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Protective effect of 5,6,7,8-trtrahydroxyflavone against acute hypobaric hypoxia induced-oxidative stress in mice

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Abstract: Severe oxidative stress triggered by acute hypobaric hypoxia (AHH) is harmful for lots of organs in body, especial brain and heart. Flavonoids with antioxidant properties can protect organs from oxidative stress. Our previous study found that 5,6,7,8-trtrahydroxyflavone (5,6,7,8-THF), a flavones with four consecutive hydrogen group on ring A, showed excellent antioxidant properties *in vitro.* In the present study, the protective of 5,6,7,8-THF against oxidative stress caused by AHH was investigated. Mice were administered with 5,6,7,8-THF(500mg/kg) for 5 consecutive days before HH exposure. The heart rate (HR) and blood pressure (BP) was measured. The activity of SOD, CAT, GSH-Px, LDH, Na^+-K^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase and the content of H_2O_2, MDA, LD and ATP in brain and heart tissue was evaluated using commercial kit. AHH led to a significant increase in HR and decrease in BP. Pretreatment of 5,6,7,8-THF could reversed these changes. In addition, administration of 5,6,7,8-THF could significantly increase the activity of SOD, CAT and GSH-Px and decrease the content of H_2O_2 and MDA in the brain and heart of mice under AHH. Furthermore, 5,6,7,8-THF inhibited the activity of LDH, decreased the level of LD and improved ATPase activity. These results indicate that 5,6,7,8-THF may protect the mice against AHH injury via scavenging free radical, inhibiting lipid peroxidation, enhancing antioxidant enzyme activity, preserving energy metabolism and can be further explored as an excellent anti-hypoxia agent for preventing acute mountain sickness.

Keywords: 5,6,7,8-trtrahydroxyflavone, oxidative stress, acute hypobaric hypoxia, antioxidant enzyme, energy metabolism.

INTRODUCTION

Hypobaric hypoxia (HH), characterized by decreased atmospheric pressure, is a stressful environmental condition during ascent to high altitude (Maity *et al.*, 2013). Acute hypobaric hypoxia (AHH), encountered when people travel too fast to high altitude, is considered as one of the main causes of high altitude illness, including acute mountain sickness (AMS), high-altitude pulmonary edema (HAPE) and high-altitude cerebral edema (HACE), (Bartsch and Swenson, 2013, Luks *et al.*, 2017, Koirala *et al.*, 2018). With the increasing numbers of person who travel to high altitude for leisure, sport or work, the incident of AMS is tending to rise. Although AMS is not often life threatening, it may significantly affect the work efficacy and cognitive function for workers and travelers. So many studies focus on the prophylaxis and therapy of AMS (Li *et al.*, 2018, Aksel *et al.*, 2019).

Although the pathophysiological mechanisms of AMS is still unclear, oxidative stress induced by AHH plays a vital role in the development and progression of AMS (Irarrazaval *et al.*, 2017). Exposure to AHH condition, the balance between the oxidant and the endogenous antioxidant systems, including antioxidant enzymes such as super oxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) and non-enzymatic antioxidant such as glutathione, ascorbic acid, uric acid, *β*-carotene *et al* in body (Stojkovic *et al.*, 2012) are broken, which induces the accumulation of reactive oxygen species (ROS) (Siques *et al.*, 2018). Overproduction of ROS, such as hydroxyl radical (·OH), super oxide anion($O_2^{\cdot-}$) radical, peroxyl radical ($RO_2\cdot$), alkoxylradical (RO·), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$), can trigger oxidative damage to various essential biomolecules including proteins, carbohydrates, lipids and nucleic acids, ultimately leading to cell apoptosis and many diseases (Farber, 1994). Therefore, application of antioxidants to eliminate ROS may serve as a potential way to combat the oxidative stress caused by AHH. Previous studies have reported that antioxidant supplements like vitamin E (Sharifi *et al.*, 2016), acetyl-L-carnitine (Barhwal *et al.*, 2009), erdosteine (Uzun *et al.*, 2006) and nitronyl nitroxide (Fan *et al.*, 2013) can limit hypoxia-induced injury *in vivo*.

