Bitter melon juice concentrate improves depressive symptoms in a mouse model: Possible effect on brain cortisol

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ABSTRACT

Depression is a prevalent mental disorder with detrimental impacts on quality of life and economy. Under the current COVID-19 pandemic, the incidence of depression has increased profoundly. To make matters worse, access to healthcare is challenged by the ongoing pandemic. This urges the availability of alternatives to treat depression with easy accessibility, and plant-derived substances can be good candidates. Bitter melon (*Momordica charantia* L.) has been known for its anti-inflammatory and antioxidant activities. We tested the effect of bitter melon juice concentrate (BM) in a mouse model of depression using the forced swimming test (FST) method. Male ddY mice were assigned to groups treated with daily oral vehicle, BM at 50 or 200 mg/kg, and 20 mg/kg fluoxetine hydrochloride. Immobility time during FST was observed on the 7th and 14th days of treatment. Upon completion of treatment, the mice were sacrificed and checked for hippocampal and frontal cortical cortisol levels. Results showed that BM produced a significant decrease in immobility time on both observation days. This finding was accompanied by a significant decline in cortisol levels in the brain areas of the mice treated with BM at both doses. Overall, results suggest that BM has the potential for use in the treatment of depression.

INTRODUCTION

Depression is a prevalent mental disorder and affects more than 200 million people worldwide. Most depressive patients are distributed in the regions of Southeast Asia and Western Pacific. Between 2005 and 2015, the prevalence of depressive patients increased by 18.4%, which could represent the growth in the global population. Mortality due to depression reaches 800,000 annually in the age range of 15–29 (WHO, 2020). The current COVID-19 pandemic sees a further increase in the number of cases of depression. One recent study reported that, after adjustment for the pandemic, the estimated prevalence of the major depressive disorder in 2020 was 246 million people from the previously estimated 193 million (COVID-19 Mental Disorders Collaborators, 2021). This makes depression one

disorder that needs serious attention and whose therapy is to be prioritized. The economic burden of depressive patients continued to rise over time. A recent study has shown that, in the United States, direct costs incurred by individuals with major depressive disorder rose from USD 111.1 billion in 2010 to USD 114.3 billion in 2018 (Greenberg *et al.*, 2021).

Based on the therapeutic guidelines from the American Psychological Association, the first-line drugs for the treatment of depression include tricyclic antidepressants, selective serotonin reuptake inhibitors, serotonin–norepinephrine reuptake inhibitors, or dopamine agonists, depending on the patient's clinical condition, drug side effects, and therapy costs (American Psychological Association, 2019). As many as 30% of depressive patients did not respond to drug therapy and 70% of them failed to achieve clinical cure. Compliance is an essential factor contributing to therapy success and better quality of life for depressive patients. Several studies have shown that patient compliance to antidepressant therapy was low; as much as 50% of patients quitted therapy. The reasons for the discontinuation can be divided into two factors: the patient factors, including side effects, drug dependence, and the belief that therapy is unsuccessful; the clinical factors,

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including low patient education as well as poor follow-up from health practitioners (Sawada *et al.*, 2009; Sansone and Sansone, 2012). These facts have become a justifying factor in embarking on the use of alternative therapies which are safer with easier accessibility. Plant-derived drugs may be the potential candidate in this regard (Kulkarni *et al.*, 2009).

Bitter melon (*Momordica charantia*) is a widely used medicinal plant due to its various potential health benefits. The studied biological activities include antihyperglycemic, antibacterial, antiviral, antitumor, immunomodulatory, antioxidant, antidiabetic, anthelmintic, antiulcer, antifertility, hepatoprotective, anticancer, and anti-inflammatory activities (Jia *et al.*, 2017). The antioxidant and anti-inflammatory activities open further potential for the activities on the nervous system. To this end and considering the need for alternative antidepressants, we explored the potential use of bitter melon in an animal model of depression.

