The Effects of Osmanthus Fragrans Flower Extract on Maternally Deprived Rats in Early Life

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Abstract: In the past decade, oxygen radicals have been associated with the development of depression. Osmanthus fragrans is a plant that is distributed in areas of China, Japan and Taiwan. Report show that Osmanthus fragrans flower extract (OFFE), which contains a high amount of total flavonoid and polyphenol, has a significant antioxidant effect, and even has a neuroprotective function. The present study investigated the effects of OFFE on maternally deprived rats (MDP) in early life. The oxygen radical absorbance capacity (ORAC), glutathione (GSH) measurements and forced swim test (FST) were conducted to estimate the effects of OFFE on the MDP rats.The data showed that OFFE caused a significant dose-dependent increase in ORAC and GSH in the organs of the MDP rats, including the brain. At lower doses, the specific brain regions of MDP rats, such as the hippocampus, cerebral cortex, thalamus and cerebellum, also saw a significant increase in ORAC and GSH. In addition, the immobile time in the FST of OFFE treated MDP rats fell significantly at all treated doses. Moreover, the results of lower dose treatment experiments showed a correlation between the antioxidant ability of OFFE and its antidepressant effects. The results indicate that OFFE can strengthen the ability to carry out antioxidation in MDP rats, and that the depression-like behavior of such rats can be decreased due to the antioxidant effect of OFFE.
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1. Introduction

Reactive oxygen species (ROS), such as superoxide radicals, are reactive chemical species generated during normal metabolic processes, and in excess these can damage lipids and proteins [1-3]. Under normal physiological conditions, there is a balance between the oxidative and antioxidative systems in an organism, with oxidative stress caused by an imbalance between these systems in favor of the former [1]. The causes of oxidative stress have been attributed to either increased generation of reactive oxygen species, or impaired enzymatic or nonenzymatic defenses against them. Oxidative stress is always detrimental to the proper functioning of the brain, and can adversely alter neuronal signaling and inhibit neurogenesis [4]. In recent years, oxidative stress has been shown to contribute to the etiology of depression [4], and it has been found that major depression is often accompanied by a decreased antioxidant status and by induction of oxidative pathways [5]. However, the evidence for the curative effects of antioxidants on depression is still limited, and this is one of the motivations for the current study.

Osmanthus fragrans, also known as sweet osmanthus of sweet olive, is a species of Osmanthus native to Asia, found from the eastern Himalaysa through southern China, and to Taiwan and to southern Japan [6, 7]. Previous reports have shown that the dried flowers of Osmanthus fragrans have neuroprotective, free radical scavenging and anti-oxidative effects [8, 9]. There is also evidence that the pulp of Osmanthus fragrans, which is often considered agricultural waste, may be a promising source of natural antioxidants [10]. In addition, tea made from the flowers of the plant is widely used in Asia for the treatment of menopathies [11]. However, the antioxidant effects of Osmanthus fragrans with regard to depression have received little attention by researchers.

The development of an appropriate animal model for use in the depression-related studies is essential. The treatment of maternal deprivation during early life is widely used to generate depression-like symptoms in animals [12, 13] and the 60-min maternal isolation protocol in early life is well established in our laboratory to achieve this [14,15]. Therefore, rates that underwent 60-min

maternal isolation in early life were used in the present study. In addition, the FST was carried out to evaluate the level of depression in rats, based on the index of the time spent immobile in the swimming test [16, 17].

The present study was carried out in order to assess the effects of OFFE on the depression, as well as to clarify the related mechanism in the antioxidant pathway.

2. Materials and methods

Animals

This study was conducted in conformity with the policies and procedures detailed in the "Guide for Animal Care and Use of Laboratory Animals". The animal experimental protocols were confirmed by the Institutional Animal Care and Use Committee (IACUC) of Chung-Hwa University of Medical Technology. Adult male Sprague-Dawley rats weighing 150-170g at the time of testing were housed at a constant room temperature (22±1°C) and humidity (50±10% RH) with a 12-h light/dark cycle. Food and water were available ad libitum.

