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Green tea polyphenols produce antidepressant-like effects in adult mice

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ABSTRACT

Recent studies have shown that a higher consumption of green tea leads to a lower prevalence of depressive symptoms in elderly individuals. However, no studies have explored the antidepressant-like effect of green tea in preclinical models of depression. The aim of this study was to investigate the antidepressant-like effects and the possible mechanism of action of green tea in widely used mouse models of depression. Mice were orally administered green tea polyphenols (GTP; 5, 10 and 20 mg/kg) for 7 days and assessed in the forced swimming test (FST) and tail suspension test (TST) 60 min after the last GTP administration. Serum corticosterone and adrenocorticotrophic hormone (ACTH) levels were also determined immediately after the FST. Green tea polyphenols significantly reduced immobility in both the FST and TST but did not alter locomotor activity in the open field test, suggesting that GTP has antidepressant-like effects, and this action did not induce nonspecific motor changes in mice. Green tea polyphenols also reduced serum corticosterone and ACTH levels in mice exposed to the FST. The present study demonstrated that GTP exerts antidepressant-like effects in a mouse behavioral models of depression, and the mechanism may involve inhibition of the hypothalamic–pituitary–adrenal axis.

1. Introduction

Major depression is a common psychiatric disorder that affects 17% of individuals worldwide [1]. Depression is estimated to cause approximately 1 million people to commit suicide each year, causing a major burden on society. Although antidepressants have been clinically available for several decades, most of them are not completely effective (only 33% of depressed patients are sensitive to the first antidepressant medication [2], and are associated with many serious adverse effects. Recent research has focused on traditional herbal medicines for antidepressant drug development. Natural products, especially plant polyphenols, have attracted progressively more attention as supplemental interventions to maintain health and treat diseases [3,4]. The biological activity of polyphenols in neurodegenerative disorders, inflammation, cancer, and cardiovascular diseases involves the regulation of cell growth and proliferation, enzyme activity, and the modulation of cellular signaling cascades [5,6].

Green tea (*Camelia sinensis*) is one of the most popular beverages in the world. Increasing evidence indicates that green tea has multiple health benefits, such as the anti-stress, anticancer and antioxidants effects. Green tea polyphenols (GTP), also referred to as catechins, representing 30% of the fresh leaf dry weight and are major constitute of green tea that contribute to its beneficial effects [7]. The active polyphenols in green tea primarily include (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epicatechin [8,9]. Increasing evidence shows that green tea has multiple health benefits, such as the anti-stress [10], anticancer [11,12], antioxidant [13,14] and neuroprotective effects [15].

A recent clinical trial found that higher consumption of green tea led to a lower prevalence of depressive symptoms in elderly Japanese individuals [16]. Furthermore, previous studies reported that GTP inhibits monoamine oxidase enzyme activity, consequently increasing monoamines levels in glial cells [17]. Conventional and newer antidepressants exert their effects predominantly through an increase in the synaptic concentrations of monoamines, indicating that monoamine systems play an essential role in the mechanism of action of antidepressants and the pathophysiology of depression [18–20]. Therefore, we hypothesized that GTP might play a critical role in the treatment of depression.

Stress is well known to be one of the most important factors responsible for depressive disorders. Maladaptive responses to stress cause hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis via stimulation of adrenocorticotropic hormone (ACTH)

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release and the subsequent peripheral release of steroids/cortisol from the adrenal grand [21,22]. Exposure to stress or depression induces neuronal atrophy of the adult hippocampus, which may contribute to the molecular changes observed in the pathophysiology of depression [23,24]. Based on these findings, the present study examined whether oral GTP administration produces antidepressant-like effects in validated mouse models of depression. We also assessed HPA activity by measuring serum corticosterone and ACTH levels to clarify the potential mechanism of action of GTP.

2. Materials and methods

2.1. Animals

Male ICR mice (weighing 18–22 g upon arrival) were individually housed under a constant temperature (23 ± 2 °C) and maintained on a 12 h/12 h light/dark cycle with free access to food and water. All the procedures were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, and the procedures were approved by the Local Animal Use Committee (LA2010-010). All of the behavioral tests and drug administrations were performed during the dark phase.

