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Original Article

Silymarin Potentiates the Effect of Buspirone on Anxiety and Depressive-Like Behaviors Induced by Chronic Restraint Stress in Male Mice

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Abstract

Background: Stress and its complications such as anxiety and depression continue to be regarded as health issues in human societies. Therefore, an effective treatment strategy is highly valued for dealing with stress and related disorders. In spite of studies suggesting the neuroprotective effects of silymarin and buspirone alone, the combined effects of these treatments on chronic stress have not been elucidated yet. Thus, this study aimed to investigate the effect of silymarin and buspirone (alone or in combination) on anxiety and depressive-like behaviors and to evaluate corticosterone levels in a mice model of chronic restraint stress (RS).

Methods: This study was conducted on seventy-two male BALB/c mice which were allocated in six equal groups. The animals were exposed to chronic RS (2 hours/day for 14 days) to induce a depressive-like model. An elevated plus maze (EPM) was performed to assess anxiety, while a tail suspension test (TST) was implemented to evaluate depressive-like behavior. Furthermore, the serum levels of corticosterone were measured by the enzyme-linked immunosorbent assay method.

Results: Our data demonstrated that exposure to RS resulted in prolonged immobility in the TST and reduced time spent in the open arms of the EPM test. Buspirone perorally (5 mg/kg, PO) alone and combined with silymarin (200 mg/kg, PO) increased time spent in the open arms of the EPM apparatus while attenuating immobility time in TST. There was a significant decrease in the blood corticosterone level of buspirone and silymarin co-treated animals.

Conclusion: These findings indicated that silymarin potentiates the beneficial effect of buspirone against chronic RS-induced anxiety and depressive-like behaviors in mice by lowering corticosterone serum levels. In this regard, further investigations should be undertaken to clarify the exact mechanism of the observed effects.

Keywords: Silymarin, Buspirone, Anxiety, Depression, Restraint stress

Introduction

Stress as a negative stimulus affects homeostasis, the natural regulation of living organisms, physiological responses, and adaptation.[1] Nowadays, modernization, industrialization, and changes in human lifestyle have created a particular situation that is the source of various stressors.[2] However, enduring stressors may produce chronic stress, which leads to changes in brain structure.[3] In addition, it reduces the volume of various brain areas, including the hippocampus, amygdala, and frontal cortex (areas with the highest glucocorticoid [GC] receptors and represent the most elevated stress response), which can cause reversible cognition and spatial memory impairment.[4] It has been demonstrated that prolonged exposure to stressors causes the activation of the hypothalamus-hypophyseal-adrenal (HPA) axis, a neuroendocrine pathway consisting of the hypothalamus, pituitary, and adrenal glands.[1] The end-product of the HPA axis is a GC secreting from adrenal glands.[5] GC has a prominent role in stress-related homeostasis, behavior, and cognition.[6,7] Further, elevated GC levels increase the production of free radicals and reactive oxygen species.[8,9]

Clinical and experimental research has demonstrated that stress is associated with increased psychiatric disorders such as depression and anxiety.[10-12] The most debilitating complication of stress is depression, which affects the body's overall function, including the cardiovascular,[13,14] gastrointestinal,[15] immune,[16] and brain structure and function.[9,17] In addition, depression is a profound public health concern with various physical ailments, reduced social functioning, a significant cause of disability, and increased mortality. It imposes a heavy burden on communities.[18,19]

Benzodiazepine drugs have been a choice therapy for anxiety symptoms for several decades. These drugs have well-known side effects such as drowsiness, lethargy, cognitive and motor impairment, and depression in some patients despite the effective anxiolytic impact. Hence,

benzodiazepines can be replaced by nonbenzodiazepine drugs such as buspirone without nervous system side effects.[20,21] *Buspirone* is a partial agonist of the 5-HT1A receptor prescribed to treat patients with generalized anxiety disorders and common anxiety behaviors in adults and generally in people who experience anxiety and depression at different ages.[22]

Mary thistle (*Silybum marianum* L. Gaernt.) is a medicinal herb that has been used for hepatoprotective, anti-inflammatory, and antioxidant properties.[23] Recent research has investigated the potential of silymarin in the central nervous system disorders such as Alzheimer's disease, Parkinson's disease, anxiety, and the treatment of depression by influence on catecholamine neurotransmitter systems such as serotonin, dopamine, and noradrenergic.[24-29] Silymarin extract can prevent apoptosis due to oxidative stress and delayed neuronal death in hippocampal neurons.[24,30] Recent studies on different types of stress illustrated that silymarin has beneficial effects on cognitive deficits, anxiety, and depression in long-term treatment.[31,32] Numerous studies revealed that chronic restraint stress (RS) is one of the standard ways to induce depressive-like behaviors, the consequences of chronic stress in rodents such as mice.[2,33] Therefore, the current study was conducted to investigate whether a combination of silymarin with buspirone has potentiating effects against anxiety and depression-like behaviors and regulation of corticosterone levels in RS-subjected mice.