Flavonoids are the most abundant nature antioxidants in our diets and widely distributed in many fruits and vegetables. Among them, natural flavones, as well as some of their synthetic derivatives, have been shown a wide range of pharmacological activities, including antioxidant, anticancer, anti-atherogenic, antidiabetic, anti-inflammatory, antihypertensive properties and neuroprotective activity (Singh *et al.*, 2014). In addition, previous studies have reported that flavones such as naringenin and quercetin could modulate hypobaric hypoxia induced oxidative stress (Sarkar *et al.*, 2012, Liu *et al.*, 2015). It is well accept that the antioxidant activity

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of flavonoids is related to the number and positions of the hydroxyl (OH) groups in their structure (Chen *et al.*, 2012). In our previous study, 5,6,7,8-tetrahydroxyflavone (5,6,7,8-THF), which owned four consecutive OH groups on ring A, was synthesized using chrysin as starting material and exhibited excellent antioxidant activity *in vitro* (Jing *et al.*, 2017). However, whether 5,6,7,8-THF had the protective effect on oxidant stress injury in *vivo* remains an enigma. Therefore, this study was designed to investigate the protective effect of 5,6,7,8-THF on the oxidative stress damage in AHH mice model *in vivo*. Special attention was paid to measure various oxidative stress markers and antioxidant system. It was hoped that 5,6,7,8-THFcould be used as an excellent anti-hypoxia agent in future.

MATERIALS AND METHODS

Chemicals and reagents

5,6,7,8-THF was synthesized in our lap according to our reported method (Jing *et al.*, 2017). The commercial test kit for H_2O_2, malondialdelyde (MDA), SOD, CAT, GSH-Px, lactic acid (LD), lactate dehydrogenase (LDH), ATP and ATPase were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The Bicinchoninic acid (BCA) protein assay kit was purchased from Thermo (Rockford, USA).

Animals

Male BALB/c mice (6-week-old, weight 18-22g, SPF rank) were purchased from the Center for Experimental Animals, Lanzhou Institute of Biological Products (Lanzhou, China). The mice were housed at constant temperature of 22±2°C and humidity of 40±5% with a 12h light/ dark cycle, and accessed to pellet food and tap water *ad libitum*. All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006). The experimental protocol was evaluated and approved by Animal Care and Use Committee of 940th Hospital.

Effect of 5,6,7,8-THF on survival time of mice under conditions of normobaric hypoxia

The normobaric hypoxia test was performed using our previous reported method (Ma *et al.*, 2011). After 3 days of acclimatization, The mice were randomly divided into five groups (n=10 mice in each group): model group, acetazolamide (AZ) group (300mg/kg/d), and three 5,6,7,8-THF group (125, 250, 500mg/kg/d). The 5,6,7,8-THF and AZ were intragastrically administered to the mice for 5 consecutive days. Equal volumes of distilled water were given to mice of model group in the same way. After sixty minutes of the last administration, each mouse was put into a 250ml airtight bottle containing 5g medical soda lime. In order to create a hermetic condition, the bottle neck was sealed with petroleum jelly. The survival time was defined as duration time from the bottles was sealed to the mouse stopped breathing. The anti-hypoxic activity was evaluated by the survival time and prolongation rate. The prolongation rate was calculated as (survival time of treatment group-survival time of model group)/survival time of model group × 100%.

Effect of 5,6,7,8-THF on death rate of mice under acute decompression condition

The death rate of mice under acute decompression condition was recorded according to our previous reported method (Ma *et al.*, 2011). The 5,6,7,8-THF was given at 500mg/kg/d by intragastric administration for 5d. AZ was given at 300mg/kg/d in same way. The death rate was calculated as (the number of dead mice in each group /the total number of mice in each group × 100%).

Hypobaric hypoxia treatment

The mice were randomly divided into four groups (n=10 mice in each group): Normal control group, hypobaric hypoxia (HH) group, AZ group (300mg/kg/d), and 5,6,7,8-THF group (500mg/kg/d). 5,6,7,8-THF and AZ was administrated as mentioned above. Except normal group, the mice were exposed to HH condition in an animal decompression chamber (GuizhouFenglei, China) to mimic a high altitude of 8000m (18% O_2, 0.035MPa) at a velocity of 100m/min for 12h. Humidity and temperature in the chamber was maintained at 40-50% and 23-25°C, respectively.

