

# *Hypericum perforatum* (ethanolic extract) ameliorates simulated hypobaric hypoxia induced oxidative stress and neuronal damage in brains of Balb/c mice



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## ABSTRACT

**Background:** Hypobaric hypoxia refers to lower oxygen availability at high altitudes and is the cause of high altitude illness. Drugs such as acetazolamide and dexamethasone provide symptomatic relief and are associated with undesired side effects. Plant extracts such as *Hypericum perforatum*, which are documented to have neuromodulatory role can be more beneficial in ameliorating high altitude illness. **Aims and Objective:** Progressive cognitive decline is the hallmark characteristic of hypobaric hypoxia induced neuropathology attributed to ensuing oxidative stress and subsequent hippocampal damage. We have explored the efficacy of ethanolic extracts of *Hypericum perforatum* in amelioration of hypobaric hypoxia induced oxidative stress and associated behavioral deficits in mice. **Material and Methods:** Male Balb/c mice were exposed to simulated altitude of 25,000 ft. for 7 days (6 hr. per day) in a specially designed chamber. Ethanolic extract of *Hypericum perforatum* (HPE) (25mg/kg of body weight) was given orally prior to Hypobaric exposure and effects were compared to Hypobaric and control groups. **Results:** Animals exposed to hypobaric hypoxia showed sign of cognitive deterioration at day 3 and day 7 in the Elevated Plus Maze and Passive Avoidance Step through behavioral paradigms as compare to normoxic animals. Administration of HPE was able to alleviate the amnesic effect in treatment group, indicated by reduction in transfer latencies at day 3 (IR-3 =  $-0.66 \pm 0.07$ ) and day 7 IR-7 =  $-0.81 \pm 0.06$ ) in elevated plus maze task and increased passive avoidance step through latency at day 3, (IR-3 =  $3.23 \pm 0.67$ ), as compared to hypoxic mice. Hypoxia group of animals suffered significant oxidative stress compared to normoxic mice as indicated by up-regulated malondialdehyde and total nitrite levels in hippocampal homogenates. The plasma lactate dehydrogenase activity was also increased following hypoxia indicating tissue damage. Co-treatment with HPE in simulated hypobaric hypoxia insult for seven days resulted in significant reduction in malondialdehyde, total nitrites and plasma LDH levels in animals. **Conclusion:** *Hypericum perforatum* extract improves cognitive performance in hypobaric hypoxia exposed mice with a concomitant reduction in oxidative stress burden suggesting its plausible use for preventing high altitude illness.

**Key words:** Hypobaric hypoxia, *Hypericum perforatum*, Cognitive deficits, Oxidative stress

## INTRODUCTION

Mountains have always fascinated human beings, making mountain tourism several billion dollar industry. However,

mountains have one of the harshest and challenging environments on earth. With increase in altitude, the partial pressure of oxygen falls almost linearly, leading to hypoxic conditions. Due to lack of oxygen as terminal electron

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acceptor, the reactive oxygen species (ROS) generated at mitochondrial respiratory sites has been implicated in the pathophysiology of high altitude illness.<sup>1</sup> Brain is most affected by this imbalance of redox state, owing to its higher oxygen demands and limited supply of antioxidants.<sup>2</sup> Reduced availability of oxygen at high altitudes manifests as high altitude illness which includes acute mountain sickness (AMS), high altitude cerebral edema (HACE) and/or high altitude pulmonary edema (HAPE). Although chemoreceptors sense the lack in oxygen tension and the body deploys counter responses such as hyperventilation, tachycardia, and an increased blood flow to the brain etc., they are not sufficient to combat the decline in aerobic capacity.<sup>3</sup>

Cognitive dysfunction is observed in humans as well as animals subjected to hypobaric hypoxia.<sup>4,5</sup> Hippocampus, part of the limbic system involved in learning and memory, is found to be a major structure affected by the hypobaric hypoxia induced oxidative stress leading to neuronal death and loss in cognitive functions.<sup>6</sup> Various drugs such as acetazolamide, ibuprofen and dexamethasone providing symptomatic relief have been used to alleviate HAI.<sup>7</sup> But pharmaceutical drugs always have their limitations and side effects. Therefore a natural approach of using herbal medicines such as plant extracts could be much more beneficial.

