EVALUATING PROPHYLACTIC EFFICACY OF *TERMINALIA ARJUNA* (ROXB. EX DC.) WIGHT & ARN., *SANTALUM ALBUM* L. AND *NYMPHAEA* X *RUBRA* ROXB. EX ANDREWS FOR ACUTE MOUNTAIN SICKNESS

THESIS
SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY IN PHARMACY

By

KUSHAL KUMAR
ENROLMENT NO. 146752

Department of Biotechnology & Bioinformatics

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

WAKNAGHAT, DISTRICT SOLAN, H.P., INDIA

OCTOBER 2017

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DECLARATION BY THE SCHOLAR

I hereby declare that the work reported in the Ph.D. thesis entitled "**Evaluating prophylactic efficacy of *Terminalia arjuna* (Roxb. Ex dc.) Wight & Arn.,S*antalum album* L. and N*ymphaea* x *rubra* roxb. Ex Andrews for Acute Mountain Sickness"** submitted at **Jaypee University of Information Technology, Waknaghat, India,** is an authentic record of my work carried out under the supervision of **Dr. Udayabanu Malairaman** and **Dr. Sunil Kumar Hota.** I have not submitted this work elsewhere for any other degree or diploma. I am fully responsible for the contents of my Ph.D. thesis.

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Date:

SUPERVISOR CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled "**Evaluating prophylactic efficacy of *Terminalia arjuna* (Roxb. Ex dc.) Wight & Arn., S*antalum album* L. and N*ymphaea* x *rubra* roxb. Ex Andrews for Acute Mountain Sickness"** at **Jaypee University of Information Technology, Waknaghat, India,** is a bonafide record of his original work carried out under our supervision. This work has not been submitted elsewhere for any other degree or diploma.

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KUSHAL KUMAR

ABSTRACT

Acute mountain sickness (AMS) is the highest prevalent illness occurring on rapid ascends to high altitude. The absence of the suitable animal model, clinical diagnostic markers and specific drugs to mitigate symptoms associated with AMS still remains an enigma. The present study was designed for the development of an animal model for acute hypobaric hypoxia-induced alterations in fluid and electrolyte balance, cerebral vascular leakage (CVL), and hypovolemia in rats. Further, we studied, the prophylactic efficacy of poly-herbal formulation comprising of *Terminalia arjuna (T. arjuna)*, *Santalum album (S. album)* and *Nymphaea rubra (N. rubra)* during combined hypoxia dehydration (CHD) stress. For acute hypobaric hypoxia study, the altitude was optimized by exposing animals at different altitudes viz., 10,000, 15,000, 20,000, 25,000, 27,000 and 30,000 ft. For dehydration studies, animals were exposed to dehydration stress for 72 hrs. During CHD stress animals were exposed to dehydration for 60 hrs and then the animals were exposed to acute hypobaric hypoxia at the altitude of 27,000 ft. For phyto-prophylactics studies, *T. arjuna* and *S. album* extracts were administered to hypoxic animals (27,000 ft.) at the doses of 150 and 200 mg/kg respectively and *N. rubra* extract at the dose of 200 mg/kg was administered to dehydrated animals. A poly-herbal formulation with an optimized composition of *T. arjuna, S. album* and *N. rubra* was prepared and toxicity studies were conducted according to the guidelines provided by the organization for economic corporation and development. Glomerular filtration rate was estimated by $^{Tc\text{-}99m}$DTPA, plasma volume and CVL was estimated by using Evan's blue and sodium fluorescein dye respectively. Renin, angiotensin-II, aldosterone, ANP, TNF-α, IL-16, IL-1β and S100B were estimated by ELISA kits. The expression of RAGE and NF-κB were studied by commercially available antibodies. RBC

fragility was estimated by isotonicity and flow cytometric methods. Exposure to hypobaric hypoxia was found to cause fluid and electrolyte imbalance and neuro-inflammation in rats. Dehydration was found to increase RBC fragility. During CHD exposure dehydration was found to additively working along with hypoxia in the progression of patho-physiology of AMS. *T. arjuna* extract mitigates acute hypobaric hypoxia-induced decrease in glomerular filtration rate and fluid and electrolyte imbalance. *S. album* extract was found to be effective in attenuating hypoxia-induced neuro-inflammation in rats and extract of *N. rubra* was found to decrease dehydration induced increased RBC fragility in rats. The optimized composition of the poly-herbal formulation was found to be 1:1:0.25 for *T. arjuna, S. album and N. rubra.* The optimized dose of poly-herbal formulation was 600 mg/kg and human equivalent dose was 97 mg/kg. The study concludes that dehydration along with the hypobaric hypoxia could be equally responsible the patho-physiology of AMS. The decreased glomerular filtration rate, increased neuro-inflammation and RBC fragility during CHD stress could be the specific markers associated with AMS. The poly-herbal formulation prepared from *T. arjuna, S. album and N. rubra* could be effective clinically in mitigating symptoms associated with rapid ascend to high altitude.

LIST OF ACRONYMS & ABBREVIATIONS

AMS	Acute Mountain Sickness
ANOVA	Analysis of Variance
ANP	Arterial natriuretic peptide
ANP	Atrial natriuretic peptide
b.w.	Body Weight
BBB	Blood-brain barrier
CHD	Combined hypoxia dehydration stress
CVL	Cerebral Vascular Leakage
CVL	Cerebral vascular leakage
DTPA	DiethyleneTriamine Pentaacetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
FACS	Fluorescence-activated cell sorting
FCM	Flow Cytometry
FSC	Forward Scattering
g	gram
GFR	Glomerular Filtration Rate
HA	High Altitude
HACE	High Altitude Cerebral Edema
HAPE	High Altitude Pulmonary Edema
HH	Hypobaric Hypoxia
HPLC	High-Performance Liquid Chromatography

Hyp	Hypoxic control animals
LLS	Lake Louise Score
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
N. rubra	*Nymphaea rubra*
NOAEL	No observed adverse effect levels
Nor	Normal control animals
OECD	Organisation for Economic Co-operation and Development
p.o.	*per oral*
PBS	Phosphate buffer saline
PCV	Packed Cell Volume
PFA	Para formaldehyde
RAAS	Renin–Angiotensin–Aldosterone System
RAGE	Receptors for the advanced glycation end products
RBC	Red Blood Cells
S. album	*Santalum album*
SD	Standard Deviation
SD	Standard deviation
SSC	Side Scattering
T. arjuna	*Terminalia arjuna*
WBC	White Blood Cells

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INTRDUCTION

1. INTRODUCTION

Acute mountain sickness (AMS) is the highest prevalent illness occurring on the rapid ascent to the high altitude. The decreased partial pressure of oxygen along with dehydration at high altitudes trigger a series of immediate physiological changes to cope with the hypoxic environment through the process of acclimatization. AMS typically results from failed acclimatization and may even result in fatal conditions like high altitude cerebral and pulmonary edema, if neglected. Despite AMS being the most prevalent illness on acute exposure to high altitude, the patho-physiology of AMS still remains an enigma. The absence of clinical diagnostic markers and suitable animal models for AMS is probably the limiting factor for researchers worldwide in determining the molecular mechanisms resulting in AMS at the high altitude. Though there have been several theories on physiological mechanisms leading to AMS, the matter continues to be debatable [1].

Though the exact mechanism has not been put forth, numerous hypotheses have been proposed regarding the patho-physiological mechanisms leading to AMS symptoms. One such hypothesis which indicates the role of hyper-activated adrenergic nervous system medicated imbalance of circulatory renin-angiotensin-aldosterone system (RAAS) in the patho-physiological mechanisms associated with AMS, is known as the fluid and electrolyte imbalance hypothesis of AMS [2-3]. Interestingly, there is an excessive accumulation of fluid has been found to observed in dwellers with positive symptoms of AMS, supporting hyper-activated RAAS mediated fluid imbalance hypothesis of AMS [4]. Under patho-physiological conditions, angiotension-II, a peptide hormone and a key component of RAAS amplifies sodium re-absorption and causes a significant decrease in glomerular filtration rate (GFR) [5]. In addition to this, angiotensin-II also has been found to control release of aldosterone, another key component

of RAAS [3]. This angiotensin II release is regulated by atrial natriuretic peptide (ANP), a peptide hormone, secreted from the atria, which also the controls the action of several hormones viz., angiotensin-II, aldosterone and vasopressin [6] and various other physiological parameters of fluid balance in the body like vasodilation, fluid and electrolyte balance and GFR [7-8].

Acute exposure to hypobaric hypoxia results in decreased blood supply to the brain and hyperventilation induced excessive accumulation of carbon dioxide both of which could be working synergistically in controlling cerebral flood flow at high altitude [9]. In order to compensate of decreased cerebral blood flow, vascular resistance was found to be significantly reduced in small arteries and arterioles along with an increase in cerebral blood flow [10]. Increase cerebral blood flow and vasodilatation in arteries and arterioles causes increased brain volume and intracranial pressure in the brain [11]. Increased brain volume and elevated intracranial pressure result in amplified blood-brain barrier (BBB) permeability that finally leads to augmented CVL, often referred to as 'tight fit hypothesis of symptoms associated with AMS' [12-14]. BBB, formed by the brain endothelial cells, plays a dominant role in maintaining fluid homeostasis by regulating the flux of fluids and substances between the systemic circulation and brain microenvironment and protecting the brain from harmful xenobiotics that may cause damage to neuronal and non-neuronal cells present in brain microenvironment [12-13]. Acute exposure to hypobaric hypoxia has been well reported to cause disruption of BBB permeability and is associated with increased CVL [15]. Hypobaric hypoxia-mediated increased neuro-inflammation has been reported to play a crucial role in acute hypobaric hypoxia-induced altered BBB permeability [12]. Under physiological conditions, homeostasis between pro- and anti-inflammatory cytokines play a pivotal role in maintaining the immune responses in the body [16].

However, under patho-physiological conditions, dys-regulation of homeostasis between pro- and anti-inflammatory cytokines leads to neuro-inflammation and disruption of the BBB [17].

S100 classes of proteins are the low molecular weight proteins, which have been characterized by the presence of two calcium binding sites with helix-loop-helix conformation [18]. 21 different types of S100 class of proteins have been reported in the literature and are generally considered as damage-associated molecular patterns [19]. The major function of S100 class of proteins is sensing Ca^{2+}, once activated they may interact with other proteins regulating their activity. S100B, one of the first identified S100 class of protein, has been reported to be secreted by astrocytes [20]. Secreted S100B protein may exert regulatory activities in response to intra- and extracellular signals and has been considered as a specific marker of brain injury [21-22]. More specifically elevated serum S100B has been considered as a marker of neuroinflammation associated with acute and chronic injury [23]. High serum S100B protein levels have been found in subjects exposed to acute hypobaric hypoxia with symptoms of AMS [24]. The receptor for the advanced glycation end products (RAGE), could work as signal transducer receptor and causes microglial activation resulting in neuro-inflammation through NF-κB dependent mechanisms [25]. Interestingly, dissociated NF-κB has been found to control hundred of genes and has emerged to be a mediator of inflammatory processes [26]. So, blocking of RAGE/NF-κB pathway could be beneficial in hypoxia induced neuro-inflammation and subsequent CVL in rats.