The preparation for the reagent of Osmanthus fragrans

The original extract stock of Osmanthus fragrans was obtained from the Seng-Da cGMP pharmaceutical factory in Taiwan. The extract stock was dried and condensed in a freeze dryer for over 72 hrs, in order to keep it in an anhydrous condition. The extract sample was then collected and kept in frozen storage at -20℃.

Maternal deprivation in early life protocol

The maternal deprivation protocol in early life was used to produce rats with depression-like symptoms, as described in detail previously [15]. In brief, pregnant Sprague-Dawley rats arrived at the animal facility on gestational day 12. The pups were sexed and culled into litters of 8-10 male pups and randomly assigned to maternal deprivation for 60 min per day for 14 days. When all pups were weaned, the rats were maintained four per cage at a constant room temperature of 22±1 ^{0}C in a 12-h light/dark cycle (light on at 6:00 A.M.) with free access to chow and water.

Oral administration of OFFE protocol

MDP rats were assigned into control and OFFE treated groups. After 28 days, the MDP rats were subjected to oral administration of OFFE. The extract was prepared at the doses of 0.01g/kg, 0.1g/kg, 1g/kg and 6g/kg. The MDP rats were then randomly assigned to oral administration of OFFE at various doses. From days 29 to 42, the rats were fed with OFFE via the feed trough once daily. The treated and untreated rats were then subjected to FST or antioxidant ability measurements.

Assay for oxygen radical absorbance capacity (ORAC)

Tissue excised from the rats was homogenized, and 15 µl of the homogenizer was dissolved with 100 µl of 0.1 µM β-phycoerythrin (Sigma) and 85µl of 75 mM AAPH. A ELISA reader was then used to estimate the ORAC of the tissue. The absorbance wavelength was set at 485nm, and the detection was carried out for120 min. The ORAC calculation formula is as follows:

"S = (0.5+ f 5 / f 0 + f 10 / f 0 + f 15 / f 0 …….+ f 65/ f 0)*5"

"ORAC value (µM) = 20 * k * ($S_{sample} - S_{blank}$) / ($S_{trolox} - S_{blank}$)"

where k is the sample dilution factor

The measurement for the glutathione (GSH) content

Tissue excised from the rats was homogenized, and 150 ul of the homogenizer was added to a number of vials. The samples in each vial were dissolved using 450 µL of 5% TCA solution, and a soluble extract was then obtained after pelleting the crude membrane fraction by centrifugation (1000 rpm for 10 min, 4 ℃). 30ul of the supernatant dissolved with 140ul of 0.4 M TRIS buffer and 10ul of 0.01M DTNB was resolved in a 96-well detection plate. After 5-min rest, the ELISA reader (OD: 405nm) was used to measure the GSH content of the tissues.

Forced swimming test

MDP rats were immersed in plexiglass cylinders (diameter 18 cm, height 38 cm) filled to a depth of 25 cm with water at 25°C. The FST was carried out on two consecutive days. On the first experimental day, rats were gently placed in the water for a 15 min period of habituation. On removal from the water, they were placed in a plexiglass box under a 60W bulb for 30 min to dry. The next day, they were placed in the cylinders and observed for 5 min. During this period, the total time that spent immobile (i.e., making only the movements necessary to remain afloat) was measured, and the immobility time (IMT) was calculated as the immobility time / total testing time*100%. After a 5-min test, the rats were removed from the water and placed in a plexiglass box under a 60W bulb for 30 min to dry and rest.

Statistics

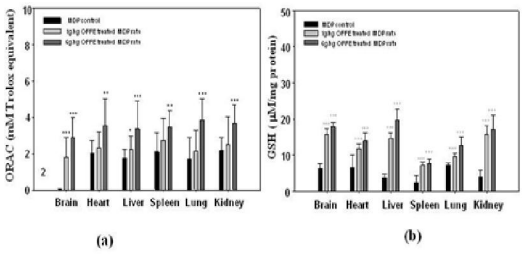
The results are expressed as mean ± SEM. Sample sizes are indicated by n.