*Hypericum perforatum*, also known as St. John's Wort, is a herb used since ancient time in treatment of many ailments such as wound healing, neuralgia, fibrositis, anxiety and other psychiatric ailments. Medicinally active constituents of St. John's Wort include phloroglucinols, naphthodianthrones, flavonoids, biflavons, fluoroglucinols, xanthenes, essential oils etc.<sup>8</sup> Extracts of *Hypericum perforatum* (HPE) possess antioxidant properties against hydrogen peroxide induced toxicity and can be effective in treating neurodegenerative diseases.<sup>9,10</sup> Flavanols such as quercetin are found to be effective in neuroprotection by preventing neuronal excitotoxicity and mitochondrial dysfunction.<sup>11</sup> *H. perforatum* was also found to inhibit monoamine oxidase-B and increase dopamine levels.<sup>12</sup> It improves spatial working memory and thus cognitive performance in animals subjected to chronic restraint stress.<sup>13</sup> Pre-treatment with standardized extracts of *H. perforatum* prevented the rotenone-induced oxidative damage in rats and increased the gene expression of antioxidant enzymes in these animals.<sup>14</sup> Therefore, we designed the following study to evaluate the effect of HPE administration on hypobaric hypoxia induced neurotoxicity. Ethanolic extract of *Hypericum perforatum* was administered to animals orally thirty minutes before subjecting them to simulated hypobaric hypoxia (6hrs/day) corresponding to 25,000ft for seven days. Cognitive functions were evaluated on behavioral paradigms such as passive avoidance step

through task and elevated plus maze task. Modulatory effect of HPE on oxidative stress markers such as malondialdehyde, total nitrites and lactate dehydrogenase activity were estimated. The study would help to devise prophylactic treatments to alleviate high altitude illness associated with hypobaric hypoxia exposure and broaden the scope of use of an already very popular and effective herbal medicine.

## Materials

### Animals

Male Balb/C mice, weighing 22-25 g, obtained from National Institute of Nutrition, Hyderabad were used for experiments at animal facility of Dr. B. R. Ambedkar Centre for Biomedical Research, University of Delhi, India. The animals had free access to food and water, maintained under 12 hour dark/light cycle, 25±2°C temperature and 50-60% humidity conditions. All experimental procedures were approved by the Institutional Animal Ethics Committee. Care was taken to use minimum number of animals and reduce their suffering as far as possible.

## MATERIALS AND METHODS

Ethanolic extract of *Hypericum perforatum* (HPE) was procured from Dr. Willmar Schwabe Pharmaceuticals, Karlsruhe, Germany. Thiobarbituric acid (TBA), IGEPAL CA 630, Tris buffer, ethylene diamine tetraacetic acid (EDTA), MgCl<sub>2</sub> and protease inhibitor cocktail (PI-P8849) were purchased from Sigma-Aldrich (St. Louis, USA). Acetic acid, pyridine, ortho-phosphoric acid, sodium nitrite, sodium chloride, potassium dihydrogen phosphate, potassium chloride were purchased from Merck Ltd. (Mumbai, India), Sodium dodecyl sulphate (SDS) was purchased from Amresco-USA. *n*-Butanol was purchased from CDH laboratories (New Delhi, India). Nitrate reductase enzyme preparation and nitrate reductase cofactor preparation were purchased from Cayman Chemical Company, USA.

### Experimental Design

Animals were randomly divided into four groups. Group I – Normoxia (N): served as normal controls. Group II - Hypoxia (H): animals were subjected to simulated hypobaric hypoxia corresponding to an altitude of 25,000 ft., in a specially designed and calibrated hypobaric chamber (Seven Stars, India, Model No.SS7001) by gradually drawing out air via vacuum pumps over a period of 1 hour. The rate of air withdrawal was maintained corresponding to ~400 ft./min. and temperature was maintained at 25±2°C. Animals were kept under these conditions for 6 hours/day for 7 days and they had free access to food and water. Group III – Hypoxia + *H. perforatum* (H+HPE): animals were

orally given 25mg/kg body weight of 70% ethanolic extract of *Hypericum perforatum* in 0.3% carboxymethylcellulose as vehicle, 30 minutes before exposing to simulated hypobaric hypoxia. Group IV- Normoxia + *H. perforatum* (N+HPE): animals were given HPE (25mg/kg) in 0.3% CMC but not exposed to hypobaric hypoxia.