Materials and Methods

Animals

Male BALB/c mice (8-10 weeks old, 28-32 g body weight) were obtained from the animal center of Tabriz University of Medical Sciences. The animals were housed in a temperature-controlled room, with 60%-65% relative humidity, a 12/12-hour dark/light cycle, and had free access to rodent diet and tap water.

Drugs Administration

After a week of environmental adaptation, the mice were randomly divided into six experimental groups of 12, including a control group that received normal saline (NS) perorally (PO) for 14 days. The other five groups underwent RS and subsequently received 14 days of treatment PO as RS + NS (10 mL/kg), RS + Bus (5 mg/kg, buspirone), RS + Sil (100 mg/kg, silymarin), RS + Sil (200 mg/kg), and RS + Bus (5 mg/kg, buspirone) + Sil (200 mg/kg). All drug solutions were prepared freshly on the day of experimentation by dissolving in NS. The determination of buspirone and silymarin dose in this study was based on previous studies, indicating that they have beneficial effects on reducing stress complications using other induction models.[24,34,35]

Chronic Restraint Stress

The animals were exposed to chronic RS by being placed in 50 mL-plastic tubes with 12 holes to keep airflow for two hours (10-12 am) once daily for 14 consecutive days. Control mice were kept in their home cages in the animal room, except for the daily handling and NS administration.

Elevated Plus Maze

The anxiety-like behaviors were measured using the elevated plus maze (EPM) test. The maze consisted of four arms (10 cm wide and 50 cm long); two opened opposite arms with 1 cm walls and two closed opposite arms are surrounded by a transverse wall 40 cm in height. These four arms form a square intersection (10 cm wide and 10 cm long), and the apparatus is elevated to a height of 55 cm above the floor. A mouse was placed at the intersection, facing the closed arm, and moved freely in different parts of the maze for five minutes during the experiment. The number of open arms entries and the time spent in the open arms were calculated as follows:

*Percentage of time spent in the opened arm = Time spent in the open arm / Total time spent in open and closed arms *100*

*Percentage of open arm entries = The number of times entered the open arm / Total number of times entered the open and closed arms *100*

The presence of a significant increase in these two parameters indicates that anxiety was lower in the mice.[36]

Tail Suspension Test

Each mouse was suspended 50 cm above the floor using adhesive tape for 6 minutes by the tail (2 cm from the end of the tail). The immobility time was calculated at the last 4 minutes. Immobility time was defined as the lack of escape-oriented behavior.[37]

Behavioral Analysis

All behavioral data were analyzed using Noldus EthoVision™ video tracking software (Noldus, The Netherlands). After testing each mouse, the device was cleaned with 10% ethanol to remove residues and olfactory clues.

Sampling and Corticosterone Evaluation

For the biochemical test, mice were anesthetized with high doses of ketamine (90 mg/kg) and xylazine (10 mg/kg) between 10 and 12 AM. 24 hours after the end of the behavioral test. The blood samples were taken from the heart and centrifuged at 1500 × g for 10 minutes at 4 °C. According to the manufacturer's instructions, serum corticosterone levels were measured by a commercially specific enzyme-linked immunosorbent assay kit (Abnova Corporation, Walnut, CA, USA).[38]

Statistical Analysis

All data are expressed as the mean ± standard error of the mean (SEM). Statistical differences between study groups

were analyzed using the analysis of variance and Tukey statistical methods and GraphPad Prism 6.01 software. Additionally, P values < 0.05 were considered statistically significant.

Results

Effects of Co-administration of Silymarin and Buspirone on Anxiety and Depressive Behaviors

The results of the EPM test revealed that there was a significant difference in the percentage of entry (open arms entries [OAE]) and the percentage of spent time (open arms times [OAT]) in the open arms between the mice of RS and NS groups ($P < 0.001$, Figures 1A and 1B, respectively). However, treatment with buspirone (5 mg/kg, PO, $P < 0.05$) and co-administration of buspirone (5 mg/kg, PO) with silymarin (200 mg/kg, PO, $P < 0.01$) represented a significant increase in the percentage of OAE and OAT in the restraint-exposed mice.

The post hoc test showed that chronic RS significantly reduced mobility time in the tail suspension test (TST) between the control and NS groups ($P < 0.001$, Figure 1C). In contrast, in buspirone-treated mice, mobility time significantly increased compared to the RS group ($P < 0.05$). Furthermore, co-administration of silymarin (200 mg/kg, PO) with buspirone (5 mg/kg, PO) had an antidepressant-like activity, which was manifested by the shortening of the duration of immobility ($P < 0.001$).

