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Original Article

Protective effects of hydrogen sulfide on anxiety in ovalbumin-induced chronic asthma

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Abstract

Introduction: Comorbidity of anxiety has been reported to aggravate the control of asthma symptoms. Considering the important role of oxidative stress in the pathophysiology of asthma and anxiety, the present study evaluated whether hydrogen sulfide (H_2S) as an antioxidant agent, has anxiolytic effects in ovalbumin (OVA)-induced chronic asthma.

Methods: BALB/c mice were randomly divided into 4 groups (n=8): control, asthma, NaHS (sodium hydrosulfide, a donor of H_2S) and ascorbic acid (as a positive control). All animals except in the control group were sensitized and challenged with ovalbumin. Mice in the NaHS group, intraperitoneally received 14µmol/kg NaHS 30min before each challenge. In the ascorbic acid group, 130mg/kg ascorbic acid was given by gavage 30min before each challenge. On the day of the last challenge, animal body weight and anxiety-related behaviors were examined.

Results: Asthma caused significant decreases in the percentages of open arm entries and spending time in open arms in the elevated plus maze as well as the spending time in the light side in the light-dark transition. Also, induction of asthma resulted in a significant decrease of the animal body weight. Administration of NaHS as well as ascorbic acid, attenuated anxiety-related behaviors and improved the body weight in asthmatic mice.

Conclusion: The current study suggested that NaHS improves anxiety-related behaviors in OVA-induced asthma same as ascorbic acid, a strong antioxidant. Therefore, NaHS appears to be effective for managing the comorbidity of anxiety with asthma.

Keywords:
Asthma;
Anxiety;
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Ascorbic acid

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Introduction

Asthma is a chronic lung disease characterized by intermittent airway obstruction, bronchial hyperresponsiveness and inflammation (Barnes, 2008; Khazdair et al., 2013). Unfortunately, the prevalence, morbidity, mortality and economic burden of asthma have been increased substantially over the past several decades worldwide (Braman, 2006). According to the pathophysiology, it is indicated that oxidative stress plays a critical role in the development of asthma (Riedl and Nel, 2008).

Anxiety is one of the biggest mental health problems

over the world associated with increased use of health care services and decreased work productivity (Somers et al., 2006). The exact mechanisms underlying anxiety are not fully understood. Nonetheless, several causal factors have been suggested to contribute to its pathogenesis including oxidative stress and decrease of plasma hydrogen sulfide (H_2S) levels (Bouayed et al., 2009). Some investigators have interestingly reported a robust link between asthma and anxiety (Dudeney et al., 2017). However, little has been known about effects of asthma on brain function and behavior (Basso et al., 2003). Because the comorbidity of anxiety leads to more difficulty in the asthma control and increased use of asthma medications, management of anxiety is a vital aspect in patients with asthma (Katon et al., 2004).

Corticosteroids are among the widely used drugs in asthma therapy (National Asthma Education and Prevention Program, 2007). Nevertheless, these drugs also contribute to a variety of the adverse events, such as mood changes and diabetes mellitus (Manson et al., 2009). On the other hand, prescription of anxiolytics to asthmatic patients may result in addiction and respiratory depressant effects (Joseph et al., 1996). Therefore, finding new and novel therapeutic strategies seems to be necessary.

H_2S is a novel gaseous signaling molecule (Wesseling et al., 2015). Several evidence suggests that H_2S has a direct antioxidant effect on scavenging and inhibiting formation of reactive oxygen species (Ju et al., 2013). Given the critical role of oxidative stress and decrease of plasma H_2S levels in the development of anxiety, the current study was designed to explore the significance of sodium hydrosulfide (NaHS, a donor of H_2S) administration on anxiety indices in ovalbumin (OVA)-induced asthmatic mice.

Materials and methods

Animals

Adult male BALB/c mice (6-8 weeks old) were purchased from Department of Pharmacology, Tehran University of Medical Sciences. Before use, animals were allowed to acclimatize to the laboratory environment for one week. Mice were maintained under controlled environmental conditions (20±2°C and 12h light-dark cycle) with free access to food and water. All experimental procedures were conducted in accordance with the Animal Ethics Committee of Tehran University of Medical Sciences.