## Behavioral experiments

### Passive avoidance step-through task (PA)

The effect of *H. perforatum* on hypobaric hypoxia induced memory deficits was assessed by observing performance of mice on passive avoidance step-through task. This behavioral test is used to study associative memory in rodents. The apparatus consists of two compartments, a light chamber (9×9×14 cm) illuminated by a light source of 60W power and a dark chamber (14×9×14 cm) separated by a sliding guillotine door. For habituation, all mice were allowed to explore both the chambers for 120 s. On day 1 (Acquisition trial), each mouse was placed in the light chamber and allowed to explore it for 5 s. Then the guillotine door was opened and time taken by the animal to enter into the dark chamber was recorded as step-through latency in seconds. In the dark chamber, the animal received an unavoidable electric shock (0.2mA, 50Hz for 2s) via a grid on the floor of the dark compartment. On the 3<sup>rd</sup> and 7<sup>th</sup> day from acquisition trial, memory retention was tested by following the same procedure, except the electric shock in the dark chamber was not given (Retention trials). The time taken by the animal to enter the dark chamber was recorded as step-through latencies for day 3 and day 7 respectively. If the animal did not enter into the dark chamber within 120 seconds, the latency was recorded as 120 s.

### Elevated plus maze task (EPM)

The spatial memory function of animals in various groups was assessed using elevated plus-maze task. It exploits the tendency of rodents to go for a closed and safer place rather than an open one. The elevated plus maze is a T-shaped apparatus with two open arms and two closed arms, facing each other, of dimensions 16 cm×5 cm×12 cm (for closed arm) having a central hub. The whole maze is raised by 25 cm from the floor. On day 1 (Acquisition trial), each mouse was placed at the end of the open arm, facing away from the central hub. The time taken by it to enter into the closed arm was recorded as transfer latency, in seconds. If the animal did not enter the closed arm for 120 s, it was gently helped into the closed arm and latency was recorded as 120s. After entering into the closed arm, the animal was allowed to explore it for 15 s, after which it was removed and placed back into the cage. On the 3<sup>rd</sup> and 7<sup>th</sup> day (retention trials), memory retention trials were done by following the same procedure as day 1.

Inflexion ratios (IR) for passive avoidance step through and elevated plus maze tasks were calculated by using the formula:

$$IR = \{(\text{Day X latency} - \text{Day 1 latency}) / \text{Day 1 latency}\}$$

If X = Day 3, Inflexion Ratio = IR-3

If X = Day 7, Inflexion Ratio = IR-7

## Biochemical experiments

### Hippocampal homogenate and plasma preparation

After behavioral studies, the animals were anesthetized using 80mg/kg b wt. of Pentothal sodium. The blood from each mouse was obtained from axillary vein and collected in centrifuge tubes containing heparin (1U/mL). It was centrifuged at 3,000 rpm for 10 minutes to obtain plasma. After taking blood, animals were euthanized and hippocampus was dissected from the brains. Hippocampus was homogenized in homogenization buffer (containing 1% IPEGAL CA 630, 10 mM Tris, 150 mM NaCl, 1.5 mM MgCl<sub>2</sub> and 1μL/100μL protease inhibitor cocktail). The homogenate was centrifuged at 4,000 g for 10 min at 4° C and the supernatant was stored at - 80°C until further used. Protein concentration was estimated by the Bradford method at 595 nm (Infinite200, Tecan).

### Lipid peroxidation

Exposure of animals to hypobaric hypoxia leads to increase in oxidative stress related lipid peroxidation. Quantification of malondialdehyde (MDA) is widely used as a marker for lipid peroxidation. The method described by Okhawa was used to estimate the MDA formed in the present study with slight modifications.<sup>15</sup> Briefly, hippocampal homogenate equivalent to 100mg protein was mixed with 1 ml of 20% v/v acetic acid, (pH 3.5), 1 ml 0.67% w/v TBA and 0.1 ml 8.1% w/v SDS. It was heated to 95°C for 60 minutes and the pink color chromogen formed was extracted with *n*-butanol and pyridine (15:1 ratio). MDA formed was estimated by recording absorbance at 532 nm and calculating the concentration in nmol/mg protein by using molar extinction coefficient of MDA, viz 1.56 × 10<sup>5</sup> M<sup>-1</sup> cm<sup>-1</sup>.