Blood pressure and heart rate

After the hypobaric hypoxia test, the altitude of the chamber was adjusted to 4500m (100m/min, 0.06 MPa). The heart rate (HR) and blood pressure (BP) of mice were recorded using BP-2010 Series Blood Pressure Meter (Softron, Japan).

Preparation of brain and heart homogenate

After testing of blood pressure and heart rate, the mice were anesthetized and sacrificed by cervical dislocation. Brain and heart were immediately collected from all of the examined mice. The brain and heart tissues were homogenized in cold potassium phosphate buffer (10.0%, w/v) and then centrifuged at 2500g for 10 min at 4°C. The supernatant was collected and used for biochemical analysis. The concentration of tissues homogenate protein was measured using a BCA kit.

H_2O_2 and Lipid per oxidation

The H_2O_2 content was determined by the commercial assay kit following the manufacturer's instructions and expressed as and mmol/mg protein. The lipid peroxidation was assessed by the content of MDA, which was determined according to the direction of the commercial assay kit and expressed as nmol/mg protein.

Antioxidant enzyme activity

To evaluate the antioxidant status, the activity of SOD,

CAT and GSH-Px were measured using the commercial assay kits according to the manufacturer's instructions. The results were expressed as U/mg protein.

Lactic acid (LD) and lactate dehydrogenase (LDH)

The level of LD and LDH was determined using commercial assay kits following the manufacturer's instructions. The result of LDH activity and LD level were expressed as U/g protein and mmol/g protein, respectively.

ATP and ATPase

ATP content was measured according to the phosphomolybdic acid colorimetry method and expressed as μmol/gprot. The K^+-Na^+-ATPase and Ca^{2+}-Mg^{2+}-ATPase activity were determined using the commercial assay kits following the instructions of the manufacturer and expressed as and μmol Pi/mg prot/h.

STATISTICAL ANALYSIS

Results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using SPSS 19.0 software by one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls tests. Difference was considered as statistically significant when $P<0.05$.

RESULTS

5,6,7,8-THF prolonged the survival time of mice in normobaric hypoxia test

To determine the optimal dosage of 5,6,7,8-THF, the dose effect of 5,6,7,8-THF on survival time in normobaric hypoxia test was investigated. As shown in table 1, pretreatment with 5,6,7,8-THF prolonged the survival time of mice in a dose-dependent manner. In the 500 mg/kg of 5,6,7,8-THF group, the longest survival time observed was 54.47±3.56min, which was nearly 1.2 times longer than that of AZ at 300mg/kg. Given this result, 500mg/kg of 5,6,7,8-THF were selected for following experiments.

5,6,7,8-THFdecrease the death rate of mice under acute decompression condition

As shown in table 1, the death rate within one hour at the simulated altitude of 10,000m was 100% and 60% for the model and the AZ group, respectively. While pretreatment with 5,6,7,8-THF could reduce the death rate to 40%, suggesting that 5,6,7,8-THF might be more effective than AZ in preventing AMS.

Effects of 5,6,7,8-THF on the heart rate and blood pressure of mice

As shown in the fig. 1, hypobaric hypoxia significantly increased the heart rate (HR), but remarkably decreased blood pressure, such as systolic blood pressure (SBP), diastolicblood pressure (DBP) and mean artery pressure (MAP). Treatment with 5,6,7,8-THF could attenuate these changes compared with model.

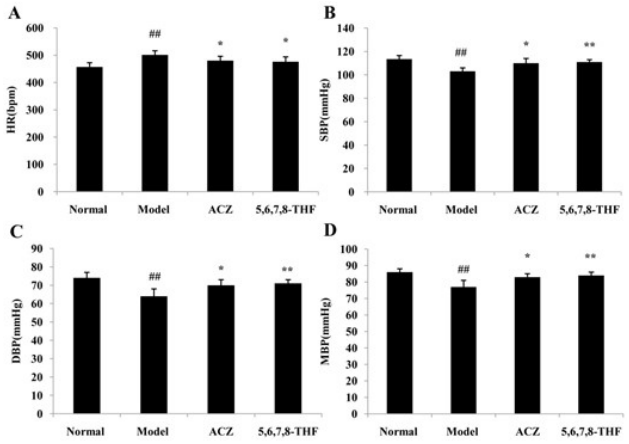

Fig. 1: Effects of 5,6,7,8-THF on heart rate and blood pressure of mice under acute hypobaric hypoxia condition. (n=6). A. Heart rate (HP, bpm); B. Systolic blood pressure (SBP, mmHg); C. Diastolic blood pressure (DBP, mmHg); D. Mean artery pressure (MAP, mmHg). Values are mean ± SD. $^{\#}P<0.05$, $^{\#\#}P<0.01$ versus control group, $*P<0.05$, $**P<0.01$ versus model group.