2.2. Drugs

Green tea polyphenols (purity >98%) were purchased from Ao-Jing Science and Technology Development Co., Ltd. (Xi'an, Shanxi Province, China). Venlafaxine (purity >99%), a serotonin and norepinephrine reuptake inhibitor, was purchased from Chengdu Daxi'nan Pharmaceutical Co., Ltd. (Chengdu, Sichuan Province, China). Venlafaxine and GTP were freshly dissolved in saline and were administered intragastric gavage (0.2 ml/10 g bodyweight) before the experiment.

2.3. Extraction of GTP

Polyphenols were prepared from green tea according to previous method [25]. The fresh green tea leaves were mixed with water (10 g green tea was mixed with 200 mL water) and then placed into a sterile beaker at 80 °C for 1 h. The mixture was filtered through a filter paper. The supernatants were collected. The precipitate was again processed at 80 °C for 1 h and was filtered. The supernatants obtained were combined and were lyophilized.

2.4. Analysis of constituents of GTP

Contents of polyphenols of green tea were determined by ferrous tartrate colorimetry with the wavelength of 350 nm through the spectrophotometer.

2.5. Forced swim test

The forced swim test (FST) was similar to a previous protocol [26]. Mice were placed in a 20 cm diameter × 35 cm height plastic cylinder filled to 20 cm with 23–25 °C water. The test was videotaped, and immobility time was measured. The definition of immobility was the absence of all movements with the exception of motions required to maintain the animal's head above the water. The results are expressed as the time spent immobile during the last 4 min of the 6 min session. Observers were blind to the group treatment of the mice.

2.6. Tail suspension test

The tail suspension test (TST) was performed according to a previous publication [27]. Briefly, mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. The test was videotaped, and immobility time was measured for 6 min. Immobility was defined as the absence of any limb or body movements, with the exception of those caused by respiration, when the mice hung passively and completely motionless. During the test, the mice were separated from each other to prevent visual and acoustic associations. The number of seconds spent immobile was recorded. Observers were blind to the group treatment of the mice.

2.7. Locomotor activity

Locomotor activity was measured using the open field test. Briefly, the apparatus consisted of a 25 cm diameter × 12 cm height circular arena divided into six equal squares on the floor of the arena. Mice were placed individually in the center of the cage, and the number of crossings to adjacent squares were counted as horizontal locomotor activity for 5 min. Observers were blind to the group treatment of the mice.

2.8. Serum corticosterone and ACTH measurement

To determine serum corticosterone and ACTH levels in mice, 1 mL of blood was collected by decapitation bleeding immediately after the 6 min FST. The blood samples were kept at room temperature for 1 h and then centrifuged at 3000 rpm for 10 min. The serum supernatant fraction was stored in another tube for the subsequent corticosterone and ACTH assays. Serum corticosterone and ACTH levels were measured using commercially available enzyme immunoassay kits (corticosterone ELISA, #2B870; ACTH ELISA, #2B350, Sun Biomedical Technology Co., Ltd., Beijing, China) according to the manufacturer's instructions. Because diurnal rhythm may induce fluctuations in hormone levels, blood samples were collected within the same time window of 4:00–5:00 pm for each mouse immediately after the 6 min FST. Data are expressed as nmol/l for corticosterone and pg/mL for ACTH.

2.9. Experimental design

2.9.1. Experiment 1: effects of GTP on immobility time in the TST in mice

In this experiment, mice were used to examine the antidepressant effect of GTP in the TST. Five groups of mice orally received saline, GTP (5, 10, and 20 mg/kg) or venlafaxine (10 mg/kg) as a positive control. The mice received the drugs orally once daily for consecutive 7 days. On day 7, 60 min after the last treatment, mice were subjected to TST for 6 min (n = 9–11 per group). Another independent five groups of mice were treated with a single dose of saline, GTP (5, 10, and 20 mg/kg) or venlafaxine (10 mg/kg) 60 min before the TST to test the acute effects on immobility (n = 8 per group).

