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Neuroprotective effect of sea buckthorn leaf extract against hypobaric hypoxia and reoxygenation induced hippocampal damage in rats.

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Abstract

Exposure to hypobaric hypoxia (HBH) and reoxygenation (H/R) causes hippocampal neuronal damage leading to memory dysfunction and mood state alteration. The present study aimed at investigating the potential neuroprotective effect of seabuckthorn leaf extract ((SBTLE)) against HBH and reoxygenation induced neuronal injury in brain. Six groups of male sprague dawley rats were taken and exposed to simulated HBH equivalent at an altitude of 7600m in an animal decompression chamber for 7 days followed by reoxygenation. Rats were supplemented with SBTLE (100mg/kgBW) 20 days prior and during hypoxic exposure. Recovery from injuries following HBH exposure and subsequent reoxygenation was carried out in separate group of animals. Our study revealed that exposure to reoxygenation stress following hypoxia enhanced hypoxia induced oxidative stress in hippocampal neurons which was reversed with SBTLE supplementation. SBTLE restored Hypoxia/Reoxygenation(H/R) induced downregulation of γ-glutamylcysteine synthetase (GCLC) enzymes responsible for glutathione biosynthesis. Post hypoxic supplementation of SBTLE decreased the reoxygenation induced enhanced oxidative markers, however, administration of SBTLE in conjunction with the inhibitor of GCLC resulted in slow recovery from H/R injuries. These results provide the first evidence of SBTLE induced increase in glutathione biosynthesis by upregulating GCLC enzyme expression and hence can be used as a promising drug to cure H/R induced neuronal damages.

Keywords: Hypobaric hypoxia, Hippocampus, Hippophae rhamnoides L, Glutathione, oxidative stress, Reoxygenation

Introduction

Hypobaric hypoxia, an environmental condition characterized by reduced partial pressure of oxygen in the atmosphere, is unique to high altitude regions. This stress has been reported to be associated with several neurophysiological disorders including alteration in higher order brain function like cognition and mood states [1]. Ascent to high altitude causes several physiological problems such as acute mountain sickness (AMS), high altitude (HA) pulmonary edema, cerebral edema (HACE) [2], memory and motor impairment [3]. Severe high altitude hypoxia causes increase in generation of free radicals leading to augmented cellular oxidative stress with consequent damage to macromolecules viz lipids, proteins, DNA and carbohydrates in cells. The oxidative stress may be one of the causative factors in high altitude (HA) induced neuronal damage in the hippocampal region of the brain [4, 5].

Hypoxia in conjunction with subsequent sudden burst of excessive oxygen induces elevated oxygen free radicals generation in the brain on descent to sea level and worsens the survivability of various cells [6]. The severities of neuronal damage are directly correlated with supranormal tissue oxygen level during reoxygenation after exposure of rat cortical brain slice with severe hypoxia [7]. The reoxygenation induced damage to neuronal tissue has been reportedly more in vivo condition compared to the ex vivo brain slices [8]. On a neurochemical level, hyperoxic reperfusion worsens the post-ischemic oxidized shift in tissue redox state and exacerbates brain lipid as well as protein oxidation. Excessive free radicals cause the cellular macromolecular damages of lipids, proteins, and nucleic acids; thus, they play a crucial role in ischemic/reperfusion brain damage. During reperfusion, the antioxidative defense mechanism is compromised when oxygen radicals are over-produced, in addition to the inactivation of detoxification systems and consumption of antioxidants in post-ischemic tissue [9].

Recovery from damage following long term exposure to hypobaric hypoxia and reoxygenation has been a major concern in high altitude research. Hypobaric hypoxic damage is a reversible phenomenon which tends to normalise gradually after the stress is over. But recent reports have shown that sometime it takes too long time to revert back to absolute normal condition whereas in many other cases the damages is not completely recovered. Therefore it is necessary to develop some intervention which could make the process of hypoxic recovery rapid alongwith protection during the stress exposure. Previous report from our lab have shown that inhibition of glial glutamate uptake by ceftriaxone [10], calcium channel blockers [11] and acetyl cholinesterase inhibitor like phytostigmine and galantamine have promising potential in protecting the neuron from hypoxic damage [12]. However, few studies have been done in term of recovery after hypoxic damages. The present study was therefore aimed at investigating the neuroprotective effect of seabuckthorn leaf extract to ameliorate the posthypoxic reoxygentation stress induced damage to hippocampal tissue. Sea buckthorn (Hippophae rhamnoides L., Elaeagnaceae) is a thorny nitrogen fixing deciduous shrub used in traditional medicines in Tibet and Mongolia [13]. It is a rich source of vitamins, trace elements, amino acids and other bioactive substances. Seabuckthorn contains potent polyphenolic antioxidants and has shown to be protective to glial cell [14]. Molecular mechanism underlying the protective effect of seabuckthorn leaf extract also has been investigated in the present study.

Material And Methods

Chemicals and Reagents

All the standards as well as the chemicals used for RP-HPLC were procured from Sigma Aldrich (USA) if otherwise mentioned. The antibodies used was procured from the SantaCruz biotechnologies,USA. Glutathione reductase activity was measured using the RANDOX kit purchased from the RANDOX laboratory, USA. Buthionine sulfoximine was purchased from sigma-aldrich (USA).

