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Research article

Effect of butylphthalide on prevention and treatment of high altitude cerebral edema in rats

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ABSTRACT

3-*n*-butylphthalide (NBP) contains one of the main active ingredients of celery seed. It has a series of pharmacological mechanisms, including reconstitution of microcirculation, protection of mitochondrial function, inhibition of oxidative stress, and inhibition of neuronal apoptosis. Based on the complex multi-targeting of NBP pharmacological mechanisms, the clinical applications of NBP are increasing, and more and more clinical studies and animal experiments have focused on NBP. In this study, we used male Sprague Dawley rats as an animal model to elucidate the intervention effect of butylphthalide on high altitude cerebral edema (HACE), and also compared the effect of butylphthalide and rhodiola rosea on HACE. Firstly, we measured the changes of body weight and brain water content and observed the pathological changes of brain tissues. In addition, the contents of inflammatory factors, oxidative stress and brain neurotransmitters were assessed by enzyme-linked immunoassay kits, and finally, the expression of apoptotic proteins in brain tissues was determined by western blotting. The results showed that NBP reduced brain water content, attenuated brain tissue damage, altered inflammatory factors, oxidative stress indicators, and brain neurotransmitter levels, and in addition NBP inhibited the expression of Caspase-related apoptotic proteins. Therefore, NBP has the potential to treat and prevent HACE.

1. Introduction

Currently, millions of people are at risk of acute mountain sickness (AMS) due to rapid travel to high altitudes [1,2]. At moderate altitudes of 2000–3000 m, the prevalence of AMS in the general tourist population is 15–25%. Acute low-pressure hypoxia (AHH) occurs frequently during ascent to high altitude (3500 m) and is one of the environmental factors of AMS, high altitude cerebral edema (HACE), and high altitude pulmonary edema (HAPE) [3,4]. The main symptoms of AMS are headache, nausea, vomiting, malaise, dizziness, and sleep disturbances. HACE is considered to be the end stage of severe AMS and may be life-threatening. Although AMS has been recognized for the past two centuries, little is known about the underlying causes of these symptoms [5]. High altitude environments can cause altitude sickness and the incidence of altitude sickness is increasing as the number of people working, traveling and living at high altitude continues to grow [6,7]. High altitude illnesses include a range of diseases that can occur in individuals traveling to high altitudes, including acute mountain sickness, high altitude cerebral edema, and high altitude pulmonary

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edema [8]. High altitude cerebral edema (HACE) is a life-threatening condition caused by rapid elevation to high altitude; although the pathogenesis is unknown, the condition has a high mortality rate. Many studies have used simple low-pressure chamber decompression as a model for HACE.

Butylphthalide has pharmacological effects such as reconstitution of microcirculation, protection of mitochondrial function, inhibition of oxidative stress, and inhibition of neuronal apoptosis [2,9,10]. NBP is now increasingly used clinically in the treatment of ischemic stroke. Secondly, it has been found that NBP has corresponding therapeutic effects on some neurodegenerative diseases such as Alzheimer's disease (AD), dementia, amyotrophic lateral sclerosis, movement disorders such as Parkinson's disease, and other neurological diseases such as carbon monoxide (CO) poisoning, traumatic central nervous system injury, autoimmune diseases and seizures, but the clinical application of NBP in the above diseases is less [11]. Therefore, there is still much room for its development in clinical applications. It is due to the diverse targets of NBP that more and more researchers are focusing their attention on its animal and clinical experiments.

The most effective treatment for HACE is oxygen administration and immediate descent to a lower altitude. However, descending to altitude is not advisable due to mission constraints [12]. Therefore, dexamethasone and acetazolamide are usually effective in the prevention and treatment of HACE [13]. However, these drugs have clear contraindications or adverse effects [14]. As the number of people exposed to high altitude for work, travel, or other reasons continues to increase, there is an urgent need to investigate other safer and better medications for the treatment and prevention of HACE.

