Life Sciences 216 (2019) 119–128

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Anxiolytic effects of γ-oryzanol in chronically- stressed mice are related to monoamine levels in the brain

Salina Akter, Hiroyuki Sasaki, Kazi Rasel Uddin, Yuko Ikeda, Hiroki Miyakawa, Shigenobu Shibata*

Laboratory of Physiology and Pharmacology, School of Advanced Science and Engineering, Waseda University, Tokyo, Japan

ARTICLE INFO

Keywords:
γ-oryzanol
Anxiety
Monoamines
Amygdala

ABSTRACT

Aims: The present study was aimed to investigate the anxiolytic effect of γ-oryzanol (GORZ) during chronic restraint stress treatment (CRST), which is a well-documented model of stress-related disorders, like anxiety, and its potential molecular mechanisms.

Materials and methods: In this experiment, 5-week-old male ICR mice were used and the concentration of GORZ was fixed at 0.5% in the mouse standard diet (14% casein, AIN 93 M). Mice were immobilized daily for 3 h from ZT 2.5 to 5.5 (ZT0 was designated as light-on time) for 20 consecutive days, followed by behavioral testing, including the open field test (OFT) and elevated plus maze (EPM) test. The concentration of serum corticosterone (CORT) was measured. In addition, the expression of central monoamine neurotransmitters with their metabolites in the hippocampus, cerebral cortex, and amygdala of the brain were examined.

Key findings: 0.5% GORZ partially blocked stress-induced reduction of body weight gain while stressed mice had significantly lower body weights during the entire experimental period. Further, 0.5% GORZ treatment could significantly improve the main behavioral parameters even in CRST situations. The significant increase in serum CORT levels indicated CRST-induced stress, which was almost unaffected by 0.5% GORZ treatment. Moreover, 0.5% GORZ also supported the anxiolytic mechanism with enhancement of 5-HIAA and NE levels in the amygdala of brain after CRST.

Significance: Taken together, our studies suggested that 0.5% GORZ is a potential therapeutic drug candidate against anxiety under chronic stress conditions.

1. Introduction

Stress can be defined as any uncomfortable emotional experience, accompanied by predictable biochemical, physiological, and behavioral changes [1]. Stress affects people of all ages, sexes, and circumstances, leading to both physical and psychological health issues. Chronic stress is thought to be associated with structural degeneration and impaired functioning of the brain, which may account for the increased risk of developing neuropsychiatric disorders like anxiety, depression, and dementia [2]. Among all stress-related neuropsychiatric disorders, anxiety disorders are the most common [3–6], which affect nearly one in five adults in the U.S. alone [7]. Yet, the precise mechanism underlying stress-related behavioral disorders is still unclear. Currently, γ-oryzanol (GORZ) has received considerable attention as an anxiolytic drug; however, very little is known about its effect.

Rice is the staple food of more than half of the world's population, with more than 3.5 billion people depending on rice for more than 20% of their daily calories. Nearly 90% of the world's rice is produced and consumed in Asia.

Rice Bran oil (RBO) is an important byproduct of rice bran, which is believed to be a healthy edible oil [8]. It contains a balanced fatty acid (FA) profile that includes 47% monounsaturated fats, 33% polyunsaturated fats, and 20% saturated fats. The fat content is composed of triacylglycerols (87%) and free fatty acids (8%), and the remaining 5% of constituents, like GORZ, tocotrienols, tocopherols, and squalene, have proven nutritional benefits [9,10]. Globally the consumption of RBO remains is increasing day by day due to its health benefits and the high production rate of rice.

Chemically, GORZ is mainly composed of a mixture of trans-ferulic acid esters (trans-hydroxycinnamic acid) and phytosterols (sterols and triterpenic alcohols), such as cycloartenol, β-sitosterol, 24-methylenecycloartenol, and campesterol [11]. GORZ is a strong antioxidant

* Corresponding author at: Laboratory of Physiology and Pharmacology, School of Advanced Science and Engineering, Waseda University, Shinjuku-Ku, Tokyo 162-8480, Japan.
E-mail address: shibatas@waseda.jp (S. Shibata).

https://doi.org/10.1016/j.lfs.2018.11.042
Received 7 June 2018; Received in revised form 19 November 2018; Accepted 19 November 2018
Available online 20 November 2018

compound that decreases plasma and serum cholesterol levels by reducing the absorption of cholesterol from foods [12]. It also inhibits tumor promotion [13], protects against liver injury [14] and decreases platelet aggression [15]. Moreover, it has been classically used by some researchers to increase testosterone and growth hormone levels and treat hyperlipidemia [16] and symptoms associated with menopause [17].

Thus, the present study aimed to clarify the anti-anxiety role of 0.5% GORZ and establish anxiolytic regulatory mechanisms on chronic restraint stress treatment (CRST)-induced behavioral changes. Firstly, we evaluated the anxiolytic-like effects of GORZ by behavioral tests, including the open-field test (OFT) and elevated plus-maze test (EPMT). Following that, based on the significance of corticosterone (CORT) and monoamines in the pathological mechanism of anxiety, the levels of serum CORT and monoamines in brain were determined to establish the possible anxiolytic-like mechanism of GORZ.

2. Materials and methods

2.1. Animals and experimental design

Male 5-week-old ICR mice were purchased from Tokyo Laboratory Animals Science Co., Ltd., Tokyo, Japan and were given 24 h recovery time after transportation. Mice were randomly divided into four groups (Control (CTL), GORZ, Control + CRST and GORZ + CRST). The unstressed mice were housed for 20 days with free access to food and tap water in a temperature- and humidity-controlled (22 ± 2 °C, 60 ± 5%) environment, under a 12-h light/dark cycle. Stressed mice were subjected to restraint stress using a wire-mesh bag (3 × 6 × 12 cm) clipped to their home cage daily for 3 h from ZT 2.5 to 5.5 (ZT 0 was designated as the light-on time) without free access to food and water. The first phase chronic restraint stress treatment (CRST) was applied for 14 consecutive days (D 14) from the beginning of the experiment and the second phase CRST was applied again for 5 consecutive days from Day 16 to day 20 followed by 24 h recovery period i.e. no scheduled CRST on Day 15 under the same experimental conditions (Fig. 1). After being restrained, the mice were returned to the home cage and provided food and water ad libitum. The morning time was considered most suitable for CRST as mice are habituated to sleep in this time and avoid eating. Therefore, food deprivation in this time period may not affect greatly. No considerable aggression was observed after returning to home cage. The body weight and food consumption of the mice were monitored daily (ZT 1.5 to 2) before restraint stress exposure. In this experimental design, a total of 10–12 mice were housed in a group and each cage of mice of a given treatment functioned as an experimental unit. Therefore, we were unable to measure the food intake volume in individual mice. Mice were group housed throughout studies to avoid unnecessary stressful circumstance in accordance with the guidelines from the animal care committee. This is why we measured food consumption as a group. Mice were also transferred to new cages with new fresh wood shaving every week. The food and tap water were also changed once a week. The procedures conformed to the "Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions" (published by the Ministry of Education, Culture, Sports, Science and Technology, Japan) and were approved by the Committee for Animal Experimentation of the School of Science and Engineering at Waseda University (permission # 2017-A074).

2.2. Application arrangement of γ-oryzanol (GORZ)

GORZ was obtained from Sigma-Aldrich (St. Louis, MO, USA). In the current study, 0.5% GORZ was administered via the mouse standard diet (14% casein, AIN 93 M). Numerous in vivo animal studies have been performed with different GORZ dosages. 0.5% GORZ was most commonly used and highly effective dose in rodents [18].

2.3. Behavioral testing

The behavioral tests, including the open field test (OFT) and elevated plus maze test (EPM), were conducted on days 15 and 20, respectively on the light intensity of 100–150 lx, to examine the effects of 0.5% GORZ on anxiety-like behaviors (Fig. 1).