Animals

All the experimental protocols followed in this experiment were approved by the ethical committee of the institute following the guidelines of "Committee for the Purpose of Control and Supervision of Experiments on Animals" of Govt. of India. The experiments were conducted on male Sprague–Dawley albino rats weighing 180-200g maintained at 25±2°C with food and water ad libitum. The rats were maintained on 12h day and night cycle.

Experimental Design

The study was carried out in three phases. The phase I study aimed at exploring the effect of sea buckthorn leaf extract supplementation to ameliorate the hypobaric hypoxia/reoxygenation induced oxidative stress. The rats (n=10/group) were distributed in six groups normoxia, normoxia+SBT, hypoxia+ vehicle, hypoxia+SBT, hypoxia + reoxygenation + Vehicle, hypoxia + reoxygenation + SBT.

Phase II: The objective of the study was to evaluate the effect of SBTLE supplementation on the rate of recovery after post hypoxic rexoygenation injury. The rats (n=50) were divided into two group (treated and non-treated) and each group consist of five subgroup. (n=5/group). Subgroup I was designated as control rest four were divided into 3 days, 6 days,12 days and 14 days of recovery for each group.

Phase III: The study aimed to unfold the modulatory role of SBTLE on endogenous antioxidant level like glutathione during hypoxia/post hypoxic reoxygenation stress. Rats(n= 25) were divided into five group where group I served as normoxic control, other groups were designated as Hypoxia +Reox.+ Veh, Hypoxia + 6d Recovery + buthionine Sulfoximine, hypoxia + 6d recovery + SBT, Hypoxia+ 6d recovery + SBT + buthionine Sulfoximine.

Preparation of Drug and pharmacological intervention

The SBT leaf extract was obtained from the phytoanalysis laboratory in DIPAS. The animal were administered with alcoholic SBT leaf extract orally at a dose of 100mg/kg body weight (BW) orally with the help of gastric cannula and the control group was maintained on saline containing 0.1% Tween-80. The rats were fed with SBT leaf extract for 20 day before exposure to hypobaric hypoxia and the drug feeding was continued during the 7 days of hypoxic exposure. Feeding of SBT leaf extract started immediately after the end of exposure to hypobaric hypoxia and continued till 14 days for recovery study. Buthionine Sulfoximine (3mM/ kg BW, i.p) was administered at alternative day immediately after the exposure to hypobaric hypoxia during six day period of recovery from post hypoxic reoxygenation stress.

Hypoxic exposure

Animals designated to be exposed for 7 days to hypobaric hypoxia were inducted to a simulated altitude of 7600 m (25,000 ft, 282 mm Hg) in a specially designed animal decompression chamber where altitude could be maintained by reducing the ambient barometric pressure with 1h reoxygenation. Fresh air was continuously flushed at a rate of 8 L/min to prevent accumulation of carbon dioxide within the chamber. The temperature and humidity in the chamber were maintained precisely at 28±2°C and 55±5% respectively. The rate of ascent and descent to hypobaric conditions was maintained at 300 m/min. The hypobaric hypoxic exposure was continuous for the stipulated period except for a 10–15 min interval each day for replenishment of food and water, drug administration and changing the cages housing the animals.

Sample preparation

On completion of stipulated period of exposure to hypobaric hypoxia, the animals were anaesthetized and sacrificed. Then hippocampal tissue was isolated from the brain and stored at –80°C for further analysis.The tissue was then homogenized in 0.15 M KCL and 1mM phenyl methyl sulphonyl fluoride (PMSF) to produce 10% homogenate. The homogenate was centrifuged at 5000 rpm at 4°C for 10 minutes and supernatant was collected.

Identification and quantification of marker compounds by RP-HPLC

A simple and gradient elution-based RP-HPLC method was used for the analysis of Quercetin-3-galactoside, kaempferol and isorhamnetin in the extracts obtained from phytoanalysis lab in the Institute [15]. Solvent system consisting of 0.3% ortho phosphoric acid in water and acetonitrile:methanol (75:25) was used to resolve the key components like Quercetin -3-galactoside, Kaempferol and Isorhamnetin. Identification of compounds was performed on the basis of the retention time, co injections and spectral matching with standard. For quantification, standard stock solutions of Quercetin-3-galactoside, Kaempferol and Isorhamnetin (1mg/2mL) was prepared in ethanol filtered through 0.22um filters (Millipore) and serially diluted (0.01-100ug/mL) to obtain the desired concentrations.

Biochemical analysis

Oxidative stress markers

Free Radical Estimation

The free radicals were estimated spectrofluorimetrically by 2',7'-dichlorofluorescein (DCFHDA) by the method of LeBel [16] and modified by Maiti et al., (2006) [4]. The readings were expressed as fluorescent units per mg of protein and converted to percentage by taking normoxic value as 100%.