MATERIALS AND METHODS

Animals

Male Deutschland Denken Yoken (ddY) mice (purchased from PT Biofarma Bandung Indonesia), aged 6–8 weeks, weighing 25–35 g at the beginning of the experiment, were used. They were kept at a constant ambient temperature of 25 ± 3ºC under a 12 hours light/dark cycle with free access to food and water except during behavioral observations. All animal treatments were approved by the Ethical Committee for the Use and Care of Laboratory Animals, Institut Teknologi Bandung (ethical approval reference: 09/KEPHP-ITB/03-2021).

Drugs

Plant determination was carried out in the *Herbarium Bandungense* at the School of Life Sciences and Technology, Institut Teknologi Bandung (determined as *M. charantia* L.). Bitter melon fruits harvested on the 28th day were washed, chopped with a food chopper, added to water in a ratio of 1:10, and then filtered. The filtrate was freeze-dried and kept until use. The yield of this process was 4.3%. Fluoxetine was suspended in 1% sodium carboxymethyl cellulose. All drugs were given orally using oral gavage.

Antidepressant activity test

The FST (Porsolt *et al.*, 1977) was utilized to model depression-like behavior. The mouse was forced to swim in a water-filled glass cylinder (30 cm high, 20 cm wide, allowing 15 cm deep water). The immobility time was recorded for each mouse during the last 4 minutes of a total 6-minute observation period. Test substances were administered orally, once daily for 14 days. The bitter melon juice was given at the dose of 50 or 200 mg/kg, while the reference drug fluoxetine was administered at 20 mg/kg. Observations of immobility time, as the index of depression, were carried out on days 7 and 14.

Measurement of cortisol

Following FST on day 14, the mice were sacrificed by cervical dislocation. Samples from the hippocampal and frontal cortical area of the brain were collected, washed with phosphate buffer saline pH 7.4, weighed, and homogenized. The homogenate was then centrifuged at 15,000 rpm for 15 minutes. The supernatant was collected and stored until use at −20ºC. Measurement of cortisol was carried out using ELISA (Bioassay Technology Laboratory) in accordance with the manufacturer's protocol.

Statistical analyses

In the statistical analyses, one-way analysis of variance (ANOVA) was used to compare means, and LSD tests were used for *post-hoc* pairwise comparisons. A difference was considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

To the authors' knowledge, the current work is the first to study the effect of bitter melon on an animal model of depression. Results on the profile of immobility time in FST following BM treatment are shown in Figure 1. The data showed that BM dose-dependently decreased immobility time on both observation days, significantly different compared to vehicle. On day 7, the immobility time observed after the doses of 50 and 200 mg/kg was 95.67 ± 31.94 sec and 84.00 ± 11.40 sec, respectively, while the time after vehicle was 145.00 ± 14.17 sec (p-value for the difference between BM and vehicle = <0.01 and <0.001, respectively; ANOVA; and *post hoc* LSD). On day 14, treatment of the doses of 50 and 200 mg/kg resulted in respective immobility times of 79.33 ± 30.66 sec and 39.00 ± 17.45 seconds, with the time after the vehicle of 116.00 ± 13.95 sec (p-value for the difference between BM and vehicle = 0.08 and <0.01, respectively.; ANOVA; and *post hoc* LSD). The model of depression used in this study was FST with immobility time as the index. FST is one of the most commonly used methods to assess depressive-like behavior in rodents. Immobility during testing is regarded as a measure of behavioral despair, and the method has been repeatedly shown to be sensitive to a wide range of antidepressants (Yankelevitch-Yahav *et al.*, 2015), as shown in the present result.