2.9.2. Experiment 2: effects of GTP on the locomotor activity in OFT and the immobility in FST in mice

A separate group of mice were divided into five groups: saline, GTP (5, 10, and 20 mg/kg) or positive reference venlafaxine (10 mg/kg). The mice received the drugs orally once daily for 7 consecutive days. On day 7, 55 min after the last treatment, crossings in the open field test were measured for 5 min, reflecting locomotor activity (n = 10–11 per group). Immediately after the open field test, the mice were subjected to the FST for 6 min (n = 10–11 per group). Another independent five groups of mice were treated with a single

dose of saline, GTP (5, 10, and 20 mg/kg) or venlafaxine (10 mg/kg) 1 h before the FST to test the acute effects on immobility ($n = 8$ per group). Furthermore, in order to exclude the effect of OFT on the behavioral responses in FST, we replicated the FST without previous OFT. Five separate groups of mice were treated orally once daily for 7 consecutive days with saline, GTP (5, 10, and 20 mg/kg) or venlafaxine (10 mg/kg) 1 h before the FST with no exposure to the OFT ($n = 8$ per group).

2.9.3. Experiment 3: effects of GTP on serum corticosterone and ACTH levels after exposure to the FST

Immediately after the FST, mice were sacrificed by decapitation, and the blood was collected for assessment of corticosterone and ACTH concentrations ($n = 6$ per group).

2.9.4. Experiment 4: effects of GTP on the normal physiological state of serum corticosterone and ACTH levels

In order to determine whether GTP can help to maintain the HPA axis activities at a relatively low level, we performed acute (1 day) and sub-chronic (7 day) GTP injections and sacrificed the mice without the forced swim test, and checked if GTP affects the baseline corticosterone and ACTH levels.

2.10. Data analysis

Data are expressed as mean ± SEM. The statistical analyses of the behavioral and biochemical data in control and drug-treated mice were performed using one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Sub-chronic GTP administration reduced immobility time in tail suspension test

One-way ANOVA of the TST data (Fig. 1A) revealed that GTP treatment for 7 days at doses of 10 mg/kg ($p < 0.05$) and 20 mg/kg ($p < 0.05$) significantly reduced immobility time, but the 5 mg/kg dose had no effects ($p > 0.05$) on the immobility compared with saline-treated control mice. The positive control venlafaxine (10 mg/kg) significantly reduced immobility time in the TST ($p < 0.01$) compared with control mice. While the acute treatment with a single dose of GTP (5, 10 and 20 mg/kg) had no effects on the immobility time in the TST (Fig. 1B).

3.2. Sub-chronic GTP treatment decreased immobility time in the forced swim test

One-way ANOVA showed that oral treatment with GTP for 7 days significantly reduced immobility time at doses of 5 mg/kg ($p < 0.05$), 10 mg/kg ($p < 0.05$), and 20 mg/kg ($p < 0.01$) compared with saline-treated control mice. The positive control venlafaxine decreased immobility time in the FST at a dose of 10 mg/kg after 7-day administration ($p < 0.05$; Fig. 2A). Conversely, the acute treatment with a single dose of GTP (5, 10 and 20 mg/kg) had no effects on the immobility time in the FST (Fig. 2B).

3.3. Sub-chronic treatment with GTP had no effects on the locomotor activity in the open field test

To exclude the possibility that GTP induces locomotor alterations in the FST, we measured the effects of GTP on locomotor activity 5 min before the FST. Mice treated with GTP (5, 10, and 20 mg/kg) and the positive control venlafaxine (10 mg/kg) for both 7 day and 1 day did not differ from mice treated with saline in the number of crossings in the open field test ($p > 0.05$; Fig. 3A and B), indicating that the antidepressant-like effects of GTP are not attributable to a stimulatory effect on locomotor function.