Lipid peroxidation

Lipid peroxidation was measured by quantitating the amount of malondialdehyde (MDA) that is produced as an end product as described by Utley et al (1967) [17] in the hippocampal homogenates and expressed as µmol/mg protein. The results thus obtained were then converted to percentage considering normoxic value as 100%.

Antioxidant status

Reduced Glutathione Level

The reduced glutathione (GSH) levels were measured from the crude homogenate according to the procedure followed by Hissin and Hilf (1976) [18]. The amount of GSH was calculated from a standard curve and expressed in µmol/ml and converted to percentage taking normoxic value as 100%.

Glutathione Reductase activity

GR activity was measured by the method followed by Pinto and Bartley (1969) [19] and the values obtained were expressed in µmol of NADPH oxidized/mg/g tissue and converted to percentage taking normoxic value as 100%.

Expression of GCLC in Hippocampus by Western blotting

The hippocampus was dissected out at 4 °C and homogenized in ice-cold lysis buffer (0.01 M Tris HCl, pH 7.6, 0.1 M NaCl, 0.1 M dithiothreitol, 1 mM EDTA, 0.1% NaN3, PMSF, Protease inhibitor cocktail). The homogenate was centrifuged at 10,000 g for 10 min at 4 °C and the supernatant was used for protein expression analysis. 12% SDS-PAGE was run in duplicates depending upon the molecular weight of the protein of interest. 50 µg of sample protein was resolved by SDS-PAGE and transferred to nitrocellulose membranes presoaked in transfer buffer (20% methanol, 0.3% Tris, 1.44% glycine) using a semidry transblot module (BIORAD).The transfer of the protein bands to the membrane was verified by Ponceau S staining. The membrane was then blocked with 5% Blotto (Santa Cruz) for 1 h and then washed with PBST (0.01 M phosphate buffer saline, pH 7.4, 0.1% Tween 20). The membranes were then probed overnight with goat polyclonal GCLC specific antibodies (Santa Cruz). Subsequently, the membranes were washed with PBST thrice 10 min each and were incubated with suitable secondary anti IgG HRP conjugated antibody for 2–3 h. The membranes were then developed using chemiluminiscent peroxidase substrate kit (Sigma). The membranes were then stripped using stripping buffer (Bio-Rad) and probed for β-actin expression. The protein expression in each group was quantified by densitometric analysis.

Statistical Analysis

Data are presented as mean ± SEM unless otherwise stated. One-way ANOVA was used for analysis of the biochemical parameters. This was followed by post hoc student's Newman–Keul's test. Probability values less than 5% (0.05) were considered significant for all statistical analysis.

Result and Discussion

Chronic exposure to hypobaric hypoxia has been reported to cause neuronal damage by induction of oxidative stress in human, depending on the severity of the exposure [1]. It has been

established that hippocampus, one of the region concerned with learning and memory is highly susceptible to hypoxic damage [20]. The damage to neuronal cells in the hippocampal tissue results in many neurophysiological disorders leading to functional deformity of higher order brain function including memory and mood state [21]. Reoxygenation on descent to sea level leads to a sudden burst of oxygen which generates huge reactive oxygen species in various tissues including the brain [22]. The neuronal tissues are more susceptible to hypoxic reoxygenation stress owing to its higher metabolic requirement of oxygen and abundant polyunsaturated fatty acid content. The compromised neuronal antioxidant defense system and high level of transition metals like copper and iron causing augmented hydroxyl radical formation by Fenton reaction further aggravate the oxidative stress in brain. The present study therefore aims at exploring the neuroprotective effect of SBT leaf extract following chronic exposure to hypobaric hypoxia and subsequent reoxygentaion. The studies also delineate duration dependent effect of SBT leaf extract on the recovery from post-hypoxic reoxygenation injuries in term of free radicals generation and lipid peroxidation. Possible mechanism underlying the neuroprotection and free radicals scavenging potential of SBT leaf extract has also been investigated in the present study.

Exposure to hypobaric hypoxia for 7 days elevated the reactive oxygen species generation in the hippocampus in the present study which corroborate with the previous findings reporting the increase in free radical generation in rats under hypoxic and ischemic condition [23,5]. Supplementation of SBT leaf extract 20 days prior to hypobaric hypoxia stress exposure effectively decreased reactive oxygen species level in hippocampus. SBT leaf extract is rich in several components with antioxidant properties and redox potential that can scavenge the free radicals generated during hypoxic stress exposure. Several studies demonstrate the free radical scavenging activity of phenolic fractions in SBT extract [24], with the predominate polyphenols being flavonols and catechins and phenolic acids representing minor portions. Supporting the present findings, similar studies in glial and lymphocyte cells showed that pretreatment of SBT leaf extract reduces free radical generation under the condition of chromium and sodium nitroprusside induced oxidative stress [25, 26].