Data on changes in cortisol concentration accompanying behavioral expression is shown in Figure 2. BM treatment was shown to significantly decrease the level of cortisol in the hippocampal and frontal cortical areas of the brain. At the dose of 50 mg/kg, BM produced the lowest level of cortisol (39.75 ± 4.59 ng/ml, $p < 0.05$ versus vehicle, ANOVA, and *post hoc* LSD), but was not significantly different compared to that after the dose of 200 mg/kg, which produced the level of 41.96 ± 4.63 ng/ml ($p < 0.05$ versus vehicle, ANOVA, and *post hoc* LSD) or 20 mg/kg fluoxetine. Hypersecretion of cortisol has been regarded as a risk factor for depression (Cosgriff *et al.*, 1990). In addition, it was demonstrated that cortisol hypersecretion found in depressed patients was associated with stress experienced by the subjects (Nemeroff *et al.*, 1996). The hippocampus and frontal cortex, structurally, have been shown to associate with depression in human subjects (Belleau *et al.*, 2019). Furthermore, it was revealed that the morbid hippocampal and frontal cortical condition in depressive patients was associated with elevated levels of baseline cortisol as well as cortisol/dehydroepiandrosterone ratio (Jin *et al.*, 2016; Travis *et al.*, 2016).

To further confirm the antidepressant activity, an assessment of changes in neurotransmitter-related depressive symptoms should be performed. In fact, most pharmacological therapies for the treatment of depressive symptoms are directed toward the normalization of biogenic amine levels (Dulawa *et al.*,
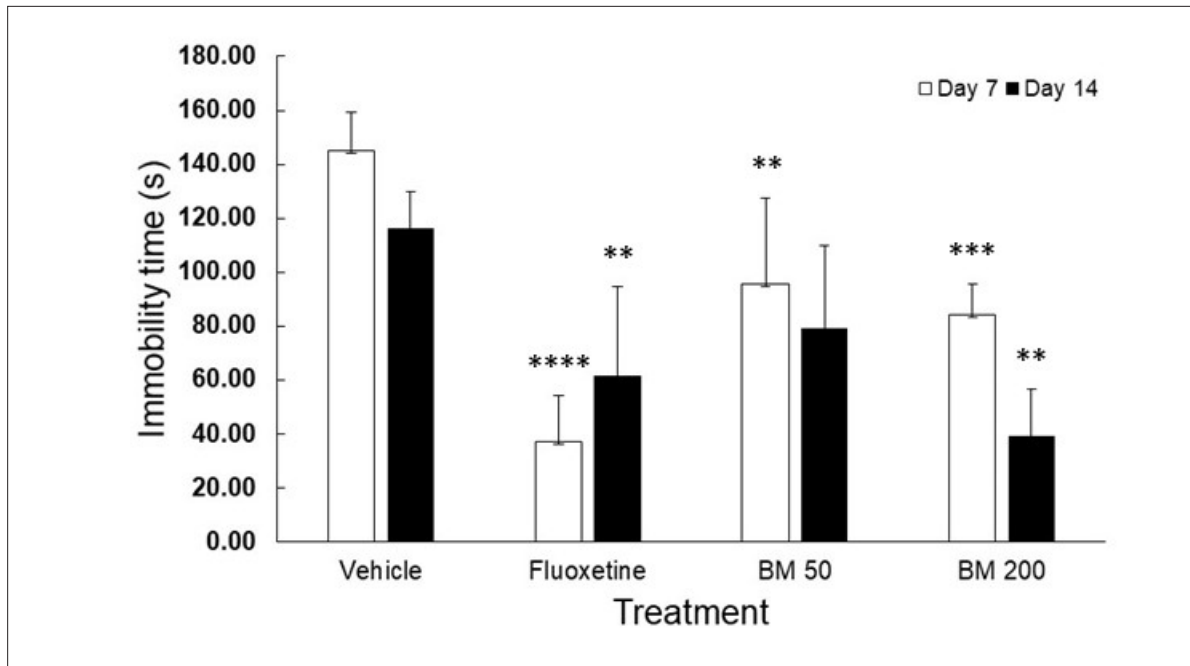
Figure 1. Effects of bitter melon juice concentrate (BM) on immobility time in FST. The vehicle, fluoxetine (30 mg/kg), and BM (50 or 200 mg/kg) were administered orally for 14 days. Immobility time was recorded on days 7 and 14. Data represents average + SD of threee to four mice. [**, ***, ****]$p < 0.01$, 0.001, and 0.0001 versus vehicle, one-way ANOVA, and *post hoc* LSD.
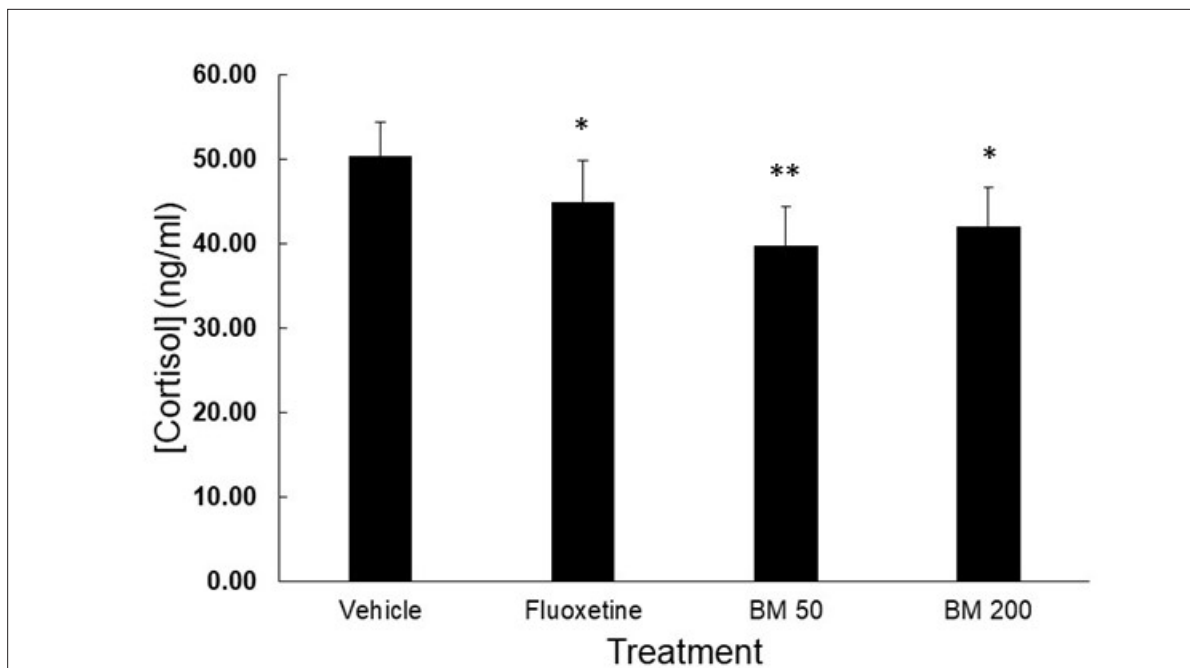