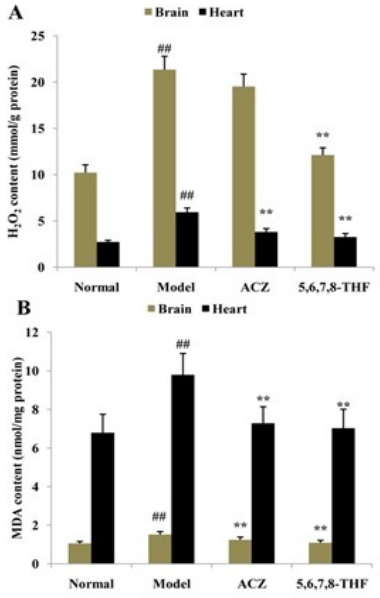

Fig. 2: Effects of 5,6,7,8-THF on H_2O_2(A) and MDA(B) content in brain and heart of mice under acute hypobaric hypoxia condition. Values are mean ± SD. $^{\#}P<0.05$, $^{\#\#}P<0.01$ versus control group, $*P<0.05$, $**P<0.01$ versus model group.

Effects of 5,6,7,8-THF on oxidative stress markers in brain and heart

According to the results given in fig. 2, a significant increase in H_2O_2 and MDA content was observed in the brain and heart of mice after AHH exposure for 12h. Pre-treatment of 5,6,7,8-THF significantly attenuated AHH induced H_2O_2 and MDA generation indicating reduced lipid per oxidation and oxidative stress.

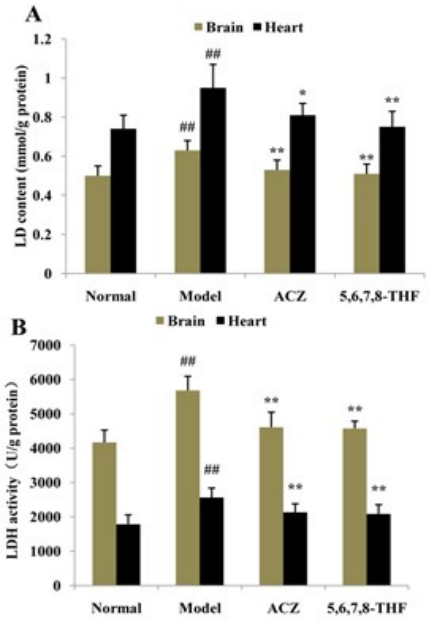

Fig. 3: Effects of 5,6,7,8-THF on LD(A) content and LDH(B) activity in brain and heart of mice under acute hypobaric hypoxia condition. Values are mean ± SD. $^{\#}P$<0.05, $^{\#\#}P$<0.01 versus control group, *P<0.05, **P<0.01 versus model group.

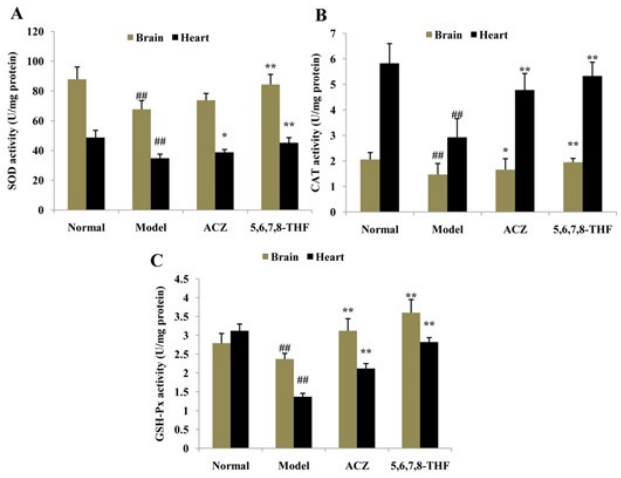

Fig. 4: Effects of 5,6,7,8-THF on the activities of SOD (A), CAT (B)and GSH-Px(C) in brain and heart of mice under acute hypobaric hypoxia condition. Values are mean ± SD. $^{\#}P$<0.05, $^{\#\#}P$<0.01 versus control group, *P<0.05, **P<0.01 versus model group.

