Research Article

Antidepressant effects of γ-oryzanol combined with curcumin in dexamethasone-induced depressive-like behaviours in rats

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Abstract

Major depressive disorder (MDD) is a serious health condition associated with neuroanatomical and functional alterations in many brain areas. As the active compound of rice bran oil, γ-oryzanol has been reported as having antioxidant, anti-inflammation, and neuroprotective effects, which protect the neurotransmitter deficits. Moreover, curcumin has been reported in antidepressant therapeutic properties. Therefore, this study aimed to determine the effects of γ-oryzanol combined with curcumin on dexamethasone-induced depressive-like behaviours in rats and hippocampal histological changes. Male *Sprague-Dawley* rats were randomly divided into 5 groups; Group 1, rats received reverse osmosis water (R.O.) as control, group 2 rats underwent dexamethasone-induced depression, group 3 and 4 depression-treated with γ-oryzanol combined with curcumin Formulation-1 (GOCur Form1) and Formulation-2 (GOCur Form2) respectively and group5, depression-treated with fluoxetine. All groups were evaluated the depressive–like behaviors by the forced-swimming and sucrose preference tests. Dexamethasone injection in the rats resulted in significant reductions in body weight, sucrose consumption and active time in the forced swim test (FST) when compared with control. Administration of GOCur Form1 and GOCur Form2 reversed the depressive behaviours by increasing body weight and sucrose consumption and decreasing the immobility period. Furthermore, GOCur Form2 can restore histological changes in hippocampal damage from dexamethasone. These results indicate the potential of γ-oryzanol combined with curcumin for preventing and treating the depressive symptoms.

Keywords: γ-oryzanol, Curcumin, Depression, Behavioral tests

Introduction

Major depressive disorder (MDD) is a common psychiatric disorder which affects people all around the world. According to the World Health Organization (WHO), MDD affects more than 350 million people worldwide [1]. Generally, the population between 10% and 15% may undergo a depressive episode in their lifetime [2]. Furthermore, the lifetime prevalence of depression ranges from 20% to 25% in women and 7% up to 12% in men [3]. MDD has various definitions and manifestations that have adverse consequences on an individual's quality of life, and it is among the most prevalent forms of mental illnesses. There are various symptoms of MDD including anhedonia (loss of pleasure), disrupted sleep, lack of motivation, or emotional distress. Depression shows a good response to pharmacological treatments among the various antidepressant drugs, selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine that is the first line and most widely prescribed medications. However, it has a lot of adverse effects in long term use. Additionally, treatment with fluoxetine has some problems such as delayed effects, sexual dysfunctions [4], reduced platelet aggregation, and prolonged bleeding [5]. Therefore, the study of effective antidepressants with fewer adverse effects is needed for providing alternative treatment for MDD.

A various number of active ingredients from plants or herbs have been investigated as potential anti-depressants for neuroprotection, as well as promoting health because they are naturally derived. Rice is a staple food of millions of people worldwide. The most consumable form is white rice in which the husk, bran, and germ are removed. Rice bran oil, an oil extracted from the bran and rice germ, contains high nutrition especially γ-oryzanol that has been reported to have antioxidation [6,7,8,9,10], anti-inflammation [11], [12], antihypertension [13], and reducing stress [14]. Evidence has been provided to support γ-oryzanol as being safe for consumption with no serious effect and has been demonstrated to be effective in many pathological conditions including age-related neurodegenerative disease and neurodegenerative diseases caused by oxidative injuries [14], [15], [16]. In a more recent study, virgin rice bran oil that contains γ-oryzanol at the dose of 5 ml/kg, given orally, was shown to improve and enhance learning and memory in ICR mice [17]. γ-oryzanol has also been reported to increase the level of monoamine neurotransmitter activity to assist in overcoming anxiety disorders in chronically stressed mice models [18]. Furthermore, turmeric has been commonly used as a spice and medicinal herb that contains the active compound curcumin, an essential curcuminoid which has been traditionally used in anti-fluctuant and wound healing. When curcumin is prepared in nanoparticle formulations, the dose at 5 mg/kg and 20 mg/kg were shown to overcome depressive-like behaviour in rats [19]. Curcumin has also been studied for its potential as an antioxidant [20], [21], anti- inflammation [22], and their impact on antidepressant effects by increasing serotonin and dopamine levels in the nerve endings which mediate by inhibiting the monoamine oxidase (MAO) activity in the brain [23,24]. All of these experimental outcomes indicate that γ-oryzanol and curcumin improve monoamine neurotransmitter deficits causing depression. Although both the γ-oryzanol and curcumin are shown to have antioxidative and anti-inflammation effects, the antidepressant effect is still needed to be elucidated. It has been hypothesized that the combination of γ-oryzanol and curcumin can