In addition to the low partial pressure of oxygen, high altitude environment is also characterized by low absolute humidity. Though the existing hypothesis on AMS considerably focuses on physiological changes associated with hypobaric hypoxia, the possible role of low humidity induced dehydration in triggering AMS has not been adequately explained in the

existing literature. Water is an essential nutrient and water deprivation of only 2% can result in impaired physical and mental performance [27]. Dehydration is referred to as a state of deficiency of body water, which may be due to the excessive loss of water and/or inadequate intake of water, resulting in hypo-hydration in organs and tissues [28]. Previous reports suggest that dehydration results in fluid and electrolyte imbalance dependent increased plasma osmolality [29]. Dehydration-induced increased plasma osmolality has been well reported to cause increased red blood cell (RBC) fragility [30]. Magnesium has been reported to play a dominant role in maintaining cellular functions and deficiency of magnesium is associated with many neurological and cardiac dysfunctions [31]. In addition to this, there is a direct link of magnesium with endurance and performance. It has been reported that animals deficient in magnesium perform at a snail's pace in trade mill test [32]. Magnesium also has a role in chronic fatigue syndrome. The patients with chronic fatigue syndrome have low red blood cells magnesium, further supporting the link of magnesium with performance and endurance [33]. Exposure to dehydration has been previously reported to decrease serum magnesium concentration [34]. Previous reports on shifting of magnesium ions from serum into RBC during marathon provided a plausible explanation for the decrease in serum magnesium concentration [35-36]. Studies by De Franceschi et al., (1997) show that supplementation of magnesium reduces RBC dehydration duringsickle cell disease [37]. Thus, studying the effect of dehydration on RBC fragility and prevention of dehydration induced RBC fragility could indirectly mitigate the symptoms associated with AMS.

The prophylactics recommended for AMS are limited to acetazolamide, a potent carbonic anhydrase inhibitor, which is generally used for both prophylactic as well as curative management of symptoms associated with AMS, high altitude pulmonary and cerebral edema

[38]. However, acetazolamide is associated with many adverse effects viz., tinnitus, numbness, motor weakness, and fluid and electrolyte imbalance [39]. Herbal drugs and/or formulations developed from herbal drugs have been widely reported traditionally for their potential to manage maladies associated with high altitude. Highly advantageous over allopathic medicines, the herbal formulations are free of side effects associated with allopathic medicines. Considering the side effects associated with the usage of acetazolamide, there is a necessity for identifying alternative prophylactics preferably from herbal resources, to prevent an occurrence of AMS on acute exposure to high altitude.

Terminalia arjuna (Roxb. ex DC.) Wight & Arn. *(T. arjuna)*, a deciduous plant of genus *Terminalia*, belongs to the *Combretaceae* family. This plant is abundantly present in many parts of India and arjun tree in India [40]. In the Ayurvedic system of medicine, *T. arjuna* has been reported for its activity in providing beneficial effects in cardiac disorders [41]. It is now clinically proven drug agent has an efficacy in various cardiac disorders [42]. Even stem bark of arjun tree has been reported to provide beneficial effects against acute renal failure [43].

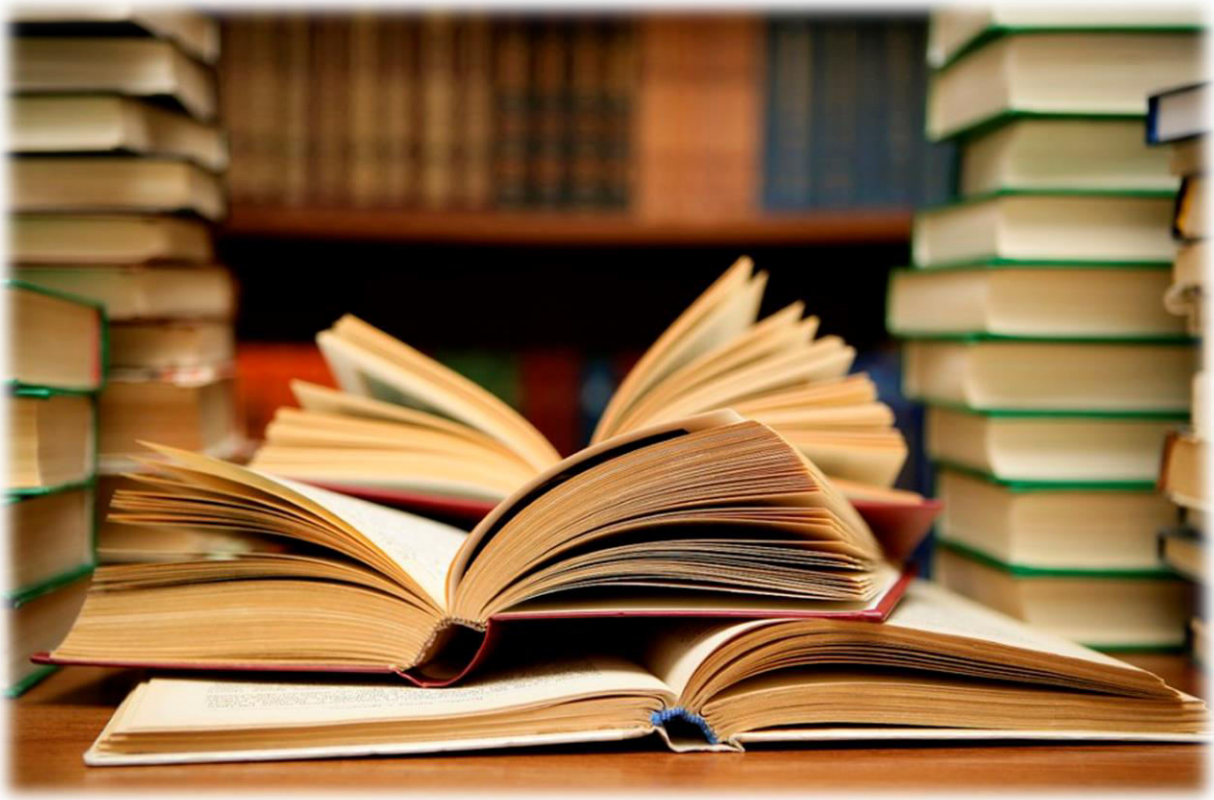
Sandalwood, the dried heartwood of *Santalum album* L., belonging to the family *Santalaceae*, has been known as the green gold [44]. It predominantly contains sesquiterpenes viz., α- and β-santalol with the percentage of 53% and 23% respectively [45]. It has been reported in the literature to have sedative, anti-diarrohoeal, diuretic, antibacterial, and antiviral activities [44, 46]. In Ayurveda, a traditional Indian medical system, sandalwood has been reported as an anti-inflammatory drug and used traditionally to treat chronic inflammation of bladder and pyelitis [47]. Existence literature, however, does not report on the anti-inflammatory and neuroprotective potential of sandalwood extract in acute hypobaric hypoxia induced neuro-inflammation in rats.

Nymphaea x *rubra* Roxb. ex Andrews (*N. rubra*), with local name kumuda, is an aquatic perennial herb belonging to family *Nymphaeaceae*. Recently, it has been found that *N. rubra* is a hybrid produced by the cross between *N. lotus* and *N. pubescens* as a parental species [48]. It grows on the shores of lakes, rivers and water bodies. The plant can grow to heights up to 6 to 7 feet in the water and has green colored leaves floating on water and pink colored flowers. The tubers are vertically covered with hairs. The plant is naturally found in Asia and in India mainly in Odisha and West Bengal [49]. In the traditional medicine system, various parts of *N. rubra* have been reported for treating disorders associated with blood [50]. In addition to this, pulverized rhizomes mixed with honey have been used as a therapeutic agent for bleeding nose, piles, dysentery and as cardiac tonic [51]. Pharmacologically, this whole plant is known to have protective effect on various disorders viz., anthelmintic, immuno-modulation, insulin resistance, anti-hyperglycemic, anti-dyslipidemic, anti-inflammatory, anti-pyretic, hepato-protective and free radical scavenging activity [49, 52-54]. The rhizomes of *N. rubra* have also been reported for their anti-oxidant efficacy and total phenolic and flavonoids in the methanolic extract were found to be in ample amount [55]. Phytochemical analysis of flowers of *N. rubra* reveals the presence of polyphenolic compounds viz., rutin, quercetin, scopoletin and kaempferol [54]. Literature is sparse on extensive toxicological evaluation, phytochemical analysis, and therapeutic potential of *N. rubra* rhizome extract in dehydration-induced increased RBC fragility in rats.

The world's toughest, coldest, highest and bravest battle is being fought on Siachen Glacier for over the last 25 years. This is the most glaciated area outside the polar region comprising 22 glaciers. Troop deployment on the forward post is from 18, 000 ft. to 21, 000 ft. AMS has been widely reported in the troops and these symptoms may be fatal if not treated timely. With the availability of scientific data on the use of *T. arjuna*, *N. rubra* and *S. album* for

various ailments in traditional medicinal systems, their bio-safety is well established since ages. However, their implications in preventing causative factors for AMS viz., sodium retention, CVL, and hypovolemia have never been explored. No herbal formulation specifically preventing AMS in humans has either been reported in the traditional literature or modern medicine. Though some studies have been carried on plants like *Rhodiola rosea* and *Panax ginseng* for high altitude-related ailments, their efficacy for preventing symptoms associated with AMS still remains debatable. Due to absence of animal model for AMS, pre-clinical research on therapeutic and prophylactic potential of pharmaceutical and bioactive compounds against AMS is sparse. The patho-physiological markers associated with AMS also remain to be conclusively identified.