2. Materials and methods

2.1. Chemicals and regents

3-Butylphthalide (Butylphthalide, No. 518190403, Fig. 1) was purchased from Shih-Pharm Group Enbip Co Ltd., Rhodiola Capsules (Military Medicine Z2006001) was provided by the General Hospital of Tibet Military Region, Ethyl Carbamate (alias Uratan, No. P2091859) was purchased from Titan Technology Exploration Platform, Kits Superoxide Dismutase (SOD), Malondialdehyde (MDA), Glutathione peroxidase (GSH-Px) were purchased from Shanghai Enzyme Link Biotechnology Co Ltd (Shanghai, China), and the Kits Endothelin (ET-1), Vascular Endothelial growth factor (VEGF), Interleukin 1β (IL-1β), Interleukin 6 (IL-6) were purchased from Beijing Dongge Boye Biotechnology Co Ltd (Bejing, China), Nitric Oxide (NO), Thromboxane B2 (TXB2), Norepinephrine (NE), 5-hydroxytryptamine (5-HT), Dopamine (DA), Acetylcholinesterase (AchE) and Calcitonin gene-related (CGRP) peptide were purchased from Wuhan Huamei Bioengineering Co Ltd (Wuhan, China), antibody Hypoxia inducible factor-1α from Abcam (Cambridge Science Park, UK), antibody Caspase-3, and β-actin from Cell Signaling Technology (Danforth, USA), goat anti-rabbit IgG secondary antibody from Beijing BoaoSen Biotechnology Co Ltd (Beijing, China).

2.2. Animals

60 SPF grade male SD rats (6 weeks old, weighing 180–220 g) were purchased from the Animal Experiment Center of Xinjiang Medical University (Urumqi, China). Maintain rats at room temperature of 18–22 °C and relative humidity of 50%–60% for 12 h of light dark cycle and random diet. Before the experiment, allow the rats to adapt to the above conditions for at least 3 days. All experiments were approved by the Animal Ethics and Use Committee of the Xinjiang Military Region General Hospital (Urumqi, China).

2.3. Drug preparation

Butylphthalein was dissolved in 2 % Tween 80 solution to make a 15 mg mL^{-1} drug solution; Rhodiola rosea was dissolved in distilled water to make a 52.5 mg mL^{-1} solution; the animals were administered by gavage.

2.4. Experimental groups

All rats (n = 60) were randomly divided into 4 groups of 15 rats each: Normoxia group, HACE group, HACE + NBP (120 mg kg^{-1}) group [15], HACE + Sal (420 mg kg^{-1}) group [16]. Drug was administered by gavage for 7 days (4 days before HH exposure and continued for 3 days under HH conditions, once daily). The normoxic and HH groups received the same volume of distilled water containing 2% Tween 80. Rats were gavaged and given water and bedding changed daily at 10:00 a.m. at a reduced elevation of 800 m.

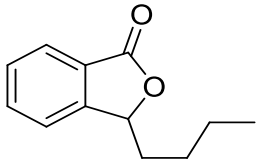

Fig. 1. Chemical structure formula of 3-butylphthalide (butylphthalide).

2.5. Establishment of rat HACE model [2,10,17]

The HACE rat model was established according to the literature. Rats were exposed to a large low-pressure hypoxic animal chamber (Fenglei, Guizhou, Xinjiang, China) at a speed of 10 m min^{-1} to simulate an environment of 6000 m (9.9 kPa, 9.8% O_2) for 72 h. The ambient temperature in the chamber fluctuated periodically from 12 °C (8:00 20:00) to 2 °C (20:00 8:00 the next day) to mimic the temperature difference between day and night. Normoxia group were housed under normal laboratory conditions (800 m above sea level; temperature 25 ± 2 °C) and had free access to standard rodent food and water.

2.6. Hematological analysis of rats

Rats (n = 8) were anesthetized with 10% uratan (5 mL kg^{-1}), fixed in supine position on a small animal operating table, the abdominal cavity was opened, the inferior vena cava was isolated, and 2 mL of blood was collected from the inferior vena cava of rats with a disposable venous blood collection needle and a disposable vacuum blood collection tube (purple tube), and all samples were analyzed within 4 h using a calibrated hematology analyzer (BC-2800Vet, Shenzhen Merry).

2.7. Determination of brain water content (BWC) in rats

Rats (n = 8) were anesthetized with 10% uratan (5 ml kg^{-1}) and executed with cervical dislocation. Brain tissue was immediately collected and divided into two hemispheres. The right hemisphere was isolated from hippocampal tissue and stored at −80 °C until further analysis. The left hemisphere was quickly weighed using a precision electronic balance and then placed in an electric oven at 80 °C for 72 h to achieve a constant weight. The BWC was then calculated as follows: Brain water content (%) = (wet weight - dry weight)/wet weight × 100%.

2.8. HE staining analysis

Whole brains of rats were removed and then fixed at 4 °C with 10 % neutral formaldehyde. After 48 h of fixation, the brains were dehydrated in ethanol and paraffin-embedded. Then 5-μm-thick sections were prepared for hematoxylin and eosin (HE) staining. Histopathological changes were observed under an Olympus BX41 microscope (Olympus, Tokyo, Japan).