provide a synergistic antidepressant effect mediating through the neurotransmission. Therefore, this study aimed to assess the antidepressant-like activity of γ-oryzanol combined with curcumin in two formulations.

Materials and methods

Animal

Male 6-week-old Sprague-Dawley rats were acclimatized for 5 days before starting the experiment. They were housed under standard laboratory following conditions; the temperature at 22±1˚C, humidity 55±10% and were maintained on a 12-hour day-night cycle. Rats were allowed to freely access food and water. The protocol was approved through the Ethics of Naresuan University Animal Care and Use Committee, the project number NU-AE 620513. The body weight of the rats was recorded daily. All experiments were done in 5 groups with 5 animals in each group.

Chemicals and drug treatment

γ-oryzanol and curcumin were prepared using nanotechnology as Formulation-1 and Formulation-2. The dosages of the two formulations used in this study were based on our preliminary results *in vitro* study. Fluoxetine, which is the first-line drug for treating depression, was prepared in a dose of 10 mg/kg and then dissolved in distilled water given to the rats orally. The preparation of dexamethasone was done as suggested in the company guidelines (APExBIO Technology LLC, USA).

Treatment protocol

Group 1 (vehicle or control group); (C): 0.9% Normal saline (1 ml/kg) in the morning (09.00 a.m.), R.O. water 1 ml/kg in the afternoon (01.00 p.m.).

Group 2 (Dx): Dexamethasone 1.5 mg/kg via subcutaneous injection (s.c.) in the morning (09.00 a.m.) for producing depressive-like symptoms. Then R.O. water (1 ml/kg) at 01.00 p.m.

Group 3 (Dx-GOCur Form1): Dexamethasone 1.5 mg/kg (s.c.) in the morning (09.00 a.m.) and γ-oryzanol combined with curcumin Formulation-1 (GOCur Form1) containing γ-oryzanol 5 mg/kg and curcumin 25 mg/kg given orally in the afternoon (01.00 p.m.) for 28 days.

Group 4 (Dx-GOCur Form2): Dexamethasone 1.5 mg/kg (s.c.) in the morning (09.00 a.m.) and γ-oryzanol combined with curcumin Formulation-2 (GOCur Form2) containing γ-oryzanol 10 mg/kg and curcumin 50 mg/kg given orally in the afternoon (01.00 p.m.) for 28 days.

Group 5 (Dx-Flu): Dexamethasone 1.5 mg/kg (s.c.) in the morning (09.00 a.m.) and fluoxetine given orally at a dose of 10 mg/kg in the afternoon (01.00 p.m.) for 28 days.

Behavioral tests

Open-field test (OFT)

Locomotor activity was performed before the forced swimming test. The rats were placed in the middle of the open field in a square box (76 cm x 76 cm x 42 cm) with a floor divided into 25 equal rectangles. The rats were allowed to habituate by moving freely within the open field for 10 min. During the last 5 min the behaviours were recorded through video. [25], [26] The number of squares that the rats crossed with four paws was recorded manually and total travelled distance (cm) was computed by video software tracking (Smart Junior

3.0, Panlab, Spain). The apparatus was thoroughly cleaned with 10 % ethanol after each trial in the OFT.