REVIEW OF LITERATURE



2. REVIEW OF LITERATURE

2.1. Introduction of AMS

Rapid ascend to high altitude in non -acclimatized individuals may lead to AMS, usually begins within the few hours of ascend and cardinal manifestations consists of headache, nausea, vomiting, insomnia, fatigue and dizziness [3]. The occurrence and severity depends on rate of ascend, the altitude attained, altitude at which dwellers sleep and individual vulnerability to the cardinal manifestations associated with AMS [56]. Depending upon the severity and treatment, the symptoms coupled with AMS may lasts from few days to week [57]. AMS may be graded from grade 1 to 4 depending upon the severity of symptoms, whereas, grade1 and 2 patient has the minimal symptoms of AMS and can continue to trek with some precautionary measures, grade 3 patient has the severe manifestations and cannot continue to trek and requires rest and grade 4 the patient cannot ambulate, symptoms are progressive and disturbance of consciousness or gait may be present and required prompt medical treatment with oxygen and descent. Grade 3 and 4 patient, severity of symptoms may be incapacitating and could be associated with HAPE and HACE [58].

2.2. History of AMS

In 37-32 BC, the symptoms of high altitude ailments were earliest reported by a Chinese official of the Western Han Dynasty, Too Kin during the reign of Chung Li [59]. In 1590, priest Jose de Acosta also had given portrayal about the symptoms allied with ascent to the Andean range in Peru (4800 m) [60-61]. Interestingly with the discovery of existence of oxygen gas in late 1772 by a Swedish chemist, first scientific expedition to Mount Blanc was accomplished by Horace-Benedict de Saussure and documented pulse and respiration at various altitudes made clear

possible role of oxygen underlying the symptoms of AMS [62-63]. In 1862, two balloonist died, while ascend to 29, 000 ft. and death was due to extreme altitude sickness [64]. In 1891, H. Kronecker, had started working on decompression chamber taking two people at a time to a pressure equivalent to 13000 ft. [65].

With the end on nineteenth century, Paul Bert made a remarkable logical achievement by finding the mechanism of oxygen carrying capacity of hemoglobin and its relationship with the partial pressure of oxygen by using decompression chamber as a model of high altitude and AMS symptoms and was considered to be father of high altitude physiology [66]. At the end of nineteenth century, researcher's now had started to understand the AMS as well as association of its symptoms with fall in partial pressure of oxygen. Joseph Barcroft, a British physiologist, one another pioneer in the field of high altitude physiology, has started doing experiment with him own as a subject in a sealed room for 6 days simulating the altitude of 18,000 ft. In 1922, first systematic study on AMS was conducted by the International High Altitude Expedition and expanded the knowledge about the high altitude physiology and concluded impairment of both physical and mental abilities during high altitude ascend [67].

Alexander M. Kellas, a British physiologist, attracted attention of researcher's towards the Himalayas, by his conclusive review of Mt. Everest, "Mount Everest could be ascended by a man of excellent physical and mental constitution in first-rate training, without adventitious aids if the physical difficulties of the mountain are not too great" [68]. In 1966, a clinical study published in New England Journal of Medicine gain popularity for providing the direct correlation between hypoxia and the symptoms of AMS [4].

HAPE and HACE, both pathologically and clinically considered being an extension of AMS [69]. Mounting evidences advocating the progression of AMS to HAPE and HAPE with 36

h and symptoms HAPE and HACE occurs concomitantly or may progress individually [58]. Historically, the theory of alteration in neurological functions during rapid ascend to high altitude was first proposed in 1898, by, Mosso and further confirmed in 1965, by, Singh [4, 70]. While, 1911, post-mortem studies of a doctor died during expedition to Mont Blanc and subsequently in 1913, TH Ravenhill, high altitude researcher, provided with diagnostic framework of HAPE [71].

2.3. Symptoms of AMS

The most leading complication of AMS is headache and it is estimated that 25, 80 and 100% non-acclimatized dwellers experience headache at the altitude of 2750, 3000 and 4500 m respectively [72]. Additional non-specific cardinal manifestations include lassitude, fatigue, malaise, dizziness and nausea [73]. In addition to this, insomnia has been found to be very prevalent in individuals at high altitude and has been considered as major symptoms of AMS [74].

Rapid ascend to high altitude has been considered as main risk factor of headache associated with high altitude [75]. Headache has been found to appear as isolated symptoms with the few hours of high altitude exposure in non-acclimatized individuals can be constitutively present with other non-specific symptoms of AMS [75]. Headache associated with AMS has commonly found to be diffuse and steady, and non-acclimatized sojourns may experience pain in the frontal, frontoparietal or holocranial regions [76-77]. Headache at high altitude may be due to the activation of pain receptors of large blood vessels of trigeminal ganglia that projects to the cortex and innervates meninges [78]. The activation of pain receptors may be due to the increased intracranial pressure, brain swelling or due to release of nociceptive substances [73].

Loss of appetite has been observed in non-acclimatized dwellers due to hypobaric hypoxia [79]. Reductions in appetite result in the reduction of caloric and protein intake to 30 and 40% respectively [80]. The possible molecular mechanism is the increased activity of hypoxia

inducible factor 1 in the hypothalamus resulting in increased expression of leptin, a responsible protein for control of appetite [81]. Nausea and vomiting has been reported to cause by rhythmic labyrinthine stimulation of afferent neurons during motion sickness [58].

Data emerges from studies provides an evidence of altered sleep cycle and deep sleep that can cause insomnia [82]. In addition to this, insomnia at high altitude has been positively correlated with symptoms of anxiety [83]. Hypobaric hypoxia induced decreased plasma volume and excessive ventilation induced decreased cerebral blood flow could be the reason for central cardinal manifestations of AMS viz., dizziness, faintness, mental confusion and ataxia [58]. Thus, describing AMS as more of cerebral and above mentioned neurological symptoms along with deficit in learning and memory, focusing and impaired finger tapping speed [57, 84]. Magnetic resonance imaging reveals edema in globus pallidus and cortical dysfunctions [85].

2.4. Questionnaire for AMS

In 1991, a simplified AMS questionnaire, Lake Louise Score (LLS), had been proposed and named after the venue of International Hypoxia Conference that was held at Lake Lousie, Canada [86]. LLS have been validated clinically and now days this questionnaire has been widely used for assessment of incidence and symptoms associated with AMS [87-88]. LLS has 5 questions with 4 point scale and total score of patient with 3-5 has mild symptoms while score more than 6 has severe symptoms of AMS [86] (Table 1).

Table 2.1: Lake Louise Score for Acute Mountain Sickness

Symptom		Score
Headache	No	0
	Mild	1
	Severe	2
	Incapacitating	3
Gastrointestinal symptoms	None	0
	Poor appetite or nausea	1
	Moderate nausea or nausea	2
	Severe nausea and or vomiting	3
Fatigue and or weakness	No tired or weak	0
	Mild fatigue/ weakness	1
	Moderate fatigue/ weakness	2
	Severe fatigue/ weakness	3
Dizziness/ light headedness	No dizziness	0
	Mild dizziness	1
	Dizziness/ light headedness	2
	Severe dizziness	3
Difficulty in sleeping	Slept as well as usual	0
	Do not sleep as well as usual	1
	Woke many times, poor sleep	2
	Could not sleep et all	3

2.5. Pathophysiology of AMS

Indeed, the exact pathophysiolological processes that cause symptoms of AMS in dwellers are still not clear [56]. There are many proposed hypothesis describing multiple factors correlated with the cardinal manifestations of AMS [73]. More specifically, symptoms associated with AMS have been found to be cumulative of various hypothesis viz., fluid and electrolyte imbalance, cerebral swelling induced by vasodilatation and cellular edema, release of local mediators and increased blood-brain barrier permeability and hypovolemia hypothesis of AMS [58, 89].

2.5.1. Fluid and electrolyte imbalance hypothesis

Fluid and electrolyte balance plays a dominant role in optimal health maintenance [3, 90]. Approximately, 70% of body weight of an individual is considered to be the total body water present in human body and is distributed between intra and extracellular compartments. Intracellular fluid is 2/3rd of total body water consists of potassium, magnesium and phosphates (ATP, ADP and AMP) as major ions while extracellular fluid constitutes 1/3rd of total body water and consists of sodium, biocarbonate and chloride as major ions system [91].

Though the exact mechanism has not been put forth, numerous hypotheses have been proposed regarding the pathophysiological mechanisms leading to AMS symptoms. One such hypothesis which indicates the role of hyper-activated adrenergic nervous system medicated imbalance of circulatory renin-angiotensin-aldosterone system (RAAS) in the pathophysiological mechanisms associated with AMS, is known as the fluid and electrolyte imbalance hypothesis of AMS [1-3]. Interestingly, there is an excessive accumulation of fluid has been found to observed in dwellers with positive symptoms of AMS, supporting hyper-activated RAAS mediated fluid imbalance hypothesis of AMS [4]. Under pathophysiological conditions, angiotension-II, a peptide hormone and a key component of RAAS amplifies sodium re-absorption and causes significant decrease in GFR [5]. In addition to this, angiotension-II also has been found to control release of aldosterone, another key component of RAAS [3]. This angiotensin II release is regulated by Atrial natriuretic peptide (ANP), a peptide hormone, secreted from the atria, which also controls the action of several hormones viz., angiotensin-II, aldosterone and vasopressin [6] and various other physiological parameters of fluid balance in the body like vasodilation, fluid and electrolyte balance and GFR [7-8]. Excessive accumulation of fluid and electrolyte could be

the reason for edema of lung and brain, pointing an arrow towards advancement of AMS towards HAPE and HACE if AMS remains untreated [3] (Figure 2.1).

