Supplementary Materials

*Oroxylum indicum* Seed Extract as a Potential Antidepressant in Chronic Stress Mouse Model of Depression

Chorpeth Chalermwongkul1, Charinya Khamphukdee2, Supawadee Daodee3, Orawan Monthakantirat3, Chantana Boonyarat3, Yutthana Chotritthirong1, Juthamart Maneenet3,4, Suresh Awale4, Anake Kijjoa2,5, Yaowared Chulikhit3\*

1 Graduated School of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand; c.chorpeth@kkumail.com, yutthana\_ch@kkumail.com

2 Division of Pharmacognosy and Toxicology, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand; charkh@kku.ac.th

3 Division of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, 40002, Thailand; csupawad@kku.ac.th, oramon@kku.ac.th, chaboo@kku.ac.th, juthamart\_pp@hotmail.com

4 Natural Drug Discovery Laboratory, Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama, 930-0154, Japan.; suresh@inm.u-toyama.ac.jp

5 ICBAS-Instituto de Ciências Biomédicas Abel Salazar and CIIMAR, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal; ankijjoa@icbas.up.pt

**\*** Correspondence: yaosum@kku.ac.th; Tel.: +66-81-3802357

*Statistical Analysis of Effect of the OIS extract on UCMS-Induced Anhedonia Behavior Using Sucrose Preference Test (SPT)*

**Table S1.** paired Student’s *t*-test andOne-way analysis of variance (ANOVA) test of SPT

|  |  |
| --- | --- |
| **Group comparison** | **ANOVA followed by Tukey’s post hoc test** |
| ***P*** | **F(DFbetween group, DFresidual)** |
| **Week 0** |
| non-stress group vs. UCMS + vehicle group | 0.994 |  |
| All UCMS-induced groups | 0.980 | F(3,36) = 0.0619 |
| **Week 1** |
| non-stress group vs. UCMS + vehicle group | 0.946 |  |
| All UCMS-induced groups | 0.812 | F(3,36) = 0.318 |
| **Week 2** |
| non-stress group vs. UCMS + vehicle group | 0.069 |  |
| All UCMS-induced groups | 0.274 | F(3,36) = 1.347 |
| **Week 3** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | 0.613 | F(3,36) = 0.609 |
| **Week 4** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,36) = 7.192 |
| UMCS + vehicle group vs. UCMS + IMP20 group | 0.005 |
| UMCS + vehicle group vs. UCMS + OIS100 group | 0.233 |
| UMCS + vehicle group vs. UCMS + OIS500 group | 0.001 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | 0.078 |
| **Week 5** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,36) = 10.134 |
| UMCS + vehicle group vs. UCMS + IMP20 group | < 0.001 |
| UMCS + vehicle group vs. UCMS + OIS100 group | 0.008 |
| UMCS + vehicle group vs. UCMS + OIS500 group | < 0.001 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | 0.685 |
| **Week 6** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,36) = 12.664 |
| UMCS + vehicle group vs. UCMS + IMP20 group | < 0.001 |
| UMCS + vehicle group vs. UCMS + OIS100 group | 0.003 |
| UMCS + vehicle group vs. UCMS + OIS500 group | < 0.001 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | 0.351 |

*Statistical Analysis of Effect of the OIS extract on UCMS-Induced Despair Behaviors Using Tail Suspension Test (TST) and Forced Swimming Test (FST)*

**Table S2.** paired Student’s *t*-test andOne-way analysis of variance (ANOVA) test of TST

|  |  |
| --- | --- |
| **Group comparison** | **ANOVA followed by Tukey’s post hoc test** |
| ***P*** | **F(DFbetween group, DFresidual)** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,36) = 23.118 |
| UMCS + vehicle group vs. UCMS + IMP20 group | < 0.001 |
| UMCS + vehicle group vs. UCMS + OIS100 group | 0.035 |
| UMCS + vehicle group vs. UCMS + OIS500 group | < 0.001 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | 0.009 |

**Table S3.** paired Student’s *t*-test andOne-way analysis of variance (ANOVA) test of FST

|  |  |
| --- | --- |
| **Group comparison** | **ANOVA followed by Tukey’s post hoc test** |
| ***P*** | **F(DFbetween group, DFresidual)** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,36) = 13.250 |
| UMCS + vehicle group vs. UCMS + IMP20 group | < 0.001 |
| UMCS + vehicle group vs. UCMS + OIS100 group | 0.013 |
| UMCS + vehicle group vs. UCMS + OIS500 group | < 0.001 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | 0.043 |