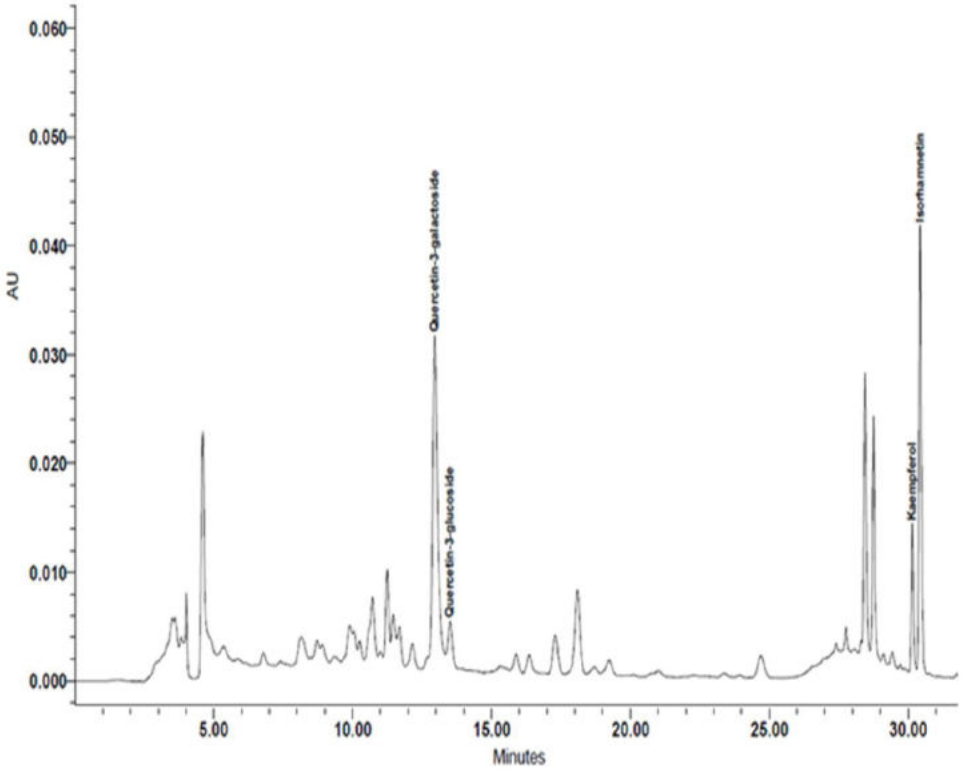
Reoxygenation following hypoxia has been reportedly accompanied with rapid transient increase in reactive oxygen species (ROS) generation in brain [27] and exacerbate hypoxia induced damage to neuronal and several other tissues. Our study reports an elevated reactive oxygen species generation in hippocampal tissue following exposure to 7 days hypobaric hypoxia and one hour reoxygenation. The increase in ROS formation could be due to incomplete reduction of O2 to superoxide (O2-) or hydrogen peroxide (H2O2). H2O2 in turn can lead to production of highly reactive hydroxyl radicals (OH-) that damage cells by the oxidization of essential cellular lipids, proteins, and nucleic acids [28]. ROS overproduction occurs during reoxygenation because hypoxia causes an accumulation of reducing equivalents (NADH, FADH2) in the mitochondria which rapidly undergo oxidation in presence of abundant oxygen resulting in the formation of oxygen free radical [29]. Supplementation of SBT leaf extract before exposure to hypobaric hypoxia/reoxygenation stress reduced the reactive oxygen species level following exposure to such stress. The observed decrease in free radical level following hypoxia/ reoxygenation could be accounted to the free radical scavenging effect of the various components like catechin, quercetin and flavanoids in SBT leaf extract, which can act as strong antioxidants. However, the reactive oxygen species level still remain higher compared to normoxic group following hypoxia/reoxygenation exposure indicating that there was a rapid burst of free radical generation following reoxygenation. Overproduction of reactive oxygen species which react with lipid membranes in neuronal cells leads to lipid peroxidation through chain reaction. Our study reports an elevated level of lipid peroxidation following exposure to hypobaric hypoxia for 7 days. This result supports the previous finding on increased lipid peroxidation in different brain region following chronic exposure to hypoxic condition [4, 30]. Prefeeding rats with SBT leaf extract for 20 days prior to exposure to hypobaric hypoxia reduced the lipid peroxidation effectively. The protective effect of SBT leaf extract against hypoxic insult leading to lipid peroxidation in macrophages has also been reported by Geetha et al. (2003) [26] but no work has been done to evaluate its effect in neuronal tissue. On the contrary, reoxygenation following 7 days of exposure to hypobaric hypoxia showed robust increase in lipid peroxidation in hippocampus. These findings are in accordance with previous work by Chuanyu [31] who have reported an elevation in lipid peroxidation following hypoxia/reoxygenation stress because of higher level of reactive oxygen species generation. Prefeeding rats with SBT leaf extract effectively diminished the lipid peroxidation indicating its strong antioxidants contents which scavenge the free radical generated and hence blocked further enhancement of damage to lipid bilayer through peroxidation. Endogenous antioxidants are very critical for the survivability of the cells under hypoxic stress [32]. Cells have their own antioxidant as well as free radical scavenging enzyme systems. Reduced glutathione level plays a very crucial role among them. Chronic exposure to hypobaric hypoxia, in the present study, decreased the reduced glutathione level as well as the glutathione reductase activity that rescues the reduced glutathione from its oxidized state. Reduced availability of NADPH that serves as the reducing power in conversion of oxidized glutathione to reduced glutathione as a result of dysfunctions in the electron transport chain in mitochondria is a plausible reason for the reduced GR activity [33]. Previous findings also report the reduction of glutathione level as well as GR activity following chronic exposure to hypobaric hypoxia [21]. This could be due to inhibition of GSH synthesis and increased utilization of GSH for detoxification of hypoxia induced free radical leading to increase in GSSG synthesis. However, supplementing SBT leaf extract following hypobaric hypoxia enhanced the GR activity which may lead to the increase in reduced glutathione levels in brain. There are numerous reports that the flavonoids have free radical scavenging activity [34].The increase in the reduced glutathione level on supplementation of the SBT leaf extract corroborate with observed upregulation of the γ-glutamylcysteine