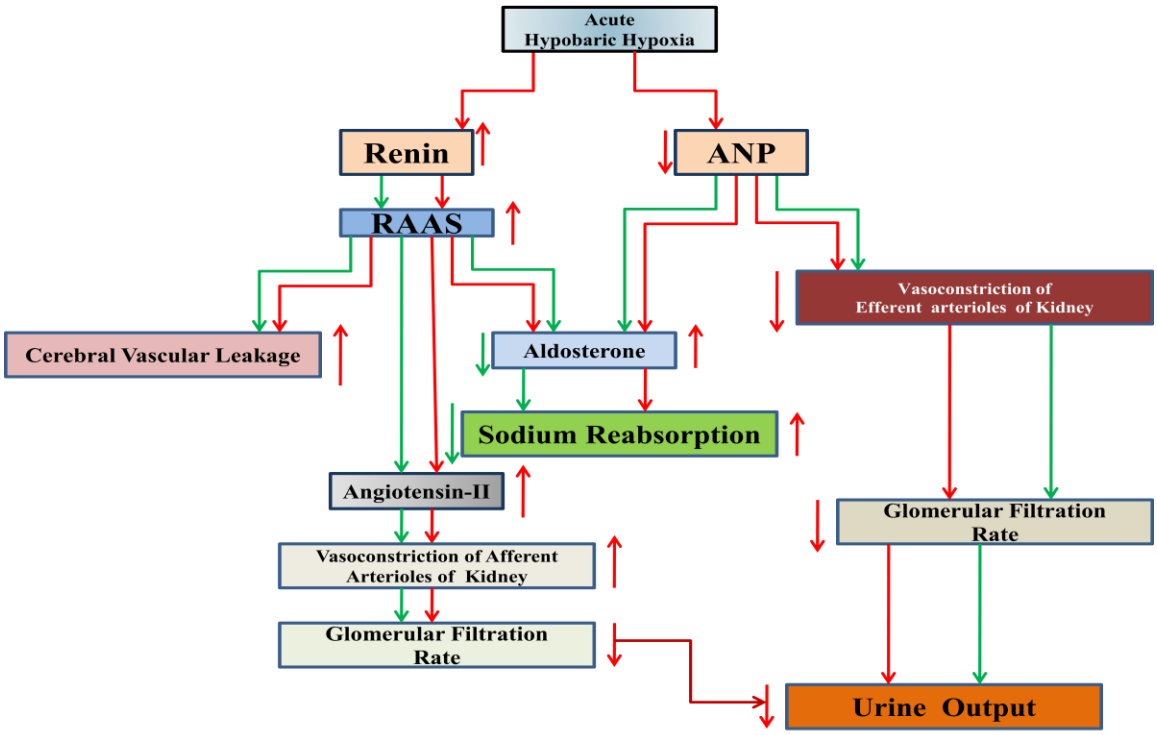


Figure 2.1: Possible molecular mechanism of hypobaric hypoxia induced altered dieresis, sodium retention and fluid accumulation in AMS positive patients.

ANP: Arterial natriuretic peptide; RAAS: Renin angiotension aldosterone system

2.5.2. Vasogenic hypothesis of AMS

High metabolic rate and restricted substrate storage capacity of brain has led brain to accurately auto-regulate blood flow in order to maintain constant supply of nutrients and oxygen [92]. In has been reported that, altered oxygen supply to brain, partial pressure of carbon dioxide, mean

arterial pressure and autonomic nervous system majorly regulate cerebral blood flow [92-93]. Acute exposure to hypobaric hypoxia result in decreased blood supply to the brain and hyperventilation induced excessive accumulation of carbon dioxide could be working synergistically in controlling cerebral flood flow at high altitude [9, 93]. In order to compensate with decreased cerebral blood flow, vascular resistance has been found to significantly reduce in small arteries and arterioles and increase in cerebral blood flow [10]. Increase cerebral blood flow and

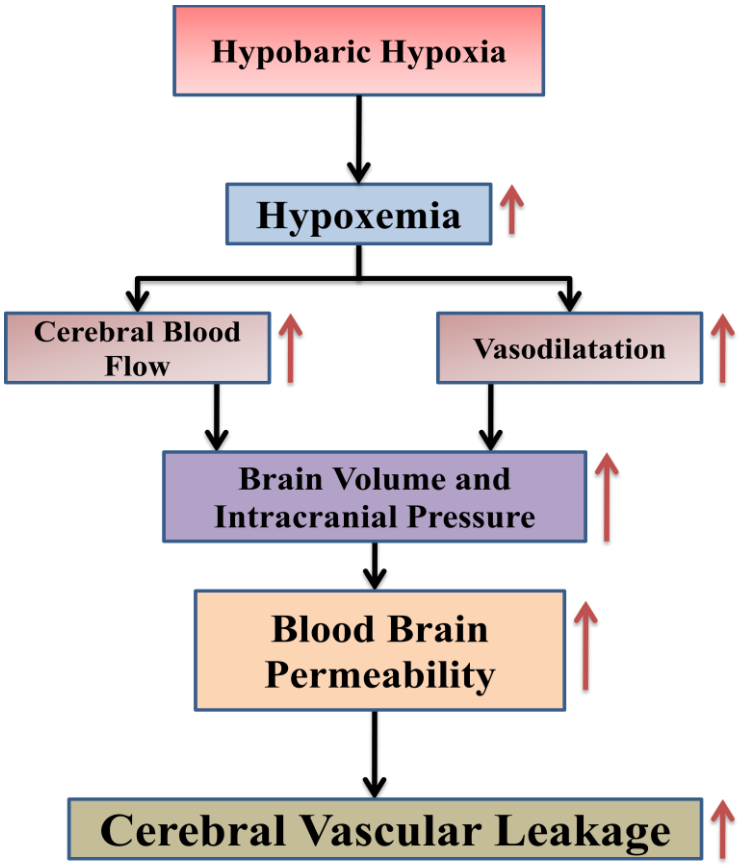


Figure 2.2: Acute hypobaric hypoxia induced altered blood-brain permeability and CVL

vasodilatation in arteries and arterioles causes increased brain volume and intracranial pressure in brain [94]. Augmented cerebral blood flow and vasodilatation may overcome

capillary vasoconstriction, increase blood-brain permeability that finally resulting in CVL [12, 95-96]. Increased brain volume and elevated intracranial pressure result in amplified blood-brain barrier (BBB) permeability that finally leads to augmented CVL referred to as 'tight fit hypotheses of symptoms associated with AMS [12, 14]. BBB, forms by the brain endothelial cells, plays a dominant role in maintaining fluid homeostasis by regulating the flux of fluid and substances between the systemic circulation and brain microenvironment and protecting the brain form harmful xenobiotics that may cause damage to neuronal and non neuronal cells present in brain microenvironment [12-13]. Acute exposure to hypobaric hypoxia has been well reported to cause disruption of BBB permeability and is associated with increased CVL [15]. Hypobaric hypoxia-mediated increased neuro-inflammation has been reported to plays a role in acute hypobaric hypoxia-induced altered BBB permeability [12]. Under physiological conditions, homeostasis between pro- and anti-inflammatory cytokines play a pivotal role in maintaining the immune responses in the body [16]. However, under pathophysiological conditions, dysregulation of homeostasis between pro- and anti-inflammatory cytokines leads to neuro-inflammation and disruption BBB [17] (Figure 2.2)

S100 classes of proteins are the low molecular weight proteins have been characterized by their two calcium binding sites with helix-loop-helix conformation [18]. 21 different types of S100 class of proteins has been reported in the literature and considered as DAMPs [19]. The major function of S100 class of proteins is their function as Ca^{2+} sensing and once activated they may interact with other proteins resulting in regulation of their activity. S100B firstly identified S100 class of protein has been reported to secrete by astrocytes [20]. Secreted S100B protein may exert regulatory activities intra- and extracellular signals and has been considered as a specific marker of brain injury [21-22]. More specifically elevated serum

S100B has been considered as a marker of neuro-inflammation associated with acute and chronic injury [23].

High serum S100B protein levels have been found in subjects exposed to acute hypobaric hypoxia with symptoms of AMS [24]. However, the exact molecular mechanism

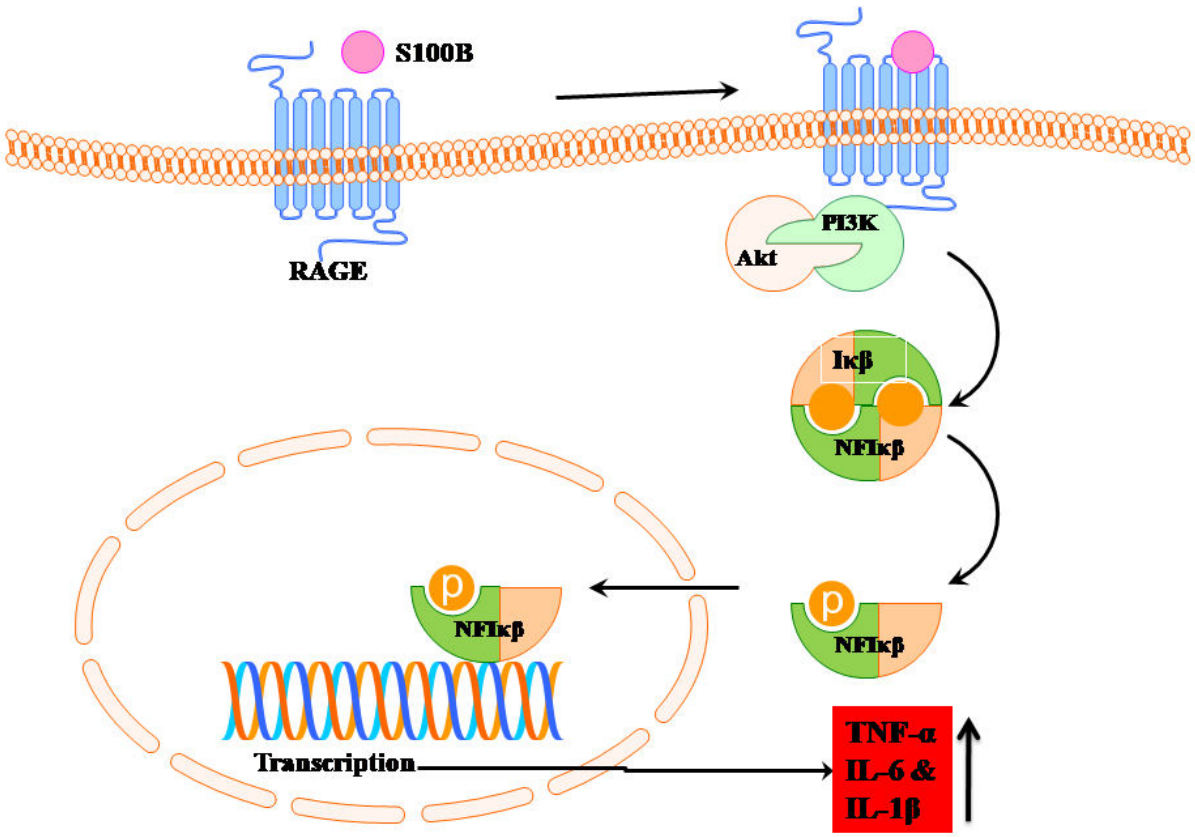


Figure 2.3: Role of S100B protein in neuro-inflammation.

S100B: S100 calcium-binding protein B; RAGE: Receptor for the advanced glycation end products; PKB: Protein kinase B; PI3K: Phosphatidylinositol-4,5-bisphosphate 3-kinase; Iκβ: Inhibitor of kappa B; Nuclear factor kappa-light-chain-enhancer of activated B cells; TNF-α: Tumor necrosis factor α; IL-1: Interleukin 6; IL-1β: Interleukin 1β

of S100B dependent regulation of inflammatory pathways is not clear during hypobaric hypoxia. The receptor the advanced glycation endproducts (RAGE), could work as signal traducer receptor and causes microglia activation result in neuro-inflammation through NF-κB dependent mechanisms [25]. Interestingly, dissociated NF-κB has been found in the literature to controls hundred of genes emerged to be a mediator of inflammatory processes [26] (Figure 2.3).