2.3.1. Open-field test (OFT)

On day 15, mice were introduced to an open field test at ZT 5.5 to 7.5 after 3 h of CRST following 24 h of interval period from CRST. Each mouse was placed at the center (23 ∗ 12 cm) of the open field apparatus (35 ∗ 24 ∗ 24 cm) and the behavior was monitored for 5 min using a charged-couple device (CCD) camera. Several parameters including the time in the center zone (s), total distance travelled (m), and number of entries into the center zone were measured to assess anxiety-like behavior and were analyzed using ANYMAZE software (Stoelting, IL, USA). The apparatus was thoroughly cleaned with 70% ethanol after each trial in the OFT.

2.3.2. Elevated plus maze (EPM) test

On day 20, anxiety related behavior was assessed by using the EPM test at ZT 5.5 to 7.5 after 3 h of CRST following 0–2 h of interval period from CRST. The EPM consists of two opposite open arms (29 × 7 cm) and two opposite closed arms (29 ∗ 19 ∗ 7 cm), which are connected by a 7-cm square center zone, elevated approximately 41 cm above the floor, and located in an appropriate observation room. The walls on the closed arms were opaque. Each mouse was placed at the center zone facing the open arm and was allowed to move freely for 5 min. A CCD camera was used to record the behavior. Important parameters like the time (s) in the open and closed arm and the distance travelled (m) in the open and closed arm and the number of entries in the open and closed arm were calculated and analyzed using ANYMAZE software (Stoelting, IL, USA). The ANYMAZE detects the mouse arm entry when the center of mouse body enters the open or closed arm of the apparatus. The apparatuses were also cleaned with 70% ethanol after each trial in EPM test.

2.4. Animal termination and sample collection

On day 20, after the 3-h CRST exposure and EPM test, mice were sacrificed at ZT 5.5 to 7.5. (Fig. 1). Mice were anesthetized using midazolam/xylazine and trunk blood samples were collected after decapitation. The samples were kept at room temperature for 1 h, centrifuged at 3000 rpm for 15 min to obtain serum, and stored at −80 °C until further analysis. Whole brains were also rapidly removed. Frontal brain slices (2-mm thick), which included the hippocampus, cerebral cortex, and amygdala, were prepared using a brain matrix (# 0530; Bioresearch Center Co., Nagoya, Japan). The various brain regions were sliced and tissues were dissected based on the neuroanatomical landmarks from the brain atlas corresponding to bregma −0.5 to bregma −2.5 [19] approximately. The hippocampus (mean wet weight, 17.1 mg) and the amygdala (mean wet weight, 14.9 mg) were dissected free hand. The cerebral cortex (mean wet weight, 17.9 mg) was dissected free hand from the upper regions of the dorsal hippocampus. The brain tissues were immediately stored at −80 °C until further analysis. The detailed protocol has been published in our previous paper [20].

2.5. Enzyme-linked immunosorbent assay

Levels of serum corticosterone (CORT) were measured through an enzyme-linked immunosorbent assay (ELISA), using a commercial ELISA kit (ASSAYPRO, St. Charles, MO) according to the manufacturer's instructions. Absorbance was measured at 450 and 570 nm; the reading at 570 nm was subtracted from those at 450 nm to correct optical imperfections. The detailed protocol has been published in our previous paper [21].

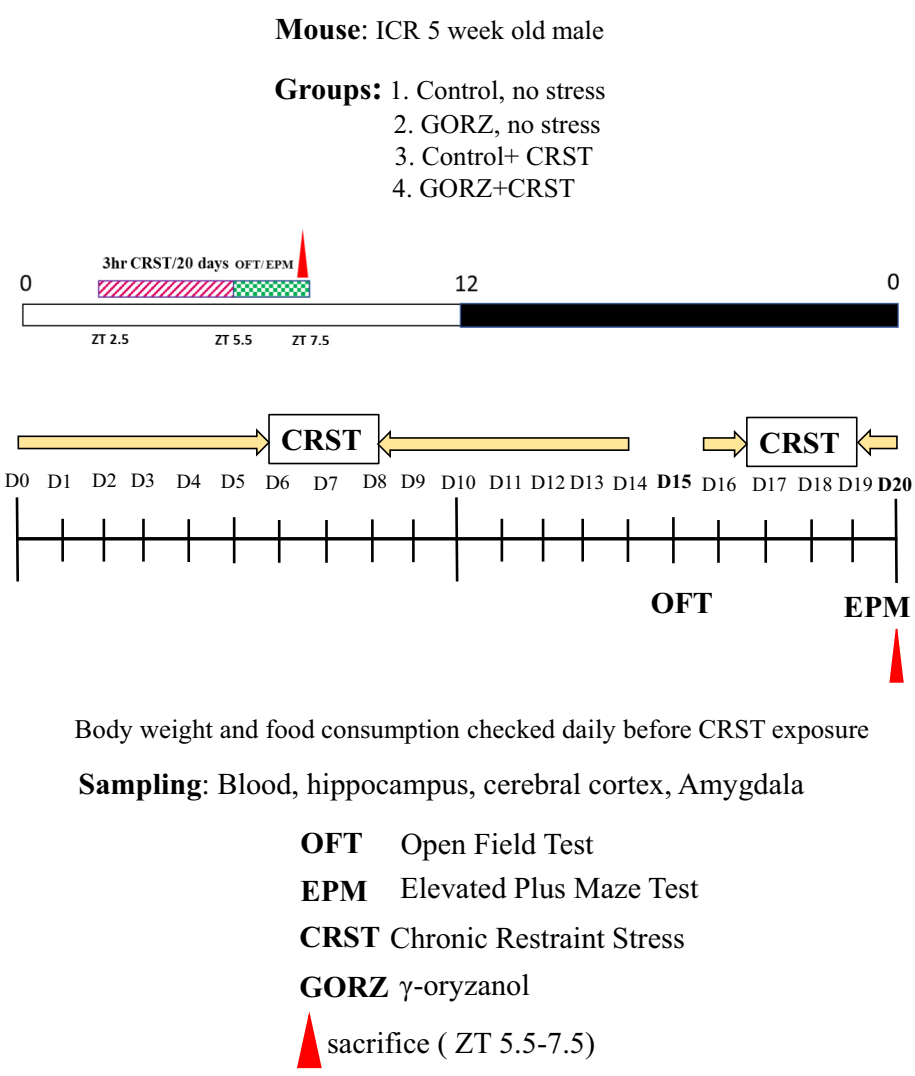


Fig. 1. Experimental design and protocol.

2.6. High-performance liquid chromatography-electrochemical detection (HPLC-ECD)

To explore the detailed neurochemical mechanisms involved in anxiolytic-like effects of 0.5% GORZ, the levels of central monoamine neurotransmitters, i.e., 5-hydroxy tryptamine (5-HT) and norepinephrine (NE), and their metabolites, 5-hydroxyindole acetic acid (5-HIAA) and 3-methoxy-4-hydroxyphenylglycol (MHPG), in the hippocampus, cerebral cortex, and amygdala were detected using HPLC-ECD (HTEC 500; Eicom, Kyoto, Japan) after the behavioral assessments. Perchloric acid (0.2 M, including 100 μM EDTA·2Na) and 20 ng of isoproterenol (internal standard) were added to each sample. Samples were homogenized using an ultrasonic-homogenizer and were centrifuged at 15,000 rpm at 4 °C for 15 min. The supernatants collected for each sample were filtered using a 0.45-μm filter. The quantity of monoamine in each 20 μL sample was measured using HPLC-ECD with the following conditions: the transfer phase consisted of 85% 0.1 M acetate citric acid buffer (pH 3.5) containing 5 mg/L EDTA·2Na, 190 mg/L 1-octanesulfonic acid sodium salt, and 15% methanol (99% purity); the velocity of the flow was 500 μL/min; the column temperature was set to 25 °C; the applied voltage was set to +750 mV versus Ag/AgCl. Data were analyzed using EPC-300 software (Eicom). The detailed protocol has been published in our previous paper [21].