Establishment of a murine model of chronic asthma and treatment regimen

Mice were randomly divided into four groups (n=8) including control group (non-sensitized and non-challenged animals), asthma group (sensitized and challenged animals), NaHS group (sensitized and challenged animals treated with NaHS) and ascorbic acid group (sensitized and challenged animals treated with ascorbic acid, as a positive control). Except animals in the control group, others were sensitized by intraperitoneal injection of 10mg OVA (grade V; Sigma, USA) and 2mg aluminum hydroxide (Sigma, USA) on days 0 and 14. After one week, the sensitized mice were exposed to aerosolized 3% OVA in a closed Plexiglas chamber (dimensions 40×40×70cm) using a nebulizer (beurer, Germany) for 30min per day on three days a week for eight weeks (Mohammadian et al., 2016). In the NaHS group, 14µmol/kg NaHS (Sigma, USA) was intraperitoneally given 30min before each challenge (Zhang et al., 2013). Animals in the ascorbic acid group received 130mg/kg ascorbic acid (Sigma, USA) orally 30min before each challenge (Chang et al., 2009) (Fig. 1).

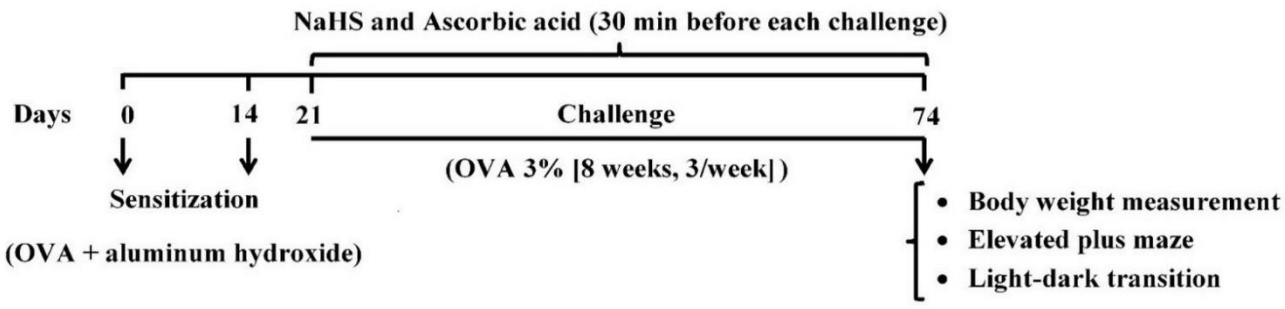

Fig.1. The experimental protocol of study. OVA: ovalbumin, NaHS: sodium hydrosulfide

Measurement of body weight

After the last challenge, animal body weight was measured using a digital electronic balance (Vibra, SJ 620 model, Japan).

Elevated plus maze

To examine anxiety-related behaviors, mice in all groups were subjected to the elevated plus maze test (Pellow and File, 1986). The maze consisted of four arms (35cm long and 5cm wide); two arms had 15-cm-high dark walls (closed arms) and two arms had 0.5-cm-high ledges (open arms). The height of the maze from floor was 50cm high. For testing, on the day of the last challenge, mice were placed individually in the center of the maze facing an open arm. The number of entries and the spending time in the open or closed arms during next 5min were recorded. After each test session, the maze was cleaned to prevent a bias based on olfactory cues.

The percentages of open arm entries (open/total entries ×100) and spending time in open arms (open/[open+ closed arm time] ×100) were calculated for each animal. Increased open arm activity (entry and time) demonstrates reduced anxiety-related behaviors. The total entries (sum of number of entries into open and closed arms) were evaluated as a locomotor activity indicator.

Light-dark transition

The light-dark transition paradigm was also used to evaluate the anxiety-related behaviors (Zuluaga et al., 2005). The apparatus included a Plexiglas box with two equal compartments (30×40×40cm), one of which with white walls and floor and illuminated by a 60-watt light from above, while another compartment was black and had a lid so it was not illuminated. Each animal was placed on the light compartment of the box. The spending time in light side was recorded over next 5min. The increased time in the light compartment indicates decreased anxiety-related behaviors. After each test session, the apparatus was cleaned.