### Total Nitrites

Nitric oxide (NO) is a very reactive molecule and gets converted to more stable nitrite/nitrate forms in cell. Greiss assay has been used to estimate the amount of total nitrites in a biological sample as an indication of NO produced. Total nitrates in the hippocampal homogenate were reduced to nitrites by Nitrate Reductase enzyme. Nitrites were then quantified by Greiss reagent (1% sulphanilamide, 0.1% naphthyl ethylene diamine dihydrochloride in 5% phosphoric acid). A standard curve using various concentrations of Sodium nitrite (0.625-100 nM) was prepared and the nitrite levels in the test samples were estimated.

### LDH activity

Hypobaric hypoxia induced release of LDH is well documented and is a marker for neuronal cytotoxicity.<sup>6</sup> LDH activity in blood plasma was measured using LDH-Cytotoxicity Assay Kit (BioVision, USA). LDH activity was determined by measuring the increase in the amount of formazan as per the manufacturer's specification. The water-soluble formazan dye was detected spectrophotometrically at 500 nm.

### Statistical analysis

Each value represents mean $\pm$ SEM calculated from 5-6 animals per group. One way ANOVA followed by Tukey's posttest analysis was applied and p value less than 0.05 were considered significant.

## RESULTS

### Memory retention in mice exposed to hypobaric hypoxia and co-treated with *Hypericum perforatum*

#### Passive Avoidance task

Average transfer latency in the normoxia group was significantly longer at day 3 (IR-3 = 5.12 $\pm$ 1.47) and day 7 (IR-7 = 4.9 $\pm$ 1.34) as compared to day 1. In the hypoxic group, there was no significant difference in day 3 (IR-3 = -0.73 $\pm$ 0.05) and day 7 (IR-7 = -0.27 $\pm$ 0.04) latencies as compared to day 1. The step-through latencies in the hypoxic compared to normoxic animals showed significant decrement on day 3 (mean difference = 67.71s, p<0.001) as well as day 7 (mean difference = 59.01s, p<0.001). In animals that received *H. perforatum* prior to hypoxia exposure, the latencies and inflexion ratios were comparable to normoxic controls at day 3 (IR-3 = 3.23 $\pm$ 0.67) as well as day 7 (IR-7 = 3.22 $\pm$ 1.17), these differences were not statistically significant from day 1. However, a significant increment in inflexion ratio at day 3 in H+HPE treated mice was observed compared to hypoxia exposed mice. In the normoxic animals treated with *H. perforatum*, the inflexion ratios at day 3 and day 7 were 1.7 $\pm$ 0.67 and 1.83 $\pm$ 0.67 respectively (Figure 1).

#### Elevated Plus Maze task

Learning in mice is indicated in the elevated plus maze task by reduction in the time taken by the animal to reach the closed arm in the retrieval trials. In the normoxia group, the animals took lesser time on day 3 (IR-3 = -0.63 $\pm$ 0.06) and on day 7 (IR-7 = -0.26 $\pm$ 0.09) as compared to day 1. The animals exposed to hypobaric hypoxia took longer time to reach the closed arm, both at day 3 (IR-3 = 0.6 $\pm$ 0.22) and at day 7 (IR-7 = 0.38 $\pm$ 0.15) as compared to day 1. The inflexion ratios at both day 3 (mean difference = 1.23, p<0.001) and day 7 (mean difference = 0.65, p<0.05) were significantly different among the normoxic and hypoxic animals. The mice exposed to simulated hypobaric

hypoxia co-administered with *H. perforatum* for seven days, showed significant decrease in time taken to enter the closed arm both at day 3 (IR-3 = -0.66 $\pm$ 0.07) and day 7 (IR-7 = -0.81 $\pm$ 0.06) as compared to non-treated hypoxic animals both on day 3 (mean difference = 1.29, p<0.001) and day 7 (mean difference = 1.20, p<0.001). Animals in group IV (N+HPE) showed decreased transfer latencies as compared to day 1, albeit statistically significant only at day 7 (IR-7 = -0.67 $\pm$ 0.11) (Figure 2). The inflexion ratios in elevated plus maze task were comparable in *H. perforatum* treated hypobaric hypoxia animals and normoxic controls.