synthetase expression in the hippocampal region. The GCLC is the key regulatory enzyme in the glutathione biosynthesis and reported to be modulated by several factor like Nrf2 during hypoxic exposure. Previous studies reports increased level of glutamate in hippocampal region on exposure to hypobaric hypoxia [35] leading to reduction in the cysteine uptake to the neuronal cells which may effect the glutathione biosynthesis. The results are in accordance with previous findings that SBT leaf extract increase reduced glutathione under various other stress condition [26]. On the other hand, exposure to hypoxia/reoxygenation stress decreased the reduced glutathione level in hippocampus which supports the pervious findings that GSH levels were decreased in brain regions during reperfusion for 1 hr after moderate or severe ischemia for 0.5 hr [36]. Supplementing SBT leaf extract prior to hypoxia/reoxygenation exposure however maintained higher level of reduced glutathione level. This effect of SBT leaf extract may be accounted for the increased glutathione reductase activities as well as the biosynthetic enzyme γ-glutamylcysteine synthetase whose activity was induced on supplementation of leaf extract following hypoxia/reoxygenation exposure.

Recovery from stress induced damage to body systems through supplementation of herbal drugs has been widely reported. In the present study, we assessed duration dependent changes in the generation of reactive oxygen species, consequent lipid peroxidation and endogenous reduced glutathione level to find out recovery rate from hypoxia reoxygenation induced damage to rat brain. The study showed a rapid recovery from the hypoxia/reoxygenation injury upon supplementation of the SBT leaf extract within 6 days compared to 14 days without any supplementation. The rapid recovery from hypoxia/reoxygenation could be because of the enhanced production of reduced glutathione by increasing the γ-glutamylcysteine synthetase enzyme activity in addition to the several components with high anti-oxidant activity in SBT leaf extract.

To unfold whether antioxidants components alone are the key factor in the neuroprotective effect of SBT leaf extract, we block the γ-glutamylcysteine synthetase (GCLC) activity by using buthionine sulfoximine along with SBT leaf extract supplementation. Our study showed that leaf extract supplementation was not that effective in reducing the reactive oxygen species as well as the lipid peroxidation compared to the rats supplemented with SBT leaf extract alone. The observed finding indicate that in addition to its antioxidant components, SBT leaf extract also modulates the endogenous antioxidant system like glutathione by upregulating the γ-glutamylcysteine synthatase expression in the hippocampal region. Upregulation of γ- glutamylcysteine synthatase could be mediated by the Nrf2 mediated pathways. There are several reports that Nrf2 regulates the expression of γ-glutamylcysteine synthetase expression and oxidative stress following hypoxic insult effect the Nrf2 expression [37, 38].

1. A.



B.

Table 1

Determination of Quercetin-3-galactoside, kaempferol and isorhamnetin in seabuckthorn leaves by HPLC.

Method of extraction Extraction	Temperature (°C)	Quercetin-3-galactoside (µg/g) ± SD* of dry raw material	Kaempferol (µg/g) ± SD* of dry raw material	Isorhamnetin (µg/g) ± SD* of dry raw material
Subcritical water	150	447.39 ±9.43	48.43 ±3.21	124.5 ±6.87

* Data expressed as mean ± standard deviation (SD) of three replicates.

Figure.1.A. Representative Chromatograph showing the peak for quercetin, kampherols and isorhamnetins in the leaf extract of SBT identified using their respective standards. B. Quantitative representation of the key antioxidants in alcoholic fraction of SBT leaf extract estimated by RP-HPLC.