Statistical analysis

All values are expressed as the mean±SEM. Differences between experimental groups were calculated by one-way ANOVA followed by the Tukey test. Statistical significance was accepted at $P<0.05$.

Results

Effect of NaHS on open arm activity in the elevated plus maze

There was a significant decrease in the percentage of open arm entries in the asthma group compared to the control group (0.96±0.96 vs. 41.53±3.86%, $P<0.0001$; Fig. 2A). Administration of NaHS and ascorbic acid significantly increased the percentage of open arm entries in comparison with the asthma group (30.33±1.94 vs. 0.96±0.96%, $P<0.0001$ and 21.26±9.1 vs. 0.96±0.96%, $P<0.05$, respectively; Fig. 2A).

There was a significant decrease in the percentage of the spending time in open arms in the asthma group compared to the control group (0.12±0.12 vs. 9.76±2.12%, $P<0.03$; Fig. 2B). Administration of NaHS and ascorbic acid significantly increased the percentage of the spending time in open arms in comparison with the asthma group (11.55±2.6 vs. 0.12±0.12%, $P<0.05$ and 16.55±14.54 vs. 0.12±0.12 %, $P<0.02$, respectively; Fig. 2B). There were not significant differences in total entries between animals in the groups of control, asthma, NaHS and ascorbic acid (16.7±1.01, 12.3±1.55, 16.86±0.85 and 12.57±1.91, respectively; Fig. 2C).

Effect of NaHS on the spending time in the light side in the light-dark transition

There was a significant decrease of the spending time in the light side in the asthma group compared to the control group (20.72±5.1 vs. 35.87±2.43 seconds, $P<0.05$; Fig. 3). Administration of NaHS and ascorbic acid significantly increased the spending time in the light side in comparison with the asthma group (38.41±4.8 vs. 20.72±5.1 seconds, $P<0.05$ and 39.85±0.06 vs. 20.72±5.1%, $P<0.05$, respectively; Fig. 3).

Effect of NaHS on body weight

In the beginning of the study, there were not significant differences in the body weight between animals in the groups of control, asthma, NaHS and ascorbic acid (19±1.08, 18±1.50, 18±0.55 and 19±0.91g, respectively). After the last challenge, there was a significant decrease of body weight in the asthma group compared to the control group (32±1.41 vs. 40±0.84g, $P<0.001$; Fig. 4).