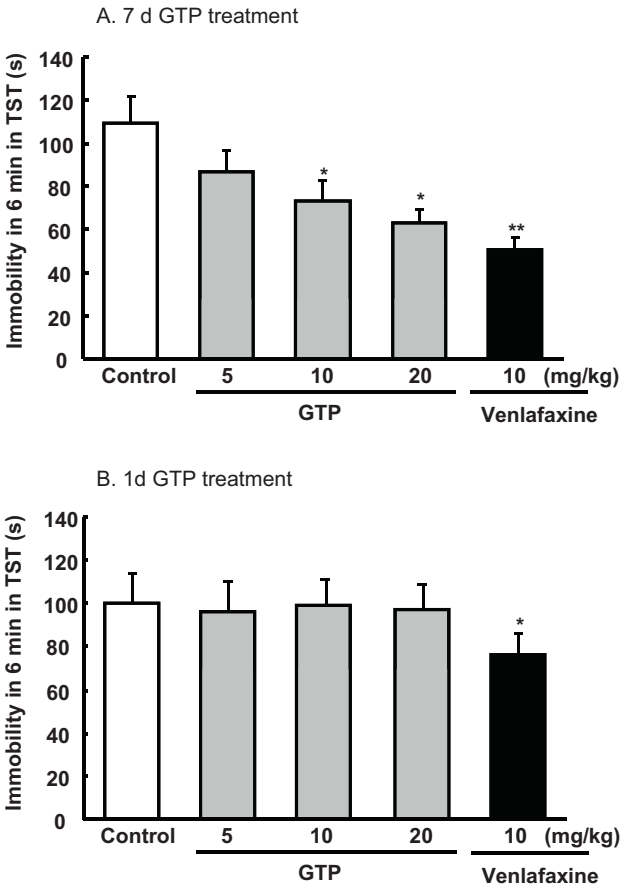

Fig. 1. Antidepressant effect of GTP in the tail suspension test (TST) in mice. Oral administration with GTP (10 and 20 mg/kg) and venlafaxine (10 mg/kg) for 7 days reduced the immobility time ($n = 9$–11 per group) (A), while single dose of GTP did not alter the immobility time ($n = 8$ per group) (B). Sixty minutes after the last treatment, the mice were exposed to the TST. Data are expressed as mean ± SEM. Differences between control and GTP or venlafaxine were assessed using Tukey's *post hoc* test. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with control mice.

3.4. GTP produced an antidepressant effect in FST without preceding OFT

In the current experiment, the OFT was conducted 5 min before the FST. Although the OFT apparatus is a novel environment for the mice, it is only a mild stressor without effects on the normal behaviors. In order to exclude the effect of OFT on the behavioral responses in FST, we replicated the antidepressant effect of 7-day GTP treatment in the FST without previous OFT. The data showed oral treatment with GTP for 7 days significantly reduced immobility time at doses of 5 mg/kg ($p < 0.05$), 10 mg/kg ($p < 0.01$), and 20 mg/kg ($p < 0.01$) compared with saline-treated control mice (Fig. 4).

3.5. GTP inhibited the serum corticosterone levels in mice exposed to FST

The ELISA data showed that 7-day administration of GTP at doses of 5, 10 and 20 mg/kg significantly inhibited corticosterone secretion induced by FST exposure compared with saline-treated controls ($F_{4,25} = 14.13$, $p < 0.001$; Fig. 5A). Additionally, both acute (1 day) and sub-chronic (7 days) GTP injections without the forced

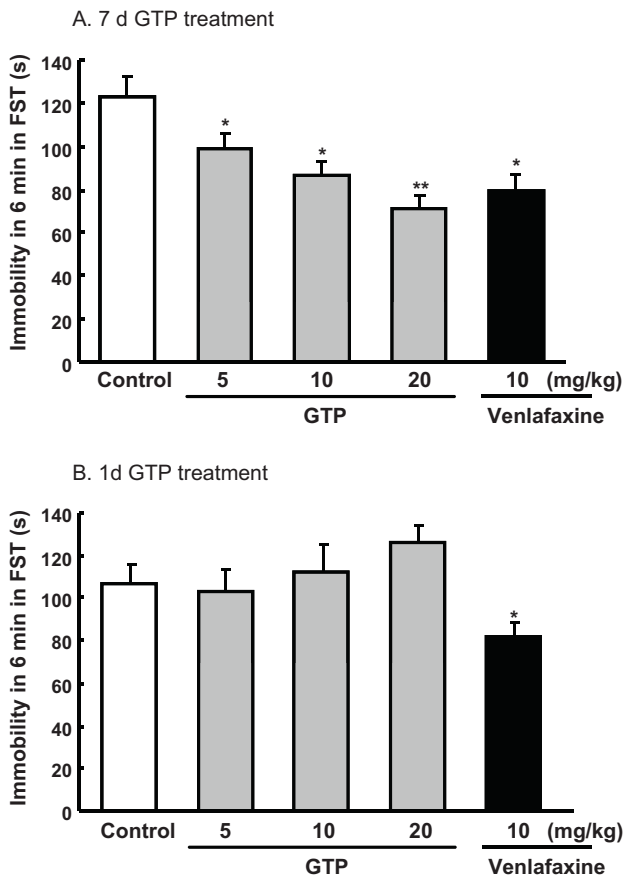

Fig. 2. Antidepressant effect of GTP in the forced swim test (FST) in mice. GTP (5, 10, and 20 mg/kg) and venlafaxine (10 mg/kg) was administered orally for 7 days decreased immobility time (n = 10–11 per group) (A), while single dose of GTP did not reduce the immobility time (n = 8 per group) (B). Sixty minutes after the last treatment, the mice were exposed to the FST. Data are expressed as mean ± SEM. Differences between control and GTP or venlafaxine were assessed using Tukey's *post hoc* test. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with control mice.

