

Effects of (–)-Sesamin on Chronic Stress-Induced Anxiety Disorders in Mice

Ting Ting Zhao^{1,2} · Keon Sung Shin¹ · Hyun Jin Park^{1,2} · Bo Ram Yi¹ ·
Kyung Eun Lee¹ · Myung Koo Lee^{1,2}

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Abstract This study investigated the effects of (–)-sesamin on chronic electric footshock (EF) stress-induced anxiety disorders in mice. Mice were treated with (–)-sesamin (25 and 50 mg/kg) orally once a day for 21 days prior to exposure to EF stress (0.6 mA, 1 s every 5 s, 3 min). Mice treated with (–)-sesamin (25 and 50 mg/kg) exhibited less severe decreases in the number of open arm entries and time spent on open arms in the elevated plus-maze test and the distance traveled in the open field test following exposure to chronic EF stress. Similarly, mice treated with (–)-sesamin exhibited significantly less severe decreases in brain levels of dopamine, norepinephrine, and serotonin following exposure to chronic EF stress. Increases in serum levels of corticosterone and expression of c-Fos were also less pronounced in mice treated with (–)-sesamin (25 and 50 mg/kg). These results suggest that (–)-sesamin may protect against the effects of chronic EF stress-induced anxiety disorders by modulating dopamine, norepinephrine, and serotonin levels, c-Fos expression, and corticosterone levels.

Keywords (–)-Sesamin · Chronic stress · Anxiety-like disorders · Dopamine and norepinephrine · Serotonin · Corticosterone · c-Fos

Introduction

Repeated or chronic exposure to stress is associated with physiological changes that occur in response to novel or threatening stimuli. Chronic stress has been linked to a number of psychiatric conditions, including anxiety disorders and depression [1].

Research has indicated that disorders of anxiety and depression secondary to chronic stress are associated with decreased levels of dopamine, norepinephrine, and serotonin in the brain [2, 3]. Chronic stress also results in an elevation of serum glucocorticoids such as corticosterone and cortisol due to the involvement of the hypothalamic–pituitary–adrenal (HPA) axis [3], which significantly influences dopamine activity [3], thereby leading to clinical levels of anxiety and depression [4]. In addition, expression of c-Fos protein, which is the product of an immediate early gene, increases in various brain regions of mice and rats exposed to chronic stress, especially in the paraventricular nuclei (PVN) of the hypothalamus [5, 6].

The PVN region plays a vital role in the synthesis of corticotropin-releasing hormone (CRH) responsible for the control of acute and chronic stress-induced HPA activation [5, 6]. The levels of dopamine, norepinephrine, and serotonin may implicate in the acute and chronic stress responses in the PVN region [7]. The changes of dopamine and serotonin lead to anxiety-like behaviors in the elevated plus-maze and open field tests [8–10].

(–)-Sesamin, which is an epimeric isomer lignans with (+)-sesamin, is a major lignan constituent of *Asiasari Radix* (*Asiasarum heterotropoides* F. Maekawa var. *mandshuricum* F. Maekawa, *Aristolochiaceae*) and has beneficial effects on nitric oxide production, and levels of cholesterol and triglycerides [11, 12]. The water extract of *A. Radix* has inhibitory effects on hyperalgesia in mice [13].

✉ Myung Koo Lee
myklee@chungbuk.ac.kr

¹ Department of Pharmacy, Chungbuk National University, Cheongju 28644, Republic of Korea

² Research Center for Bioresource and Health, College of Pharmacy, Chungbuk National University, Cheongju 28644, Republic of Korea

The methanol extract of *A. Radix* also ameliorates memory deficits in rats [14]. In addition, (–)-sesamin exerts protective effects against 6-hydroxydopamine (6-OHDA)-induced neurotoxicity in PC12 and dopaminergic neuronal cells in a rat model of Parkinson's disease (PD) [15]. (–)-Sesamin reduces anxiety-like behaviors induced by chronic pain in mice [16]. Sesamol, a lignin derivative of sesamin and a major compound of Sesame seeds, also has ameliorating effects on depression by modulating central oxidative stress and inflammation in mice [17].