2.7. Statistical analysis

Data are presented as mean ± standard error of mean (SEM). Statistical analyses were performed using Graph Pad Prism version 6.03 (Graph Pad Software, San Diego, CA). The data were tested for normal distribution, and equal or biased variation using the D'Agostino-Pearson test and Bartlett's test, respectively. Parametric analysis was conducted using two-way analysis of variance (ANOVA) with Sidak test for post-hoc analysis. If normal distribution was absent, the data were tested by mathematical transformation (logarithmic) and subsequently by two-way analysis of variance (ANOVA) with Sidak test for post-hoc analysis.

3. Results

All data of statistical analysis are listed in the Supplemental Table 1. At first, a preliminary behavioral study including the OFT and EPM test were conducted to examine the anxiolytic effect of 1% GORZ. In this experiment, 6 week old C57BL/6 J male mice were used and randomly divided into two groups, control (14% casein diet, n = 3) and 1% GORZ (14% casein diet + 1% GORZ, n = 5) and housed for 17 weeks with ad libitum food and tap water under 12 h light dark cycle. For the OFT, time in the center zone (s) in control and 1% GORZ mice were 67 ± 19.38 (s) and 67.14 ± 9.93 (s) respectively. For the EPM test, the important behavioral parameters including the time in the open arm (s) and the distance travelled in the open arm (m) in control and

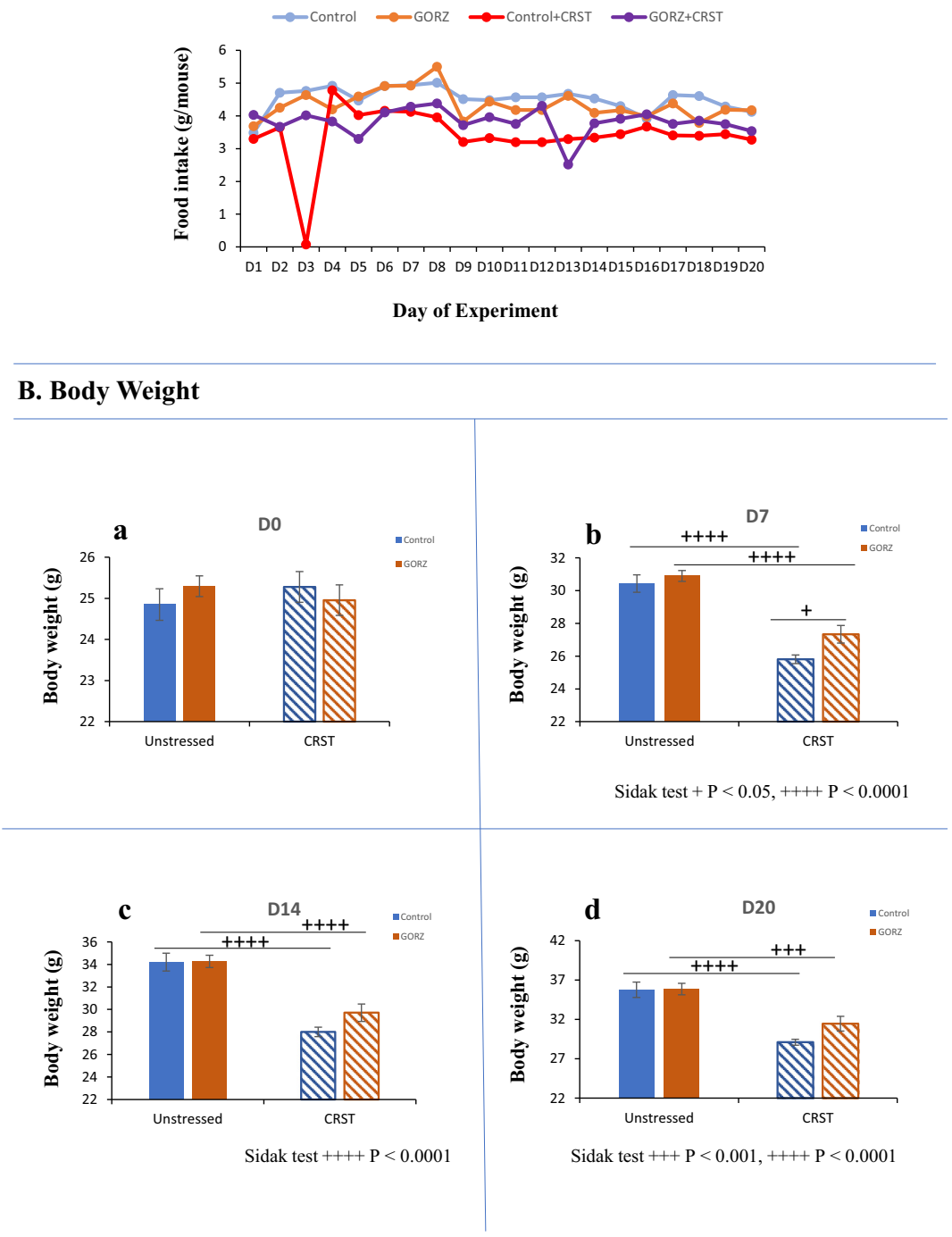


Fig. 2. Effect of chronic restraint stress treatment (CRST) on food intake and body weight. **(A)** daily food intake (g/mouse) of mice, **(B)** Body weight (g) of all groups from baseline i.e., **B(a)** day 0 (D0), **B(b)** day 7 (D7), **B(c)** day 14 (D14), **B(d)** Day 20 (D20) to the end of experiment. Data are presented as mean ± standard error (SE); n = 10–12 mice per group. Statistical differences were evaluated using two-way analysis of variance (ANOVA) with Sidak test (+, $P < 0.05$; +++, $P < 0.001$; ++++, $P < 0.0001$).

1% GORZ group mice were (91.33 ± 8.08; 90.48 ± 45.43 (s), respectively) and (1.301 ± 0.14; 0.6554 ± 0.21 (m), respectively). No significant changes of important behavioral parameters recorded in OFT and EPM test in this preliminary experiment inspired us to set the present study design with 5 week old ICR male mice with 0.5% GORZ dietary treatment.

3.1. Effects of CRST on food intake and body weight

The effects of daily 3-h CRST for 20 days on food intake and body weight are shown in Fig. 2. The daily food intake of the stressed mice dropped sharply during the first few days of CRST, but gradually recovered substantially (Fig. 2A). As shown in Fig. 2B (a), there was no significant difference in body weight per group of mice before the CRST