2.5.3. Hypovolemia hypothesis of AMS

Exposure to hypobaric hypoxia has been found to decrease plasma volume and has been positively correlated with the symptoms of AMS [97]. The mechanism behind high altitude induced dehydration and hypovolemia is not clear. It could be due to the cold environment at high altitude that leads to excessive diuresis and poor availability of water [97-98]. In addition to this dehydration at high altitude has been found to compromise physical performance, this could be a reason behind positive correlation of AMS symptoms with dehydration [89]. However, further studies are required in this direction to propose molecular mechanisms to justify the correlation between dehydration and manifestations of AMS.

Magnesium has been reported to play a dominant role in maintaining cellular functions and deficiency of magnesium has been associated with many of neurological and cardiac dysfunction [31]. In addition to this, there is a direct link of magnesium with endurance and performance. It has been reported that animals deficient in magnesium performs at a snail's pace in trade mill test [32]. Magnesium also has a role in chronic fatigue syndrome. The patients with chronic fatigue syndrome have low RBC magnesium, further supporting the link of magnesium with performance and endurance [33]. Exposure to dehydration has been previously reported to decrease serum magnesium concentration [34]. Previous studies report on shifting of magnesium ions from serum into RBC during marathon providing a plausible explanation for decrease in

serum Magnesium concentration [35-36]. Studies by Franceschi et al., (1997) show that supplementation of magnesium reduces RBC dehydration in sickle cell disease [37].

2.6. Non-pharmacological and pharmacological measures for preventing AMS

There no specific drug molecule has been identified to treat symptoms pertaining to AMS. This may be because of non-selective symptoms associated with pathophysiology of AMS. However, the symptoms could be preventively managed non-pharmacologically and pharmacologically [73].

2.6.1. Non-pharmacological measures for AMS

Majorly there are three factors that describe incidence and severity of AMS viz., speed of ascent, altitude attained and the previous acclimatization [58]. Rapid ascent to high altitude has been found to be a major contributor to the symptoms of AMS [99]. It has been reported that about 25% of dwellers experience symptoms of AMS following rapid ascend to high altitude [58]. So, by controlling speed of ascent in terms of meters gain per day has been found to be the best method to control symptoms associated with AMS [73, 100]. In addition to this, sleep at particular altitude gain in a day has been additionally considered for preventing symptoms [100].

Prevalence and severity of cardinal manifestations associated with AMS has been found to increase with altitude attained [58]. A study conducted by Montgomery et al. have found that 25% of individual experiences symptoms of AMS at the altitude of 2000m [101]. Subsequently, 79% of subjects were found AMS positive at the altitude of 3660m and approximately 96% of subjects had symptoms of AMS at the altitude of 4, 232 m [59, 102].

Table 2.2: Description about non-pharmacological measures of Acute Mountain Sickness

Measures	Description
Speed of ascend	300-500m/day ascend with 1 day rest every 3-4 days
Altitude attained	The percentage prevalence of AMS is above 90% at the altitude more than 4000
Pre-acclimatization	5 or more days above the altitude of 3000m in last 2 months

Pre-exposure to high altitude in last few months has also been found to reduce the incidence of AMS in subjects [103]. Interestingly, pre-acclimatization reduces the symptoms up to 50% by spending 5 or more days above the altitude of 3000m in the last 2 months [104]. So, taking consideration into rate of ascend, the particular altitude attained and pre-acclimatization could be a preventive measure for preventing symptoms of AMS (Table 2).

2.6.2. Pharmacological measures for AMS

Acetazolamide, a carbonic anhydrase inhibitor, at the dose of 125mg *bis a day,* has been suggested sufficient for preventing symptomatic AMS [58]. Acetazolamide has been reported to cause bicarbonate dieresis and metabolic acidosis resulting in stimulation of ventilatory responses to hypobaric hypoxia during high altitude exposure [75]. On the other hand, intravenous therapy of acetazolamide has also been found to increase cerebral blood flow and respiration by causing carbonic acidosis in brain tissue [38]. Dexamethasone, a synthetic glucocorticoid, has been reported to be the best alternative to acetazolamide [73]. The molecular mechanism of dexamethasone has found to be acting on reduction of capillary bed permeability by inhibiting production of prostaglandins [105]. The use of dexamethasone over acetazolamide is still

debatable [106]. However, dexamethasone has been found highly effective as compare to acetazolamide in cases where rapid ascend for short period is required [107]. Non-steroidal-anti-inflammatory drug viz., ibuprofen has also been found to be effective for preventing symptoms associated with AMS [73, 108].

2.7. Drawbacks with existing prophylactics for AMS

As discussed earlier, there is no specific drug discovered for mitigating AMS symptoms. Acetazolamide and dexamethasone have been well reported in the previous literature for preventing manifestations associated with AMS [58]. Common adverse effects associated with the acetazolamide is nausea and vomiting and has been proved to synergistically increases the severity of nausea and vomiting in subjects exposed to acute hypobaric hypoxia [109]. In addition to this, acetazolamide has been found un-effective during rapid ascend has been required with less acclimatization protocols [107]. Previous published literature advocates avoidance of dexamethasone use for prevention of AMS [106]. Dexamethasone has been reported to cause rebound to symptoms associated with AMS viz., fatigue, insomnia and depression after discontinuation of therapy [110].

2.8. Selection of plants for preventing AMS

2.8.1. Selection of *Terminalia arjuna* (Roxb. Ex DC.) Wight & Arn.

Terminalia arjuna (Roxb. ex DC.) Wight & Arn. *(T. arjuna)*, a deciduous plant of genus *Terminalia*, belongs to the *Combretaceae* family. This plant is abundantly present in many parts of India and arjun tree in India [40]. In arurvedic system of medicine, *T. arjuna* has been reported for its activity in providing beneficial effects in cardiac disorders [41-42]. It is now clinical proven drug agent has an efficacy in various cardiac disorders [42]. Even stem bark of arjun tree has been reported to provide beneficial effects against acute renal failure [43] (Figure 2.4).

Figure 2.4: *Terminalia arjuna* plant. (http://www.planetayurveda.com/media/wysiwyg/planet/terminalia-arjuna.png)

2.8.2. Selection of *Santalum album* L.

Sandalwood, the dried heartwood of *Santalum album* L., belongs to the family Santalaceae, has been known as green gold [44]. It is widely distributed in India, Malaysia and Australia [47]. It predominantly contains sesquiterpenes viz., α- and β-santalol with percentage of 53 and 23 respectively [45].

Figure 2.5: *Santalum album* plant (http://www.planetayurveda.com/media/wysiwyg/planet/santalum-album.jpg)

It has been reported in the literature as sedative, anti-diarrohoeal, diuretic, antibacterial and antiviral activities [44, 46]. In Ayurveda, a traditional Indian medicinal system, sandalwood has been reported as anti-inflammatory drug and used traditionally to treat chronic inflammation of bladder and pyelitis [47] (Figure 2.5).

2.8.3. Selection of *Nymphaea* x *rubra* Roxb. Ex Adnrews

Nymphaea x *rubra* Roxb. ex Andrews, with local name kumuda, is an aquatic perennial herb belonging to family Nymphaeaceae. A recent study has found that, *Nymphaea* x *rubra* is a hybrid produced by cross between *N. lotus* and *N. pubescens* as a parental species [48]. It grows on the shores of lakes, rivers and water bodies.

Figure 2.6: *Nymphaea rubra* plant (https://pxhere.com/en/photo/652241)

The plant can grow to heights upto 6 to 7 feet in water and has green colored leaves floating on water and pink colored flowers. The tubers are vertically covered with hairs. The plant is naturally found in Asia and in India mainly in Odisha and West Bengal [49]. In the traditional medicine system, various parts of *Nympaea rubra* has been reported to treat various types of bleeding disorders [50].

In addition to this, pulverized rhizomes mixed with honey have been used as therapeutic agent for bleeding nose, piles, dysentery and as cardiotonic [51]. Pharmacologically, this whole plant is known to have protective effect on various disorders viz., anthelmintic, immunomodulation, insulin resistance, anti-hyperglycemic, anti-dyslipidemic, anti-inflammatory, anti-pyretic, hepatoprotective and free radical scavenging activity [49, 52-54] (Figure 2.6).

AIM AND OBJECTIVES

3. OBECTIVES OF THE STUDY

The present work was designed to investigate:

Objective 1. Development of an animal model for acute hypobaric hypoxia-induced alterations in fluid and electrolyte balance, CVL, and hypovolemia in Sprague Dawley (SD) rats

Phase 1. Development of an animal model to study the effect of acute exposure to hypobaric hypoxia on renal function and CVL in SD rats

Phase 2. To study the effect of dehydration on hematology, plasma volume and GFR in SD rats

Phase 3. To study the effect of combined hypoxia dehydration (CHD) stress on renal function, neuroinflammation, plasma volume, and hematology in SD rats

Objective 2. Phyto-prophylactics for the prevention of AMS

Phase 1. To study the effect of *Terminalia arjuna* bark extract on acute hypobaric hypoxia-induced decrease GFR in SD rats

Phase 2. To study the effect of *Santalum album* heartwood extract on acute hypobaric hypoxia-induced CVL and neuroinflammation in SD rats

Phase 3. To study the effect of *Nymphaea rubra* rhizome extract on dehydration-induced increased RBC fragility in SD rats

Objective 3. To study the prophylactic efficacy of poly-herbal formulation comprising of *Terminalia arjuna*, *Santalum album* and *Nymphaea rubra* during CHD stress in SD rats

MATERIAL AND METHODS

RESULTS

RESULTS

5. RESULTS

5.1. Development of an animal model for acute hypobaric hypoxia-induced alterations in fluid and electrolyte balance, CVL, and hypovolemia in SD rats

5.1.1. Development of an animal model to study the effect of acute exposure to hypobaric hypoxia on renal function and CVL in SD rats

a). Effect of acute exposure to hypobaric hypoxia on ANP concentration, GFR, and aldosterone concentration during simulated hypobaric hypoxia exposure

Initially, at the altitudes of 10,000 and 15,000 ft. of acute hypobaric hypoxia, there was the slight but non-significant increase in GFR was observed in hypoxic animals as compared to the normal control animal. However, as the altitude increases, the decrease in GFR