The present study aims to investigate the pharmacological effects of (–)-sesamin on chronic electric footshock (EF) stress-induced anxiety as assessed using the elevated plus-maze, as well as to examine the influence of such treatment on levels of dopamine, norepinephrine, and serotonin, c-Fos expression, and corticosterone levels in mice.

Materials and Methods

Materials

(–)-Sesamin was isolated from *A. heteropoides* and identified according to a previously described protocol [18]. A voucher specimen was deposited in the herbarium of the College of Pharmacy at Chungbuk National University.

Dopamine, norepinephrine, serotonin, isoproterenol, and 5-hydroxyindoleacetic acid (HIAA) were purchased from Sigma (St. Louis, MO, USA). c-Fos antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). A corticosterone assay kit was obtained from USCN Life Sciences (E0504m, Wuhan, China). All other chemicals were of analytical grade.

Animals

Male ICR mice (weight: 20–25 g) were obtained from Samtako Co. (Osan, Korea). Mice were housed two per cage in a temperature and humidity controlled environment (12-h light/dark cycle, $23 \pm 2^\circ\text{C}$, $50 \pm 2\%$ humidity), with ad libitum access to standard mouse food and water. This study was approved by the Animal Ethics Committee of Chungbuk National University (Approval No., CBNU-728-14-01), and all experiments were performed in accordance with the guidelines of the NIH for Care and Use of Laboratory Animals and Chungbuk National University Laboratory Animal Research Center.

Experimental Design and Chronic EF Stress

Mice were randomly divided into three subsets and each subset has four groups (each group, $n = 8\text{--}10$) for determining (1) dopamine, norepinephrine, and corticosterone

levels including the elevated plus-maze test, (2) serotonin levels including the open field test, and (3) c-Fos immunohistochemical analysis. Animals in the control group received saline (0.9%). Mice in the chronic EF stress group (chronic EF stress) received saline (0.9%) and were subjected to unavoidable EF stress alone (intensity: 0.6 mA; shock duration: 1 s every 5 s; period: 3 min) once a day (at 14:00) for 21 days to induce anxiety disorders using an Electric Shock Generator (Seil Electric Co., Taejeon, Korea) [19]. Mice in the chronic EF stress plus (–)-sesamin groups received (–)-sesamin (25 or 50 mg/kg, p.o., feeding needle, length 50 mm, tip 2.0 mm, Fuchigami, Japan) once a day for 21 days [15], in addition to receiving the same EF stress [Chronic EF stress + (–)-Sesamin]. (–)-Sesamin administered 4 h prior to EF stress exposure on each testing day. On day 21, all experimental groups were subjected to elevated plus-maze testing, following which the mice were sacrificed using Zoletil 50 (100 mg/kg, i.p.; Virbac, Carros, France) in order to obtain brain tissue and serum for biochemical and immunohistochemical analyses [15, 20]. The injection of Zoletil 50 showed no influence with the brain levels of dopamine, norepinephrine, and serotonin.

The Elevated Plus-Maze Test

The elevated plus-maze test was used to evaluate the anxiety behaviors [21]. The elevated plus-maze apparatus consisted of four arms: two open arms (30×5 cm) and two closed arms of the same size, with 16-cm-high black walls elevated 45 cm above the floor. The open and closed arms were connected via a central square (5×5 cm) to form a plus sign. Following a 1-min adaptation period, the number of open arm entries and the time spent on the open arms during a 5-min test period were recorded using a video camera connected to a SMART video-tracking system (Panlab S.I., Barcelona, Spain) [21]. In addition, the distances traveled in 5 min were recorded to measure the locomotion [20].

The Open Field Test

The open field test was used to test distance traveled. The apparatus (ENV-520, Med Associates Inc., St. Albans, VT, USA) consists of one white floor and four transparent walls (30×30×15 cm). The mice were removed to experimental room at least 24 h prior to the test to adapt the novel environment. Each mouse was put in the center of the open field box and was tested for 5 min to record the distance traveled [20].