Figure 2. Effects of bitter melon juice concentrate (BM) on cortisol levels in the hippocampal and frontal cortical areas of the brain. The vehicle, fluoxetine (30 mg/kg), and BM (50 or 200 mg/kg) were administered orally for 14 days. Extraction of the brain areas for cortisol level measurements was carried out soon after the last behavioral assessment. Data represents average + SD of four mice. [*, **]$p < 0.05$ and 0.01 versus vehicle, one-way ANOVA, and *post hoc* LSD.

2004; Safhi *et al.*, 2019). However, cortisol has been indicated to be the predictor of psychological therapy response in depressive disorder (Fischer *et al.*, 2017). Furthermore, López *et al.* (1997) have put forward a hypothesis that the association between changes in 5-HT receptors with suicidal ideation and act might be attributed to or exacerbated by overactivity of the hypothalamus–pituitary–adrenal axis, leading to an elevated level of cortisol. In addition, chronic stress was shown to reduce 5-HT1A receptor mRNA

levels, as well as 5-HT1A binding densities in the hippocampus (López *et al.*, 1998). Taking this data into consideration, therefore, one might suggest that cortisol modulates 5-HT receptors, which in turn controls affective states as expressed in the change in mood of depressive patients, further articulating the essential of cortisol in the pathology of depression with hippocampus as a key substrate.