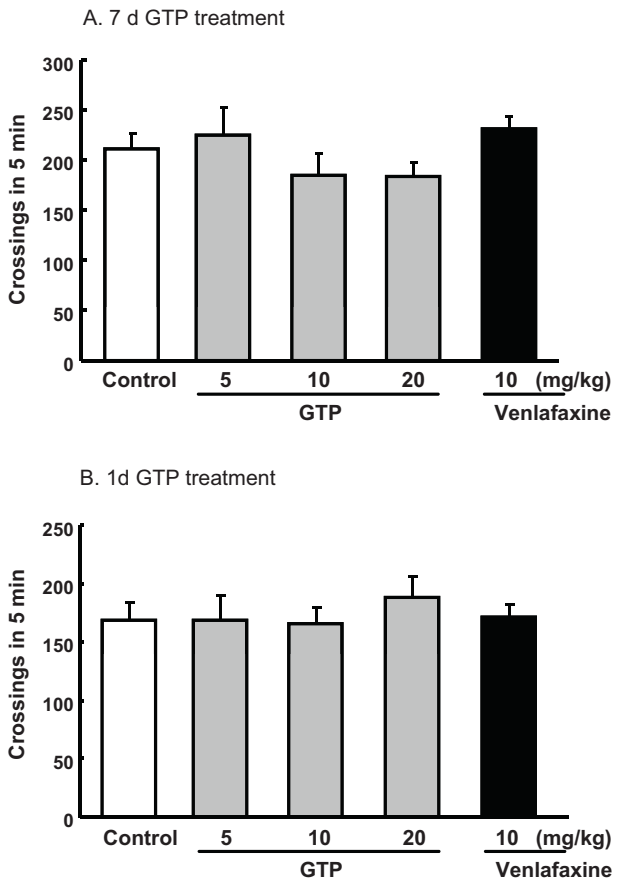

Fig. 3. The effect of GTP on the locomotor activity in the open field test. Both 7 day (A) and 1 day (B) GTP treatment had no effects on the number of crossings in the open field test. GTP and venlafaxine were administered orally for 7 days or a single dose respectively, 55 min after the last treatment, mice were exposed to the open field test (n = 10–11 per group for 7 day; n = 8 per group for 1 day). Data are expressed as mean ± SEM.

swimming test did not affect the baseline corticosterone levels (Fig. 5B and C).

3.6. GTP inhibited the serum ACTH levels in mice exposed to FST

One-way ANOVA revealed that mice treated with GTP (5, 10, and 20 mg/kg) exhibited decreased serum ACTH levels compared with control mice ($F_{4,25}$ = 19.95, $p < 0.001$; Fig. 6A). While both acute (1 day) and sub-chronic (7 days) GTP injections with no forced swim test had no effects on the baseline ACTH levels (Fig. 6B and C). These findings suggested that GTP specifically regulated the activated HPA axis but not normal physiological state of HPA system.

4. Discussion

The TST and FST are two validated models used to assess putative antidepressants compounds. Immobility time in these two paradigms reflects antidepressant-like activity. In the present study, oral treatment with GTP for 7 days but not for 1 day significantly reduced immobility time in both the TST and FST, suggesting that GTP has antidepressant-like effects in mice. The findings that acute GTP did not reduce the immobility in TST and FST suggested that only repeated treatment with GTP to keep a stable blood concentration can produce antidepressant properties. To exclude the possibility that the antidepressant-like effects of GTP are attributable to stimulatory effects on locomotor function, we evaluated GTP-induced spontaneous activity in the open field test. Green tea polyphenols-treated mice exhibited no alterations in spontaneous activity in the open field test, indicating that the decreases in immobility in the FST and TST were not caused by motor stimulation but rather to increases in active movements, such as struggling and swimming.