### Lipid peroxidation in hippocampal homogenates:

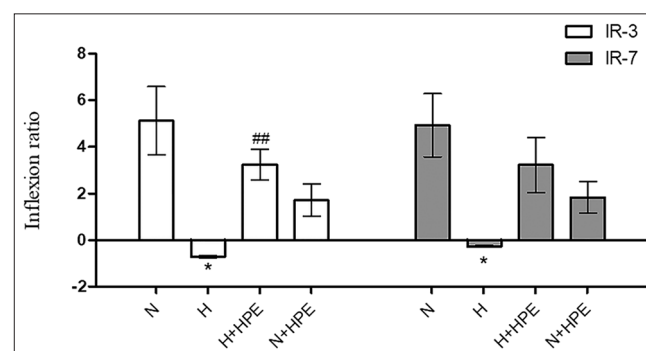
The animals in the hypoxia group had significantly higher levels of malondialdehyde in hippocampal homogenates as compared to normoxic animals (593.6 $\pm$ 34.42nmol/mg protein, p<0.001). Co-administration of *H. perforatum* to hypoxia exposed animals for 7 days brought down the levels of MDA in hippocampal homogenates to 194.8 $\pm$ 29.32 nmol/mg protein (p<0.001). There was not much difference in the MDA levels of animals in normoxia and normoxia treatment groups (Figure 3).

### Total Nitrite levels in hippocampal homogenates

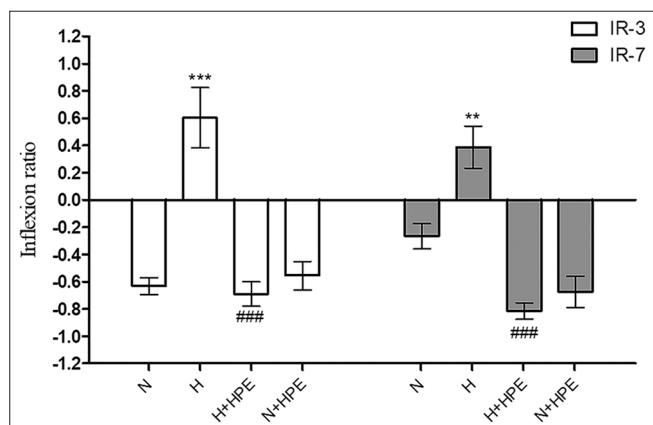
There was significant increase in total nitrite levels in the hypobaric hypoxia group (32.99 $\pm$ 7.25 $\mu$ M/mg protein) as compared to the normoxic animals (14.02 $\pm$ 2.47  $\mu$ M/mg protein, p<0.05). Treatment with *H. perforatum* brought down the nitrite levels to 15.6 $\pm$ 1.72 $\mu$ M/mg protein, p<0.05 (Figure 4).

### Lactate dehydrogenase activity

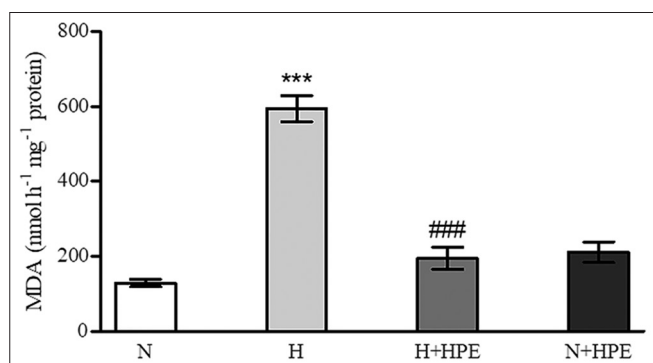
LDH activity is a measure of cytotoxicity. Animals in the hypoxia group had significantly higher LDH activity as compared to normoxic animals (76.61 $\pm$ 23.86%, p<0.05). Whereas three fold less plasma LDH activity was



**Figure 1:** Modulatory effect of HPE on hypobaric hypoxia induced associative memory changes observed by passive avoidance step through task in mice. Results are shown as mean Inflexion ratios  $\pm$ SEM at subsequent retrieval trials at day 3 and day 7. IR-3 = Inflexion ratio at day 3, IR-7 = Inflexion ratio at day 7. One way ANOVA followed by Tukey's multiple comparison test was applied for IR-3 and IR-7 separately. For Day 3- \*p<0.05 N vs. H; \*\*p<0.05 H vs. H+HPE. For Day 7- \*p<0.05 N vs. H. N=6