2.9. Measurement of oxidative stress levels in hippocampal tissues

Hippocampal tissues from each group of rats were homogenized in PBS (pH = 7.4) solution and formed into a 10% (w/v) homogenate. The homogenates were then centrifuged at 4 °C for 30 min (3000 r·min^{-1}). The supernatant was used to detect biochemical markers related to oxidative stress. The levels of SOD, MDA and GSH-Px were determined using commercially available kits (Shanghai Enzyme Link Biotechnology Co., Ltd.) according to the manufacturer's instructions.

2.10. Determination of inflammatory factor content in hippocampal tissues

Hippocampal tissues from each group of rats were homogenized in PBS (pH = 7.4) solution and formed into 10% (w/v) homogenates. The homogenates were then centrifuged at 4 °C for 30 min (3000 r·min^{-1}). The supernatant was used to detect biochemical markers related to inflammatory factor content. The levels of ET-1, VEGF, IL-1β and IL-6 were determined according to the manufacturer's instructions using commercially available kits (Beijing Dongge Boye Biotechnology Co., Ltd).

2.11. Determination of neurotransmitter expression in hippocampal tissues

Hippocampal tissues from each group of rats were homogenized in PBS (pH = 7.4) solution and formed into 10% (w/v) homogenates. The homogenates were then centrifuged at 4 °C for 30 min (3000 r·min^{-1}). The supernatant was used to detect biochemical markers related to oxidative stress. 5-HT, DA, CGRP and AchE levels were determined using commercially available kits (Wuhan Huamei Bioengineering Co., Ltd.) according to the manufacturer's instructions.

2.12. Determination of vasodilatation-related factor content in hippocampal tissues

Hippocampal tissues from each group of rats were homogenized in PBS (pH = 7.4) solution and formed into 10% (w/v) homogenates. The homogenates were then centrifuged at 4 °C for 30 min (3000 r·min^{-1}). The supernatant was used to detect biochemical markers related to oxidative stress. The levels of NO, NE and TXB2 were determined using commercially available kits (Wuhan Huamei Bioengineering Co., Ltd.) according to the manufacturer's instructions.

2.13. Western blot analysis

Hippocampal tissues from each group of rats were washed and homogenized in RIPA lysate. After centrifugation at 12,000 r·min^{-1} for 10 min, the clarified supernatant was obtained. The total protein content was determined using the BCA Protein Assay Kit (Beijing

Solabao Biotechnology Co., Ltd., Beijing, China). Equal amounts of protein were then loaded on sodium dodecyl sulfate polyacrylamide gels (SDA-PAGE) for electrophoretic separation. The protein bands were transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Schwallbach, Germany) and closed in 5% (w/v) skimmed milk in TBST buffer for 2 h. Primary antibodies of HIF-1α (Abcam, 1:1000), Caspase-3 (CST, 1:1000)and β-actin (CST, 1:1000) were incubated overnight at 4 °C. Subsequently, the membranes were incubated with goat anti-rabbit or IgG HRP-coupled secondary antibodies for 2 h at room temperature. Specific bands were detected by the ECL Western Blotting Detection System. ChemiDoc-It 510 imager (UVP, USA) used development was performed. The intensity of the bands was quantified and β-actin was selected as an internal reference.

2.14. Statistical analysis

All values are expressed as the Mean ± SD in this study. Significant differences between two groups were calculated using analysis of variance (ANOVA) and Tukey's multiple comparison test and a minimum probability value of $P < 0.01$ was considered statistically significant.

3. Results

3.1. Hematological analysis of butylphthalide on rats with plateau brain edema

As shown in Table 1, the hematological analysis in blood samples of rats from each group is listed. After 72 h hypoxic exposure compared with normoxia group, there was a significant increase in leukocytes, lymphocytes, monocytes, granulocytes and hemoglobin in HACE group after 72 h exposure compared with Normoxia group, $P < 0.01$, and no significant difference in erythrocytes and platelets, $P > 0.05$; while HACE + Sal group and HACE + NBP group, there was a significant decrease in leukocytes, lymphocytes, monocytes and granulocytes, $P < 0.01$, and no significant difference in erythrocytes, hemoglobin and platelets, $P > 0.05$.

3.2. Effect of butylphthalide on brain water content of rats with plateau brain edema

As shown in Fig. 2, the changes in brain tissue water content of rats before and after hypoxia exposure in each group were expressed. Compared with the Normoxia group, there was a significant increase in brain water content in the HACE group and a significant decrease in brain water content in the HACE + Sal group and the HACE + NBP group after 72 h of exposure, $P < 0.01$.