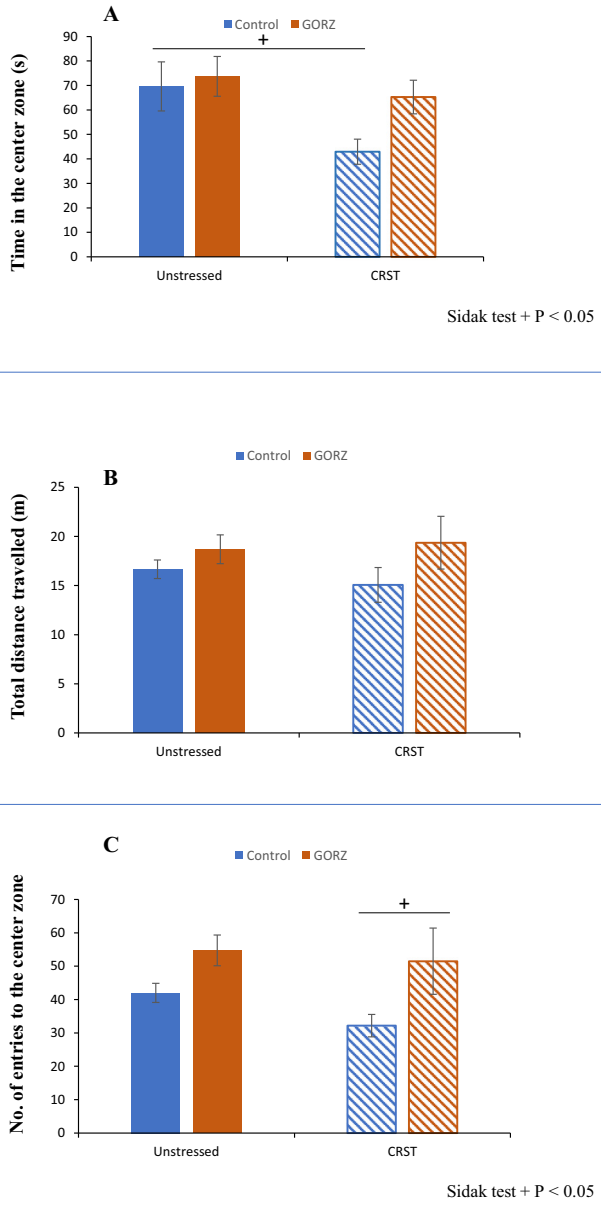


Fig. 3. Effect of 0.5% γ-oryzanol (GORZ) treatment on chronic restraint stress (CRST)-induced behavioral changes in the open field test (OFT). OFT was conducted on day 15 at ZT 5.5 to 7.5, and the **(A)** time in the center zone (s), **(B)** total distance travelled (m), and **(C)** number of entries into the center zone were determined. Values are presented as mean ± standard error (SE); n = 10–12 mice per group. Statistical differences were evaluated by two-way analysis of variance (ANOVA) with Sidak test (+, $P < 0.05$).

procedure was performed. However, as a consequence of CRST, the stressed mice had significantly ($P < 0.001$, $P < 0.0001$) lower body weights than the unstressed control and GORZ mice (Fig. 2B (b, c, d)) during the entire experimental period. To some extent, these effects of CRST can be reduced by concurrent treatment with 0.5% GORZ in the diet. Fig. 2B (b) clearly showed that 0.5% GORZ treatment remarkably ($P < 0.05$) blocked the stress-induced reduction of body weight gain.

3.2. Effect of 0.5% γ-oryzanol treatment on chronic restraint stress-induced behavioral changes in OFT

Anxiety related behavior in the OFT is shown in Fig. 3. As shown in Fig. 3A, the CRST-exposed mice spent significantly less time in the center zone (s) as compared to the unstressed control mice ($P < 0.05$), indicating that CRST induced an anxiety-like phenotype. Although significant differences were not observed, GORZ treatments slightly reversed the CRST-induced decrease in the time spent in the center zone (s) Fig. 3A.

Fig. 3B shows that the total distance travelled (m) was slightly increased in the GORZ-treated mice under both unstressed and CRST conditions. As shown in Fig. 3C, it is clear that 0.5% GORZ treatment significantly increased the number of entries into the center zone ($P < 0.05$) under CRST conditions. These results support the idea that 0.5% GORZ feeding decreases anxiety-like behaviors.

3.3. Effect of 0.5% γ-oryzanol treatment on chronic restraint CRST induced behavioral changes in EPM test

The EPM test (Fig. 4) demonstrated that CRST could affect the main behavioral parameters slightly as compared to mice from the control group (unstressed versus CRST). For the EPM test, as shown in Fig. 4A and B, mice treated with 0.5% GORZ showed a significant increase in the time in the open arm (s), ($P < 0.01$) and distance travelled in the open arm (m), ($P < 0.05$), even when they underwent CRST. The time

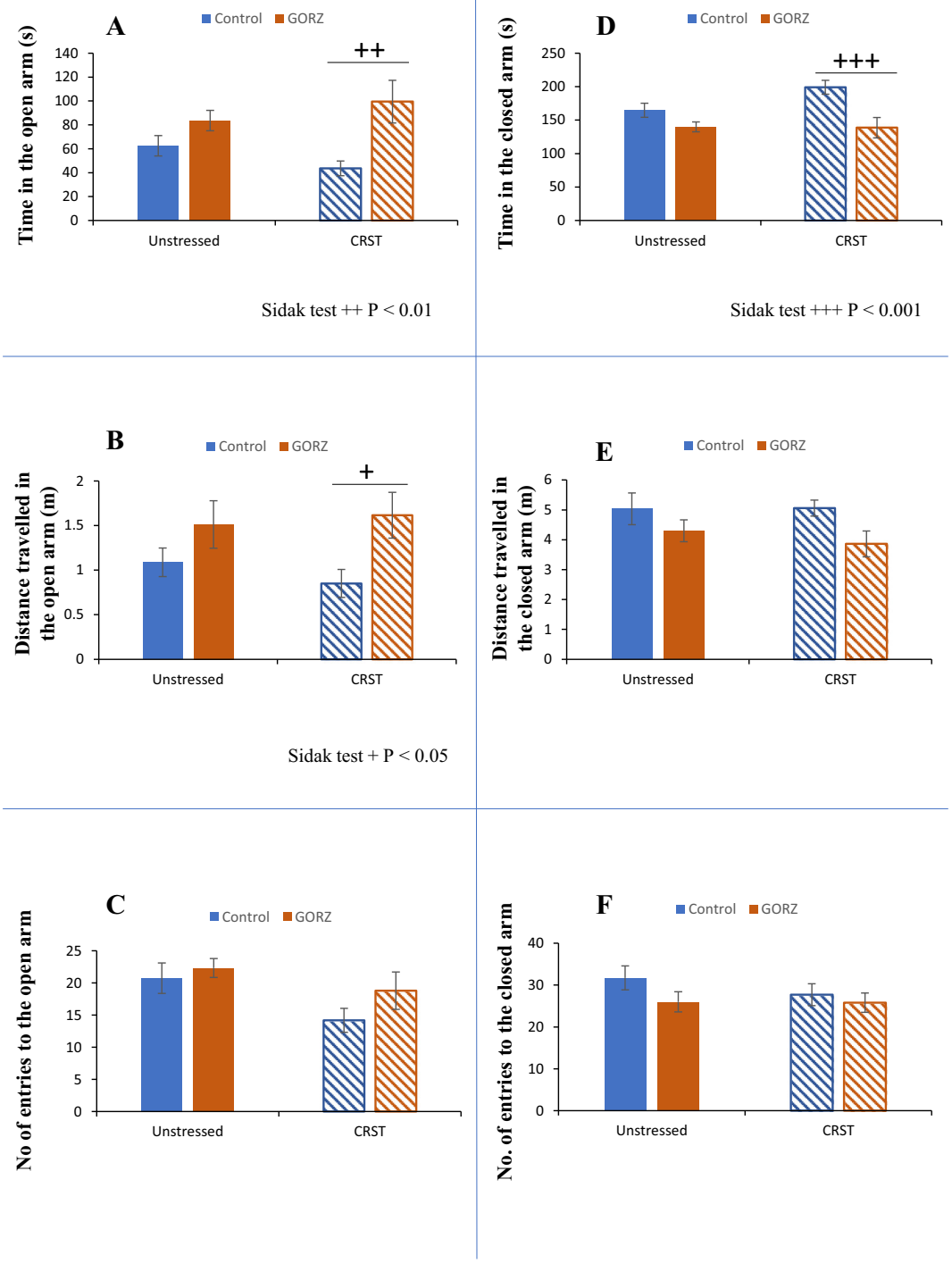


Fig. 4. Effect of 0.5% γ-oryzanol (GORZ) treatment on chronic restraint stress treatment (CRST)-induced behavioral changes in the elevated plus maze (EPM) test. The EPM test was conducted on day 20 from ZT 5.5 to 7.5 to measure anxiety-related behavior. **(A)** The time in the open arm (s), **(B)** distance travelled in the open arm (m), **(C)** number of entries into the open arm, **(D)** time in the closed arm (s), **(E)** distance travelled in the closed arm (m), and **(F)** number of entries into the closed arm were measured. All values are expressed as mean ± standard error (SE); n = 10–12 mice per group. Statistical differences were evaluated by two-way analysis of variance (ANOVA) with Sidak test (+, $P < 0.05$; ++, $P < 0.01$; +++, $P < 0.001$).

in the closed arm (s) ($P < 0.001$) was also significantly decreased in GORZ + CRST mice as compared to Control + CRST mice (Fig. 4D). GORZ treatment also slightly decreased the distance travelled (m) in the closed arm (Fig. 4E). No, significant differences in the number of open to closed arm entries were noted among the groups under CRST situations (Fig. 4C and F).