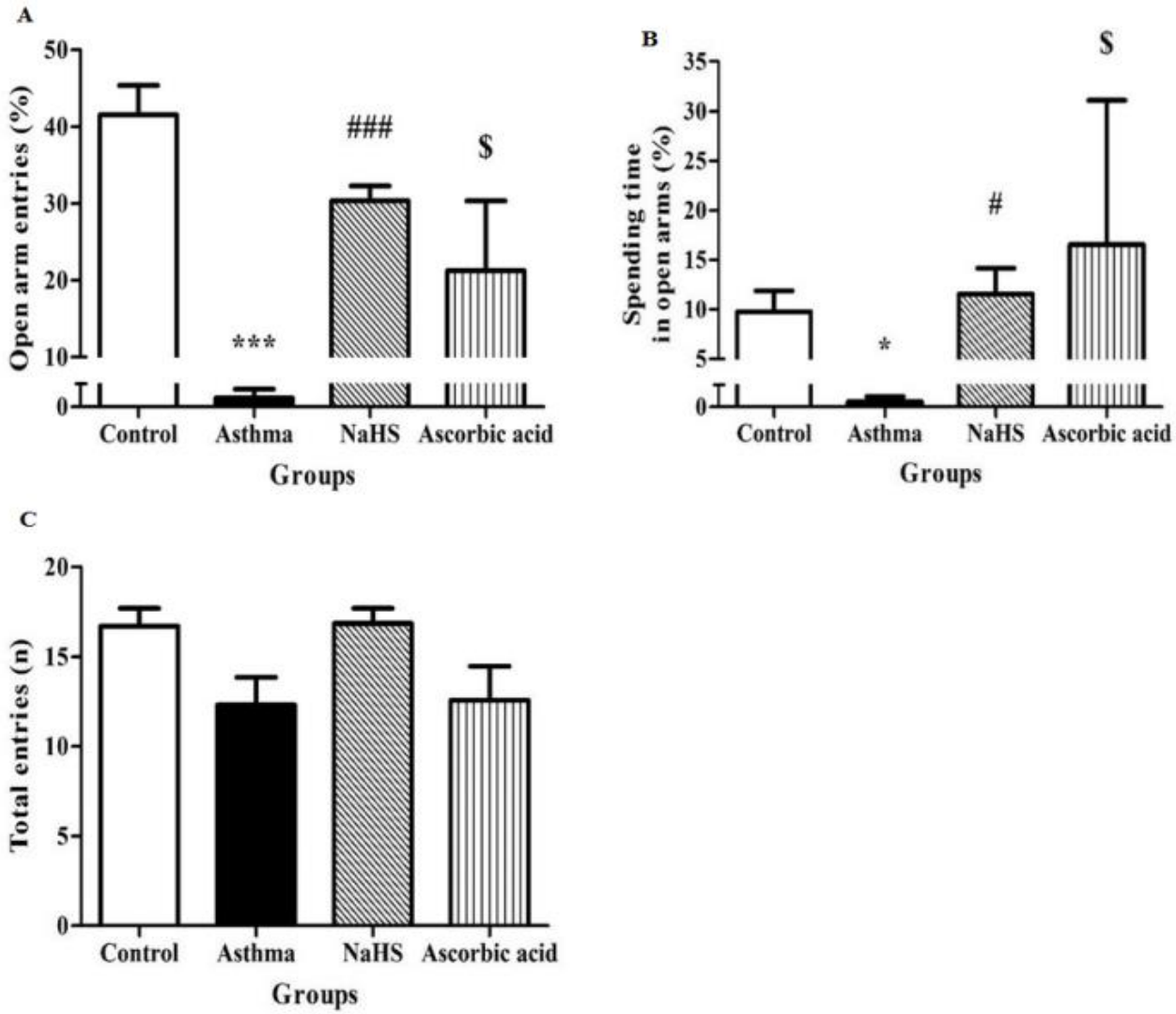

Fig.2. Changes in the percentages of open arm entries (A) and spending time in open arms (B) and total entries (C) in different groups. The data are expressed as mean±SEM. $^{*}P$<0.05 and $^{***}P$<0.001 versus the control group. $^{\#}P$<0.05 and $^{\#\#\#}P$<0.001 versus the asthma group. $^{\$}P$<0.05 versus the asthma group.

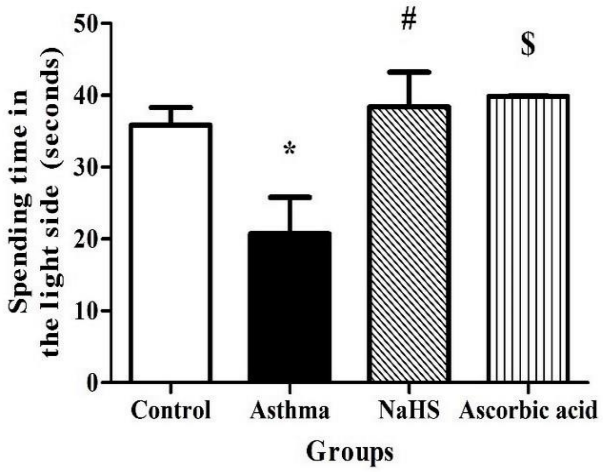

Fig.3. Changes in the spending time in the light side in different groups. Data are expressed as mean±SEM. $^{*}P$<0.05 versus the control group. $^{\#}P$<0.05 versus the asthma group. $^{\$}P$<0.05 versus the asthma group.

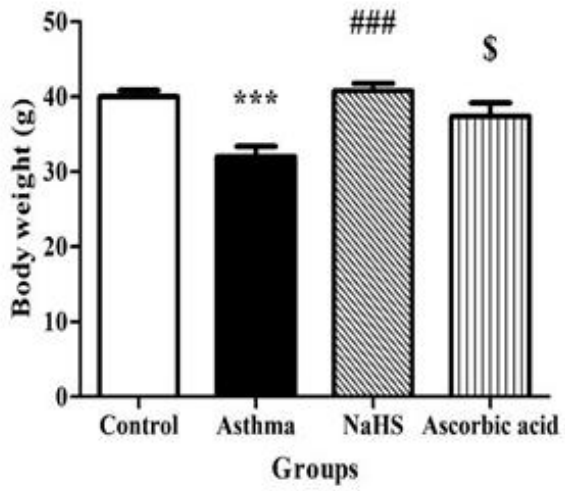

Fig.4. Changes in body weight in different groups. Data are expressed as mean±SEM. $^{***}P<0.001$ versus the control group. $^{###}P<0.001$ versus the asthma group. $^{\$}P<0.05$ versus the asthma group.