**Figure 2:** Effect of *H. perforatum* co-administration on elevated plus maze task performance of mice exposed to hypobaric hypoxia. Results are shown as inflexion ratios  $\pm$ SEM, IF-3 = Inflexion ratio at day 3, IF-7 = Inflexion ratio at day 7. One way ANOVA followed by Tukey's Multiple Comparison Test was applied for IR-3 and IR-7 separately. For Day 3- \*\*\*  $p < 0.001$  N vs. H; ###  $p < 0.001$  H vs. H+HPE. For Day 7- \*\*  $p < 0.05$  N vs. H; ###  $p < 0.001$  H vs. H+HPE. N=6



**Figure 3:** *H. perforatum* mediated modulation in hippocampal Malondialdehyde (MDA) levels in hypobaric hypoxia exposed mice compared to pair matched controls. Amount of MDA formed in nmol  $\text{mg}^{-1}$  protein by using molar extinction coefficient of MDA-TBA complex ( $1.56 \times 10^5 \text{ cm}^2 \text{ mmol}^{-1}$ ). Results expressed as means  $\pm$  SEM. \*\*\*  $p < 0.05$  N vs. H; ###  $p < 0.05$  H vs. H+HPE. N=6

observed in the hypoxic animals receiving *H. perforatum* ( $24.86 \pm 31.43\%$ ). Animals in group IV did not show any increased cytotoxicity (Figure 5).

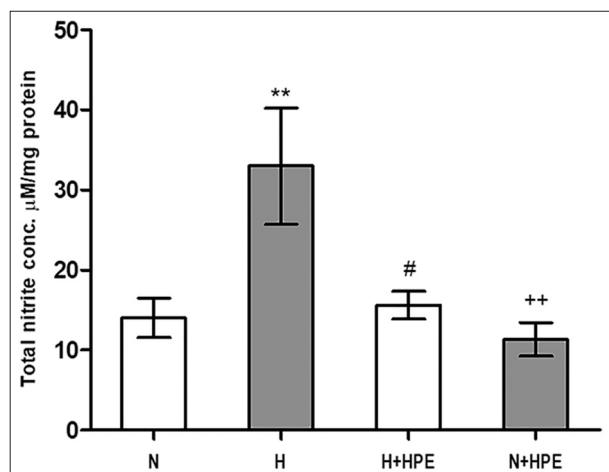
## DISCUSSION

In the present study, we have speculated the use of *Hypericum perforatum*, a popular and widely used medicinal plant, in ameliorating hypobaric Hypoxia induced oxidative stress and cognitive dysfunction in mice. As the altitude increases, the partial pressure of gases is reduced linearly. At  $\sim 8000$  m, the partial pressure of oxygen is 30% of its value at sea level.<sup>35</sup> The reactive oxygen species (ROS) generated as a result of less oxygen have been implicated in the pathophysiology of intermittent hypobaric hypoxia. Animals exposed to a regimen of intermittent hypobaric hypoxia had elevated levels of various oxidative stress

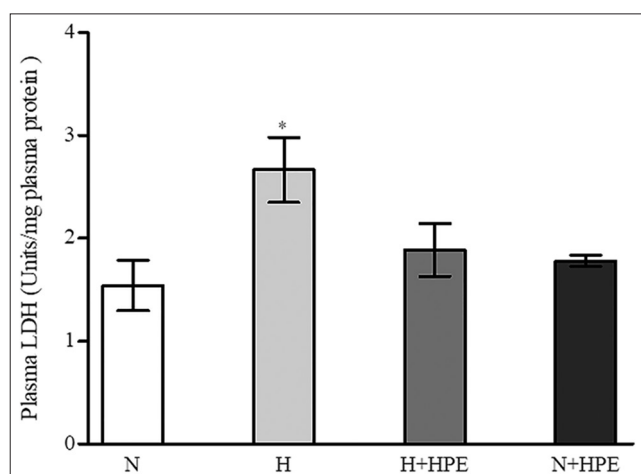
indices.<sup>16</sup> Thus, maintaining the redox balance seems imperative in preventing hypobaric hypoxia induced pathology.