Levels of Dopamine, Norepinephrine, Serotonin, and Corticosterone

For the determination of dopamine and norepinephrine levels, whole mouse brains were homogenized in perchloric acid (300 μ l, 1 M) and isoproterenol (100 pmol, internal standard), and dopamine levels were measured using an HPLC system [2]. For the determination of serotonin levels, brain samples were homogenized in trichloroacetic acid (500 μ l, 0.3 M) and HIAA (300 pmol, internal standard), and serotonin levels were measured using an HPLC system [19].

Blood was collected from the hearts of sacrificed mice and subsequently centrifuged to obtain a serum (13,000 \times g, 15 min, 4 °C). Corticosterone levels were assessed using an enzyme-linked immunosorbent assay kit [19].

Immunohistochemistry of c-Fos

Mice were intracardially perfused with a paraformaldehyde solution (4% in 0.1 M phosphate buffered saline, pH 7.4), following which the brains were removed. Coronal section (35 μ m) at the PVN regions were processed for c-Fos immunocytochemistry using polyclonal rabbit anti-c-Fos antibody (1:500) and biotinylated goat anti-rabbit antibody (1:1000; Vector Laboratories, Burlingame, CA) according to a previously described protocol [2]. Cells positive for c-Fos in the PVN were counted using an image analysis system (Axiovision software, Carl Zeiss MicroImaging, GmbH, Jena, Germany) and microscope (\times 100 magnification) (Zeiss Axiophot, Carl Zeiss MicroImaging).

Statistical Analysis

Data were analyzed using one-way analyses of variance (ANOVA) followed by Tukey's test. All data are represented as means \pm SEM with p values <0.05 being considered statistically significant.

Results

Effects of (–)-Sesamin in the Elevated Plus-Maze Test

Following 21 days of exposure to chronic EF stress, the number of open arm entries and time spent on the open arms were decreased to 64.2% (df=19, $F=30.2$, $p<0.01$, $n=10$) and 63.0% (df=19, $F=36.5$, $p<0.01$, $n=10$), respectively, in the chronic EF stress group compared to the control group (Fig. 1a, b). However, less severe decreases in the number of open arm entries were observed in the chronic EF stress + (–)-sesamin (25 mg/kg) (76.8, df=19, $F=9.08$, $p<0.05$, $n=10$) and chronic EF

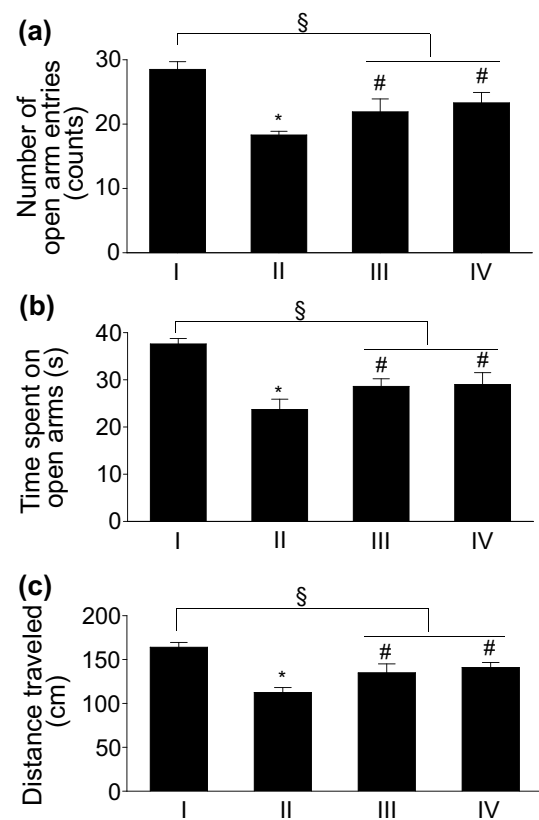


Fig. 1 Effects of (–)-sesamin on the number of open arm entries (a), the time spent on open arms (b), and distance traveled (c) in the elevated plus-maze test. Control group (I), chronic EF stress (II), chronic EF stress + (–)-sesamin (25 mg/kg) (III), chronic EF stress + (–)-sesamin (50 mg/kg) (IV). The results are expressed as the mean \pm SEM for ten animals per group. * $p<0.01$ compared to the control group; # $p<0.05$ compared to the chronic EF stress group; § $p<0.05$, compared between the control group and chronic EF stress + (–)-sesamin (25 and 50 mg/kg) groups