Amid the current COVID-19 pandemic, when household income declines, challenging the provision of healthcare services and their accessibility (Núñez *et al.*, 2021), natural product-derived alternatives become an avenue that people turn to. Thus, depressive patients could take the benefit from consuming bitter melon juice to help improve their symptoms. It has been demonstrated that infections were followed by a battery of body responses, and depression was one of them (Hart, 1988). Indeed, an increased level of cortisol has been observed in COVID-19 patients (Yavropoulou *et al.*, 2022).

The antidepressant effect has been shown in a number of plants of the family Cucurbitaceae, to which bitter melon also belongs. Randawa *et al.* (2015) demonstrated that methanol extract of *Coccinia indica* at 400 mg/kg produced behavioral improvement in an animal model using FST. Meanwhile, methanol extract of *Cucurbita pepo* at 100 mg/kg lowered immobility time in an FST experiment, and the effect was comparable to imipramine at 30 mg/kg (Umadevi *et al.*, 2011). In addition, methanol extract of *Benincasa hispida* at 100 mg/kg was shown to significantly decrease the immobility time, with MAO-A inhibition as a possible mechanism (Dhingra and Joshi, 2012). Hydroalcoholic extract of *Benincasa hispida* was studied for its effect on head twitch, a behavioral model associated with the serotoninergic activity. At the dose of 30 mg/kg, the extract was found to dampen the increase in head twitch (Shakya *et al.*, 2019). Another study on a plant from the family Cucurbitaceae was carried out by Rahman *et al.* (2013), showing that the n-hexane extract *Citrullus lanatus* at 250 and 500 mg/kg given for 7 days lowered immobility times as tested by FST and tail suspension test (TST), which was comparable to a dose of 10 mg/kg of imipramine. The methanol extract of *Lagenaria siceraria* at 100 and 200 mg/kg was demonstrated to decrease immobility time which was stronger than the reference drug imipramine at 12.5 mg/kg (Prajapati *et al.*, 2011). Aqueous extract of *Cucurbita maxima* at 100 mg/kg was demonstrated to blunt immobility time in FST and TST, the activity which was comparable to imipramine at 30 mg/kg (George and Nazmi, 2012). Lastly, *Cucurbita moschata* water extract was also reported to produce an improvement in the behavioral index in the FST model, which was in line with the increase in blood serotonin, a neurotransmitter essentially affected by depression (Kim *et al.*, 2016).

Antidepressant activity in a plant from the family Cucurbitaceae has also been studied at the level of isolate. The most detailed work in this respect was that on gypenosides, a saponin isolated from the plant *Gynostemma pentaphyllum*. Gypenosides administered orally for 4 weeks at 50 and 100 mg/kg significantly dampened immobility time in FST testing, and the activities were comparable to that of fluoxetine (Mu *et al.*, 2016). With regard to bitter melon, phytochemical analyses have unveiled alkaloids, flavonoids, glycosides, saponins, steroids, and tannins as the bioactive components (Mada *et al.*, 2013; Oragwa *et al.*, 2013). Based on the chemotaxonomic approach, further studies on the saponin component might be an interesting research focus.

CONCLUSION

Results of the present study demonstrated a decrease in immobility time in a mouse model of depression accompanied by decreased cortisol level after BM treatment. This implies that bitter melon juice concentrate has antidepressant potential, which could be utilized in patients with depressive symptoms. As a "do it yourself" alternative, its use is of particular benefit during the condition in which access to therapy is limited.

AUTHORS' CONTRIBUTIONS

All authors contributed substantially to the design and implementation of this work, including data collection and analysis and preparation of the manuscript.

CONFLICT OF INTERESTS

The authors report no conflict of interests in this work.

ETHICAL APPROVAL

The Ethical Committee for the Use and Care of Laboratory Animals, Institut Teknologi Bandung (ethical approval number: 09/KEPHP-ITB/03-2021) has approved the study protocol.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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