Hippocampus is involved in many aspects of memory formation and any damage to this region can be attributed to memory impairment.<sup>17</sup> Animals exposed to HH were in fact found to have damaged neurons in the hippocampal region.<sup>6</sup> In the present study, the learning ability of the animals exposed to hypobaric hypoxia was decreased, as reflected by increased transfer latencies and decreased escape latencies of day 3 and day 7 in EPM and PA respectively.

EPM is used to study memory and learning in rodents effectively as there is no need to manipulate appetitive behavior or use aversive stimuli. Transfer latency in this behavioral paradigm decreases as the animal learns the spatial orientation of the maze. In the present study, the normoxic animals took significantly lesser time in reaching the closed arm in 3<sup>rd</sup> day and 7<sup>th</sup> day retrieval trials. In contrast, in the hypoxic group, the transfer latency was increased both at day 3 and day 7, indicating impairment in memory formation in these animals. Treatment with *H. perforatum* could ameliorate this amnesic behavior as the transfer latency was decreased at day 3 and day 7 compared to day 1. In passive avoidance task also, the normoxic animals demonstrate intact learning mechanism as indicated by increase in the transfer latencies at day 3 and day 7. They were able to associate the aversive electric stimuli with the dark chamber and therefore were hesitant in entering it. Animals in the hypoxia group had little or no such association established as indicated by the decreased step-through latencies at day 3 and day 7. Treatment with *H. perforatum* increased the time taken by animal to enter the dark chamber, where it had received a noxious stimulus, in retrieval trials of day 3 and day 7, indicating memory consolidation and retrieval. Klusa et al showed that the 25mg/kg body weight dose of ethanolic extract of *H. perforatum*, although effective in preventing depressive symptoms in chronic restraint test, did not show any significant improvements in passive avoidance task in mice administered with scopolamine.<sup>18</sup> However, *Hypericum* extracts were able to improve performances in passive avoidance task and other behavioral tasks in animal subjected to chronic restraint stress.<sup>19</sup> Ethanolic extracts of Indian *Hypericum perforatum* have been shown to reverse scopolamine induced memory dysfunction in mice, albeit at much higher doses than 25mg/kg dose used in the present study.<sup>19</sup> This dose is equivalent to a dose of 1800 mg/kg b wt. in humans, which is the maximum tolerated dose in depressed patients.<sup>20</sup> Therefore, it is encouraging to see the nootropic effect of *Hypericum perforatum* extracts at 25 mg/kg b wt. which can further be extrapolated to a prophylactic dose for tolerable use in



**Figure 4:** *H. perforatum* ameliorates the hypobaric hypoxia mediated rise in hippocampal. Concentration of total nitrite in hippocampal homogenate was calculated by using different dilutions of  $\text{NaNO}_2$  as standards (1.5-100µM). Data represented as mmole/mg protein (Mean±SEM). \*\*p<0.05N vs. H; #p<0.05H vs. H+HPE, \*\*p<0.05 H vs. N+HPE. N=6



**Figure 5:** Lactate dehydrogenase (LDH) activity in blood plasma in *H. perforatum* treated hypoxic animals compared to pair matched controls. Results expressed as units (means ±SEM). \*p<0.05 Nvs.H. N=6

human subjects exposed to hypobaric hypoxia. Hyperforin, one of the active constituent of *Hypericum* extracts has been shown to elevate the neurotransmitter levels in neurons in atypical ways by targeting the transient receptor potential channel protein 6 (TRPC6) and increasing the  $\text{Na}^+$  and  $\text{Ca}^{2+}$  concentrations.<sup>21</sup> The nootropic effect of *Hypericum* can be one of the outcomes of this altered ionic balance, especially the calcium ion.