stress + (–)-sesamin (50 mg/kg) (81.8%, df=19, $F=15.7$, $p<0.05$, $n=10$) groups (Fig. 1a) when compared to the chronic EF stress group. Similarly, less severe decreases were also observed in the amount of time spent on open arms in both the chronic EF stress + (–)-sesamin (25 mg/kg) (76.1%, df=19, $F=4.65$, $p<0.05$, $n=10$) and chronic EF stress + (–)-sesamin (50 mg/kg) (77.4%, df=19, $F=5.00$, $p<0.05$, $n=10$) groups (Fig. 1b). In addition, the number of open arm entries (df=29, $F=17.9$, $p<0.05$, $n=10$) and time spent on the open arms (df=29, $F=16.1$, $p<0.05$, $n=10$) in chronic EF stress + (–)-sesamin (25 and 50 mg/kg) groups were significant compared to the control group following exposure to chronic EF stress for 21 days (Fig. 1a, b).

The distance traveled in the elevated plus-maze test was significantly decreased to 68.6% (df=19, $F=45.2$, $p<0.01$, $n=10$) in the chronic EF stress group compared to the control group following 21 days of exposure to chronic

EF stress (Fig. 1c). However, the decreases of distance traveled were significantly less pronounced in the chronic EF stress + (–)-sesamin (25 mg/kg) (82.1%, $df=19$, $F=7.70$, $p<0.05$, $n=10$) and chronic EF stress + (–)-sesamin (50 mg/kg) (85.9%, $df=19$, $F=16.9$, $p<0.05$, $n=10$) groups (Fig. 1c), compared to the chronic EF stress group. The distance traveled in chronic EF stress + (–)-sesamin (25 and 50 mg/kg) groups ($df=29$, $F=15.8$, $p<0.05$, $n=10$) was also significant compared to the control group following exposure to chronic EF stress for 21 days (Fig. 1c).

Effects of (–)-Sesamin in the Open Field Test

The distance traveled in the open field test was significantly decreased to 64.4% ($df=19$, $F=48.1$, $p<0.01$, $n=10$) in the chronic EF stress group compared to the control group after 21 days of exposure to chronic EF stress (Fig. 2). However, less severe decreases in the distance traveled were observed in the chronic EF stress + (–)-sesamin (25 mg/kg) (80.2%, $df=19$, $F=11.2$, $p<0.05$, $n=10$) and chronic EF stress + (–)-sesamin (50 mg/kg) (82.2%, $df=19$, $F=12.2$, $p<0.05$, $n=10$) groups when compared to the chronic EF stress group (Fig. 2). In addition, the distance traveled in chronic EF stress + (–)-sesamin (25 and 50 mg/kg) groups ($df=29$, $F=31.3$, $p<0.01$, $n=10$) was significant compared to the control group following exposure to chronic EF stress for 21 days (Fig. 2).

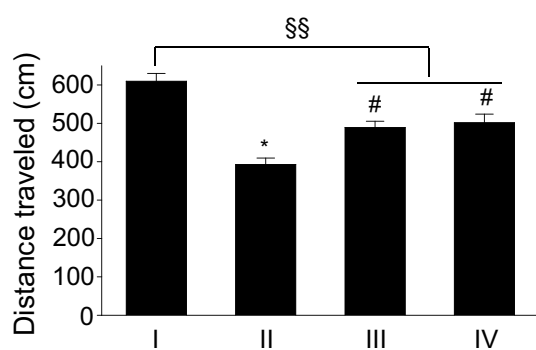


Fig. 2 Effects of (–)-sesamin on distance traveled in the open field test. Control group (I), chronic EF stress (II), chronic EF stress + (–)-sesamin (25 mg/kg) (III), chronic EF stress + (–)-sesamin (50 mg/kg) (IV). The results are expressed as the mean \pm SEM for ten animals per group. * $p<0.01$ compared to the control group; # $p<0.05$ compared to the chronic EF stress group; §§ $p<0.01$ compared between the control group and chronic EF stress + (–)-sesamin (25 and 50 mg/kg) groups