*Statistical Analysis of Effect of the OIS extract on UCMS-Induced Hypersecretion of Glucocorticoids Using Serum Corticosterone (CORT) Level*

**Table S4.** paired Student’s *t*-test andOne-way analysis of variance (ANOVA) test of serum CORT level

|  |  |
| --- | --- |
| **Group comparison** | **ANOVA followed by Tukey’s post hoc test** |
| ***P*** | **F(DFbetween group, DFresidual)** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,16) = 18.231 |
| UMCS + vehicle group vs. UCMS + IMP20 group | < 0.001 |
| UMCS + vehicle group vs. UCMS + OIS100 group | < 0.001 |
| UMCS + vehicle group vs. UCMS + OIS500 group | < 0.001 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | 0.695 |

*Statistical Analysis of Effect of the OIS extract on UCMS-Induced Hyperactivation of* *Hypothalamic-pituitary-adrenal (HPA) axis in Frontal Cortex and Hippocampus Using Quantitative real-time polymerase chain reaction (qPCR)*

**Table S5.** paired Student’s *t*-test andOne-way analysis of variance (ANOVA) test of FK506 binding protein 51 (FKBP5) in frontal cortex and hippocampus

|  |  |
| --- | --- |
| **Group comparison** | **ANOVA followed by Tukey’s post hoc test** |
| ***P*** | **F(DFbetween group, DFresidual)** |
| **Frontal cortex** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,20) = 23.573 |
| UMCS + vehicle group vs. UCMS + IMP20 group | < 0.001 |
| UMCS + vehicle group vs. UCMS + OIS100 group | 0.002 |
| UMCS + vehicle group vs. UCMS + OIS500 group | < 0.001 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | 0.009 |
| **Hippocampus** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,20) = 21.625 |
| UMCS + vehicle group vs. UCMS + IMP20 group | < 0.001 |
| UMCS + vehicle group vs. UCMS + OIS100 group | 0.006 |
| UMCS + vehicle group vs. UCMS + OIS500 group | < 0.001 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | 0.009 |

**Table S6.** paired Student’s *t*-test andOne-way analysis of variance (ANOVA) test of serine/threonine-protein kinase 1 (SGK-1) in frontal cortex and hippocampus

|  |  |
| --- | --- |
| **Group comparison** | **ANOVA followed by Tukey’s post hoc test** |
| ***P*** | **F(DFbetween group, DFresidual)** |
| **Frontal cortex** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,20) = 93.881 |
| UMCS + vehicle group vs. UCMS + IMP20 group | < 0.001 |
| UMCS + vehicle group vs. UCMS + OIS100 group | < 0.001 |
| UMCS + vehicle group vs. UCMS + OIS500 group | < 0.001 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | <0.001 |
| **Hippocampus** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,20) = 21.625 |
| UMCS + vehicle group vs. UCMS + IMP20 group | < 0.001 |
| UMCS + vehicle group vs. UCMS + OIS100 group | 0.003 |
| UMCS + vehicle group vs. UCMS + OIS500 group | < 0.001 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | 0.029 |

**Table S7.** paired Student’s *t*-test andOne-way analysis of variance (ANOVA) test of glucocorticoid receptor (GR) in frontal cortex and hippocampus

|  |  |
| --- | --- |
| **Group comparison** | **ANOVA followed by Tukey’s post hoc test** |
| ***P*** | **F(DFbetween group, DFresidual)** |
| **Frontal cortex** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,20) = 27.307 |
| UMCS + vehicle group vs. UCMS + IMP20 group | < 0.001 |
| UMCS + vehicle group vs. UCMS + OIS100 group | 0.007 |
| UMCS + vehicle group vs. UCMS + OIS500 group | < 0.001 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | 0.001 |
| **Hippocampus** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,20) = 8.894 |
| UMCS + vehicle group vs. UCMS + IMP20 group | 0.005 |
| UMCS + vehicle group vs. UCMS + OIS100 group | 0.363 |
| UMCS + vehicle group vs. UCMS + OIS500 group | < 0.001 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | 0.034 |

*Statistical Analysis of Effect of the OIS extract on UCMS-Induced Impaired Neurogenesis in Frontal Cortex and Hippocampus Using Quantitative real-time polymerase chain reaction (qPCR)*