The Effect of Co-administration of Silymarin and Buspirone on Serum Corticosterone Levels

As shown in Figure 2, the serum corticosterone level was significantly increased in the RS group compared to the control group ($P < 0.001$). Co-administration of silymarin (200 mg/kg, PO) as the effective dose with buspirone (5 mg/kg, PO) markedly decreased the corticosterone level when compared with the RS + NS group ($P < 0.001$). Furthermore, the corticosterone levels were significantly decreased in buspirone (5 mg/kg, PO) and RS-exposed mice ($P < 0.01$).

Discussion

There is growing evidence indicating an increase in combination drugs used to treat psychiatric and physical symptoms and diseases. On the other hand, herbal remedies are traditionally employed to treat diseases experimentally without having scientific information about their effects and side effects on patients.[39,40]

The present study investigated the effect of two doses of silymarin (200 and 100 mg/kg) and co-administration with buspirone (5 mg/kg) on anxiety and depression behaviors due to chronic RS in male mice. The results demonstrated that treatment with buspirone for 14 days in chronic RS mice improved anxiety and depressive-like behaviors by the increasing number of entries and spending more extended time in open arms of the EPM apparatus. Moreover, mobility time in the TST task was longer than in the RS group. The combination of buspirone with silymarin (200 mg/kg) more potentiated their protective effect, suggesting the therapeutic potential against RS-induced, anxiety, and depression-like behaviors. In addition, serum corticosterone levels remarkably decreased in combination treatment and buspirone alone in RS-subjected mice. These findings indicated the anxiolytic and anti-depressant effects of buspirone and a combination of silymarin and buspirone. However, the co-administration of these two drugs was more effective than each drug alone.

As one of the standards and accepted methods of stress induction in rodents, RS is a widely applied stress model. This method can be utilized inexpensively and quickly and does not harm the animal physically.[41] It also causes biochemical, neurophysiological, and behavioral changes in laboratory animals, and the results in the clinic can be generalized to human cases.[42] Furthermore, the findings revealed that exposure to acute or chronic RS causes anxiety and depression in rodents.[43] In the current study, movement restriction due to RS (for 14 consecutive days and 2 hours per day) could lead to anxiety and depression. This finding is in line with those of some studies, indicating that inducing RS not only causes depressive and anxiety behaviors but can also be associated with complications such as cognitive impairment and appetite.[42,44]

Buspirone is administrated to treat anxiety and

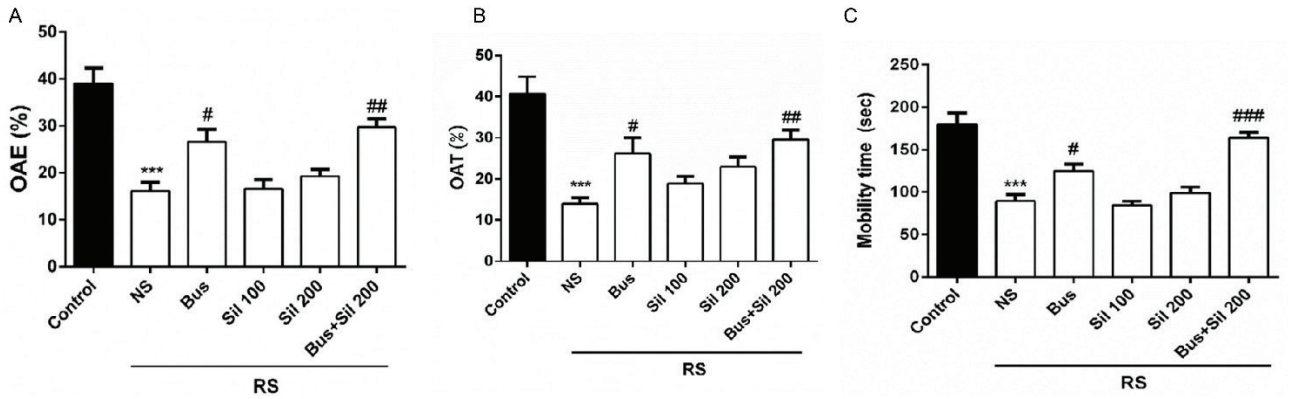

Figure 1. Effects of Co-administration of Buspirone and Silymarin on (A) the %OAE, (B) %OAT in the EPM, and (C) Immobility Time in TST. *Note*. Data are presented as the mean ± SEM (n = 12 in each group). $^{***}P < 0.001$ in comparison with control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$ in comparison with the NS group. SEM: Standard error of the mean; Bus: Buspirone, Sil: Silymarin, %OAT: Time spent in open arms; %OAE: Open arm entries, EPM: Elevated plus, TST: Tail suspension test, NS: Normal saline