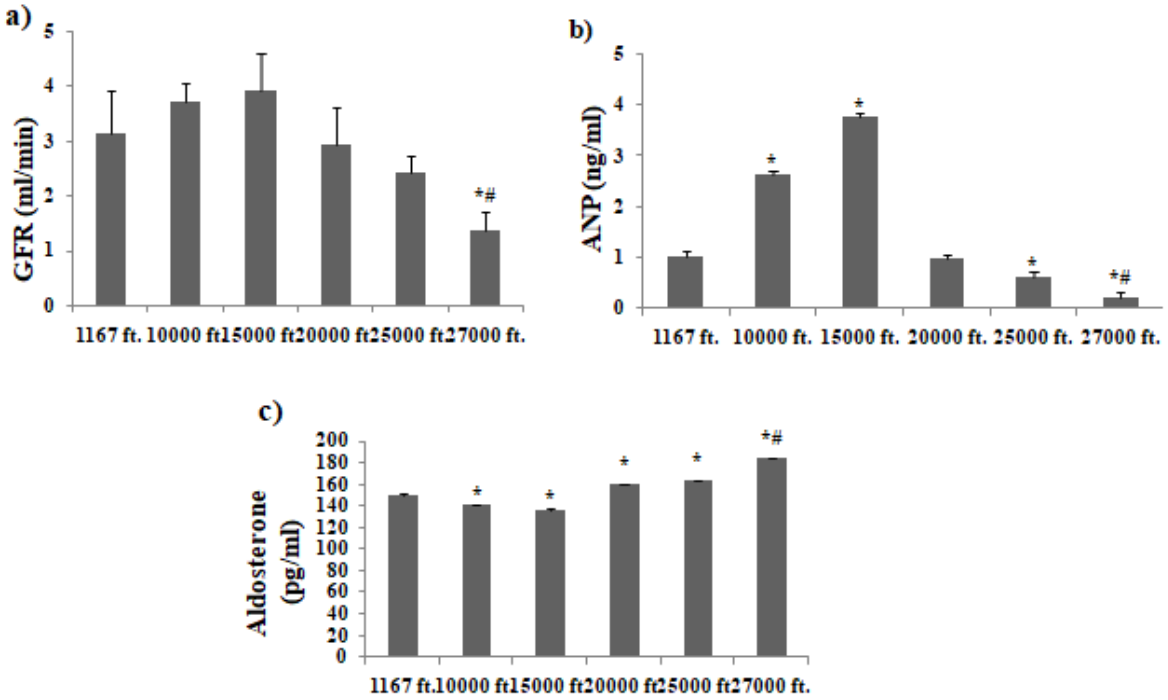


Figure 5.1: Graphs denoting mean ±SD of a) glomerular filtration rate, b) atrial natriuretic peptide concentration and c) aldosterone concentration in rat serum, following different hypobaric hypoxia viz., 10,000, 15,000, 20,000, 25,000 and 27,000 ft. exposure. * denotes $p<0.05$ when compared to Nor + Veh (1167 ft.) and # denotes $p<0.05$ when compared to Hyp + Veh (25,000 ft.). GFR: Glomerular Filtration Rate, ANP: Atrial Natriuretic Peptide, Nor: Normoxia, Hyp: Hypoxia, Veh: Vehicle

was observed and significant decreased GFR was observed in animals exposed at the altitude of 27,000 ft. as compared to the normal control animals (Figure 5.1. a).

ANP levels were found to significantly increased up-to the altitude of 15,000 ft. as compared to the normal control animals. However, afterward, the ANP levels were found to be significantly reduced as compared to the normal control animals. There was also significantly low ANP levels in rat serum exposed at the altitude of 27,000 ft. as compared to the normal control animals (Figure 5.1. b). However, on the contrary, the significant decrease in aldosterone levels was observed up to the altitude of 15,000 ft., as compared to the normal control animals. Serum aldosterone level significantly increased with the increase in high altitude exposure (Figure 5.1. c).

b). Effect of acute exposure to hypobaric hypoxia on S100B levels, CVL and percentage prevalence of CVL in rats.

With the increases in high altitude exposure, the serum S100B levels, CVL and percentage prevalence of CVL was found to increase as exposure to simulated hypoxia increased. In addition to this, a significant increase in serum S100B levels and CVL was observed at the altitude of 25,000 and 27,000 ft. In addition to this, we observed a significant increase in serum S100B levels and CVL was observed in animals exposed at the altitude of 27,000 ft. as compared to the animals exposed at the altitude of 25,000 ft. (Figure 5.2. a and b).

Percentage prevalence of CVL was also found to increase with the increase in altitude up to the altitude of 27,000 ft., unfortunately, none of the experimental animals were able to survive at the altitude of 30,000 ft., (Figure 5.2. c).

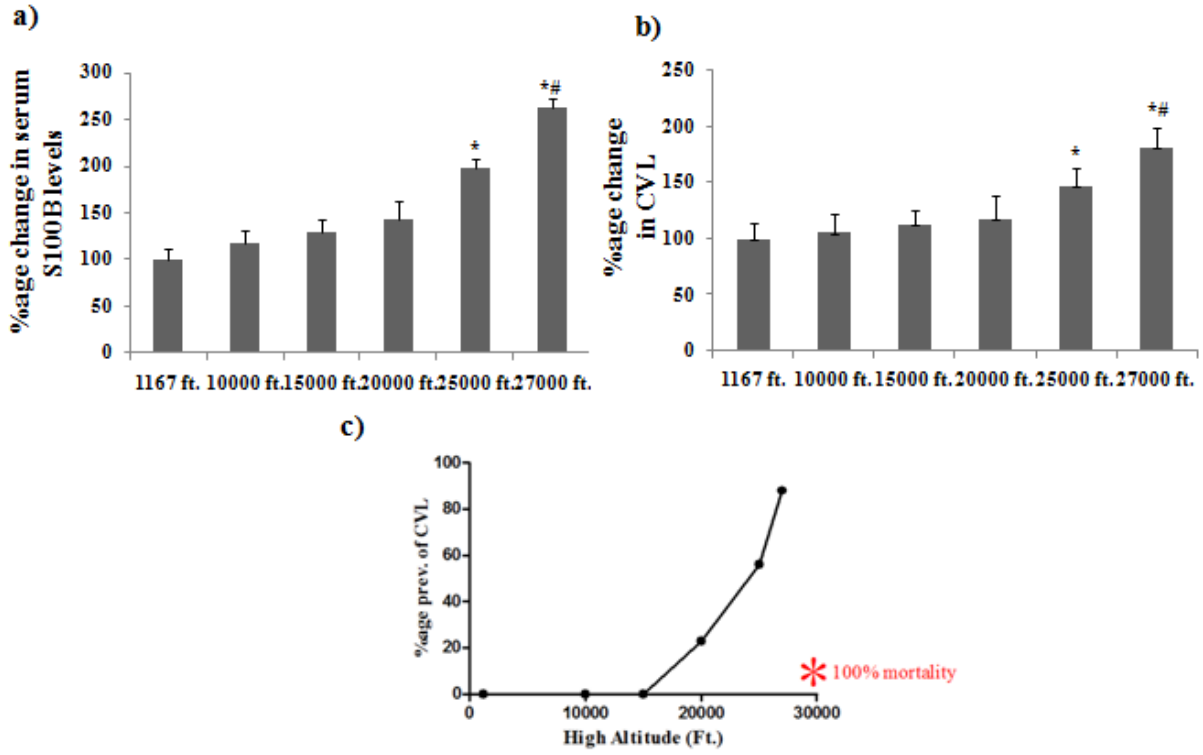


Figure 5.2: Graph denoting mean ± SD of a) S100B conc., b) CVL (CVL) and c) %age prevalence of CVL following different hypobaric hypoxia viz., 10,000, 15,000, 20,000, 25,000 and 27,000 ft. exposure. * denotes $p<0.05$ when compared to Nor + Veh (1167 ft.) and # denotes $p<0.05$ when compared to Hyp + Veh (25,000 ft.). Nor: Normoxia, Hyp: Hypoxia, Veh: Vehicle; CVL: CVL

c). Effect of acute exposure to hypobaric hypoxia on hematological parameters, plasma volume and serum electrolytes in rats

Exposure to simulated high altitude of 27,000 ft. in animals did not showed any significant difference in hematological parameters and plasma volume. However, hypoxia-exposed animals showed the significant increase in serum Na^+ levels and decrease in serum K^+ levels, as compared to the normal control animals (Table 5.1).

Table 5.1: Denoting effect of acute exposure to hypobaric hypoxia on haematology and serum electrolytes in rats. Nor: Normoxia; Hyp: Hypoxia. * denotes $p<0.05$ when compared to Nor (1167 ft.). RBC: Red blood cells; PCV: Packed cell volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration

Parameters	Nor (1167 ft.)	Hyp (27000 ft.)
RBC's (10^6/ mm^3)	7.88 ± 0.31	8.31 ± 0.33
Hemoglobin (g/dl)	14.8 ± 0.27	16.5 ± 0.24
PCV (%age)	40.67 ± 0.76	42.32 ± 1.82
MCV (fl)	51.4 ± 1.20	49.32 ± 2.11
Plasma Volume (ml/100g)	9.044±0.47	9.217±0.31
Sodium (mmol/ltr)	127.1±6.45	137.9±3.08*
Potassium (mmol/ltr)	5.087±0.60	4.532±0.56*

5.1.2. Effect of dehydration on hematological parameters, plasma volume and serum electrolytes in rats

Exposure to dehydration results in significant increase in RBC's number, hemoglobin concentration and PCV as compared to the normal control animals. However, no significant difference in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration was observed in dehydrated animals as compared with normal control animals. Significant decreased in plasma volume, body weight and GFR levels were observed in dehydrated animals as compared with normal control animals (Table 5.2).