Comparisons between groups were carried out with a one- or two-way analysis of variance (ANOVA). Differences between two groups were compared using the unpaired Student's t-test, with $p<0.05$ considered statistically significant.

3.Results

Clarifying the antioxidant effects of OFFE on control MDP rats

After 14 days of OFFE oral treatment, 4-week-old MDP rats were sacrificed for organ tissue analysis with regard to ORAC and GSH. Specifically, the organ tissues, including the brain, heart, liver, spleen, lung and kidney, were obtained for analysis. In figure 1A, the data show that the organ tissues taken from 1g/kg OFFE treated MDP rats had no significant enhancement in ORAC, except for the brain and the liver (figure 1a , the brain: control, 0.02%±0.04; 1g/kg OFFE treated MDP, 1.80%±1.11 ; $p<0.001$ compared to control; the liver: control, 1.76%±0.49; 1g/kg OFE treated MDP, 2.22%±0.78 ; $p<0.05$ compared to control, n=6 in each group). However, all the organ tissues taken from 6g/kg OFFE treated MDP rats showed significant enhancement in ORAC (figure 1a , the brain, the liver, the lung and the kidney groups, $p<0.001$ compared to control, n=6 in each group; the heart and the spleen groups; $p<0.01$ compared to control, n=6 in each group). Figure 1b shows the results for the GSH content. The data showed that all the organ tissues taken from 1g/kg or 6g/kg OFFE treated MDP rats expressed a significant increase in the amount of GSH compared to the control MDP rats (figure 1b, the brain, the liver, the spleen, the lung and the kidney groups; $p<0.001$ compared to control, n=6 in each group).



(a) (b)

Figure 1. Estimation of the antioxidant effects of OFFE on the organs of MDP control rats

The MDP control rats treated with OFFE orally for 14 days. Then the rats were sacrificed and the organs (brain, heart, liver, spleen, lung, kidney) were taken out and homogenized for the measurement of ORAC and GSH. (a) At the dose of 1g/kg OFFE treatment, the brain expressed a significant increase in ORAC (versus control, $p<0.001$), and the liver also showed a lower, but still significant, increase in ORAC. At the dose of 6g/kg OFFE, all the screened organs expressed a significant increase in ORAC (versus control, $p<0.001$). (b) The quantitative measurement of GSH in screened organ tissues was also carried out. Two doses, 1g/kg and 6g/kg of OFFE, were also examined here. The data showed that all the screened organ tissues produced significantly increased amounts of GSH at the two doses of 1g/kg and 6g/kg of OFFE (versus control, $p<0.001$).

Estimation for the depression-like behavior of OFFE treated MDP rats

After 1g/kg and 6g/kg OFFE oral treatment for 14 days, MDP rats were subjected to the FST. The data showed that MDP rats treated with 1g/kg and 6g/kg OFFE has an obvious reduction in the IMT in FST (figure 2, naive, 126.2%±4.2; control, 191.5%±12.9; sham, 171.0%±14.7; 1g/kg OFFE treated MDP, 61.2%±16.9; 6g/kg OFE treated MDP, 55.5%±13.3 ; $p<0.001$ compared to sham, n=6 in each group).

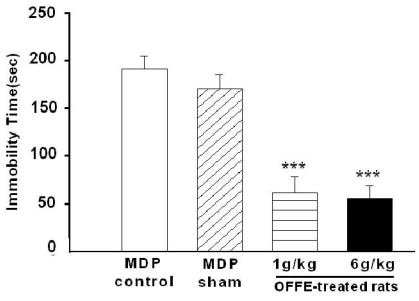


Figure 2. The depression-like behaviour of OFFE treated MDP rats was estimated by the forced swim test. MDP rats administered orally with OFFE at 1g/kg and 6g/kg for 14 days were subjected to the forced swim test. The data showed that MDP rats, treated with OFFE at 1g/kg and 6g/kg, produced significant and equivalent reductions in the immobility time in the FST.