Forced swimming test (FST)

The FST was carried out as explained earlier [27], [28] and performed at the end of drug administration. The rats were placed individually into a glass cylinder (Diameter 21 cm, Height 50 cm) containing 30 cm of room temperature water. The rat was left there for 6 min. After the first 1 min the total duration of immobility time was measured during the following 5 min test. The rats were dried and returned to their home cages later.

Sucrose preference test

Sucrose consumption of animals can indicate anhedonia state. Before testing, the animals were trained to habituate to sucrose solution (1%, w/v) for 30 hrs. After that R.O. water was given to rats for 16 hrs., the sucrose preference baseline test was performed. This process was carried out every week for 4 weeks. On the test day, each rat was given a free choice with two bottles, one bottle containing 100 ml with 1% sucrose solution and another filling with R.O. water, which were placed in each cage for 1 h. The water and sucrose solution intake were measured, and sucrose preference was calculated as a percentage of the sucrose intake over the total intake [%sucrose preference = [sucrose solution consumption (ml) / sucrose solution consumption (ml) + water consumption (ml)] x 100.

Histological study

After the last dose of drug administration, all the rats were sacrificed and then the hippocampus was dissected and kept in 10% neutral buffer formalin. The fixed tissues were dehydrated and embedded in paraffin, following which 5-μm sections were cut and stained with hematoxylin & Eosin and mounted on glass slides. Stained tissues were visualized using light microscope for the histological changes. The pictures were analyzed using image analysis software (ImageJ, US National Institutes of Health)

Statistical analysis

The results of behavioural tests were expressed as mean ± SEM. Data were analyzed by One-way ANOVA followed by Tukey's post-hoc test. P-Value ≤ 0.05 was considered as statistically significant.

Results

Effect of GOCur Form1 and GOCur Form2 on dexamethasone-induced depressive behavioural change in body weight

The result of the mean body weight in dexamethasone group (Dx) was significantly decreased ($p<0.05$) in 14^{th}, 21^{st}, and 28^{th} day when compared with control **(Figure 1)** The depressive rat group was received GOCur Form2 showed a significant increase ($p<0.05$) in body weight compared with fluoxetine-treat (Dx-Flu) on day 21 and 28

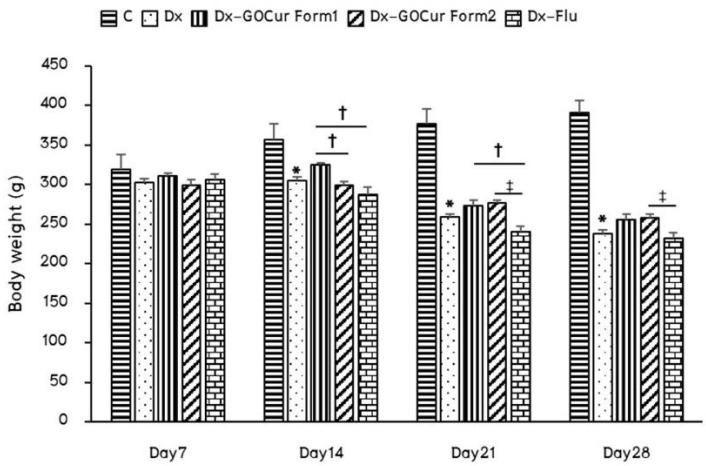

Figure 1 Effect of GOCur Form1 and GOCur Form2 on body weight. Data are shown as mean ± SEM (*P < 0.05 when compared with control (C), †P< 0.05 when compared with Dx-GOCur Form1, and ‡ P< 0.05 when compared with Dx-GOCur Form2)

Effect of GOCur Form1 and GOCur Form2 on dexamethasone-induced depressive behavioural change in locomotor activity

Locomotor activity was tested to confirm normal spontaneous activity before the forced swimming test. The administration of Dx, GOCur Form1, GOCur Form2, and Flu had no significant difference in all group when compared with control (C). These data reflect that GOCur Form1, GOCur Form2, did not alter the total distance and the number of squares crossed of the open field apparatus. As shown in **Figure 2 A and B**