**Table S8.** paired Student’s *t*-test andOne-way analysis of variance (ANOVA) test of brain-derived neurotrophic factor (BDNF) in frontal cortex and hippocampus

|  |  |
| --- | --- |
| **Group comparison** | **ANOVA followed by Tukey’s post hoc test** |
| ***P*** | **F(DFbetween group, DFresidual)** |
| **Frontal cortex** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,20) = 27.347 |
| UMCS + vehicle group vs. UCMS + IMP20 group | < 0.001 |
| UMCS + vehicle group vs. UCMS + OIS100 group | 0.100 |
| UMCS + vehicle group vs. UCMS + OIS500 group | < 0.001 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | < 0.001 |
| **Hippocampus** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,20) = 10.661 |
| UMCS + vehicle group vs. UCMS + IMP20 group | 0.007 |
| UMCS + vehicle group vs. UCMS + OIS100 group | 0.750 |
| UMCS + vehicle group vs. UCMS + OIS500 group | < 0.001 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | 0.004 |

**Table S9.** paired Student’s *t*-test andOne-way analysis of variance (ANOVA) test of cyclic AMP-responsive element-binding protein (CREB) in frontal cortex and hippocampus

|  |  |
| --- | --- |
| **Group comparison** | **ANOVA followed by Tukey’s post hoc test** |
| ***P*** | **F(DFbetween group, DFresidual)** |
| **Frontal cortex** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,20) = 13.720 |
| UMCS + vehicle group vs. UCMS + IMP20 group | 0.005 |
| UMCS + vehicle group vs. UCMS + OIS100 group | 0.814 |
| UMCS + vehicle group vs. UCMS + OIS500 group | < 0.001 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | < 0.001 |
| **Hippocampus** |
| non-stress group vs. UCMS + vehicle group | < 0.001 |  |
| All UCMS-induced groups | < 0.001 | F(3,20) = 7.058 |
| UMCS + vehicle group vs. UCMS + IMP20 group | 0.031 |
| UMCS + vehicle group vs. UCMS + OIS100 group | 0.911 |
| UMCS + vehicle group vs. UCMS + OIS500 group | 0.004 |
| UCMS + OIS100 group vs. UCMS + OIS500 group | 0.018 |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Baicalin | Baicalein | Chrysin | Oroxylin A |
| LOQ | **Concentration (µg/ml)** | 1.0 | 2.5 | 1 | 2.5 |
| **S/N** | 9.83 ± 0.20 | 9.90 ± 0.19 | 9.84 ± 0.10 | 9.91 ± 0.13 |
| Linearity | **Range (µg/ml)** | 1 – 6 | 2.5 – 15 | 2.5 – 15 | 2.5 – 15 |
| **Equation** | y = 54.636x - 21.493 | y = 51.014x - 54.144 | y = 88.454x - 45.318 | y = 72.566x - 76.601 |
| **Coefficient determination (R2)** | 0.9997 | 0.9996 | 0.9991 | 0.9997 |
| Precision | **Repeatability****(within day)****RSD** | 0.26 – 0.92% | 0.1 – 1.59% | 0.28 – 0.89% | 0.08 – 0.80% |
| **Intermediate precision****(between day)****RSD** | 0.30 – 3.77% | 0.20 – 1.32% | 0.08 – 0.95% | 0.14 – 1.43% |
| Accuracy(%recovery) | **Low concentration** | 104.8 ± 1.43 | 101.66 ± 0.86 | 104.99 ± 0.71 | 101.40 ± 0.65 |
| **Medium concentration** | 99.39 ± 0.12 | 99.95 ± 0.52 | 100.87 ± 0.094 | 100.66 ± 0.23 |
| **High concentration** | 100.13 ± 0.15 | 99.92 ± 0.15 | 99.41 ± 0.085 | 99.48 ± 0.14 |

 **Table S10.** Validation result of the HPLC method for determination of baicalin, baicalein, chrysin, and oroxylin A

**Figure S1.** Inhibitory effect of *O. indicum* seed on MAO-A and MAO-B (panel A and B, respectively). The inhibition graph was plot between log(concentration) (X-axis) and %inhibition (Y-axis).

**Figure S2.** Inhibitory effect of Clorgyline on MAO-A and MAO-B (panel A and B, respectively). The inhibition graph was plot between log(concentration) (X-axis) and %inhibition (Y-axis)

**Figure S3.** Inhibitory effect of Deprenyl on MAO-A and MAO-B (panel A and B, respectively). The inhibition graph was plot between log(concentration) (X-axis) and %inhibition (Y-axis)