Effects of 5,6,7,8-THF on LD content and LDH activity in brain and heart

The level of LD and LDH in brain and heart of mice exposed to AHH showed a significant increase as compared to normal group. Pre-treatment with 5,6,7,8-THF decreased the LD content and LDH activity (fig. 3). This result indicated that the metabolic acidosis and energy metabolism disorder caused by AHH could be inhibited by 5,6,7,8-THF.

Effects of 5,6,7,8-THF on the activity of antioxidant enzyme in brain and heart

As shown in fig. 4, AHH induce a dramatically decreased in the activities of SOD, CAT, and GSH-Px both in brain and heart compared to the normal group. Pre-treatment with 5,6,7,8-THF could reverse these change and enhance activities of SOD, CAT and GSH-Px to normal level, which might provide an effective defense from the damaging effects of ROS induced by HH.

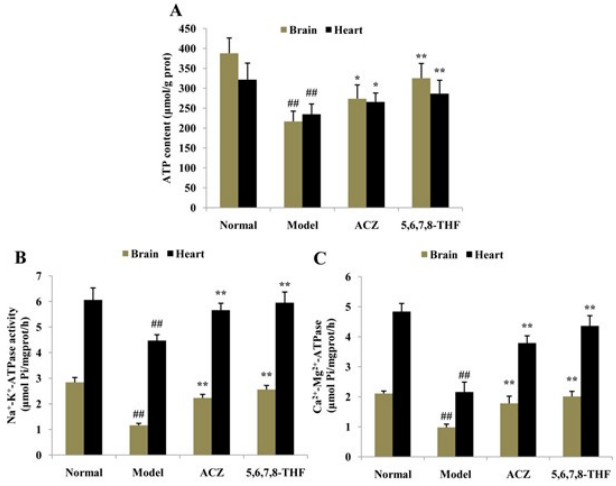

Fig. 5: Effects of 5,6,7,8-THF on ATP (A) content and Na^{+}-K^{+}-ATPase (B). Ca^{2+}-Mg^{2+}-ATPase (C)activity in brain and heart of mice under acute hypobaric hypoxia condition. Values are mean ± SD. $^{\#}P$<0.05, $^{\#\#}P$<0.01 versus control group, *P<0.05, **P<0.01 versus model group.

Effects of 5,6,7,8-THF on ATP content and ATPase activity in brain and heart

To investigate effect mitochondrial function in mice brain, ATP content and ATPase activity were measured. As shown in fig. 5, the ATP level in mice brain was decreased after 12h of AHH. However, 5,6,7,8-THF significantly increased the ATP levels when compared with the AHH group. The activities of Na^{+}-K^{+}-ATPase in mice brain was significantly reduced in AHH group compared with normoxic group. On the contrary, 5,6,7,8-THF lessened the decrement in mice brain compare to HH group. The change of Ca^{2+}-Mg^{2+}-ATPase activity in mice brain showed a similar trend as Na^{+}-K^{+}-ATPase.

DISCUSSION

It is known that AHH induced oxidative stress impaired many important organs, especially brain and heart. The brain is much more susceptible than other organs to hypoxic stress due to many factors including the high rate of oxygen consumption, high concentration of polyunsaturated fatty acids and metal iron as well as low antioxidant capacity (Rauchova *et al.*, 2012). Previous report have improved that AHH can severely affects cognitive, memory and behavior functions (Sharma *et al.*,

Table 1: Effects of 5,6,7,8-THF on the survival time of mice under normobaric hypoxia condition and the death rate of mice under acute decompression condition.

Group	n	Dose (mg/kg)	Survival time(min)	Prolongation rate (%)	Number of dead mice	Death rate (%)
Model	10	---	34.65±0.88		10	100
Acetazolamide	10	300	44.76±2.13**	22.59%	6	60**
	10	125	39.24±1.56*	11.70%	---	---
5,6,7,8-THF	10	250	47.41±2.59**	26.91%	---	---
	10	500	54.47±3.56**	36.39%	4	40**

Each group represents the mean ± SD.*P<0.05, **P<0.01 versus model group

2011, Koester-Hegmann *et al.*, 2018). The heart also easily affected by AHH, since it is an obligate aerobic organ and constant supply of oxygen is needed for sustaining cardiac function and viability. Under AHH condition, the energy produced by the heart muscle cannot match myocardial demand, resulting in cardiac contractile dysfunction and cell apoptosis and necrosis (Karar *et al.*, 2007). Therefore, the protective effect of 5,6,7,8-THF against AHH induced oxidative stress in brain and heart was investigated in current study.