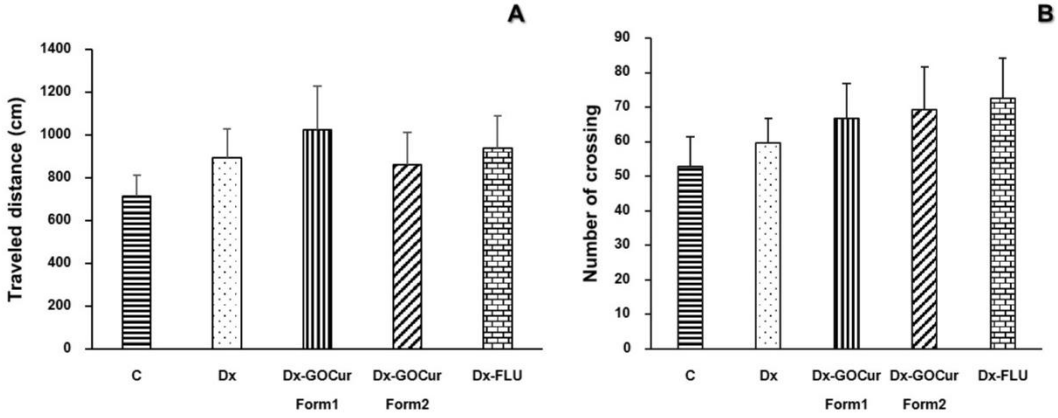

Figure 2 Effect of GOCur Form1 and GOCur Form2 on locomotor activity. Total distance travelled (A), number of square rats crossed (B). Data are shown as mean ± SEM.

Effect of GOCur Form1 and GOCur Form2 on dexamethasone-induced depressive behavioural change in forced swimming test

The immobility time was increased in Dx group as compared to the control group. Although, Dx group was no significant difference in the duration of immobility, the result showed a tendency in depressive symptom. Among the treatment groups, GOCur Form2-treatment group showed the most reduction in immobility time in the forced swim test.

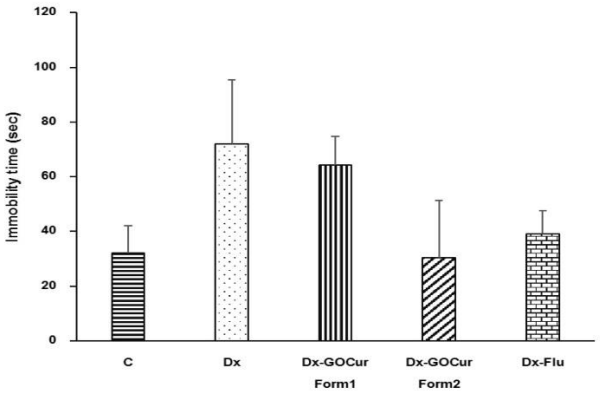

Figure 3 Effect of GOCur Form1 and GOCur Form2 on forced swimming test. Data are shown as mean ± SEM.

Effect of GOCur Form1 and GOCur Form2 on dexamethasone-induced depressive behavioural change in sucrose preference test

The reduced sucrose preference is an indicator of anhedonia- like behavioural change. The animals treated with dexamethasone for 3 weeks showed a significant decrease in sucrose consumption when compared with the control group ($p<0.05$). It can indicate the depressive-like symptom. As demonstrated in **Figure 4,** One-way ANOVA presented a significantly increased in sucrose intake at the fourth week [$F(2,12) = 7.113$, $p = 0.009$]. *Post-hoc* analysis showed a significant increase in sucrose intake in Dx-GoCur From2 compared to the Dx group.