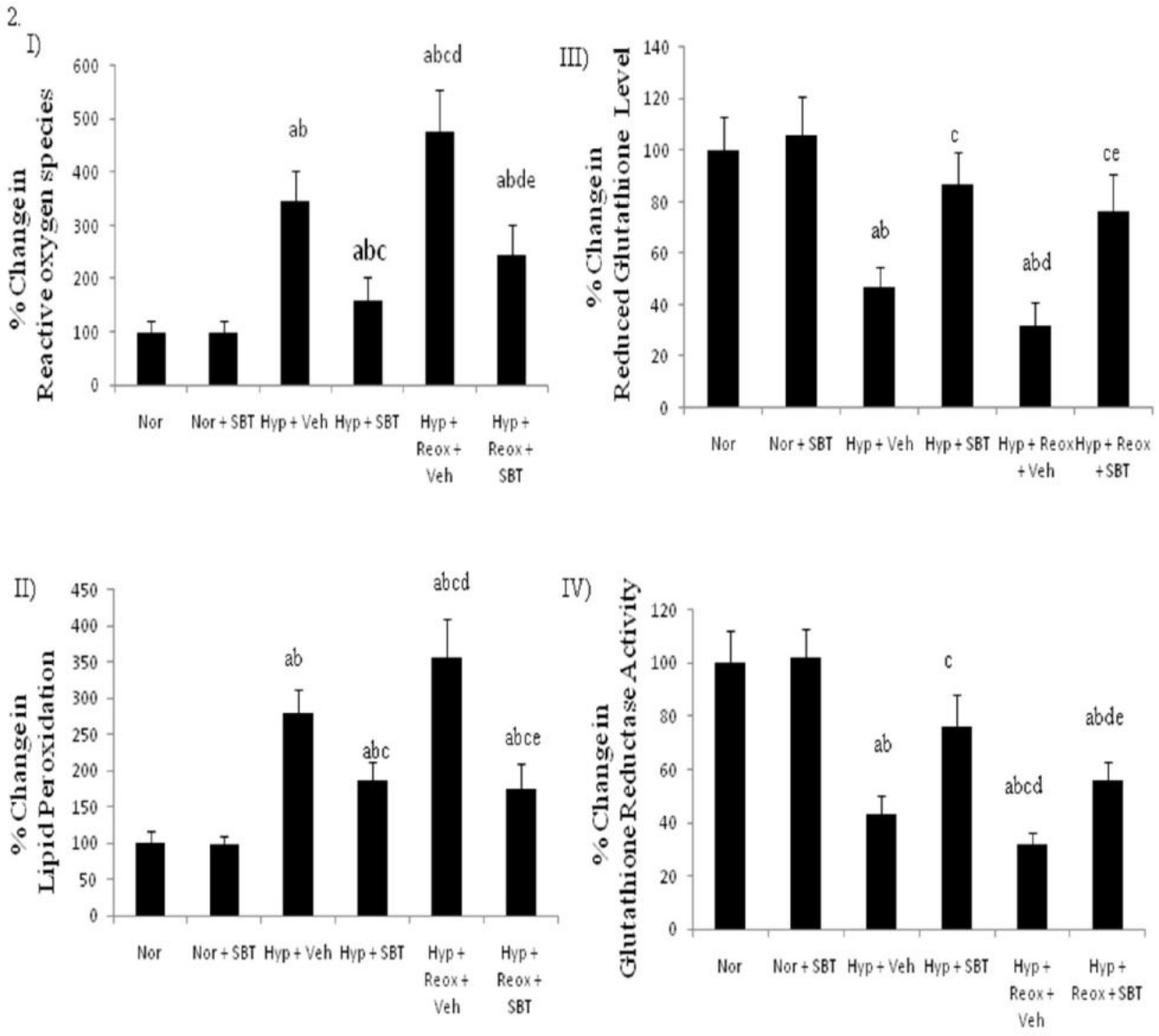


Figure.2. Prefeeding SBT leaf extract decreases (i) Reactive Oxygen species generation and (ii) Lipid Peroxidation whereas (iii) the reduced glutathione level and (iv) glutathione reductase activity increase in hippocampal tissue following hypoxia and hypoxia/reoxygenetion stress. . 'a' denotes p< 0.05 vs when compared with normoxia, 'b' denotes p<0.05 vs when compared with Nor + SBT, 'c' denotes p< 0.05 Vs when compared to hypoxia+ Vehicle, 'd' denotes p< 0.05 Vs when compared with hypoxia+ Reoxygenation+ Vehicle, 'e' denotes p< 0.05 Vs when compared to hypoxia+ Reoxygenation+SBT.