Administration of NaHS and ascorbic acid significantly increased the body weight of animals in comparison with the asthma group (40.75±1.03 vs. 32±1.41g, $P<0.001$ and 37.4±1.77 vs. 32±1.41g, $P<0.05$, respectively; Fig. 4).

Discussion

The results of the present study showed significant increases in anxiety-related behaviors with induction of asthma. In addition, it revealed that administration of NaHS and ascorbic acid significantly attenuated these behaviors. This study demonstrated that there was a considerable increase in anxiety-related behaviors in asthmatic mice. Similar to this result, Basso et al. (2003) study found that induction of asthma caused increased levels of anxiety in animals. The current study used the tests of elevated plus maze and light-dark transition as they are valuable models for evaluating anxiety-related behaviors (Pellow and File, 1986; Zuluaga et al., 2005).

The underlying molecular mechanisms linking anxiety and asthma are not fully elucidated (Thomas et al., 2011). However, in asthma disease, the balance of oxidants and antioxidants is disturbed which results in oxidative stress (Riedl and Nel, 2008). Since oxidative stress is indicated to cause anxiety, it is reasonable to suppose that asthma-induced oxidative stress may lead to this disorder (Guney et al., 2014). Therefore, administration of antioxidants appears to be effective. Since ascorbic acid is one of the most important and well-known antioxidants (Chambial et al., 2013), in this study, we decided to insert this vitamin as a positive control and evaluate its protective effects on anxiety-related behaviors in asthmatic mice. The present study showed that ascorbic acid administration significantly reduced the levels of anxiety in asthmatic mice. These protective effects may possibly be through scavenging reactive oxygen and nitrogen species and modulating the activity of γ-aminobutyric acid (GABA) receptor by conversion from the last GABA-bound closed state to the open state (Hu and Chen, 2010; Calero et al., 2011) (Fig. 5). This is in agreement with another study that supplementation with this vitamin caused a considerable decrease of anxiety levels in diabetic patients (Mazloom et al., 2013).

In the current study, the protective effects of NaHS as a potent antioxidant agent examined on anxiety indices in asthma. This study indicated that NaHS administration caused a considerable reduction of anxiety-related behaviors in asthmatic animals. Similar to this result, another study reported that administration of NaHS reduced anxiety-related behaviors in both mice and rats (Chen et al., 2013). However, the precise signaling pathways involved in mediating the protective effects of NaHS are unclear. It has been well known that NaHS prevents oxidative stress by scavenging and inhibiting formation of reactive oxygen species (Ju et al., 2013). Therefore, NaHS is recognized as an important cytoprotective agent. In addition, NaHS has non-antioxidant functions like increased mRNA and protein levels of