So, the OFT and EPM test results together corroborated the idea that 0.5% GORZ treatment had importantly anxiolytic effect.

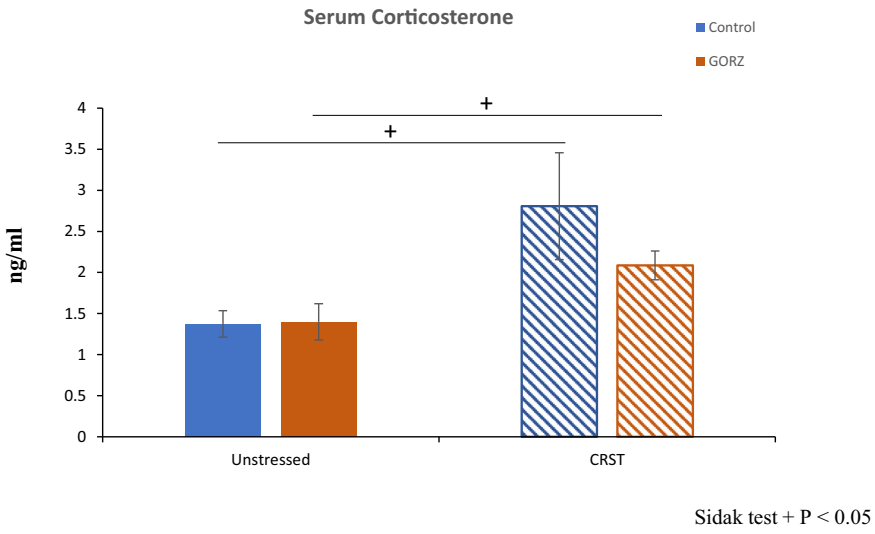


Fig. 5. Effect of 0.5% γ-oryzanol (GORZ) treatment on serum corticosterone (CORT). Data are presented as mean ± standard error (SE); n = 10–12 mice per group. Statistical differences were evaluated by two-way analysis of variance (ANOVA) with Sidak test (+, $P < 0.05$).

3.4. Effect of 0.5% γ-oryzanol treatment on serum corticosterone (CORT)

Serum corticosterone (CORT) was measured using ELISA. A significant increase ($P < 0.05$) in the levels of serum CORT was observed in both the control and GORZ mice that underwent CRST as compared to respective unstressed control mice from both GORZ-treated and non-treated groups. These findings indicate that CRST produced a stress response, which was almost unaffected by the 0.5% GORZ treatment (Fig. 5). Collectively, the results indicated that the anti-anxiety effect mediated by 0.5% GORZ may be independent of the anti-stress effect of CRST.

3.5. Effect of 0.5% γ-oryzanol on brain monoamine neurotransmitters and their metabolites

Anxiety-related neurotransmitters 5-HT and NE with their metabolites, i.e., 5-HIAA and MHPG, respectively, were also measured in the hippocampus, cerebral cortex, and amygdala to evaluate the possible anti-anxiety effect of 0.5% GORZ (Fig. 6A–L). As shown in Fig. 6A–H, CRST led to an increase in anxiety-like behaviors, and caused a slight reduction in the levels of the neurotransmitters and their metabolites in the hippocampus and cerebral cortex of brain. In addition, CRST decreased the 5-HT and 5-HIAA levels without significance in the amygdala (Fig. 6I and J). CRST exposure is expected to yield higher anxiety level in mice due to trend of decrease in the NE level in the amygdala ($P = 0.076$) in the Control + CRST group versus the unstressed control group (Fig. 6K).

As shown in Fig. 6A–H, in the hippocampus and cerebral cortex, intake of 0.5% GORZ food restored the slightly decreased levels of 5-HT, NE and their metabolites.

In the amygdala (Fig. 6J), in comparison to the control group, mice treated with 0.5% GORZ showed a trend of increase in 5-HIAA which is the metabolite of 5-HT, an important neurotransmitter ($P = 0.083$), under CRST exposure. Additionally, as shown in Fig. 6K, mice treated with 0.5% GORZ showed significantly higher NE ($P < 0.05$) values under CRST exposure as compared to those without 0.5% GORZ treatment. In addition, an increase ($P = 0.057$) in the level of MHPG, an NE metabolite was observed in the GORZ group without stress (Fig. 6L).

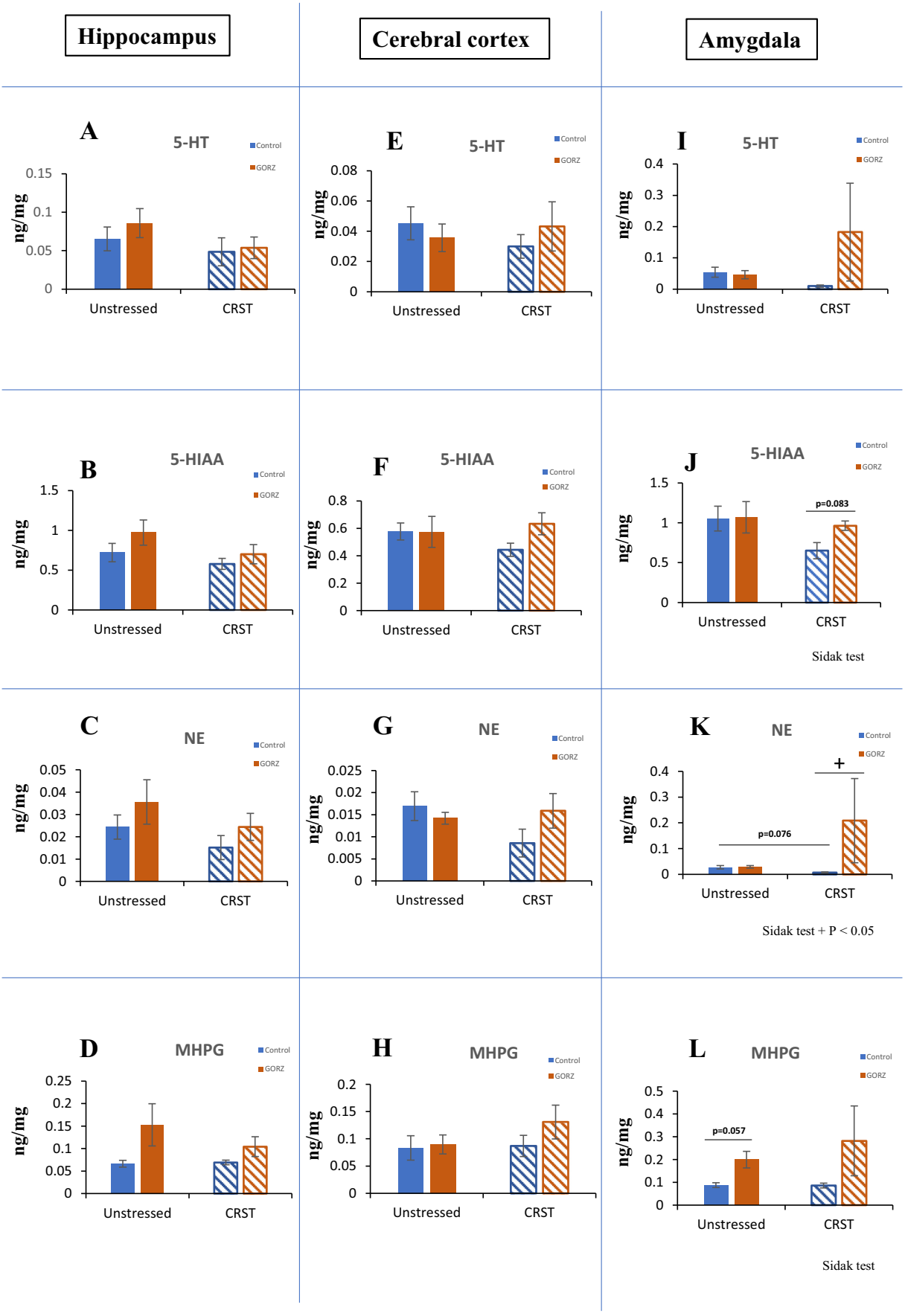
Thus, the HPLC-EC results demonstrated the anxiolytic effect of 0.5% GORZ under conditions of chronic stress.