In order to evaluating for the anti-hypoxic activity of 5,6,7,8-THF, the animal model of normobaric hypoxia and acute decompression hypoxia were used in present study. A longer survival time and a lower death rate indicated a better anti-hypoxic activity of the drugs. We found that mice administrated with 5,6,7,8-THF could survive longer in normobaric hypoxia test and die lower in acute decompression hypoxia test than ACZ, which is the only drug approved by United States Food and Drug Administration (FDA) for treatment of AMS.

In this study, we also found that AHH induced some physiological parameters including HR, SBP, MAP and DBP significantly increased. The elevated HR and BP of the mice could be due to a compensatory mechanism to increase oxygen supplement. Pre-treatment with 5,6,7,8-THFcould attenuated these changes, which suggesting that the unbalance of oxygen demand-supply in mica under AHH has been ameliorated.

As a common fact, AHH induced oxidative stress damage is related to the ROS accumulation and per oxidation (Bakonyi and Radak, 2004, Murray and Horscroft, 2016). The ROS level was monitored by the content of H_2O_2, which is a most stable form of ROS. Comparing to control group, AHH-induced oxidative stress stimulated H_2O_2 production in brain and heart of mice. Treatment with 5,6,7,8-THF decreased H_2O_2 content in mice. Lipid per oxidation is another important indicator for the assessment of oxidative stress (Celep *et al.*, 2013). MDA, one of the end product of lipid per oxidation, can be used as a sign of lipid per oxidation (Suji and Sivakami, 2008). In our finding, HH exposure significantly increased the MDA content, which indicated that lipid peroxidation occurred, while 5,6,7,8-THF significantly reduced the MDA level in brain and heart of mice in AHH group. Normally, cellular antioxidant systems, such as SOD, CAT and GSH-Px could effectively remove ROS. SOD reacts with O_2^- produced during cellular respiration to generate H_2O_2, which is subsequently broken down by CAT and GSH-Px. However, hypoxic induced ROS production that overwhelms antioxidant capacities, leading to cellular damage. So maintaining the activity of these antioxidant enzymes is very important in the metabolic pathway of free radicals. Previous study has shown that the activity and effectiveness of antioxidant enzyme systems would decrease under HH condition (Baitharu *et al.*, 2013, Gong *et al.*, 2018). Similar results also observed in the present study, while 5,6,7,8-THF can increase these antioxidant enzyme activates in mice brain and heart to the normal level. These results obtained indicated that 5,6,7,8-THF had the ability of inhibiting oxidant stress induced by acute HH *in vivo.*

LD content and LDH activity are important biochemical indicators of glycometabolism and energy metabolism. The increase of LD indicated metabolic disorders and a clear response against energy depletion. LDH is one of the cytotoxic marker enzymes, which may increase, in anaerobic condition. Our results show that the LD level and LDH activity in brain and heart tissue was significant increased in AHH group compared to normal control. Pre-treatment with 5,6,7,8-THF reversed these changes in brain and heart to the normal level (P>0.05) and ameliorated the impairment of energy metabolism induced by AHH.

Mitochondria are central for various cellular processes that include ATP production, intracellular Ca^{2+} signaling, and generation of reactive oxygen species (Kann and Kovacs, 2007, Murray and Horscroft, 2016). The mitochondrial function can be reflected via the changes of the ATP levels. Hypoxia may induce the mitochondrial damage through overproduction of free radicals and calcium overload, as a result of decreasing of ATP content and ATPase activity (Fan *et al.*, 2015, Jain *et al.*, 2015). In agreement with this, our result indicated that the activity of ATPase and ATP content in mice brain and heart were significantly decreased in brain and heart after

AHH for 12h. Treatment of 5,6,7,8-THF could reverse these changes and make these indexes close to normal level, which suggested that 5,6,7,8-THF protected mitochondria in hypobaric hypoxia treatment.