Effects of (–)-Sesamin on Levels of Dopamine, Norepinephrine, and Serotonin in the Brain

Significantly decreased levels of dopamine were observed in the chronic EF group (66.5%, $df=15$, $F=15.0$, $p<0.01$, $n=8$) relative to controls following 21 days of exposure to chronic EF stress (Fig. 3a). In the chronic EF stress + (–)-sesamin (25 mg/kg) and chronic EF stress + (–)-sesamin (50 mg/kg) groups, however, such decreases were significantly less pronounced (78.6%, $df=15$, $F=5.75$, $p<0.05$, $n=8$; 81.7%, $df=15$, $F=7.53$, $p<0.05$, $n=8$, respectively) than in the chronic EF stress group (Fig. 3a). The levels of dopamine in chronic EF stress + (–)-sesamin (25 and 50 mg/kg) groups ($df=23$, $F=5.11$, $p<0.05$, $n=8$) were also significant compared to the control group following exposure to chronic EF stress for 21 days (Fig. 3a).

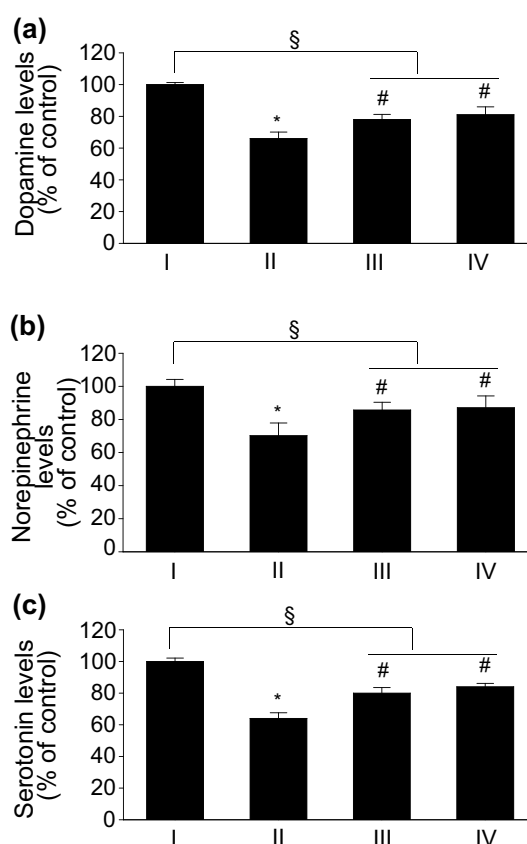


Fig. 3 Effects of (–)-sesamin on dopamine (a), norepinephrine (b), and serotonin (c) levels in the brain. Control group (I), chronic EF stress (II), chronic EF stress + (–)-sesamin (25 mg/kg) (III), chronic EF stress + (–)-sesamin (50 mg/kg) (IV). Dopamine, norepinephrine, and serotonin levels in the control group (ng/mg of tissue) were 5.1 ± 1.2 , 2.1 ± 0.7 , and 3.2 ± 1.1 , respectively. The results are expressed as the mean \pm SEM for eight animals per group. * $p<0.01$ compared to the control group; # $p<0.05$ compared to the chronic EF stress group; § $p<0.05$, compared between the control group and chronic EF stress + (–)-sesamin (25 and 50 mg/kg) groups