3.3. Effect of butylphthalide on brain histopathology in rats with plateau brain edema

As shown in Fig. 3, the histopathological examination of rat brain is depicted in the figure. Normoxia group showed neuronal cell arrangement in hippocampus, while HACE group with 72 h hypoxia exposure showed disorganized cell arrangement, decreased neuronal cell number, increased cell swelling and even nuclear consolidation in hippocampal area, HACE + NBP group and HACE + Sal group both showed better improvement in brain tissue, which basically changed the cell swelling in the HACE group.

3.4. Effect of butylphthalide on oxidative stress indexes in hippocampal tissues of rats with plateau brain edema

As shown in Fig. 4, the changes of oxidative stress levels in hippocampal tissues of rats are depicted in the figure. Compared with Normoxia group, SOD and GSH-Px were significantly decreased in HACE group after 72 h exposure, $P < 0.01$, and MDA was significantly increased, $P < 0.01$; HACE + NBP group and HACE + Sal group both significantly increased the expression of SOD and GSH-Px, $P < 0.01$, and significantly decreased the expression of MDA, $P < 0.01$.

3.5. Effect of butylphthalide on the levels of inflammatory factors in hippocampal tissues of rats with plateau brain edema

As shown in Fig. 5, the changes of inflammatory factor levels in hippocampal tissues of rats are depicted in the figure, compared with Normoxia group, IL-1β, IL-6, ET-1 and VEGF and were significantly higher in HACE group after 72 h exposure compared with

Table 1
Hematological changes in rats before and after hypoxia in all groups ($\bar{X} \pm S$).

Group	Normoxia	HACE	HACE + Sal	HACE + NBP
WBC(10^9/L)	8.413 ± 3.342	37.6 ± 11.016**	13.75 ± 12.794$^{\#}$	10.775 ± 3.523$^{\#}$
Lymphocyte (10^9/L)	6.289 ± 2.617	28.35 ± 11.112**	7.3 ± 3.459$^{\#}$	8.2 ± 3.094$^{\#}$
Monocytes (10^9/L)	0.188 ± 0.064	1.951 ± 0.756**	0.188 ± 0.099$^{\#}$	0.238 ± 0.177$^{\#}$
Granulocytes (10^9/L)	1.688 ± 0.954	7.425 ± 1.878**	1.931 ± 0.755$^{\#}$	2.139 ± 0.761$^{\#}$
RBC(10^{12}/L)	8.799 ± 0.719	9.386 ± 0.897	9.121 ± 0.547	8.929 ± 0.192
HGB (g/L)	165.375 ± 16.265	186.75 ± 17.069**	174.75 ± 6.453	176.25 ± 4.950
Platelet (10^9/L)	1033.25 ± 358.298	1195.5 ± 182.865	1182.125 ± 56.405	1057.625 ± 252.636

Hematological analysis of butylphthalide on rats with plateau brain edema; compared with normoxia group, $^{**}P < 0.01$, compared with HACE, $^{\#}P < 0.01$.

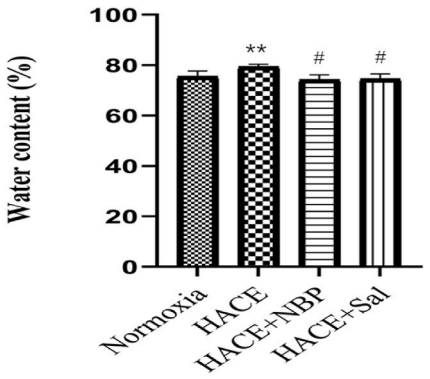

Fig. 2. Effect of butylphthalide on brain water content of rats with plateau brain edema. $^{**}P < 0.01$, $^{\#}P < 0.01$.