4. Discussion

The brain is the main target of chronic excessive stress, which can consequently lead to the development of stress-related psychiatric disorders and cognitive ambiguities [22,23]. A recent study showed that CRST induced morphological changes in the blood brain barrier, establishing a link between stress and neurodegenerative disorders [24]. Further, it is already clear that chronic stress exposure can lead to neurological, morphological, and functional changes in the brain, which is closely associated with post-traumatic stress disorders [25]. Thus, in the present experiment, we investigated the anxiolytic effects of 0.5% GORZ and demonstrated the potential molecular mechanism of its effects, following exposure to chronic and extreme forms of unescapable stress. The CRST mouse model used in the current study is the most commonly employed model in similar experiments [26].

Daily exposure to 3-h CRST, from ZT 2.5 to 5.5, for 20 days in 5-week-old male ICR mice elicited anxiety-like behavior and cognitive impairment, which corroborated with previous findings [27,28]. In-keeping with previous studies our results also demonstrated functional and structural changes of several brain regions related to behavior and cognition regulation, including the hippocampus, cerebral cortex [29–32], and amygdala [33], and a reduction in body weight gain and food intake [34–36]. These behavioral and cognitive aberrations were also paralleled by biochemical alterations, including higher levels of stress hormone, corticosterone [37], disturbance of monoamine neurotransmitters balance [38]. The results in the current study demonstrated that CRST rapidly induced a marked decrease in body weight. Previous reports described that the hypothalamic mRNA expression of food intake-related genes such as ghrelin (an orexigenic factor which strongly stimulates food intake and increases body weight, [39]) and pro-opiomelanocortin [POMC] (act anorexigenically by producing and releasing α-MSH, a peptide that activates melanocortin-3, -4 receptors and inhibits food intake [40]) showed a significant decrease and increase in mice undergoing CRST, respectively [41] which may also be the consistent fact findings of the current study. A previous report also showed that the rapid CRST-induced reduction in body weight did not recover, even after the removal of stress [35]. In the current study, however, 0.5% GORZ may act in opposition to the mechanism of lower body weight and partially reverse the CRST-induced body weight loss.

The significant increase in serum CORT levels indicated a positive correlation of CRST-induced anxiety, which was almost unaffected by 0.5% GORZ. The data presented here supported the idea that the increased serum CORT levels could be attributed to a continuous state of



(caption on next page)

Fig. 6. Effect of 0.5% γ-oryzanol (GORZ) on the levels of monoamine neurotransmitters (i.e., 5-hydroxy tryptamine [5-HT] and norepinephrine [NE]) and their metabolites (i.e., 5-hydroxyindole acetic acid [5-HIAA] and 3-methoxy-4-hydroxyphenylglycol [MHPG]), in the hippocampus (**A–D**, respectively), cerebral cortex (**E–H**, respectively), and amygdala (**I–L**, respectively). All values are expressed as mean ± standard error (SE); n = 8 mice per group. Statistical differences were evaluated using two-way analysis of variance (ANOVA) with Sidak test (+, $P < 0.05$).

stress, which may stimulate the catabolism of skeletal muscle proteins, leading to a loss of body weight [42]. Our data revealed increased level of serum CORT associated with CRST-induced stress. However, in the present study, GORZ was implicated as a weak modulator of the stress response in the HPA axis; whereas, its anxiolytic effect was mainly mediated via upregulation of the central monoaminergic system in the amygdala of the brain. Thus, GORZ may have anti-stress and anti-anxiety effects which are independent of CORT activity.

It has already been proven that chronic stress increases anxiety-like behavior [43,44]. The most widely used assays to assess anxiety-related behavior, include the OFT and EPM tests [45]. In the current study, mice that were fed 0.5% GORZ showed a decrease in anxiety-like behaviors, such as the significant increase in the number of entries into the center zone in the OFT. Additionally, these mice also displayed a similar trend for the EPM test after CRST, where they showed a significant increase in the time spent (s) and the distance travelled (m) in the open arm. Moreover, for the EPM test, mice that were administered 0.5% GORZ demonstrated a significant reduction in the time (s) spent in the closed arm, even when they underwent CRST. Therefore, the results of the OFT and EPM test together supported the idea that although the CRST caused mice to enter an anxious emotional state, 0.5% GORZ produced a measurable anxiolytic effect.

We also conducted an experiment using 5 week old ICR male mice (n = 4) to check whether GORZ had any alteration effect on locomotor activity or not. With the maintenance of 12 h LD cycle, GORZ were administered orally (0.05 ml/10 g B W) at ZT0 and the locomotion activity of mice was monitored for 2 days onward with a model SE-10 infrared radiation sensor (Akizuki Denshi Tsusho Co. Ltd., Tokyo, Japan) and analyzed using CLOCKLAB software (Actimetrics, Wilmette, IL, USA). No significant change of the total amount of locomotor activity was observed in control (339.63 ± 0.95) and GORZ (248.74 ± 0.67) group mice. Here, the results also supported the real anxiolytic effect of GORZ.

There are many hypotheses about the pathogenesis of stress related disorders, including the monoamine dysfunction hypothesis. Furthermore, the improvement of symptoms following treatment with SSRIs (Selective Serotonin Reuptake Inhibitors) has been one explanation supporting the role of serotonergic dysfunction in anxiety [46]. Based on these evidences, the role of monoamines in the anxiolytic-like effects of 0.5% GORZ was elucidated. The findings of the present study are consistent with previous reports on the implications of the central monoaminergic system, including 5-HT and NE, in the pathophysiology and therapeutic measures for emotional disorders [47,48]. In addition, the synthesis and release of neurotransmitters in the hippocampus and cerebral cortex are closely related to those during neuropsychiatric disorders [49]. Because the amygdala exerts a direct influence on stimuli [50], we focused on its monoaminergic response. Although we suggest that changes in monoamines demonstrate an anxiolytic effect of GORZ treatment, current data show only correlations but no direct causation. Therefore, further experiments using mouse models having monoamine impairments or in mice with adrenal deficits will provide further insights.

The present experimental data showed that pretreatment with 0.5% GORZ could effectively improve the levels of 5-HIAA and NE in the amygdala of brain after CRST. In addition, the enhancement of MHPG level in the amygdala of 0.5% GORZ treated mice also supported its anxiolytic mechanism without stress. In human society, anxiety is highly prevalent among young people, and rice is one of the most important staple foods. Therefore, to model the anxiety-related problems in young (school age) people, we used 5-week-old mice in the current study. However, future studies using similar experimental approaches in adult and old mice to confirm the present results will be beneficial.