In addition, significantly decreased levels of norepinephrine were observed in the chronic EF group (70.2%, $df=15$, $F=8.72$, $p<0.01$, $n=8$) relative to controls following 21 days of exposure to chronic EF stress (Fig. 3b). In the chronic EF stress + (–)-sesamin (25 mg/kg) and chronic EF stress + (–)-sesamin (50 mg/kg) groups, the decreases in norepinephrine were significantly less pronounced (85.7%, $df=15$, $F=4.71$, $p<0.05$, $n=8$; 87.1%, $df=15$, $F=5.33$, $p<0.05$, $n=8$, respectively), than in the chronic EF stress group (Fig. 3b). The levels of norepinephrine in chronic EF stress + (–)-sesamin (25 and 50 mg/kg) groups ($df=23$, $F=1.63$, $p>0.05$, $n=8$) were also not significant compared to the control group, following exposure to chronic EF stress for 21 days (Fig. 3b).

Furthermore, the significant decreased levels of serotonin were observed in the chronic EF group (64.7%, $df=15$, $F=9.63$, $p<0.01$, $n=8$) relative to controls (Fig. 3c). In the chronic EF stress + (–)-sesamin (25 mg/kg) and chronic EF stress + (–)-sesamin (50 mg/kg) groups, however, such decreases were significantly less pronounced (80.5%, $df=15$, $F=5.23$, $p<0.05$, $n=8$; 84.2%, $df=15$, $F=7.91$, $p<0.05$, $n=8$, respectively) than in the chronic EF stress group (Fig. 3c). In addition, the levels of serotonin in chronic EF stress + (–)-sesamin (25 and 50 mg/kg) groups ($df=23$, $F=6.30$, $p<0.05$, $n=8$) were significant compared to the control group, following exposure to chronic EF stress for 21 days (Fig. 3c).

Effects of (–)-Sesamin on Serum Levels of Corticosterone

Corticosterone levels in the chronic EF stress group increased to 185.9% ($df=15$, $F=79.6$, $p<0.01$, $n=8$) relative to controls (Fig. 4) following exposure to chronic EF stress. However, these increases were less pronounced in the chronic EF stress + (–)-sesamin (25 mg/kg) and chronic EF stress + (–)-sesamin (50 mg/kg) groups (150.8%, $df=15$, $F=48.1$, $p<0.01$, $n=8$ and 140.6%, $df=15$, $F=58.7$, $p<0.01$, $n=8$, respectively) than in the chronic EF stress group (Fig. 4). In addition, the corticosterone levels in chronic EF stress + (–)-sesamin (25 and 50 mg/kg) groups ($df=23$, $F=40.5$, $p<0.01$, $n=8$) was significant compared to the control group following exposure to chronic EF stress for 21 days (Fig. 4).

Effects of (–)-Sesamin on c-Fos Expression in the PVN

Expression of c-Fos positive cells significantly increased to 265.3% ($df=15$, $F=95.6$, $p<0.001$, $n=8$) relative to controls in the chronic EF stress group following exposure to chronic EF stress (Fig. 5). However, these increases were less pronounced in the chronic EF stress + (–)-sesamin (25 mg/kg) (173.6%, $df=15$, $F=45.1$, $p<0.01$, $n=8$)

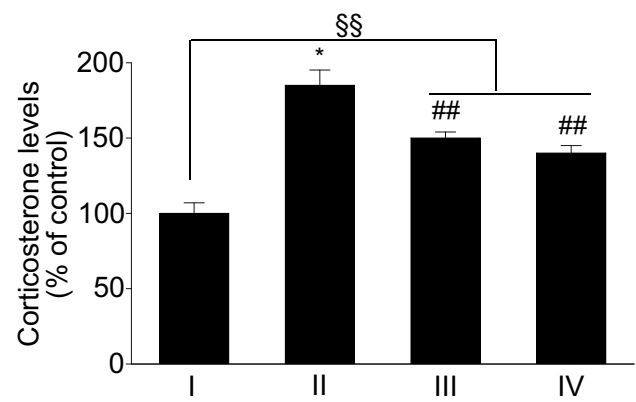


Fig. 4 Effects of (–)-sesamin on serum corticosterone levels. Control group (I), chronic EF stress (II), chronic EF stress + (–)-sesamin (25 mg/kg) (III), chronic EF stress + (–)-sesamin (50 mg/kg) (IV). Corticosterone levels in the control group were 196.2 ± 26.8 ng/ml. The results are expressed as the mean \pm SEM for eight animals per group. * $p<0.01$ compared to the control group; ## $p<0.01$ compared to the chronic EF stress group; §§ $p<0.01$ compared between the control group and chronic EF stress + (–)-sesamin (25 and 50 mg/kg) groups

