Supporting Information

Essential Oil of *Syzygium aromaticum* Reverses the Deficits of Stress-Induced Behaviors and Hippocampal p-ERK/p-CREB/Brain-Derived Neurotrophic Factor Expression

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Behavioral tests

In the experiments, we determined whether long-term (5 weeks) Tween vehicle (10% Tween 80) treatment by gavage had potential effects on depressive-like behaviors induced by CUMS in rats. Two-way ANOVA analysis with stress (control and CUMS), as the between-subject factor, and solvent (saline and 10% Tween 80), as a within-subject factor, revealed that 10% Tween 80 had no significant effect on the sucrose preference, latency to feed, and home cage feed consumption in control/CUMS rats (Fig. 1S).

Western blot

The experiment was conducted to determine whether 10% Tween 80 treatment via gavage has potential effects on hippocampal BDNF, p-ERK, and p-CREB expressions in CUMS-induced rats. Two-way ANOVA analysis with stress (control and CUMS) as the between-subject factor and solvent (saline and 10% Tween 80) as a within-subjects factor revealed that 10% Tween 80 had no significant effect on the expression of BDNF ($F(1,8) = 0.14, p > 0.05$), p-ERK
\( F(1,8) = 1.00, p > 0.05 \), and p-CREB \( F(1,8) = 0.07, p > 0.05 \) proteins in control/CUMS rats (Fig. 2S).

**Fig. 1S** Effects of long-term 10% Tween 80 treatment on depressive-like behaviors induced by CUMS in rats. (A) 10% Tween 80 treatment did not influence the decrease of sucrose preference (\%); (B) latency to feed (sec); (C) home-cage food consumption (g).
preference induced by CUMS in rats. (B) 10% Tween 80 did not influence the increase of latency to feed induced by CUMS in rats. (C) Both CUMS and 10% Tween 80 had no effect on home cage food consumption in rats. The data are expressed as mean ± SD (n = 7 per group).

Two-way ANOVA results: (A) Solvent $F(1,24) = 0.09, p > 0.05$; stress $F(1,24) = 13.17, p < 0.01$; treatment × stress interaction $F(1,24) = 0.01, p > 0.05$. (B) Solvent $F(1,24) = 0.15, p > 0.05$; stress $F(1,24) = 33.00, p < 0.01$; treatment × stress interaction $F(1,24) = 0.00, p > 0.05$. (C) Solvent $F(1,24) = 0.00, p > 0.05$; stress $F(1,24) = 0.18, p > 0.05$; treatment × stress interaction $F(1,24) = 0.22, p > 0.05$. 

Fig. 2S Effects of long-term 10% Tween 80 treatment on hippocampal brain-derived neurotrophic factor, p-ERK, and p-CREB expressions in rats exposed to CUMS. (A) Representative Western blot images of BDNF, GAPDH, p-ERK, ERK, p-CREB, and CREB are shown, and BDNF (B), p-ERK (C), and p-CREB (D) were quantified. The data are expressed as mean ± SD (n = 3 per group).

Two-way ANOVA results: (B) Solvent $F(1,8) = 0.14$, $p > 0.05$; stress $F(1,8) = 66.11$, $p < 0.01$; treatment × stress interaction $F(1,8) = 0.04$, $p > 0.05$. (C) Solvent $F(1,8) = 1.00$, $p > 0.05$; stress $F(1,8) = 26.89$, $p < 0.01$; treatment × stress interaction $F(1,8) = 0.41$, $p > 0.05$. (D) Solvent $F(1,8) = 0.07$, $p > 0.05$; stress $F(1,8) = 46.83$, $p < 0.01$; treatment × stress interaction $F(1,8) = 0.42$, $p > 0.05$.

Missing details of Western blot analysis

The missing details of Western blot analysis are as follows:

Amount of protein loaded onto the gels

BDNF: 70 µg total protein; GAPDH: 45 µg; p-ERK and ERK: 45 µg; p-CREB and CREB: 45µg.

Percent of the polyacrylamide gel separating gel: BDNF (15%), GAPDH (12%), p-ERK and ERK (12%), p-CREB and CREB (12%); stacking gel: 4% for all proteins.
Composition of the wash buffer

1L TBST buffer: 8.8 g NaCl, 20 mL 1 M Tris-HCl (pH 8.0) and 0.5 mL Tween-20.

Method used for band quantitation

Quantity One.