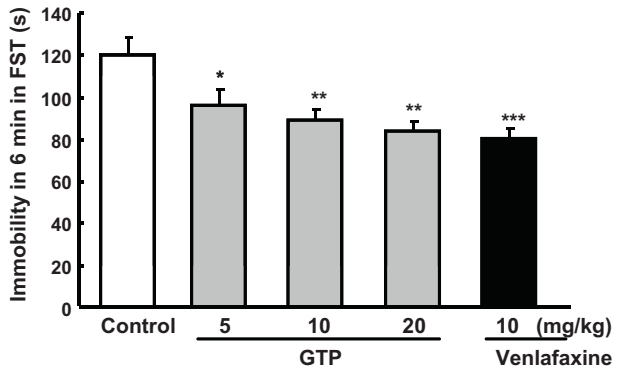

Fig. 4. The antidepressant effect of GTP and venlafaxine measured in FST without preceding OFT. GTP (5, 10, and 20 mg/kg) and venlafaxine (10 mg/kg) was administered orally for 7 days decreased immobility time in FST (n = 8 per group). Data are expressed as mean ± SEM. Differences between control and GTP or venlafaxine were assessed using Tukey's *post hoc* test. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, compared with control mice.

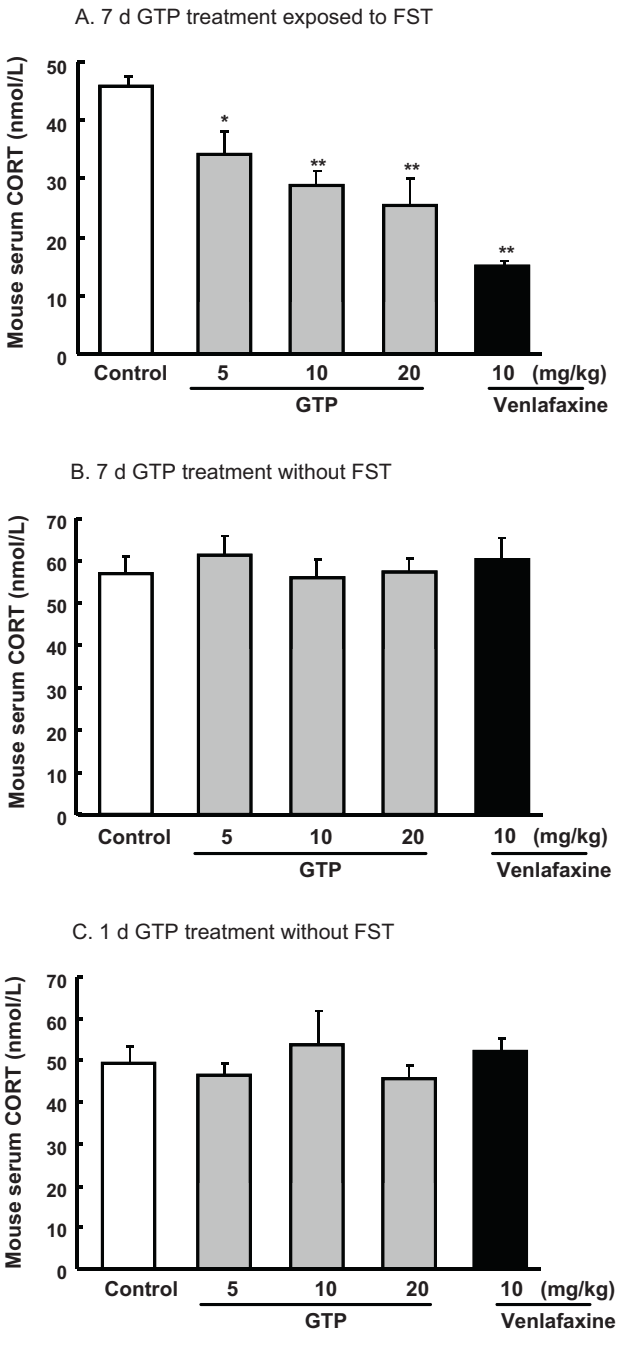

Fig. 5. The effect of GTP on the serum corticosterone (CORT) levels in mice. GTP (5, 10, and 20 mg/kg) and venlafaxine (10 mg/kg) was administered orally for 7 days decreased serum CORT levels in mice exposed to the FST (A). Immediately after the FST, blood was collected for CORT measurement using ELISA ($n = 6$ per group). Both 7 day (B) and 1 day (C) GTP treatment did not alter the serum CORT levels in mice without exposure to FST. One hour after last drug administration, blood was collected for CORT measurement using ELISA ($n = 6$ per group). Data are expressed as mean ± SEM. Differences between control and GTP or venlafaxine were assessed using Tukey's *post hoc* test. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with control mice. CORT, corticosterone.