Identifying the effects of OFFE on depression related brain regions based on the lower dose treatment

Based on the lower dose treatment, the sensitivity of the antioxidant response to OFFE in the depression related brain regions would reflect the correlation between the antioxidant effects and the anti-depression effects of OFFE. In figure 3, the data show that the depression related brain region tissues taken from 0.01g/kg, 0.1g/kg, and 1g/kg OFFE treated MDP rats expressed significant enhancement in ORAC (figure 3,

the whole brain, the cerebellum, the medulla, the hippocampus, the thalamus and the cerebral cortex groups; $p<0.001$ compared to control, n=6 in each group). Figure 4 shows the results for the amounts of GSH. The data show that the depression related brain region tissues (except for the cerebellum) taken from 0.01g/kg, 0.1g/kg, and 1g/kg OFFE treated MDP rats had a significant increase in the amount of GSH (figure 4, the whole brain, the medulla, the hippocampus, the thalamus and the cerebral cortex groups; $p<0.001$ compared to control; the cerebellum; $p>0.1$ compared to control, n=6 in each group).

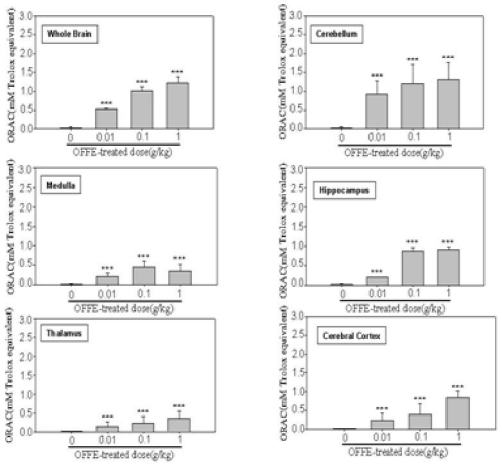


Figure 3. The estimation of the antioxidant effects of OFFE with regard to ORAC in depression related brain regions

MDP rats treated with OFFE at the doses of 0.01g/kg, 0.1g/kg and 1g/kg were sacrificed, and the depression related brain regions, the hippocampus, cerebral cortex, thalamus, cerebellum, medulla, and even the whole brain, were then dissected out. The antioxidant abilities of these brain regions were estimated by measuring the level of ORAC. The data showed that the OFFE extract could significantly increase the level of ORAC in the depression related brain regions at various doses.

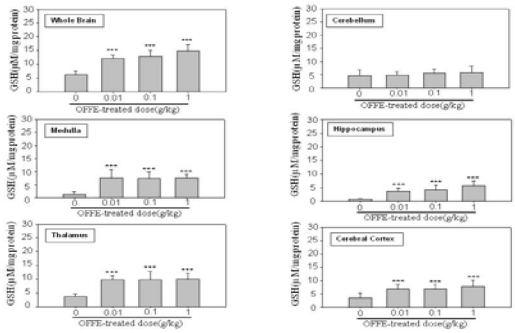


Figure 4. The estimation of the the antioxidant effect of OFFE with regard to the level of GSH in the depression related brain regions

MDP rats treated with OFFE at the doses of 0.01g/kg, 0.1g/kg and 1g/kg were sacrificed. , and the depression related brain regions, the hippocampus, cerebral cortex, thalamus, cerebellum, medulla, and even the whole brain, were then dissected out. The antioxidant abilities of these brain regions was estimated by measuring the amount of GSH. The data showed that the OFFE extract could significantly increase the amount of GSH in the depression related brain regions, except the cerebellum, at various doses.

