse	GV15003	1:1000	43 kDa	Solarbio	WB
Iba-1	Rabbit	ab178847	1:500	—	Abcam	IF
CD68	Rabbit	ab213363	1:500	—	Abcam	IF

MW, molecular weight; WB, western blotting; IF, immunofluorescence; —, not applicable.

Supplementary Table S10 Percentage of CD68/Iba-1-positive cells in the hippocampus.

Category	
Control	0.09 ± 0.02
CUMS	0.28 ± 0.03***
CUMS + Flu	0.18 ± 0.01###
CUMS + AE-D	0.15 ± 0.01###
CUMS + AE-G	0.14 ± 0.01###
p	0.001

*n = 3, Statistical significance determined by one-way ANOVA and Tukey's post hoc test, where * $p < 0.05$, ** $p < 0.01$,*

**** $p < 0.001$ compared to control group; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ compared to CUMS group.*

Supplementary Table S11 Screening of effective doses of aloe-emodin on BV2 cells

Category	
Control	100.00 ± 0.00
LPS	66.79 ± 4.27***
LPS + 0.325	90.35 ± 5.87
LPS + 1.250	91.47 ± 1.18#
LPS + 2.500	95.81 ± 3.57##
LPS + 3.125	95.48 ± 3.45###
LPS + 5.000	98.68 ± 1.40###
LPS + 10.00	91.51 ± 1.31##
LPS + 12.50	71.91 ± 0.80

n = 3, Statistical significance was determined using one-way analysis of variance (ANOVA) and Tukey's post hoc test, where **p*<0.05, ***p*<0.01, ****p*<0.001 compared with the control group; #*p*<0.05, ##*p*<0.01, ###*p*<0.001 compared with the LPS group.

Supplementary Table S12 The effect of aloe-emodin on the levels of TNF- α , IL-6 and IL-10 in the culture supernatant of LPS-induced BV2 microglial cells

Category	IL-6	TNF- α	IL-10
Control	21.70 ± 8.81	87.86 ± 13.05	18.24 ± 0.72
CUMS	5148.89 ± 111.89***	6383.24 ± 323.90***	64.97 ± 1.19***
CUMS + 1 μ M	2690.36 ± 157.16###	2396.85 ± 171.53###	100.94 ± 0.53###
CUMS + 5 μ M	2610.79 ± 97.64###	2539.56 ± 67.20###	107.76 ± 0.33###
CUMS + 10 μ M	2009.87 ± 69.36###	2438.52 ± 78.18###	118.83 ± 1.06###
<i>p</i>	0.000	0.000	0.000

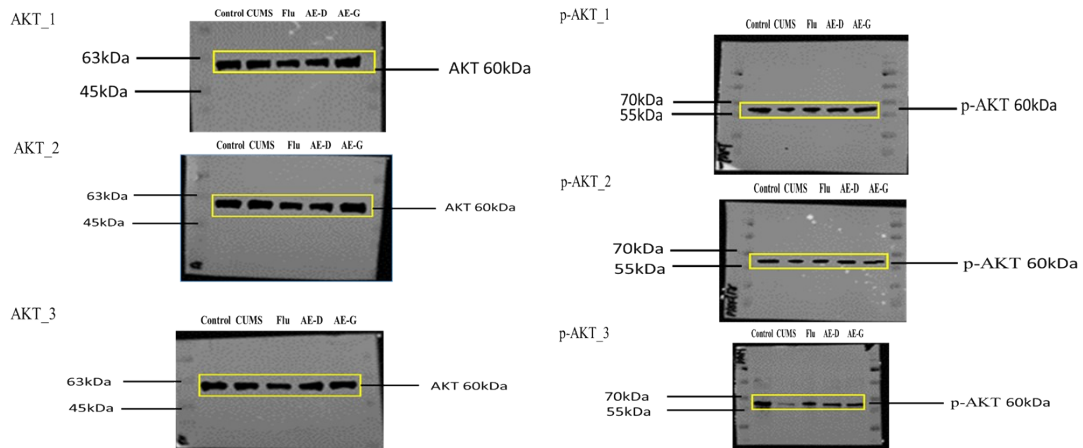
n = 3, Statistical significance was determined using one-way analysis of variance (ANOVA) and Tukey's post hoc test, where **p*<0.05, ***p*<0.01, ****p*<0.001 compared with the control group; #*p*<0.05, ##*p*<0.01, ###*p*<0.001 compared with the LPS group.