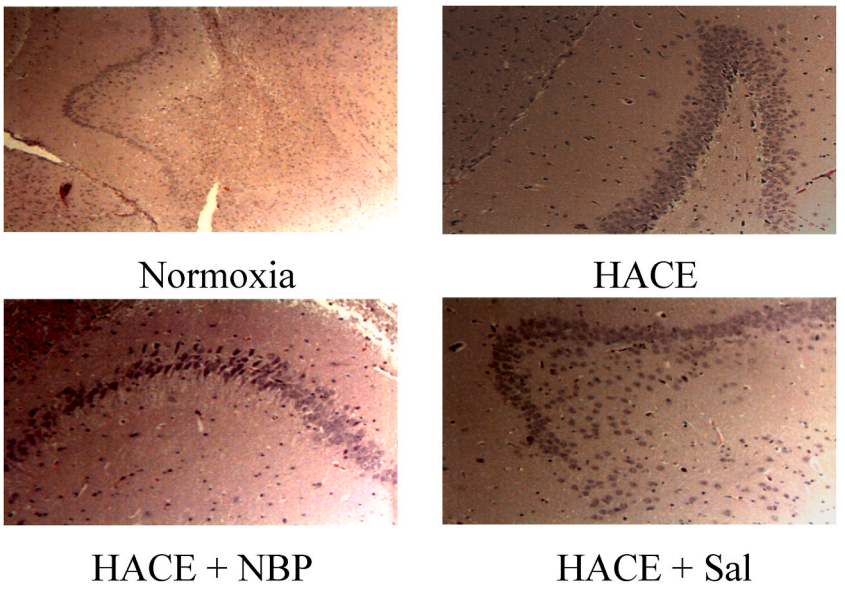

Fig. 3. Effect of hypoxic environment on HE staining of brain tissue in various groups of rats.

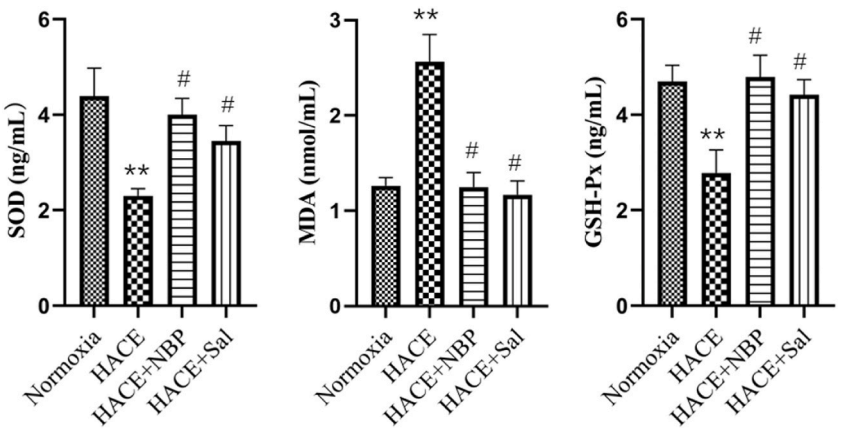

Fig. 4. The effect of hypoxic environment on the level of oxidative stress in brain tissue of various groups of rats. $^{**}P < 0.01$,$^{\#}P < 0.01$.

Normoxia group, $P < 0.01$; HACE + NBP group and HACE + Sal group both significantly reduced the expression of IL-1β, IL-6, ET-1 and VEGF in hippocampal tissue, $P < 0.01$.

3.6. Effect of butylphthalide on neurotransmitter expression in hippocampal tissues of rats with plateau brain edema

As shown in Fig. 6, which depicts the changes of neurotransmitter expression in hippocampal tissue of rats, compared with Normoxia group, AchE, DAand 5-HT were significantly increased in HACE group after 72 h exposure, $P < 0.01$, while CGRP was significantly decreased, $P < 0.01$; HACE + NBP group and HACE + Sal group both significantly decreased the expression of AchE, DA and 5-HT in hippocampal tissues, $P < 0.01$, and significantly elevated the expression of CGRP in tissues, $P < 0.01$.

3.7. Effect of butylphthalide on the level of vasodilating factors in hippocampal tissues of rats with plateau brain edema

As shown in Fig. 7, the changes of vasodilating factor table in rat hippocampal tissue are depicted in the figure, compared with Normoxia group, NEand TXB2 were significantly increased in HACE group after 72 h exposure, $P < 0.01$; both HACE + NBP group and HACE + Sal group significantly decreased the hippocampal tissue expression of NE, NO and TXB2, $P < 0.01$.

3.8. Effect of butylphthalide on the expression of HIF-1α, Caspase-3 in hippocampal tissues of rats with plateau brain edema

As shown in Fig. 8, the expression of apoptosis-related proteins in rat hippocampal tissue is depicted in the figure. Compared with the Normoxia group, the expression of HIF-1α, Caspase-3 was significantly higher in the HACE group after 72 h exposure, and their bands were found to be significantly higher by optical density analysis and corresponding quantification of Western blot data normalized to β-actin, $P < 0.01$; both HACE + NBP group and HACE + Sal group significantly decreased the expression of HIF-1α and Caspase-3 in hippocampal tissues, $P < 0.01$.