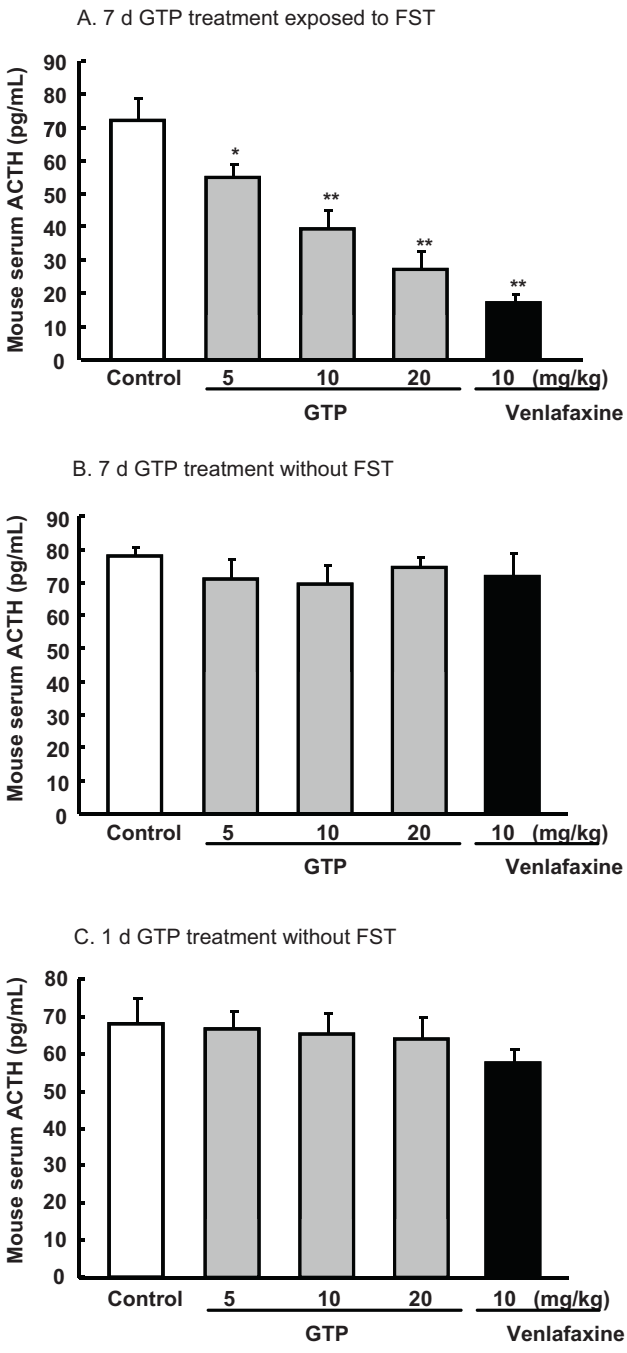

Fig. 6. The effect of GTP on the serum adrenocorticotropic hormone (ACTH) levels in mice. GTP (5, 10, and 20 mg/kg) and venlafaxine (10 mg/kg) was administered orally for 7 days reduced serum ACTH levels in mice exposed to the FST (A). Immediately after the FST, blood was collected for ACTH measurement using ELISA ($n = 6$ per group). Both 7 day (B) and 1 day (C) GTP treatment did not alter the serum CORT levels in mice without exposure to FST. One hour after last drug administration, blood was collected for CORT measurement using ELISA ($n = 6$ per group). Data are expressed as mean ± SEM. Differences between control and GTP or venlafaxine were assessed using Tukey's *post hoc* test. $^{*}p < 0.05$, $^{**}p < 0.01$, compared with control mice.