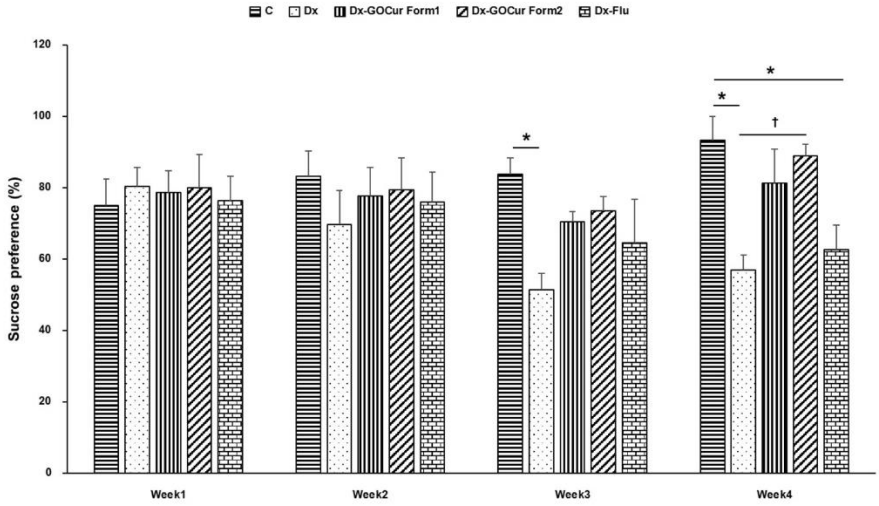

Figure 4 Effect of GOCur Form1 and GOCur Form2 on sucrose preference test. Data are shown as mean ± SEM. (*P < 0.05 when compared with control (C), † P< 0.05 when compare with Dx group)

Effect of GOCur Form2 on histological changes in hippocampus

The results of sucrose preference and forced swimming tests in GOCur Form2 showed a reversal of depressive-like behaviours. Therefore, we paid attention to a GOCur Form2 group to investigate the histological changes in the hippocampal formation. As shown in **Figure 5**, the H&E stain of the hippocampal formation demonstrated in dexamethasone group, the hippocampal pyramidal cell layer in the Cornu Ammonis (CA) and dentate gyrus (DG) **(Figure 5**

B and E), were thin; intercellular spaces were enlarged, cells were irregularly arranged, indicating that the hippocampal tissue was damaged and cell apoptosis was occurred. On the other hand, GOCur Form2 group in the CA and DG regions **(Figure 5 C and F)**, the hippocampal pyramidal cell layer was restored, and intercellular spaces were narrowed, indicating the restored effect of GOCur Form2 against damage in the hippocampus when compared with the control group **(Figure 5 A and D).**