4. Discussion

A number of studies in the literature have shown that NBP has a better improvement in cerebrovascular disease. It has also been reported that NBP has a therapeutic effect on neurodegenerative diseases. Numerous animal studies have found that NBP can also improve symptoms of other neurological disorders, such as epilepsy, cerebral edema, and cognitive decline due to severe acute carbon monoxide poisoning. In addition, NBP has therapeutic effects in diabetes, diabetes-induced cataracts, and non-neurological disorders such as atherosclerosis, and acts primarily by improving microcirculation and protecting mitochondria. Its broad range of pharmacological effects also include inhibition of oxidative stress, neuroapoptosis, inflammatory responses, and antiplatelet and antithrombotic effects [18–25]. In this study, we found that NBP pretreatment was able to play a preventive and curative role in the hypoxic environment exposure environment of the plateau, and the mechanism may be related to the reduction of brain water content, improvement of brain histopathological damage, alteration of hematological indicators, oxidative stress indicators, inflammatory

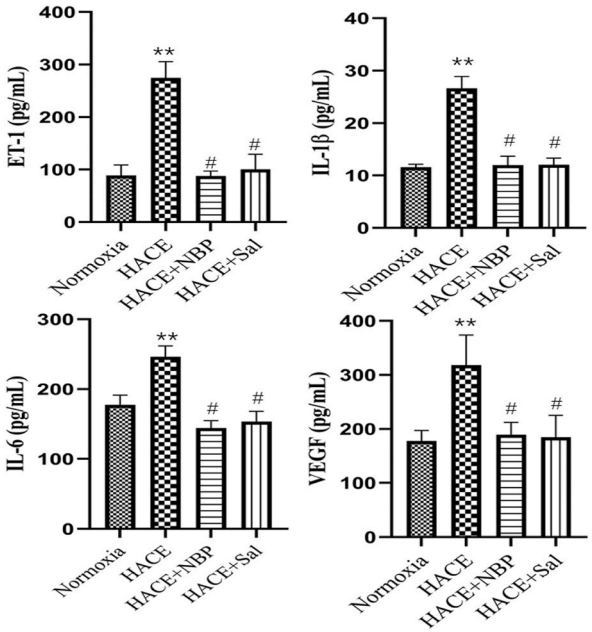

Fig. 5. Effect of hypoxia on inflammatory factors in brain tissue of various groups of rats. $^{**}P < 0.01$, $^{\#}P < 0.01$.

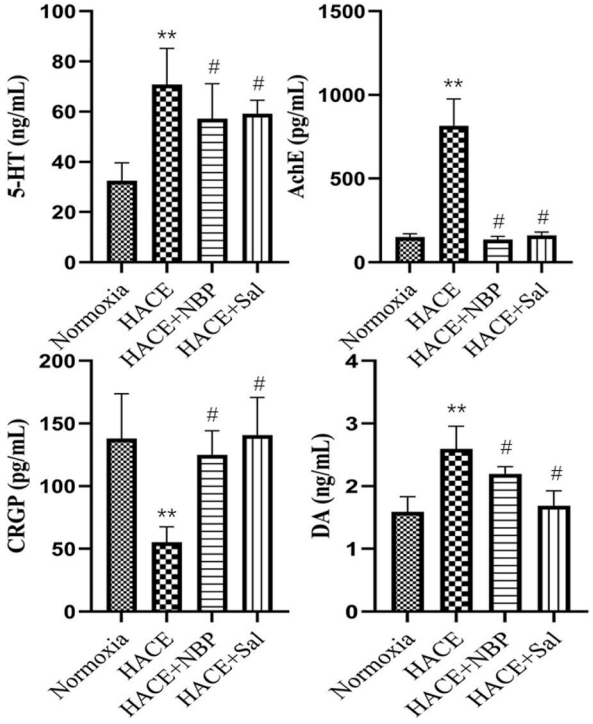

Fig. 6. Effect of hypoxia on neurotransmitter expression in rat brain tissue of various groups. $^{**}P < 0.01, ^{\#}P < 0.01$.

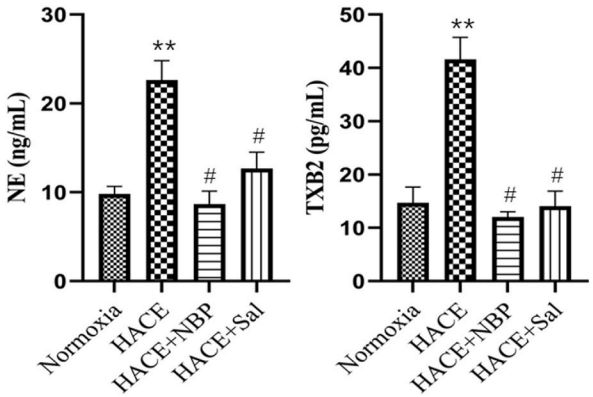

Fig. 7. Effect of hypoxia on the expression of vasodilatory factors in various groups of rats. $^{**}P < 0.01, ^{\#}P < 0.01$.

factors, expression of neurotransmitters in the brain, and inhibition of caspase-dependent apoptotic pathways, and the above results suggest that NBP is expected to be a candidate drug for the prevention and treatment of HACE.