The findings of this study suggest that 0.5% GORZ effectively reduces anxiety-like behaviors, and activation of serotonergic and norepinephrinergic systems plays a key role in mediating the antianxiety-like responses.

5. Conclusion

In summary, the present study indicated that 0.5% GORZ exerted a therapeutic effect on CRST evoked anxiety that is accompanied by (or associated with) **i)** the partial recovery of CRST induced body weight loss, **ii)** subsequent potential improvement of the main behavioral parameters in the OFT and EPM test, **iii)** slight amelioration of excessive levels of serum CORT, and **iv)** enhancement of central monoamine neurotransmitters in the amygdala of the brain. Thus, GORZ can potentially be used as a novel therapy for anxiety-related disorders. However, further research is needed to clarify the exact molecular mechanisms underlying its effects and to better understand the neuropathological changes in anxiety.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2018.11.042.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was partially supported by the Council for Science, Technology, and Innovation and the cross- ministerial Strategic Innovation Promotion Program (SIP).

Ethics approval

The Committee for Animal Experimentation of the School of Science and Engineering at Waseda University (permission # 2017-A074) approved the current animal experimental protocol.

Authors' contributions

S.A. and S.S. designed the research, analyzed the data, and wrote the manuscript. S.S. also supervised the project. S.A. performed the experiments. K.R.U. performed in mouse food preparation, behavioral tests and HPLC-ECD. H.S., Y.I., and H.M. performed the sampling.

References

[1] A. Baum, Stress, intrusive imagery, and chronic distress, Health Psychol. 9 (1990) 653–675.
[2] L. Mah, C. Szabuniewicz, A.J. Fiocco, Can anxiety damage the brain? Curr. Opin. Psychiatry 29 (2016) 56–63, https://doi.org/10.1097/yco.0000000000000223.
[3] R.C. Kessler, K.A. McGonagle, S. Zhao, C.B. Nelson, M. Hughes, S. Eshleman, H.U. Wittchen, K.S. Kendler, Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey, Arch. Gen. Psychiatry 51 (1994) 8–19.
[4] M. Fava, I. Hwang, A.J. Rush, N. Sampson, E.E. Walters, R.C. Kessler, The importance of irritability as a symptom of major depressive disorder: results from the National Comorbidity Survey Replication, Mol. Psychiatry 15 (2010) 856–867, https://doi.org/10.1038/mp.2009.20.
[5] R.C. Kessler, W.T. Chiu, O. Demler, K.R. Merikangas, E.E. Walters, Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication, Arch. Gen. Psychiatry 62 (2005) 617–627, https://doi.org/10.1001/archpsyc.62.6.617.

[6] R.C. Kessler, P. Berglund, O. Demler, R. Jin, K.R. Merikangas, E.E. Walters, Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication, Arch. Gen. Psychiatry 62 (2005) 593–602, https://doi.org/10.1001/archpsyc.62.6.593.

[7] C.P. McLean, A. Asnaani, B.T. Litz, S.G. Hofmann, Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness, J. Psychiatr. Res. 45 (2011) 1027–1035, https://doi.org/10.1016/j.jpsychires.2011.03.006.

[8] M. Sugano, E. Tsuji, Rice bran oil and cholesterol metabolism, J. Nutr. 127 (1997) 521s–524s.

[9] V. Van Hoed, J. Vila Ayala, M. Czarnowska, W. De Greyt, R. Verhé, Erratum to: optimization of physical refining to produce rice bran oil with light color and high oryzanol content, J. Am. Oil Chem. Soc. 88 (2011) 1083, https://doi.org/10.1007/s11746-011-1766-3.

[10] K. Tyagi, M.A. Ansari, S. Tyagi, A. Tyagi, A novel process for physically refining rice bran oil through degumming, Adv. Appl. Sci. Res. 3 (2012) 5.

[11] Z. Xu, J.S. Godber, Purification and identification of components of gamma-oryzanol in rice bran oil, J. Agric. Food Chem. 47 (1999) 2724–2728.

[12] A.L. Gerhardt, N.B. Gallo, Full-fat rice bran and oat bran similarly reduce hypercholesterolemia in humans, J. Nutr. 128 (1998) 865–869.

[13] H. Kai, H. Ikeda, H. Yasukawa, M. Kai, Y. Seki, F. Kuwahara, T. Ueno, K. Sugi, T. Imaizumi, Peripheral blood levels of matrix metalloproteases-2 and -9 are elevated in patients with acute coronary syndromes, J. Am. Coll. Cardiol. 32 (1998) 368–372.

[14] C. Chotimarkorn, H. Ushio, The effect of trans-ferulic acid and gamma-oryzanol on ethanol-induced liver injury in C57BL mouse, Phytomedicine 15 (2008) 951–958, https://doi.org/10.1016/j.phymed.2008.02.014.

[15] G.S. Seetharamaiah, T.P. Krishnakantha, N. Chandrasekhara, Influence of oryzanol on platelet aggregation in rats, J. Nutr. Sci. Vitaminol. 36 (1990) 291–297.

[16] S. Nakayama, A. Manabe, J. Suzuki, K. Sakamoto, T. Inagaki, Comparative effects of two forms of gamma-oryzanol in different sterol compositions on hyperlipidemia induced by cholesterol diet in rats, Jpn. J. Pharmacol. 44 (1987) 135–143.

[17] Y. Murase, H. Iishima, Clinical studies of oral administration of gamma-oryzanol on climacteric complaints and its syndrome, Obstet Gynecol Prac. 12 (1963) 3.

[18] K.A. Szczesniak, P. Ostaszewski, A. Ciecierska, T. Sadkowski, Investigation of nutriactive phytochemical - gamma-oryzanol in experimental animal models, J. Anim. Physiol. Anim. Nutr. 100 (2016) 601–617, https://doi.org/10.1111/jpn.12428.

[19] J.-P. Konsman, G. Paxinos, K.B.J. Franklin (Eds.), The Mouse Brain in Stereotaxic Coordinates: Second Edition (Deluxe), Academic Press, New York, 2001(ISBN 0-12-547637-X2003).

[20] S. Moriya, Y. Tahara, H. Sasaki, J. Ishigooka, S. Shibata, Phase-delay in the light-dark cycle impairs clock gene expression and levels of serotonin, norepinephrine, and their metabolites in the mouse hippocampus and amygdala, Sleep Med. 16 (2015) 1352–1359, https://doi.org/10.1016/j.sleep.2015.06.020.

[21] H. Sasaki, Y. Hattori, Y. Ikeda, M. Kamagata, S. Iwami, S. Yasuda, Y. Tahara, S. Shibata, Forced rather than voluntary exercise entrains peripheral clocks via a corticosterone/noradrenaline increase in PER2::LUC mice, Sci. Rep. 6 (2016) 27607, , https://doi.org/10.1038/srep27607.

[22] A. Gregus, A.J. Wintink, A.C. Davis, L.E. Kalynchuk, Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats, Behav. Brain Res. 156 (2005) 105–114, https://doi.org/10.1016/j.bbr.2004.05.013.

[23] R.L. Wright, E.N. Lightner, J.S. Harman, O.C. Meijer, C.D. Conrad, Attenuating corticosterone levels on the day of memory assessment prevents chronic stress-induced impairments in spatial memory, Eur. J. Neurosci. 24 (2006) 595–605, https://doi.org/10.1111/j.1460-9568.2006.04948.x.

[24] P. Santha, S. Veszelka, Z. Hoyk, M. Meszaros, F.R. Walter, A.E. Toth, L. Kiss, A. Kincses, Z. Olah, G. Seprenyi, G. Rakhely, A. Der, M. Pakaski, J. Kalman, A. Kittel, M.A. Deli, Restraint stress-induced morphological changes at the blood-brain barrier in adult rats, Front. Mol. Neurosci. 8 (88) (2015), https://doi.org/10.3389/fnmol.2015.00088.