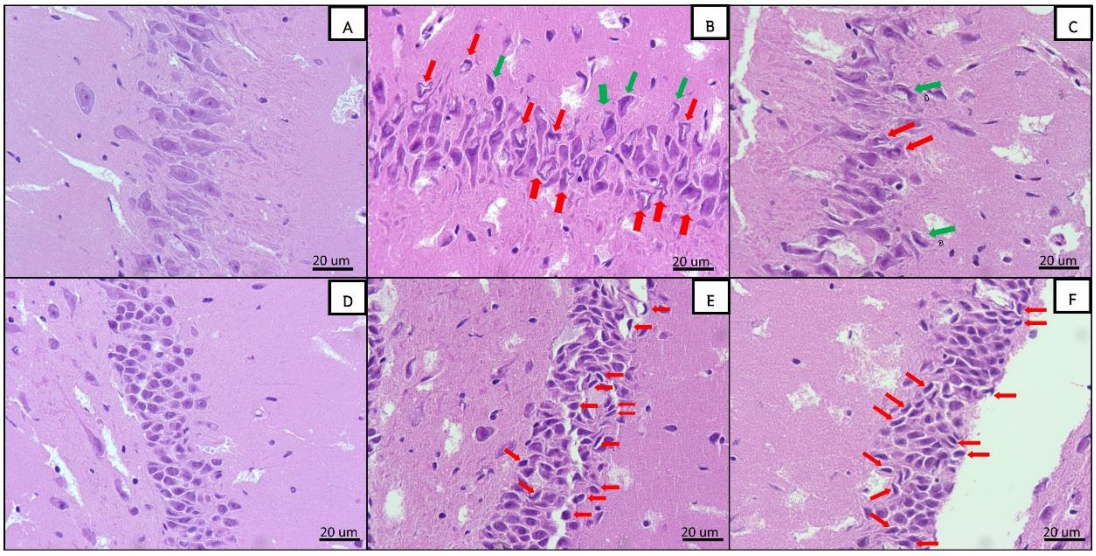

Figure 5 The effects of GOCur Form2 on the hippocampal histopathology changes in the CA region in Control (A), Dx (B), GoCur Form2 (C) groups, and in the dentate gyrus in Control (D), Dx (E), GOCur Form2 (F) groups. Red arrow indicated nuclear loss within the neuron and green arrow indicated neuron shrinkage.

Discussion and Conclusion

This present study aimed to assess the effect of γ-oryzanol combined with curcumin on dexamethasone administration induced depressive -like behaviours in rats through the open field, forced swimming, and sucrose preference tests. The histological changes in the hippocampal CA region were also determined. We found that dexamethasone could induce depressive-like behaviours including a decrease in locomotor activity and an increase in immobility time. Moreover, the sucrose preference test showed decreased sucrose consumption after 3-week of dexamethasone induction. These results were consistent with previous reports indicating that dexamethasone administration can induce a depressive-like condition [28, 29]. Dexamethasone administration has resulted in depressogenic activity accompanied by a dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis leading to disrupted cortisol rhythmicity. These hyperactive endocrine observations are consistent with clinical remarks in depressed patients [30]. The present study showed that GOCur Form1 and GOCur Form2 significantly increased sucrose consumption and body weight. Furthermore, GOCur Form2 rehabilitated depressive behaviour (sucrose consumption and body weight) in rats as well as fluoxetine. An increase in immobility time of FST was observed in dexamethasone administration group, but this did not reach significant levels compared to the control group. Interestingly, GOCUR Form2 reversed this effect. The locomotor activity showed no differences among all groups

which confirmed the results of FST of no motor deficits in agreement with findings from Sigwalt, A. et. al. [26].

Therefore, the findings in the present study indicated that GOCur Form2 had antidepressant-like action. GOCur Form2 which was a combination of γ- oryzanol and curcumin demonstrated a protective effect by preventing depressive-like symptoms. This was consistent with the previous studies on the effect of γ-oryzanol and curcumin on psychiatric symptoms. γ-oryzanol has also been reported for a therapeutic effect in preventing anxiety-like behavior. [17] Moreover, curcumin has been reported the antidepressant action. Taken together, the combination of γ-oryzanol and curcumin can be used for preventing the depressive symptoms as well as other psychiatric symptoms [22], [31], [32], [33].

Furthermore, the mechanism of curcumin has been reported for involvement in regulating changes in synaptic structural plasticity within the rat amygdala [34]. Besides, a previous study demonstrated that antidepressant- like effects of curcumin administration in a model of depression were accompanied with increased hippocampal neurogenesis similar to the effects of the antidepressant fluoxetine [35].The hippocampus is a critical structure considered to be associated with behavioural inhibition, mood regulation and plays an essential role in major depressive disorder pathogenesis [36]. Previous results found that curcumin mediated via enhanced neurogenesis in the hippocampus [37]. Our observation in hippocampal area revealed that GOCur Form2 could reverse dexamethasone-induced hippocampal CA and DG region damage. This result was consistent with a previous study that curcumin restored a decrease in hippocampal neurogenesis in chronically stressed rats [32].

In conclusion the present study shows that co-administration of GOCur Form1 and GOCur Form2 alleviated dexamethasone- induced depression. These findings suggest that GOCur Form1 and GOCur Form2 may provide a potential role in antidepressant activity. Although further studies are needed to confirm in term of mechanisms, our results provide evidence to support that the γ-oryzanol combined with curcumin can be a indicated as a dietary phytochemical supplement to prevent depression.

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