Table 5.2. Table denoting effect of dehydration on haematology, plasma volume, body weight and glomerular filtration rate in rats. * vs NC. NC: Normal control animals; DC: Dehydration control animals; RBC: Red blood cells; PCV: Packed cell volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; GFR: Glomerular filtration rate

Parameters	NC	DC
RBC's (10^6/ mm^3)	8.02 ± 0.31	8.95 ± 0.03*
Hemoglobin (g/dl)	14.47 ± 1.04	17.23 ± 0.9*
PCV (%age)	43.12 ± 2.64	48.97 ± 2.33*
MCH (pg)	18.73 ± 0.86	18.87 ± 0.7
MCHC (g/dl)	36.73 ± 1.62	37.70 ± 1.04
Plasma Volume (ml/100 g)	9.044 ± 0.47	8.31±0.35*
Body weight (g)	230.0 ± 14.14	198.5 ± 12.02*
GFR (ml/min)	2.988 ± 0.694	1.379 ± 0.348*

5.1.3. To study the effect of combined hypoxia and dehydration (CHD) exposure on renal function, neuro-inflammation, plasma volume and hematology in SD rats

In this study when animals were exposed to CHD, significantly increased S100B serum concentration and significantly decreased GFR as compared to normal control animals were observed. Increased levels of PCV and decreased levels of plasma volume were observed in the animals exposed CHD as compared with normal control animals. However, we did not observed any change in the hematological parameters, when the animals were exposed to acute hypobaric hypoxia for 12 hrs. When animals were dehydrated for 72 hrs,

Table 5.3. Table denoting effect of hypobaric hypoxia on plasma volume, glomerular filtration rate, serum S100B concentration and haematology in rats. *Vs Nor (1167ft.). Nor: Normoxia; Hyp: Hypoxia; RBC: Red blood cells; PCV: Packed cell volume; GFR: Glomerular filtration rate

Parameters	Nor (1, 167 ft.)	Hyp (17, 688 ft.)
Plasma Volume (ml/100 g)	9.044 ± 0.47	6.71±0.25*
GFR (ml/min)	3.20±0.80	0.50±0.21*
S100B (ng/µg)	14.23±3.21	36.34±4.67*
RBC (10^6/ mm^3)	6.7 ± 1.61	8.57 ± 2.03*
Haemoglobin (g/dl)	13.93 ± 4.16	18.7 ± 4.91
PCV (%age)	40.6 ± 9.69	51.6 ± 11.1*

we have observed increased PCV, decreased plasma volume and decreased GFR. But, when we exposed the animals to CHD stress, we have seen the additive effect of dehydration on acute hypobaric hypoxia (Table 5.3).

5.2. Phyto-prophylactics for the prevention of AMS

5.2.1. To study the effect of *T. arjuna* bark extract on acute hypobaric hypoxia-induced decrease GFR in SD rats

a). Phytochemical fingerprinting of T. arjuna bark extract

Phytochemical fingerprinting by RP-HPLC analysis reveals the presence of arjunolic acid, gallic acid, and catechin in the hydro-alcoholic extract by comparing the retention time of standards with the extract (Figure 5.3).

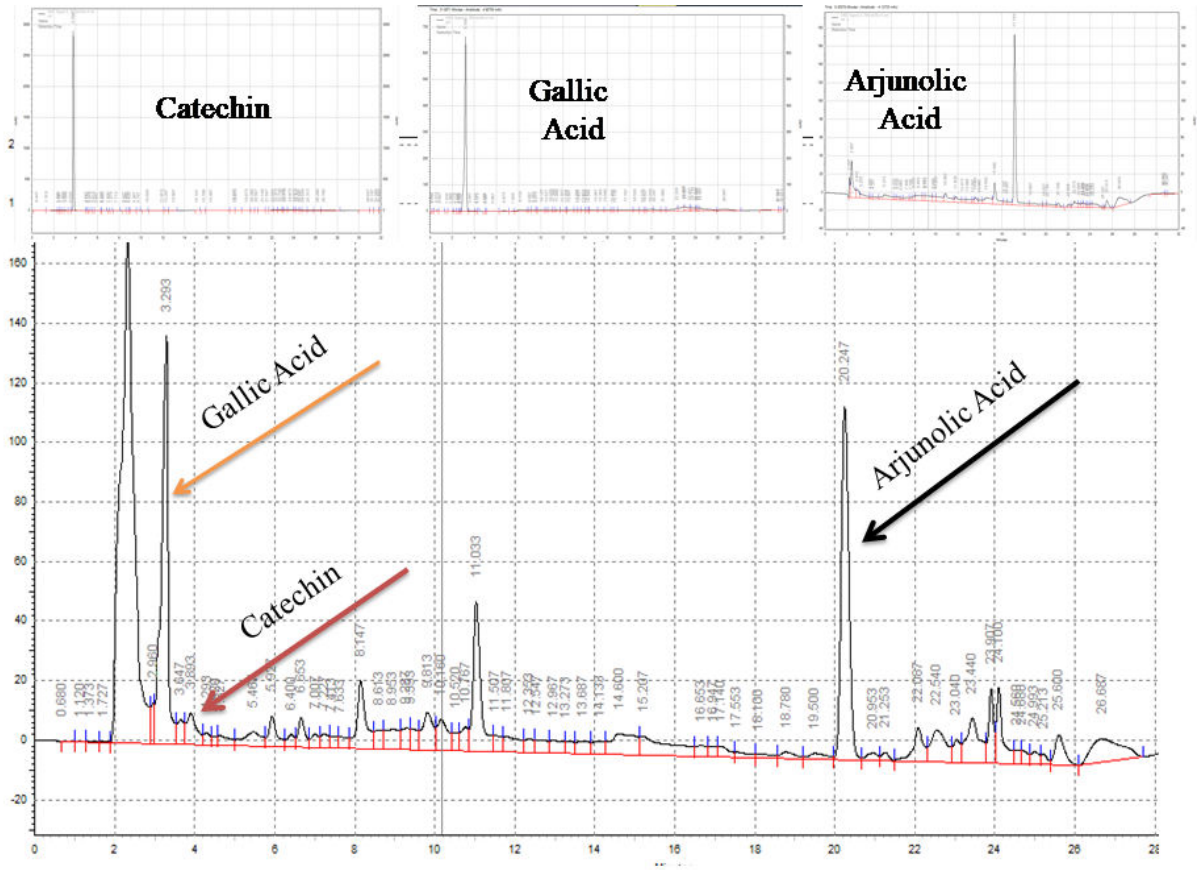


Figure 5.3: Representative HPLC-chromatogram indicating presence of Gallic acid, Catechin and Arjunolic acid with other compounds in hydro- alcoholic *T. arjuna* bark extract.

b). Effect of acute and sub-acute administration of T. arjuna bark extract

Acute and sub-acute toxicity studies were conducted according to OECD guidelines. No mortality was observed at the dose of 2,000 mg/kg of *b.w.* However, there was an increase in urine output during initial six hours of extract administration. Anatomical and histological examination revealed no adverse effect on vital organs viz., brain, lungs, kidneys, spleen, liver, and heart (Figure 5.4).

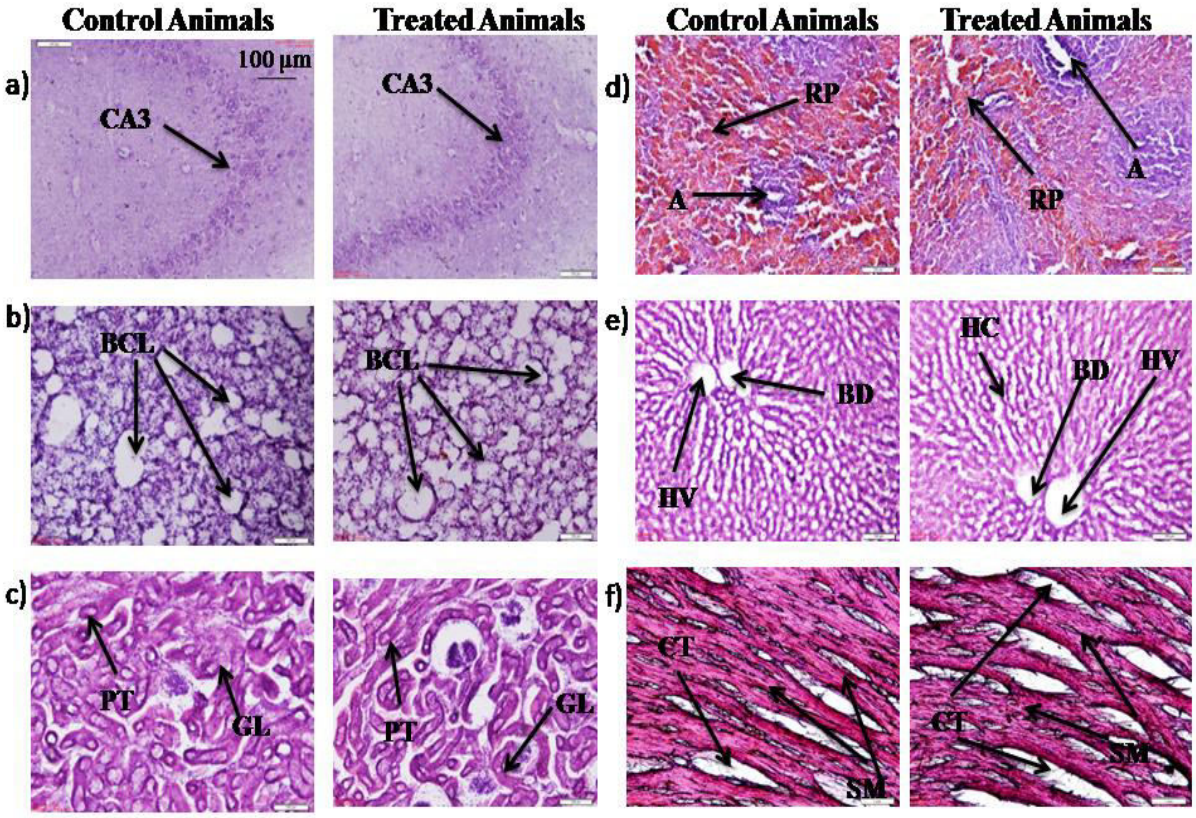


Figure 5.4: Histopathological studies for sub-acute toxicity of *T. arjuna* bark extract at dose of 2000mg/kg *b.w.* Panels showing representative histological sections of different organs of rats viz., a) Brain. Arrow denotes CA3 neurons. b) Lungs. Arrows denote bronchioles (BCL) c) Kidney. Arrows denote proximal tubules (PT) and glomerulus (GL) d) Spleen. Arrows denote red pulp (RP) and arteries (A) e) Liver. Arrows denote hepatocytes (HC), hepatic vein (HV) and bile duct (BD) f) Heart. Arrow denotes cardiac muscles (CM) and connective tissue (CT). Scale bar-100 µm.

c). Dose optimization for diuretic effect of *Terminalia arjuna* bark extract

Administration of hydro-alcoholic extract of *T. arjuna* to normoxic rats at doses of 100 mg/kg *b.w.* and above resulted in a dose-dependent increase in ANP concentration in the serum. However, no significant difference in ANP concentration was observed between animals administered with 150, 200 and 250 mg/kg *b.w.* of *T. arjuna* extract. Hence, the optimal dose for the hydro-alcoholic extract of *T. arjuna* was determined to be 150 mg/kg *b.w.*, which was used for further experimentation. In addition to elevated ANP concentration, we also observed an increase in GFR urine volume in normoxic rats administered with *T. arjuna* extract at doses above 150mg/kg *b.w.* (Figure 5.5).