Estimation for the effects of OFFE at lower doses on the depression-like behavior of MDP rats

The antioxidant effects of OFFE on depression related brain regions suggest some relationship may exists between the antioxidant and anti-depression effects of OFFE, and thus the depression-like behavior of the lower dose OFFE treated MDP rats was estimated. Rats treated with 0.01g/kg, 0.1g/kg, and 1g/kg OFFE orally for 14days were subjected to the FST. The data show that the MDP rats treated with 0.01g/kg and 0.1g/kg OFFE had a less significant reduction in IMT in the FST (figure 5, naive, 126.2%±4.2; control, 191.5%±12.9; sham, 171.0%±14.7; 0.01g/kg OFFE treated MDP, 129.3%±13.2; 0.1g/kg OFFE treated MDP, 131.6%±2.1; $p<0.05$ compared to sham, n=6 in each group). However, the rats treated with 1g/kg OFFE still had an obvious reduction in IMT in the FST (figure 4, sham, 171.0%±14.7; 1g/kg OFE treated MDP, 61.2%±16.9; $p<0.001$ compared to sham, n=6 in each group).

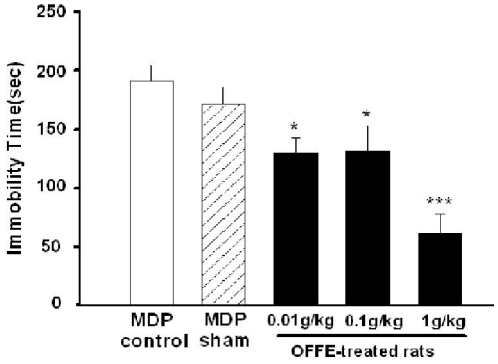


Figure 5. The effects of OFFE at the doses of 0.01g/kg, 0.1g/kg and 1g/kg on the depression-like behavior of MDP rats.

MDP rats were orally administered OFFE at 0.01g/kg, 0.1g/kg and 1g/kg respectively for 14 days and then subjected to the forced swim test. The data showed that MDP rats, treated with OFFE at various

doses, produced obvious reductions in IMT during the FST.

4. Discussion

The results of this study demonstrate that treatment with OFFE could significantly strengthen the antioxidation ability in the rats with depression-like symptoms. In addition, they show that the OFFE treatment reduced the depression-like behavior of the MDP rats. The results also suggest that this latter effect might be due to the increase in the amount of ORAC and GSH in the depression related brain regions of MDP.

Many studies have shown that the development of depression can be attributed to the damage caused to neurons by oxygen radical species (ORS)[1, 2; 4, 18]. However, whether antioxidant substrates are able to reduce depression has been unclear in the literature. Some evidence suggests that antioxidants could decrease depression-like behavior in animals, based on the results of indirect animal model experiments [19, 20]. In the present study, a more direct depression animal model, namely MDP rats, were used to assess the contribution of antioxidants to alleviating depression. The data showed that OFFE, an effective antioxidant [8, 9, 21, 22], can significantly increase the levels of ORAC and GSH in the depression related brain regions of MDP rats (figures 3 and 4). In addition, at the lower doses of OFFE treatment, the depression-like behavior of MDP rats was also significantly improved (figure 4). The results of this study thus provide convincing evidence for the contribution of antioxidants to the alleviation of depression.

It is of interest to consider whether as the antioxidation ability increases, this is accompanied by greater antidepressant effects. from the results of the current study show that the levels of ORAC in depression related brain regions show a dose-dependent increase when treated with OFFE (figure 3). Figure 4 also shows that the antidepressant effects of OFFE increase along with the treatment dose. In addition, the ceiling effect of OFFE treatment appears to be at a dose of 6g/kg, while the optimal dose is 1g/kg (figures 2 and 5). Taken together, these results suggest that the increased ability of antioxidation associated with OFFE can alleviate depression in MDP rats. In addition, the results of the lower dose treatment experiments also show a close association between the antioxidant ability of OFFE and the reduction in depression-like behavior in MDP rats. This is more evidence of the correlation between the antioxidant and antidepressant effects of OFFE, and it is likely that the antidepressant effects may be caused by the antioxidation effects.

5. Conclusions

The results of this work not only reveal the positive effects of OFFE with regard to increasing the antioxidant ability of MDP rats, but also the antidepressant effects of OFFE. Therefore, treatment with OFFE is likely to be an important candidate for the prevention or intervention of depression.

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