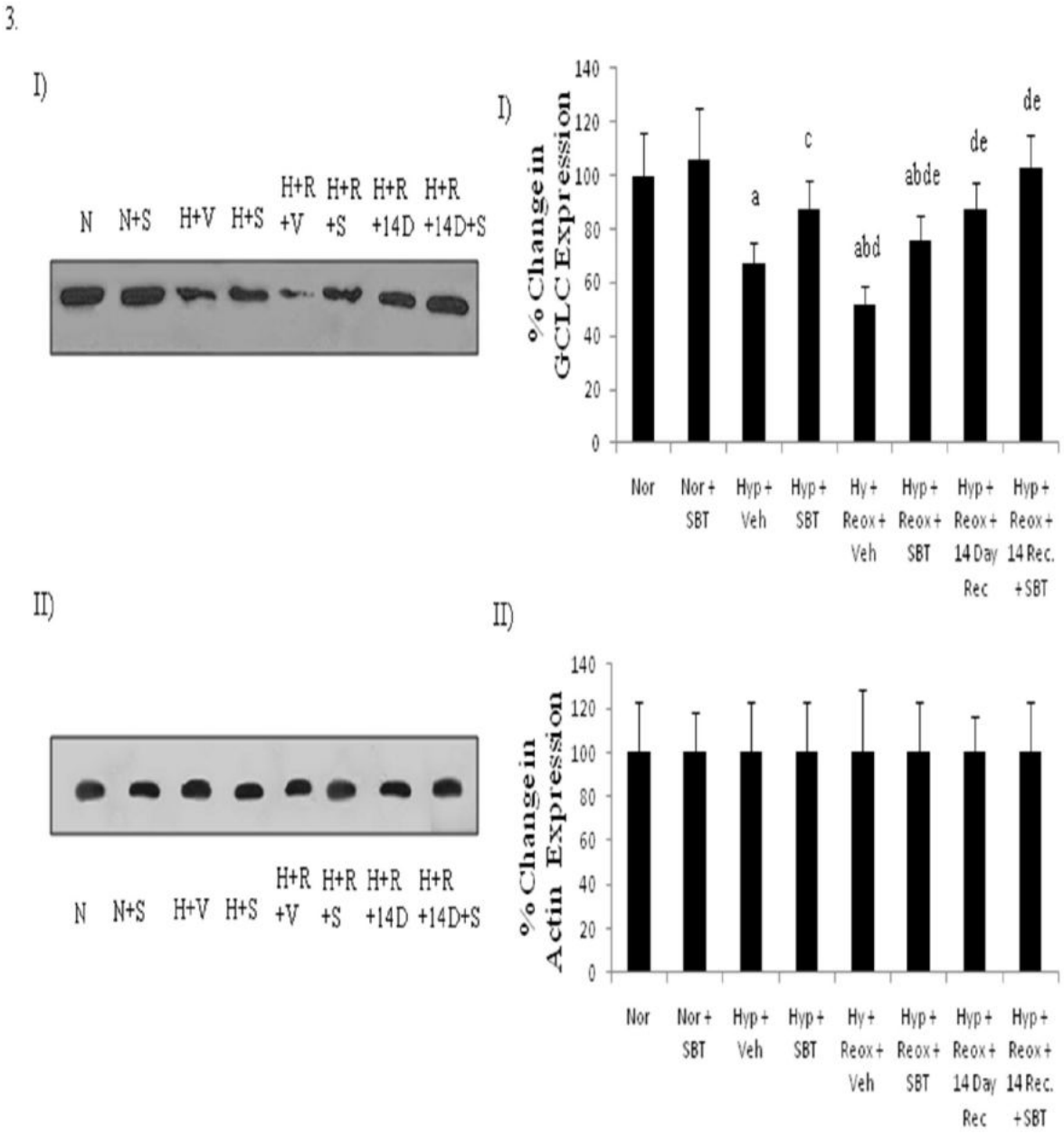


Figure.3. (i) Representative blot showing up regulation of key enzyme γ-glutamyl cysteine ligase for glutathione biosynthesis in hippocampal tissue following supplementation of SBT leaf extract prior to and during exposure to post hypoxic reoxygenation stress. (ii) β-actin was used as loading control. 'a' denotes p< 0.05 vs when compared with normoxia, 'b' denotes p<0.05 vs when compared with Nor + SBT, 'c' denotes p< 0.05 Vs when compared to hypoxia+ Vehicle, 'd' denotes p< 0.05 Vs when compared with hypoxia+ Reoxygenation+ Vehicle, 'e' denotes p< 0.05 Vs when compared to hypoxia+ Reoxygenation+SBT.