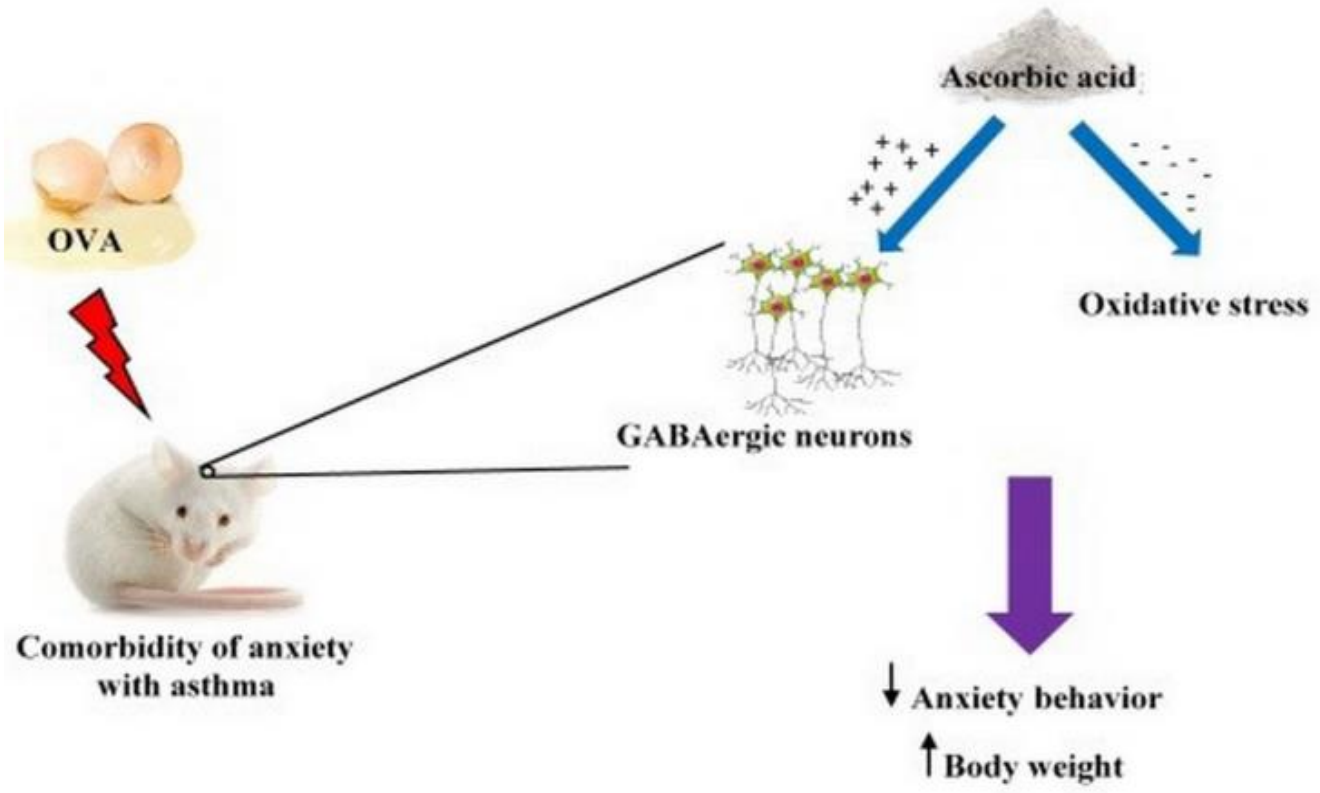

Fig.5. Possible protective effects of ascorbic acid against comorbidity of anxiety with asthma. Ascorbic acid inhibits oxidative stress and stimulates GABAergic neurons resulting in the decrease of anxiety and increase of body weight. OVA: ovalbumin, GABA: γ-aminobutyric acid.

GABA receptors (Han et al., 2005).

It was demonstrated that the comorbidity of anxiety with asthma is closely linked with clinical features and functional parameters such as reduction in body weight (Ciprandi et al., 2015). In this study, OVA-induced asthma caused a considerable decrease in animal body weight that was improved by ascorbic acid administration. This observation is in accordance with another study that ascorbic acid significantly increased body weight in an elderly population (Schorah et al., 1981). Another report found that GABAergic neurons within the hypothalamus regulate feeding behavior as well as the body weight (Meister, 2007). Since ascorbic acid has GABA agonistic activity, it is possible that its protective effects on the improvement of body weight will be through stimulating GABA receptors (Fig. 5). In this study, decreased animal body weight due to induction of asthma was improved by administration of NaHS. Since NaHS is able to increase GABA receptor levels, its protective effects on the improvement of body weight seem to be via stimulation of these receptors.

There are some limitations in the current study that might improve through mentioned suggestions in the future. Oxidative stress marker assessment in comorbidity of anxiety with asthma in addition to use of GABA agonist and antagonistic factors along with NaHS administration are proposed to understand underlying molecular mechanisms of protective effect NaHS precisely.

Conclusion

In conclusion, the present study demonstrated that NaHS attenuates anxiety-related behaviors in asthmatic mice similarly as ascorbic acid, a strong antioxidant. Thus, NaHS may be effective for managing the comorbidity of anxiety with asthma.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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