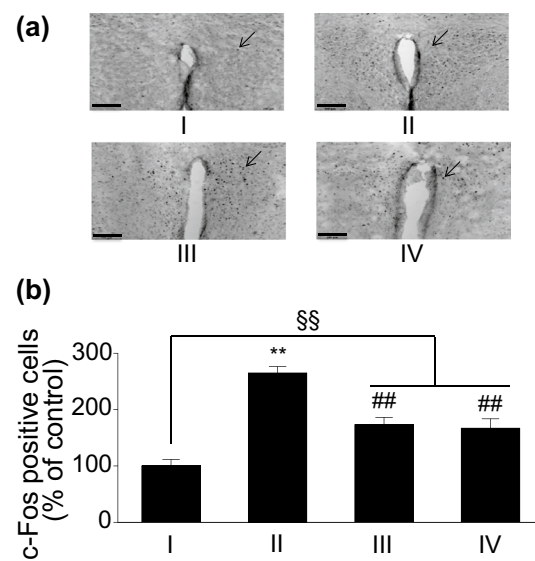


Fig. 5 Representative photographs illustrating the effects of (–)-sesamin on c-Fos positive cells in the PVN (a) and the number of c-Fos positive cells (b). Control group (I), chronic EF stress (II), chronic EF stress + (–)-sesamin (25 mg/kg) (III), chronic EF stress + (–)-sesamin (50 mg/kg) (IV). The number of c-Fos positive cells in the control group was 30 ± 6 cells per section. Arrows indicate the nuclei of c-Fos-positive neurons. Scale bar is 100 μ m. The results are expressed as the mean \pm SEM for ten animals per group. ** $p<0.001$ compared to the control group; ## $p<0.01$ compared to the chronic EF stress group; §§ $p<0.01$ compared between the control group and chronic EF stress + (–)-sesamin (25 and 50 mg/kg) groups

and chronic EF stress + (–)-sesamin (50 mg/kg) (167.9%, $df=15$, $F=51.3$, $p<0.01$, $n=8$) groups (Fig. 5). The expression of c-Fos in chronic EF stress + (–)-sesamin (25

and 50 mg/kg) groups ($df=23$, $F=46.8$, $p<0.01$, $n=8$) was also significant compared to the control group following exposure to chronic EF stress for 21 days (Fig. 5).

Discussion

The elevated plus-maze reflects passive avoidance of an animal in response to a potential threat and has become a very popular approach for the evaluation of anxiety behaviors by assessing the number of open arm entries as well as the time spent on open arms [21]. The distance traveled in the elevated plus-maze and open field tests is a common measure of exploratory and spontaneous locomotor activity in rodents [20–22]. The elevated plus-maze test is closely associated with dopamine and serotonin activities [9, 10, 23], and the open field test is also associated with serotonin activity [8]. Chronic EF stress may decrease spontaneous locomotor activity in mice [2]. In this study, the effects of (–)-sesamin on chronic EF stress-induced anxiety disorders in mice were investigated. Exposure to chronic EF stress decreased the number of open arm entries of the elevated plus-maze and the spontaneous locomotor activity by distance traveled of the elevated plus-maze and open field tests. However, these decreases were less severe in the chronic EF stress + (–)-sesamin (25 and 50 mg/kg) groups than in the chronic EF stress group (Figs. 1, 2). In addition, the decreased levels of dopamine, norepinephrine, and serotonin by chronic stress in the brain were recovered by (–)-sesamin treatment (Fig. 3a–c). These results indicate that (–)-sesamin can ameliorate anxiety-like behaviors induced by chronic EF stress.