Research on the pathogenesis of highland cerebral edema is still not well understood [26], and therefore has implications for the search for effective drugs for prevention and treatment. Some studies have shown that the occurrence of HACE is closely related to oxidative stress [27]. Acute hypoxia exposure disrupts the balance of reactive oxygen species, a process that accelerates lipid peroxidation in cell membranes and causes damage to the antioxidant system of cells, mainly through abnormal expression of endogenous enzymes and antioxidants. In the present study, 72-h hypoxia exposure decreased SOD and GSH-Px content and increased MDA content, while NBP intervention reversed these changes, indicating that NBP can mitigate the oxidative stress induced by acute hypoxia by scavenging free radicals, inhibiting lipid peroxidation and repairing damage to the antioxidant defense system.

In addition, many scholars have suggested that inflammation is an important factor affecting HACE, and one result showed that acute hypoxic treatment led to abnormal expression of VEGF, MMP-9 and NF-κβ, which induced the development of HACE [28]. Another study showed that a large number of pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6, promoted the development of HACE [29]. A recent study showed that gibberellin (GP-14) could reduce HACE-induced neuroinflammation and BBB destruction by inhibiting NF-κβ signaling pathway [30]. Previous studies have found that butylphthalide inhibits inflammation by modulating the

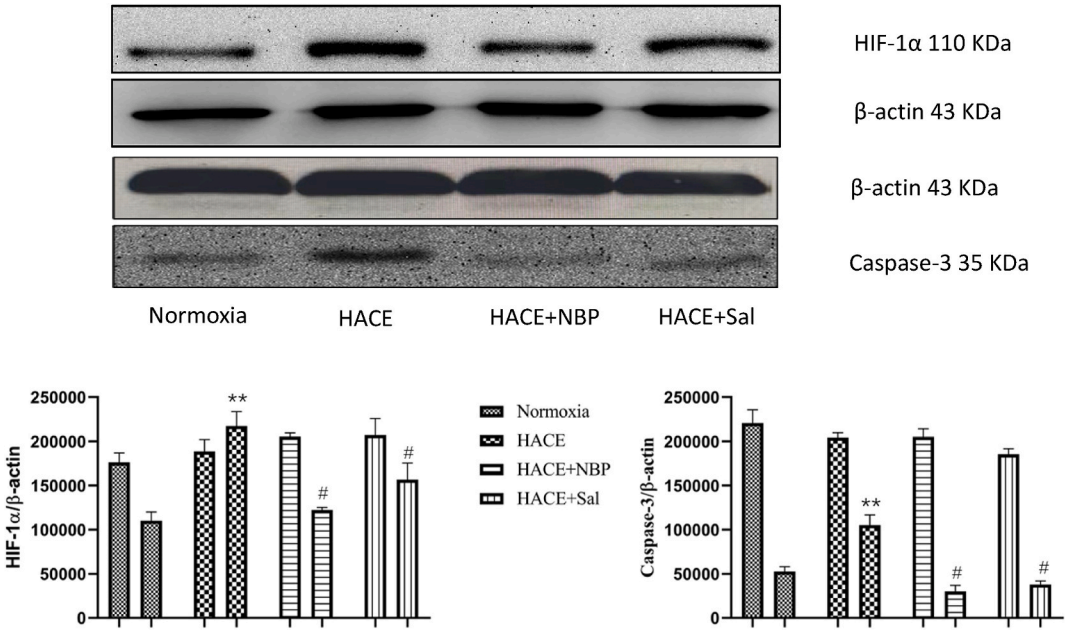

Fig. 8. Effect of hypoxia on protein expression and protein quantification in various groups of rats. The data in the figure indicates the quantification of actin and target protein in each group. $^{**}P < 0.01$,$^{\#}P < 0.01$.

Akt/Nrf2 signaling pathway [31]. In the present study, NBP intervention significantly reduced the levels of TNF-α, VEGF, IL-1β and IL-6 in brain tissues, while the expression of NE, NO and TXB2 in hypoxic brain tissues was reduced after NBP intervention, suggesting that the protective effect of NBP on HACE may be partly attributed to the anti-inflammatory activity of the drug and the maintenance of the normal physiological function of the blood-brain barrier.