The ELISA assay revealed that GTP suppressed HPA axis hyperactivity by reducing serum corticosterone and ACTH levels in mice exposed to the FST. These results reveal the previously unrecognized antidepressant-like effects of GTP in laboratory animal studies and suggest that GTP may be used to develop new pharmaceutical treatments for depression. However, GTP did not alter the serum corticosterone and ACTH levels in mice without exposure to forced swim procedure, suggesting that GTP can regulate the activated HPA axis acitivity.

Hypothalamic–pituitary–adrenal axis dysfunction, reflected by elevations in circulating glucocorticoids (corticosterone in rodents; cortisol in humans), contributes to the development of depression in humans [28,29]. Major depression patients exhibit higher plasma cortisol levels compared with healthy subjects [30,31]. Moreover, acute restraint stress increased serum corticosterone

levels and immobility in the FST and TST in mice and is associated with depressive-like symptoms [32]. To determine whether HPA axis hyperactivity induced by the FST is affected by GTP, we measured serum corticosterone and ACTH levels in mice in response to FST exposure. Green tea polyphenols reduced serum corticosterone and ACTH levels in mice exposed to the FST, suggesting that the mechanism of action of the antidepressant effects of GTP may involve the regulation of HPA homeostasis to increase the ability of mice to cope with stressful conditions. The reduced HPA responsiveness after GTP and venlafaxine treatment in the present study is consistent with previous findings, in which agents with antidepressant-like efficacy decreased the levels of glucocorticoids, such as corticosterone [33,34]. The present study raises the possibility that chronic GTP treatment can reduce HPA axis hyperactivity in response to stress. Hyperactivity of the HPA axis decreased the function of glucocorticoid receptors (GRs), particularly in the hippocampus, and consequently led to impairment of glucocorticoid feedback inhibition [35]. Dysfunction of GRs reduced neurogenesis and impaired neuroplasticity, thereby contributing to the development of depressive-like performance [36–38]. Clinically effective antidepressants exert their therapeutic actions partially by modulating GR function through the regulation of receptor expression, thereby ameliorating many of the behavioral disturbances associated with depressive-like states [39,40]. Considering the fact that GR function plays a key role in the development and treatment of depression, the regulatory effect of GTP on GR expression and the target genes involved in GTP's antidepressant-like effects is an issue that needs to be addressed in the future.

Previous studies reported that oral administration of L-theanine, a major amino acid constituent of green tea, increased serotonin and dopamine levels in the striatum, hypothalamus, and hippocampus [41]. Additionally, GTP dose-dependently inhibited monoamine oxidase B activity and increased monoamine levels in rat C6 astrocyte cells [17]. Moreover, EGC and EGCG altered monoamine metabolites in epileptic discharges induced by iron ions [42]. According to these findings, we presume that the regulation of monaminergic neurons in the central nervous system might be another mechanism involved in the antidepressant effects of GTP.

Adult hippocampal neurogenesis is one of the possible mechanisms of the pathophysiology of depression and the actions of antidepressants [43,44]. Notably, green tea extract alone induced neurite outgrowth and potentiated neuritogenesis induced by nerve growth factor [45–47]. In the present study, chronic treatment with GTP for 7 days produced significant antidepressant-like effects, whereas acute GTP treatment with a single dose failed to exert this effect (data not shown), suggesting that adaptive alterations in neuroplasticity might be involved in the antidepressant-like action of GTP. Plant polyphenols not only precipitate but also interact with various molecular targets that affect the formation of functional proteins and cellular signaling pathways. For example, EGCG, a bioactive tea polyphenols, regulates CD3-mediated T-cell leukemia receptor signaling through inhibition of tyrosine kinase ZAP-70 [48]. The present study showed the effectiveness of GTP on depressive-like behavior. Investigations of how GTP interacts with specific molecular targets will provide a better understanding of the possible mechanism of action of the antidepressant-like effects of GTP.

In conclusion, oral GTP administration exerted significant antidepressant-like effects in the FST and TST and was associated with normalization of HPA axis dysfunction induced by stress. Regular dietary intake of green tea can maintain a stable GTP concentrations in the body and may have valuable effects on health because of good oral bioavailability and few adverse effects [49–53]. These advantages of GTP provide exciting insights into the development of GTP as a potential antidepressant drug. Further research is needed to determine whether the antidepressant-like effects of GTP in mice are applicable to depressed patients. Additionally, investigations of the biologically active compounds in GTP that produce antidepressant-like effects are still needed.

Conflict of interest

The authors declare that they have no competing interests.

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