CONCLUSION

In conclusion, the protective effect of 5,6,7,8-THF against the brain and heart injury induced by AHH *in vivo* has been proved in the present study. Our finding suggested that the 5,6,7,8-THF may protect the mice against AHH injury via scavenging free radical, inhibiting lipid per oxidation, enhancing antioxidant enzyme activity, preserving energy metabolism and mitochondrial function and can be utilized as new therapeutic agent for preventing acute mountain sickness in future.

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REFERENCES

Aksel G, Corbacioglu SK and Ozen C (2019). High-altitude illness: Management approach. *Turk J. Emerg. Med.,* **19**(4): 121-126.

Baitharu I, Jain V, Deep SN, Hota KB, Hota SK, Prasad D, Ilavazhagan G (2013). *Withania somnifera* root extract ameliorates hypobaric hypoxia induced memory impairment in rats. *J. Ethnopharmacol.,* **145**(2): 431-441.

Bakonyi T, Radak Z (2004). High altitude and free radicals. *J. Sports Sci. Med.,* **3**(2): 64-69.

Barhwal K, Hota SK, Jain V, Prasad D, Singh SB, Ilavazhagan G (2009). Acetyl-l-carnitine (ALCAR) prevents hypobaric hypoxia-induced spatial memory impairment through extracellular related kinase-mediated nuclear factor erythroid 2-related factor 2 phosphorylation. *Neuroscience.,* **161**(2): 501-514.

Bartsch P, Swenson ER (2013). Acute high-altitude illnesses. *N. Engl. J. Med.,* **369**(17): 1666-1667.

Celep E, Aydin A, Kirmizibekmez H and Yesilada E (2013). Appraisal of *in vitro* and *in vivo* antioxidant activity potential of cornelian cherry leaves. *Food Chem. Toxicol.,* **62**: 448-455.

Chen YH, Yang ZS, Wen CC, Chang YS, Wang BC, Hsiao CA and Shih TL (2012). Evaluation of the structure-activity relationship of flavonoids as antioxidants and toxicants of zebrafish larvae. *Food Chem.,* **134**(2): 717-724.

Fan PC, Ma HP, Jiang W, Li L, Ren J, Jing LL and Jia ZP (2015). Anti-hypoxia activity of the Novel NO donor acetyl ferulic isosorbide mononitrate in acute high-altitude hypoxia mice. *Biol. Pharm. Bull.,* **38**(9): 1280-1289.

Fan PC, Ma HP, Jing LL, Li L and Jia ZP (2013). The antioxidative effect of a novel free radical scavenger 4'-hydroxyl-2-substituted phenylnitronyl nitroxide in acute high-altitude hypoxia mice. *Biol. Pharm. Bull.,* **36**(6): 917-924.

Farber JL (1994). Mechanisms of cell injury by activated oxygen species. *Environ. Health Perspect.,* **102**(Suppl 10): 17-24.

Gong G, Yin L, Yuan L, Sui D, Sun Y, Fu H, Chen L, Wang X (2018). Ganglioside GM1 protects against high altitude cerebral edema in rats by suppressing the oxidative stress and inflammatory response via the PI3K/AKT-Nrf2 pathway. *Mol. Immunol.,* **95**: 91-98.

Irarrazaval S, Allard C, Campodonico J, Perez D, Strobel P, Vasquez L, Urquiaga I, Echeverria G and Leighton F (2017). Oxidative stress in acute hypobaric hypoxia. *High. Alt. Med. Biol.,* **18**(2): 128-134.

Jain K, Prasad D, Singh SB and Kohli E (2015). Hypobaric hypoxia imbalances mitochondrial dynamics in rat brain hippocampus. *Neurol. Res. Int.,* **2015**: 742059.

Jing LL, Ma HP, Fan PC and Jia ZP (2017). Synthesis and antioxidant properties of 5,6,7,8-tetrahydroxyflavone. *Chem. Nat. Compd+.* **53**(2): 248-253.

Kann O and Kovacs R (2007). Mitochondria and neuronal activity. *Am. J. Physiol. Cell Physiol.,* **292**(2): C641-657.

Karar J, Dolt KS, Mishra MK, Arif E, Javed S and Pasha MA (2007). Expression and functional activity of pro-oxidants and antioxidants in murine heart exposed to acute hypobaric hypoxia. *FEBS Lett.,* **581**(24): 4577-4582.