Neurotransmitters are chemicals that act as messengers during synaptic transmission. They are critical to human health and imbalances in neurotransmitters can lead to serious central nervous system disorders such as Parkinson's disease, schizophrenia and Alzheimer's disease. Therefore, the concentrations of various neurotransmitters are important for the study and diagnosis of CNS disorders. There are many types of neurotransmitters in the brain, including γ-aminobutyric acid, 5-hydroxytryptamine, norepinephrine, glutamate, acetylcholine, and dopamine. γ-Aminobutyric acid is an important inhibitory neurotransmitter in the central nervous system and is involved in the regulation of many biological processes, such as mood, blood pressure, and sleep-wake [32]. 5-Hydroxytryptamine is involved in the development of pain and analgesia, and temperature. 5-Hydroxytryptamine is involved in the formation of pain and analgesia, regulation of body temperature and feeding behavior. Norepinephrine is involved in the transmission of upstream and downstream fibers. Glutamate has important roles in learning, memory, neuronal plasticity and brain development. Acetylcholine is an important excitatory neurotransmitter and is involved in the regulation of sensory and motor processes. Dopamine, mainly derived from dopaminergic neurons, is involved in motor coordination processes and is also associated with the excitatory state of the brain [33,34]. After brain injury, the passive release of neurotransmitters in the brain increases due to necrosis of brain tissue, so that the concentration of transmitters in the brain will first increase in the early stages and will gradually decrease as the brain tissue is repaired and will return to normal levels. If the degree of injury is more severe, the effective transmitters in the brain will continue to be lower than normal levels because of reduced transmitter synthesis, impaired release and receptor destruction [35,36]. The results of the present study showed that AchE, DA, and 5-HT release were significantly increased and CGRP synthesis was significantly decreased in the rat brain after acute hypoxia exposure; the neurotransmitters in the brain were gradually reduced to normal levels after NBP intervention, which also indicated that NBP could repair the damaged brain tissue to some extent.

Hematological parameters of the rats were also examined in this study. The data showed that 72 h hypoxia exposure resulted in significant increases in blood WBC, Lymph, Mon, Gran and HGB, while RBC and PLT did not improve significantly. This result is generally consistent with a previous study in which acute hypoxia-stimulated HACE caused significant increases in WBC as well as several other hematological parameters (RBC, HGB, HCT, and PLT) in rats [37]. According to the hematological parameters, acute hypoxia for 72 h actually increased WBC and did not affect RBC. therefore, this result suggests that WBCs may be involved in the inhibition of early inflammation and apoptosis in HACE.

HIFs act as a master switch in the human response to hypoxia. HIF-1α protein is predominantly distributed in the brain, kidney, liver and heart of mice during normoxic conditions and is highly expressed in acute hypoxic environments with organ-specific regulation [38]. It was shown that stimulation of the HIF-1α signaling pathway during acute hypoxia triggers the release of cytochrome C from the mitochondrial matrix to the cytoplasm, which binds to the HIF-1α protein and activates the production of caspase-3, and the activated caspase-3 complex induces apoptosis [10]. In the present study, HIF-1α, caspase-3 and caspase-9 were highly expressed in 72 h hypoxia-exposed brain tissue, which was significantly improved by NBP administration, a result that suggests that

NBP may exert a protective effect against plateau brain edema by inhibiting the HIF-1α/caspase apoptotic signaling pathway and thereby.

In conclusion, this study confirmed the neuroprotective effects of NBP on HACE and explored the underlying mechanisms. This suggests that NBP ameliorates the development of cerebral edema after acute hypoxic exposure by reducing brain water content, inhibiting oxidative stress and inflammatory responses, modulating hematological changes, altering the secretion of brain neurotransmitters, and the expression of proteins related to the Caspase signaling pathway, all of which evidence suggests that NBP is a candidate drug for the prevention and treatment of HACE.

Compliance with ethical standards

All procedures involving animals were approved by the Xinjiang Medical University IACUC.

Date availability statement

This project is still in the confidential stage, and the author has not obtained permission to share data.

CRediT authorship contribution statement

Bohua Ma: Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Qian Li:** Methodology, Data curation. **Meng Li:** Methodology, Data curation. **Jiangtao Wang:** Methodology. **Ning Fan:** Validation, Software. **Shanpeng Yang:** Methodology, Investigation. **Wenhui Shi:** Methodology, Investigation. **Rui Wang:** Project administration, Methodology, Investigation, Conceptualization. **Dongfeng Yin:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dongfeng Yin reports financial support was provided by the Army Logistics Research Program. Dongfeng Yin reports a relationship with The Army Logistics Research Program that includes: consulting or advisory, funding grants, speaking and lecture fees, and travel reimbursement. Dongfeng Yin has patent pending to Dongfeng Yin. There is no content that has an impact on the submitted work. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27833.

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