[25] C.S. North, A.M. Suris, R.P. Smith, R.V. King, The evolution of PTSD criteria across editions of DSM, Ann. Clin. Psychiatry 28 (2016) 197–208.

[26] T. Buynitsky, D.I. Mostofsky, Restraint stress in biobehavioral research: recent developments, Neurosci. Biobehav. Rev. 33 (2009) 1089–1098, https://doi.org/10.1016/j.neubiorev.2009.05.004.

[27] K.P. Wilder Schaaf, L.K. Artman, M.A. Peberdy, W.C. Walker, J.P. Ornato, M.R. Gossip, J.S. Kreutzer, Anxiety, depression, and PTSD following cardiac arrest: a systematic review of the literature, Resuscitation 84 (2013) 873–877, https://doi.org/10.1016/j.resuscitation.2012.11.021.

[28] A.J. Jak, L.D. Crocker, R.L. Aupperle, A. Clausen, J. Bomyea, Neurocognition in PTSD: treatment insights and implications, Curr. Top. Behav. Neurosci. (2016), https://doi.org/10.1007/7854_2016_62.

[29] L. Meng, J. Jiang, C. Jin, J. Liu, Y. Zhao, W. Wang, K. Li, Q. Gong, Trauma-specific grey matter alterations in PTSD, Sci. Rep. 6 (2016) 33748, , https://doi.org/10.1038/srep33748.

[30] J. Sheynin, I. Liberzon, Circuit dysregulation and circuit-based treatments in post-traumatic stress disorder, Neurosci. Lett. 649 (2017) 133–138, https://doi.org/10.1016/j.neulet.2016.11.014.

[31] S. Yoon, J.E. Kim, J. Hwang, I. Kang, S. Jeon, J.J. Im, B.R. Kim, S. Lee, G.H. Kim, H. Rhim, S.M. Lim, I.K. Lyoo, Recovery from posttraumatic stress requires dynamic and sequential shifts in amygdalar connectivities, Neuropsychopharmacology 42 (2017) 454–461, https://doi.org/10.1038/npp.2016.136.

[32] X. Zhu, L. Helpman, S. Papini, F. Schneier, J.C. Markowitz, P.E. Van Meter, M.A. Lindquist, T.D. Wager, Y. Neria, Altered resting state functional connectivity of fear and reward circuitry in comorbid PTSD and major depression, Depress. Anxiety 34 (2017) 641–650, https://doi.org/10.1002/da.22594.

[33] J. Laugharne, C. Kullack, C.W. Lee, T. McGuire, S. Brockman, P.D. Drummond, S. Starkstein, Amygdala volumetric change following psychotherapy for posttraumatic stress disorder, J. Neuropsychiatry Clin. Neurosci. (2016), https://doi.org/10.1176/appi.neuropsych.16010006 appineuropsych16010006.

[34] O. Marti, J. Marti, A. Armario, Effects of chronic stress on food intake in rats: influence of stressor intensity and duration of daily exposure, Physiol. Behav. 55 (1994) 747–753.

[35] R.B. Harris, J. Zhou, B.D. Youngblood, I.I. Rybkin, G.N. Smagin, D.H. Ryan, Effect of repeated stress on body weight and body composition of rats fed low- and high-fat diets, Am. J. Phys. 275 (1998) R1928–R1938.

[36] G.D. Gamaro, L.P. Manoli, I.L. Torres, R. Silveira, C. Dalmaz, Effects of chronic variate stress on feeding behavior and on monoamine levels in different rat brain structures, Neurochem. Int. 42 (2003) 107–114.

[37] A.C. Ferraz, A.M. Delattre, R.G. Almendra, M. Sonagli, C. Borges, P. Araujo, M.L. Andersen, S. Tufik, M.M. Lima, Chronic omega-3 fatty acids supplementation promotes beneficial effects on anxiety, cognitive and depressive-like behaviors in rats subjected to a restraint stress protocol, Behav. Brain Res. 219 (2011) 116–122, https://doi.org/10.1016/j.bbr.2010.12.028.

[38] M. Hamon, P. Blier, Monoamine neurocircuitry in depression and strategies for new treatments, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 45 (2013) 54–63, https://doi.org/10.1016/j.pnpbp.2013.04.009.

[39] C.B. Lawrence, A.C. Snape, F.M. Baudoin, S.M. Luckman, Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers, Endocrinology 143 (2002) 155–162, https://doi.org/10.1210/endo.143.1.8561.

[40] B. Meister, Neurotransmitters in key neurons of the hypothalamus that regulate feeding behavior and body weight, Physiol. Behav. 92 (2007) 263–271, https://doi.org/10.1016/j.physbeh.2007.05.021.

[41] J.Y. Jeong, D.H. Lee, S.S. Kang, Effects of chronic restraint stress on body weight, food intake, and hypothalamic gene expressions in mice, Endocrinol. Metab. 28 (2013) 288–296, https://doi.org/10.3803/EnM.2013.28.4.288.

[42] T. Sato, H. Yamamoto, N. Sawada, K. Nashiki, M. Tsuji, K. Muto, H. Kume, H. Sasaki, H. Arai, T. Nikawa, Y. Taketani, E. Takeda, Restraint stress alters the duodenal expression of genes important for lipid metabolism in rat, Toxicology 227 (2006) 248–261, https://doi.org/10.1016/j.tox.2006.08.009.

[43] M.C. Pardon, G.G. Gould, A. Garcia, L. Phillips, M.C. Cook, S.A. Miller, P.A. Mason, D.A. Morilak, Stress reactivity of the brain noradrenergic system in three rat strains differing in their neuroendocrine and behavioral responses to stress: implications for susceptibility to stress-related neuropsychiatric disorders, Neuroscience 115 (2002) 229–242.

[44] T. Strekalova, R. Spanagel, O. Dolgov, D. Bartsch, Stress-induced hyperlocomotion as a confounding factor in anxiety and depression models in mice, Behav. Pharmacol. 16 (2005) 171–180.

[45] A.A. Lau, A.C. Crawley, J.J. Hopwood, K.M. Hemsley, Open field locomotor activity and anxiety-related behaviors in mucopolysaccharidosis type IIIA mice, Behav. Brain Res. 191 (2008) 130–136, https://doi.org/10.1016/j.bbr.2008.03.024.

[46] A.M. Wehry, K. Beesdo-Baum, M.M. Hennelly, S.D. Connolly, J.R. Strawn, Assessment and treatment of anxiety disorders in children and adolescents, Curr. Psychiatry Rep. 17 (2015) 52, https://doi.org/10.1007/s11920-015-0591-z.

[47] C.O. Bondi, J.D. Jett, D.A. Morilak, Beneficial effects of desipramine on cognitive function of chronically stressed rats are mediated by alpha1-adrenergic receptors in medial prefrontal cortex, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 34 (2010) 913–923, https://doi.org/10.1016/j.pnpbp.2010.04.016.

[48] A.F. Arnsten, B.M. Li, Neurobiology of executive functions: catecholamine influences on prefrontal cortical functions, Biol. Psychiatry 57 (2005) 1377–1384, https://doi.org/10.1016/j.biopsych.2004.08.019.

[49] V.N. Thakare, V.D. Dhakane, B.M. Patel, Potential antidepressant-like activity of silymarin in the acute restraint stress in mice: modulation of corticosterone and oxidative stress response in cerebral cortex and hippocampus, Pharmacol. Rep. 68 (2016) 1020–1027, https://doi.org/10.1016/j.pharep.2016.06.002.

[50] E.J. Nestler, M. Barrot, R.J. DiLeone, A.J. Eisch, S.J. Gold, L.M. Monteggia, Neurobiology of depression, Neuron 34 (2002) 13–25.