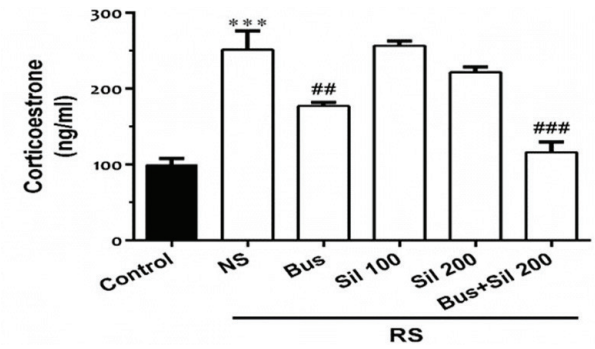

Figure 2. The Effect of Co-administration of Silymarin and Buspirone on Serum Corticosterone Levels. *Note*. Data are presented as the mean ± SEM (n = 12 in each group). $^{***}P < 0.001$ in comparison with the control group; $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ in comparison with the NS + RS group. Bus: Buspirone, Sil: Silymarin, RS: Restraint Stress, NS: Normal Saline

depression-like symptoms by the partial serotonin receptor agonist mechanism. It affects serotonin receptors located in the CA1 region of the hippocampus and cerebral cortex.[22] Animal models have shown that low doses (1 mg/kg) of this drug activate pre-synaptic autoreceptors, and high doses (2.0 mg/kg) stimulate post-synaptic receptors, thus the effects of this drug depend on the dose. It has also been indicated that high doses increase locomotion.[45,46] Some studies demonstrated that the imbalance of the serotonergic system increases anxiety; accordingly, for the treatment of anxiety symptoms, anxiolytic drugs have been used that act on the serotonin system by inhibiting serotonin reuptake and serotonin 1A receptor agonist (5-HT1AR).[32,47] Studies have documented that mice exposed to RS increased their fear. Hence, NS mice treated with serotonergic drugs moved more time in the open arm, and mobility time increased on the TST, whereas it was not observed in the NS pretreatment group.[48]

Several lines of studies in animal models have proven that silymarin (100 and 200 mg/kg) has antidepressant and neuroprotection activity. Moreover, it can pass through blood-brain barrier and affect serotonin (5-HT), dopamine, and norepinephrine receptors in the brain, increase the level of these hormones in the blood, and lessen behavioral and biochemical in ischemic rat models.[32,49] Thakare et al, in an in vivo study on acute restrained stress mice, demonstrated that after treatment with silymarin 100 and 200 mg/kg, mobility time in forced swimming test increased, and it had an antidepressant effect.[35]

The HPA axis is activated as one of the most critical neuroendocrine systems in response to stressors, leading to the release of GCs from the adrenal glands, including cortisol in humans and corticosterone in rodents.[50] Disturbances in this axis can cause disruptions in synaptic plasticity and lead to depression by exacerbating neuronal damage.[51] Previous studies have pointed out that stressful events, especially chronic RS, can cause the dysregulation of this axis by disrupting the harmful feedback activity. Hence, it leads to the overstimulation of the axis and causes an unusual increase in serum cortisol and corticosterone in humans and rodents, respectively, leading to brain damage.[52,53] The three regions of the brain such as the hippocampus, frontal cortex, and amygdala are susceptible to long-term exposure to stress, causing increased GC expression in these structures and leading to physiological changes and disorders.[54,55] In line with previous studies,[32,35,56] our result represented that exposure to chronic RS increased serum corticosterone levels in mice compared to the control group. Moreover, co-administration of buspirone and silymarin (200 mg/kg) and buspirone alone in RS-subjected mice significantly decreased corticosterone levels in the serum.

Conclusion

In general, the present findings indicated that the co-administration of silymarin and buspirone exerts a potentiating effect against biochemical and behavioral alterations induced by chronic RS stress. Nevertheless, more molecular and behavioral studies should be performed to reveal their exact antianxiety and antidepressant mechanisms.

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Project administration: Alireza Mohajjel Nayebi.
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Supervision: Alireza Mohajjel Nayebi.
Validation: Aytak Khabbaz.
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Competing Interests

The authors have no conflict of interests to disclose.

Ethical Approval

All animal experiments were performed in accordance with the guidelines for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996) and were approved by the Ethical Committee of Tabriz University of Medical Sciences (IR.TBZMED.VCR.REC.1397.318).

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