Original western blot bands :

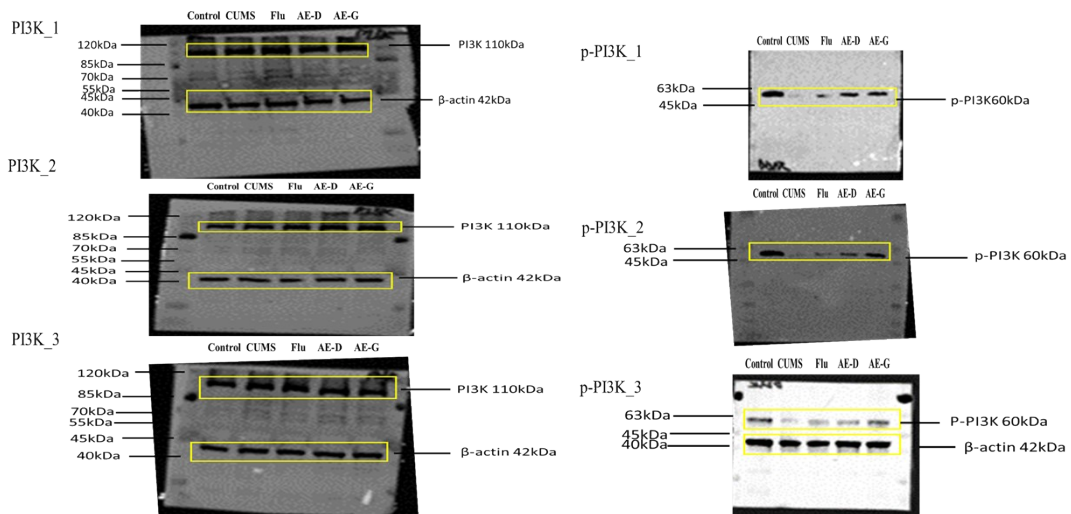
Complete Western blot bands of AKT, phosphorylated AKT, PI3K, phosphorylated PI3K, NF- κ B P65, phosphorylated NF- κ B P65, and β -actin in hippocampal tissues of different groups

Figure_5A

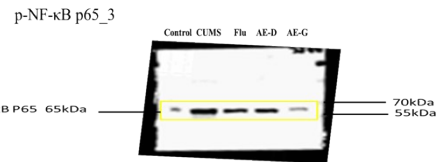
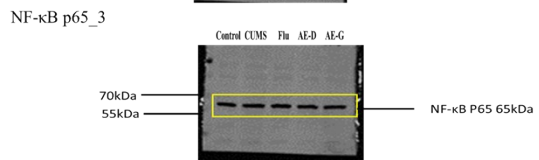
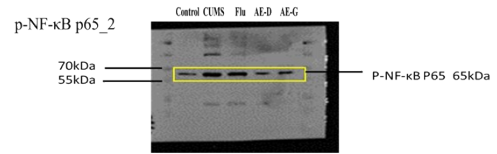
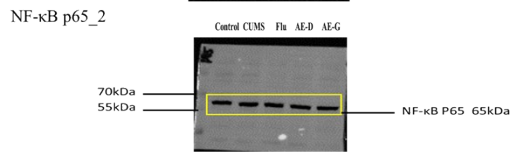
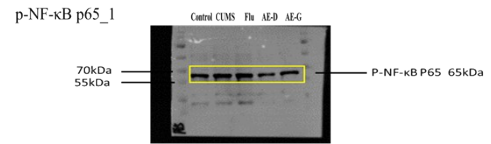
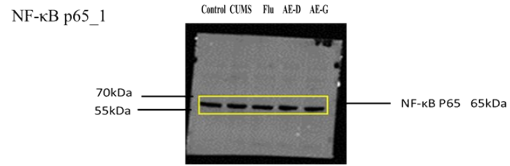
AKT ; p-AKT



PI3K ; p-PI3K



NF- κ B P65 ; p-NF- κ B P65



β-actin