Extracts of *Hypericum* rich in flavonoids demonstrate anti-oxidant properties *in vitro* against hydrogen peroxide induced toxicity.<sup>9</sup> In a recent study, the hypericin deficient and flavonoid rich methanolic extracts of *Hypericum connatum* showed promising results in stress induced behavioral abnormalities.<sup>22</sup> Flavonol quercetin has been shown to

ameliorate the neurodegenerative effects and cognitive impairment associated with hypobaric hypoxia in rats. Daily dose of quercetin during HH exposure also brought down the increased levels of various oxidative stress markers.<sup>23</sup> Zou also proposed that *H. perforatum* extracts can be used in treatment of various neurodegenerative diseases where the pathology is mediated or caused by oxidative stress.<sup>11</sup>

Increased expression of nitric oxide synthase (NOS) and subsequent rise in cellular NO was found to be correlated with hypobaric hypoxia induced oxidative stress and DNA damage in hippocampus, and use of NOS inhibitors can be useful.<sup>24</sup> Nitric oxide is a highly reactive molecule and in biological systems is converted to more stable nitrates. Animals exposed to simulated hypobaric hypoxia in Hypoxia group had increased total nitrite level as compared to normoxia. The co-treatment of *H. perforatum* extracts under simulated hypobaric hypoxia condition showed a significant less total nitrite levels in the hippocampal homogenates indicating animals that received *H. perforatum* extracts prior to hypoxic insult had lower NO production. *In vitro* studies show that flavonoids can inhibit nitric oxide (NO) production by down regulating the inducible nitric oxide synthase (iNOS) enzyme and that planar ring geometry is essential for such property.<sup>25</sup> The flavonoid quercetin and hyperoside were found to be effective inhibitors of NOS enzymes in rat cerebrum homogenate and blood. Amongst them hyperoside was found to be selectively inhibiting the neuronal NOS.<sup>26</sup>

Lipid peroxidation is one of the markers of oxidative stress. ROS attack the lipid rich cell membranes generating peroxy free radicals. These radicals attack other lipid molecules producing more free radicals, thereby starting a chain reaction and damaging the membrane.<sup>27</sup> As in any other oxidative stress mediated ailment, lipid peroxidation is also observed in hypobaric hypoxia.<sup>16</sup> In our study also, the malondialdehyde levels- marker for lipid peroxidation, were significantly higher in animals exposed to hypobaric hypoxia. Treatment with 25 mg/kg b wt *Hypericum perforatum* significantly brought these levels down. Alcoholic extracts of *Hypericum* were found to prevent lipid peroxidation induced by chemical agents such as  $\text{FeSO}_4$  and CHP as well as that induced *in vivo* by restraint stress.<sup>28</sup> Ethanolic extracts of *Hypericum* were able to reduce lipid peroxidation *in vitro* in hippocampal neurons.<sup>29</sup> *H. perforatum* extracts were also able to attenuate the lipid peroxidation in animal models of ischemia-reperfusion injury caused by bilateral occlusion of common carotid arteries.<sup>30</sup>

The key effects of ROS mediated cellular damage have been reported on plasma membrane. Release of lactate

dehydrogenase (LDH) enzyme in the cellular milieu is one the marker for assaying membrane damage. LDH assay faithfully determines the neuronal death caused by various agents.<sup>31</sup> In the hypoxic group, the plasma LDH activity was significantly more, indicating that there was increase cell death as a result of hypobaric hypoxia exposure. Treatment with *Hypericum* extracts ameliorated this cellular damage as the LDH released was significantly lowered. *Hypericum* extracts have been shown to reduce LDH release in a number of cellular stresses such as that of H<sub>2</sub>O<sub>2</sub> mediated,<sup>20</sup> ischemia/reperfusion injury, malaria infection, tert-butyl hydroperoxide (t-BHP) etc. which is in consonance with our findings.<sup>32-34</sup>

In view of the current findings, we propose that pretreatment with *Hypericum* extract can prevent adverse effects of hypobaric hypoxia. A more extensive research may lead to better understanding of interaction of *Hypericum perforatum* with major players of oxidative stress induced at high altitude.

## CONCLUSIONS

*Hypericum perforatum* could ameliorate the cognitive dysfunction caused by hypobaric hypoxia. The reduction in oxidative stress in *Hypericum* treated animals indicates that it may be acting by attenuating ROS mediated neuropathology. *Hypericum* seems to be a good candidate in preventing high altitude cognitive dysfunction.

This study establishes *Hypericum perforatum* as a potential herbal extract in treatment of high altitude illness. *Hypericum* already finds use in public medicine as an anti-depressant. This is first study to evaluate its nootropic effect in hypobaric hypoxia induced cognitive dysfunction.

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