Koester-Hegmann C, Bengoetxea H, Kosenkov D, Thiersch M, Haider T, Gassmann M and Schneider Gasser EM (2018). High-altitude cognitive impairment is prevented by enriched environment including exercise via VEGF signaling. *Front. Cell. Neurosci.,* **12**: 532.

Koirala P, Wolpin SE and Peterson JT (2018). High altitude illness: Knowledge, practice and attitudes of porters in Nepal. *Wilderness Environ. Med.,* **29**(4): 431-436.

Li Y, Zhang Y and Zhang Y (2018). Research advances in pathogenesis and prophylactic measures of acute high altitude illness. *Respir. Med.,* **145**: 145-152.

Liu P, Zou D, Yi L, Chen M, Gao Y, Zhou R, Zhang Q, Zhou Y, Zhu J, Chen K and Mi M (2015). Quercetin ameliorates hypobaric hypoxia-induced memory impairment through mitochondrial and neuron function adaptation via the PGC-1alpha pathway. *Restor. Neurol. Neurosci.,* **33**(2): 143-157.

Luks AM, Swenson ER and Bartsch P (2017). Acute

high-altitude sickness. *Eur. Respir. Rev.,* **26**(143).

Ma HP, Fan PC, Jing LL, Yao J, He XR, Yang Y, Chen KM and Jia ZP (2011). Anti-hypoxic activity at simulated high altitude was isolated in petroleum ether extract of Saussurea involucrata. *J. Ethnopharmacol.,* **137**(3): 1510-1515.

Maity C, Lahiri P, Adak A, Ghosh K, Pati BR and Mondal KC (2013). Hypobaric-hypoxia induces alteration in microbes and microbes-associated enzyme profile in rat colonic samples. *Biomed. Environ. Sci.,* **26**(10): 869-873.

Murray AJ and Horscroft JA (2016). Mitochondrial function at extreme high altitude. *J. Physiol.,* **594**(5): 1137-1149.

Rauchova H, Vokurkova M and Koudelova J (2012). Hypoxia-induced lipid peroxidation in the brain during postnatal ontogenesis. *Physiol. Res.,* **61**(Suppl 1): S89-101.

Sarkar A, Angeline MS, Anand K, Ambasta RK and Kumar P (2012). Naringenin and quercetin reverse the effect of hypobaric hypoxia and elicit neuroprotective response in the murine model. *Brain Res.,* **1481**: 59-70.

Sharifi MR, Khajali F and Hassanpour H (2016). Antioxidant supplementation of low-protein diets reduced susceptibility to pulmonary hypertension in broiler chickens raised at high altitude. *J. Anim. Physiol. Anim. Nutr. (Berl.).* **100**(1): 69-76.

Sharma NK, Sethy NK, Meena RN, Ilavazhagan G, Das M and Bhargava K (2011). Activity-dependent neuroprotective protein (ADNP)-derived peptide (NAP) ameliorates hypobaric hypoxia induced oxidative stress in rat brain. *Peptides.,* **32**(6): 1217-1224.

Singh M, Kaur M and Silakari O (2014). Flavones: An important scaffold for medicinal chemistry. *Eur. J. Med. Chem.,* **84**: 206-239.

Siques P, Brito J and Pena E (2018). Reactive oxygen species and pulmonary vasculature during hypobaric hypoxia. *Front. Physiol.,* **9**: 865.

Stojkovic T, Radonjic NV, Velimirovic M, Jevtic G, Popovic V, Doknic M and Petronijevic ND (2012). Risperidone reverses phencyclidine induced decrease in glutathione levels and alterations of antioxidant defense in rat brain. *Prog. Neuropsychopharmacol. Biol. Psychiatry.* **39**(1): 192-199.

Suji G, Sivakami S (2008). Malondialdehyde, a lipid-derived aldehyde alters the reactivity of Cys34 and the esterase activity of serum albumin. *Toxicol. In Vitro.* **22**(3): 618-624.

Uzun O, Balbay O, Ustündag Comunoglu N, Yavuz O, Nihat Annakkaya A, Guler S, Silan C, Erbas M and Arbak P (2006). Hypobaric-hypoxia-induced pulmonary damage in rats ameliorated by antioxidant erdosteine. *Acta Histochem.,* **108**(1): 59-68.