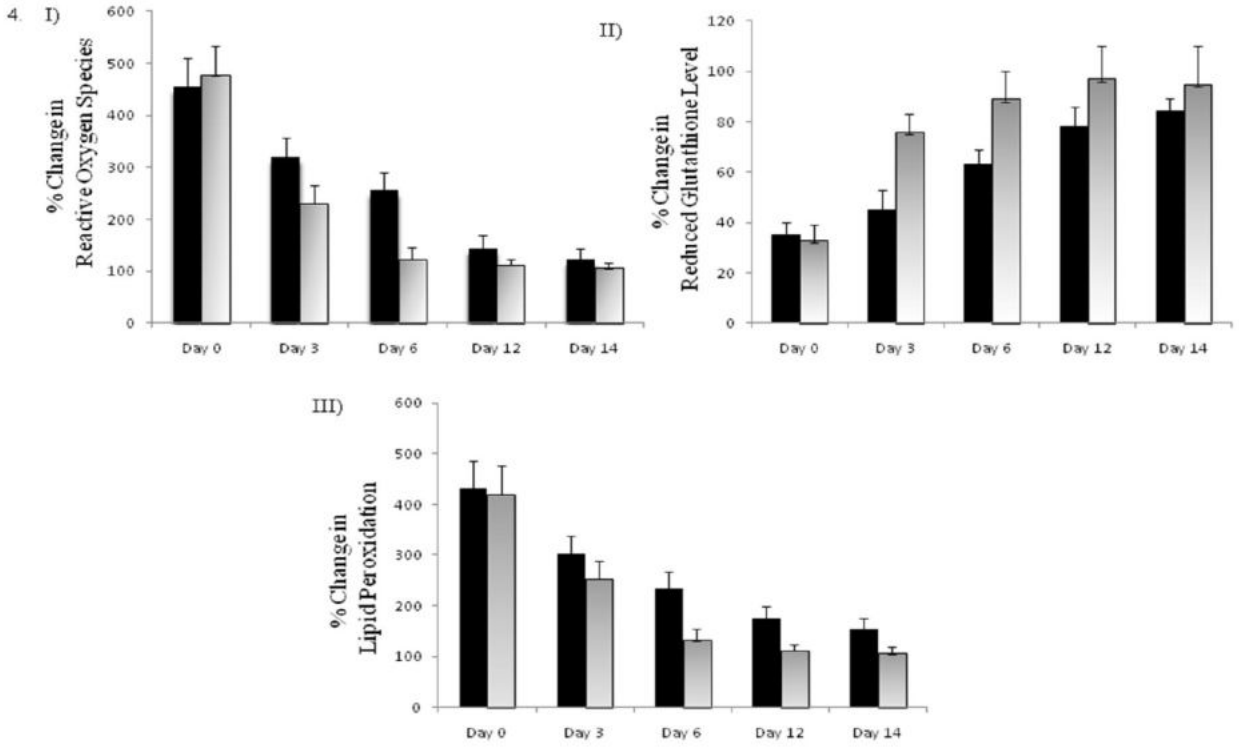


Figure.4. SBT leaf extract administration increase the rate of recovery from post hypoxic reoxygenation injury as revealed in term of (i) Reduced reactive Oxygen Species level (ii) decreased lipid peroxidation and (iii) Rapidly improved reduced glutathione level in hippocampus.

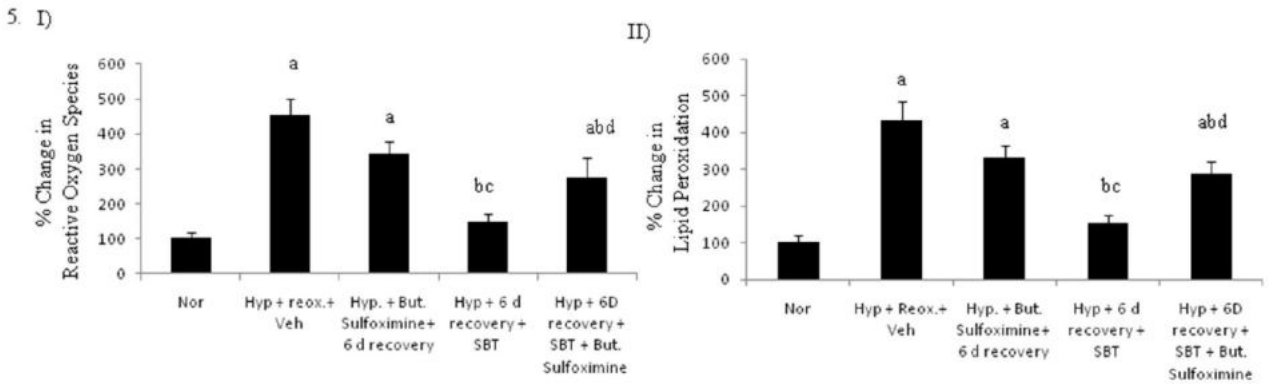


Figure.5. SBT leaf extract fail to reduce the (i) Reactive Oxygen Species and (ii) Lipid Peroxidation when administered along with buthionine sulfoximine during recovery from hypoxia and hypoxia/reoxygenation stress. 'a' denotes p< 0.05 vs when compared with normoxia, 'b' denotes p<0.05 vs when compared with hypoxia + Reoxygenation+Vehicle, 'c' denotes p< 0.05 Vs when compared to hypoxia+ but. sufoximine+ 6d recovery, 'd' denotes p< 0.05 Vs when compared with hypoxia+ 6D recovery+ SBT, 'e' denotes p< 0.05 Vs when compared to hypoxia+ 6D Recovery+SBT+ but. sulfoximine.

Conclusion

Exposure to hypobaric hypoxia and reoxygenation decreased GSH level in hippocampus by down regulating key biosynthetic enzyme γ-glutamylcysteine synthetase and enhance hypoxia and post hypoxic reperfusion injury in brain. SBT leaf extract supplementation reduced the free radical generation and consequent lipid peroxidation partially by modulating the endogenous glutathione biosynthesis in addition to its rich content of several components with potential anti-oxidant properties. Supplementations of SBT leaf extracts upregulates the expression of γ-glutamylcysteine synthetase and enhance GSH level in hippocampal region of the brain. Though the associated mechanism and signaling involved in SBTLE mediated modulation of glutathione biosynthesis and neuroprotection is not known, the observed augmented recovery from hypoxia and reoxygenation induced hippocampal oxidative stress indicate the SBTLE can be used as promising therapeutic agent to cure hypobaric hypoxia induced hippocampal damages.

Author's Contribution

The idea of the study was conceived by IB and GI, the experimental work and analysis of the data generated was done by SND and IB and the manuscript was prepared by GI and IB.

Acknowledgement

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