Under stressed conditions, changes in dopamine, norepinephrine, and serotonin are associated with behavioral disturbances including anxiety, and learning and memory [2, 20]. Chronic EF stress may induce anxiety disorders, which are closely linked to decreased levels of dopamine, norepinephrine, and serotonin in the brain [3]. In this experiment, treatment with (–)-sesamin (25 and 50 mg/kg) significantly attenuated decreases in levels of dopamine, norepinephrine, and serotonin following exposure to chronic stress (Fig. 3a–c).

Corticosteroids such as corticosterone are secreted from the adrenal glands in response to chronic stress, a process mediated by the HPA axis [4]. In chronic stress-induced anxiety-like states, increased levels of corticosterone relative to unstressed states are maintained [3]. However, levels of corticosterone associated with chronic stress may be lower than those induced by acute stress [2, 24], though persistently elevated corticosteroid levels may influence dopamine and serotonin levels [3]. The levels of dopamine, norepinephrine, and serotonin in the PVN are also enhanced by corticosterone levels, which are associated

with the release of CRH [7]. Therefore, the levels of dopamine, norepinephrine, and serotonin may be implicated in the acute and chronic stress responses in the PVN [7]. In addition, corticosterone levels have high correlation with elevated plus-maze [25] and chronic corticosterone treatment increases anxiety-like behaviors in the elevated plus maze and open field tests in rats [26]. Valtrate isolated from *Valeriana jatamansi* may also reduce both anxiety-like behaviors in the elevated plus-maze and open field tests and concurrently corticosterone levels in the serum in rats [27].

In present study, treatment with (–)-sesamin (25 and 50 mg/kg) significantly decreased the serum levels of corticosterone, which were increased due to chronic stress exposure (Fig. 4), and concurrently recovered the decreased levels of dopamine, norepinephrine, and serotonin (Fig. 3). (–)-Sesamin (25 and 50 mg/kg) also recovered decreased in the number of open arm entries and spontaneous locomotor activities in the elevated plus-maze and open field tests (Figs. 1, 2). These results showed that (–)-sesamin has anxiolytic effects by modulating corticosterone levels.

In addition, c-Fos in the PVN is an immediate early gene linked with glucocorticoid regulation whose activation is induced following exposure to chronic and acute stresses in mice and rats [5, 6]. The PVN is essential for the regulation of HPA axis hormone secretion, which may ultimately influence levels of corticosterone [28]. In this study, the increased expression of c-Fos protein following exposure to chronic EF stress was significantly less pronounced in animals treated with (–)-sesamin (25 and 50 mg/kg) (Fig. 4).

(–)-Sesamin has an inhibitory effect on LPS-induced nitric oxide production in BV-2 microglial cells, which is associated with oxidative stress and inflammation [8, 29] and exerts protective effects against 6-OHDA-induced oxidative cell death in 6-OHDA-lesioned rat models of PD during long-term L-DOPA treatment [15]. (–)-Sesamin also reduces anxiety-like behaviors induced by chronic pain via regulating the GABAergic and glutamatergic transmission in the amygdala of mice [16]. In addition, (+)-sesamin possesses reactive oxygen species scavenging activity, allowing it to modulate the ERK1/2, p38MAPK, and JNK1/2 pathways in PC12 cells [30, 31], and can scavenge free radicals and superoxide anions [32]. With respect to these studies, our findings suggest that the protective functions of (–)-sesamin against oxidative stress-induced cell death may play a role in producing the observed anxiolytic effects on chronic stress-induced anxiety-like disorders in the mouse model.

In conclusion, the present study indicate that (–)-sesamin attenuates the behavioral effects of chronic EF stress-induced anxiety disorders by modulating levels of dopamine, norepinephrine, and serotonin as well as c-Fos expression and corticosterone levels. These results suggest that (–)-sesamin may serve as a phytonutrient for the

prevention of chronic stress-induced anxiety disorders, though further clinical evaluation is required.

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Compliance with Ethical Standards

Conflict of interest There are no conflict of interest.

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