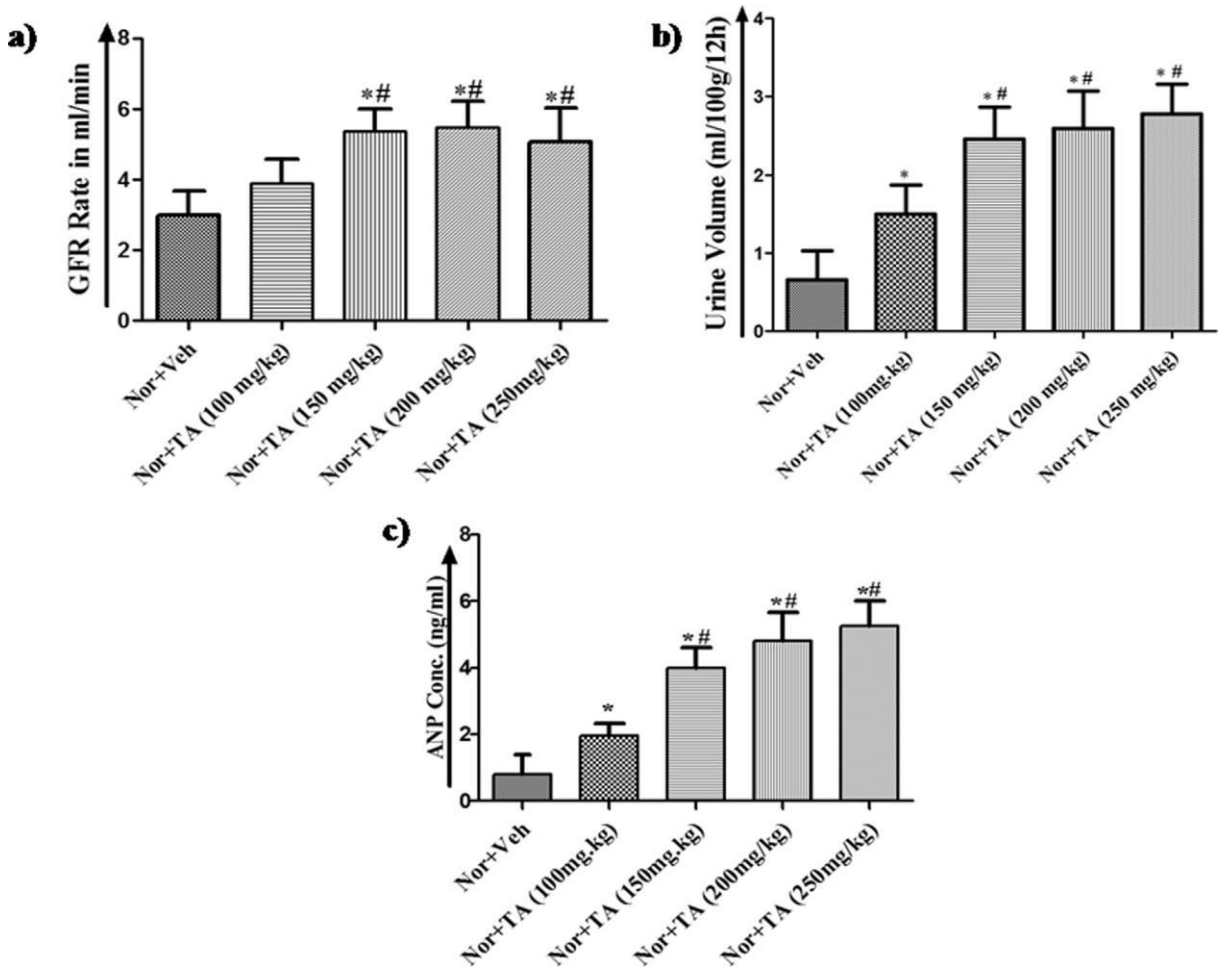


Figure 5.5: Graphs denoting mean ±SD of a) glomerular filtration rate, b) urine volume and c) atrial natriuretic peptide levels in rats following administration of hydro-alcoholic extract of *T. arjuna* in different doses viz., 100 mg/kg, 150 mg/kg, 200 mg/kg and 250 mg/kg of body weight to normoxic animals. * denotes $p<0.05$ when compared to Nor + Veh and # denotes $p<0.05$ when compared to Nor +TA (100 mg/kg). ANP: Atrial Natriuretic Peptide, GFR: Glomerular Filtration Rate, Nor: Normoxia, Veh: Vehicle, TA: *Terminalia arjuna*

d). Effect of T. arjuna bark extract administration on urine volume, CVL and serum electrolytes in rats

Exposure to hypobaric hypoxia simulated an altitude of 27,000 ft. for the duration of 12 hours resulted in CVL in 86% of vehicle-treated animals. Administration of hydro-alcoholic extract of *T. arjuna* at the dose of 150 mg/kg *b.w.* 30 minutes prior to exposure to hypobaric hypoxia

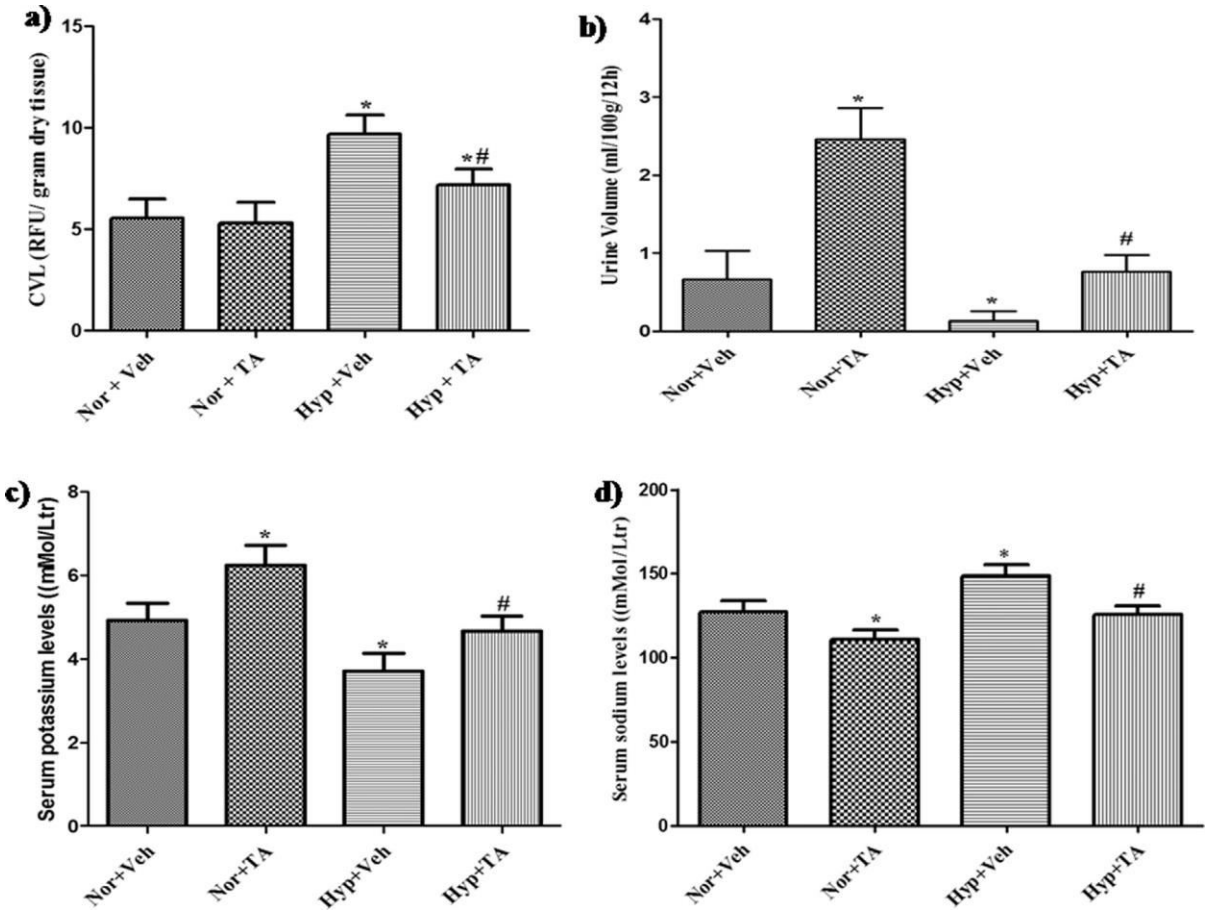


Figure 5.6: Graphs denoting mean ±SD of a)) CVL, b) urine volume, c) serum potassium levels and d) serum sodium levels in rats following administration of hydro-alcoholic extract of *T. arjuna* (150 mg/kg) to hypoxic animals. * denotes $p<0.05$ when compared to Nor + Veh and # denotes $p<0.05$ when compared to Hyp +Veh. Nor: Normoxia, Hyp: Hypoxia, TA: *Terminalia arjuna*

resulted in the prevention of CVL on exposure to simulated altitude of 27,000 ft. While exposure to hypobaric hypoxia resulted in significant decrease in urine volume in vehicle-treated group, administration of *T. arjuna* extract 30 min. prior to hypobaric hypoxia exposure maintained urine volume close to normoxic rats. In addition to maintenance of urine

volume, *T. arjuna* bark extract also ameliorated hypobaric hypoxia-induced decrease in serum potassium and increase in serum sodium, which was observed in vehicle-treated hypoxic animals (Figure 5.6).

e). Effect of T. arjuna bark extract administration on Renin-angiotensin-aldosterone system and arterial natriuretic peptide levels in rats

The plasma half-life of ^{99m}Tc provides an indirect measure of GFR. Nonlinear regression analysis for the half-life of ^{99m}Tc showed that acute exposure to hypobaric hypoxia simulating the altitude of 27,000 ft. resulted in a significant increase in plasma half-life of ^{99m}Tc as compared to normoxic vehicle-treated animals. Administration of *T. arjuna* bark extract prior to hypoxic exposure did not alter total protein concentration in the serum but decreased plasma half-life of ^{99m}Tc. *T. arjuna* induced increase in GFR attenuated by administration of ANP-receptor antagonist-anantin. Exposure to hypobaric hypoxia also resulted in increased serum renin, angiotensin-II and aldosterone concentration in vehicle-treated hypoxic groups, which was ameliorated by the administration of *T. arjuna* bark extract prior to hypoxic exposure. *T. arjuna* extract, co-administrated with anantin to hypoxic animals, failed to reduce renin, angiotensin-II and aldosterone concentration in the serum indicating ANP mediated regulation of RAAS system resulting in increased GFR in *T. arjuna* administered hypoxic animals (Figure 5.7).

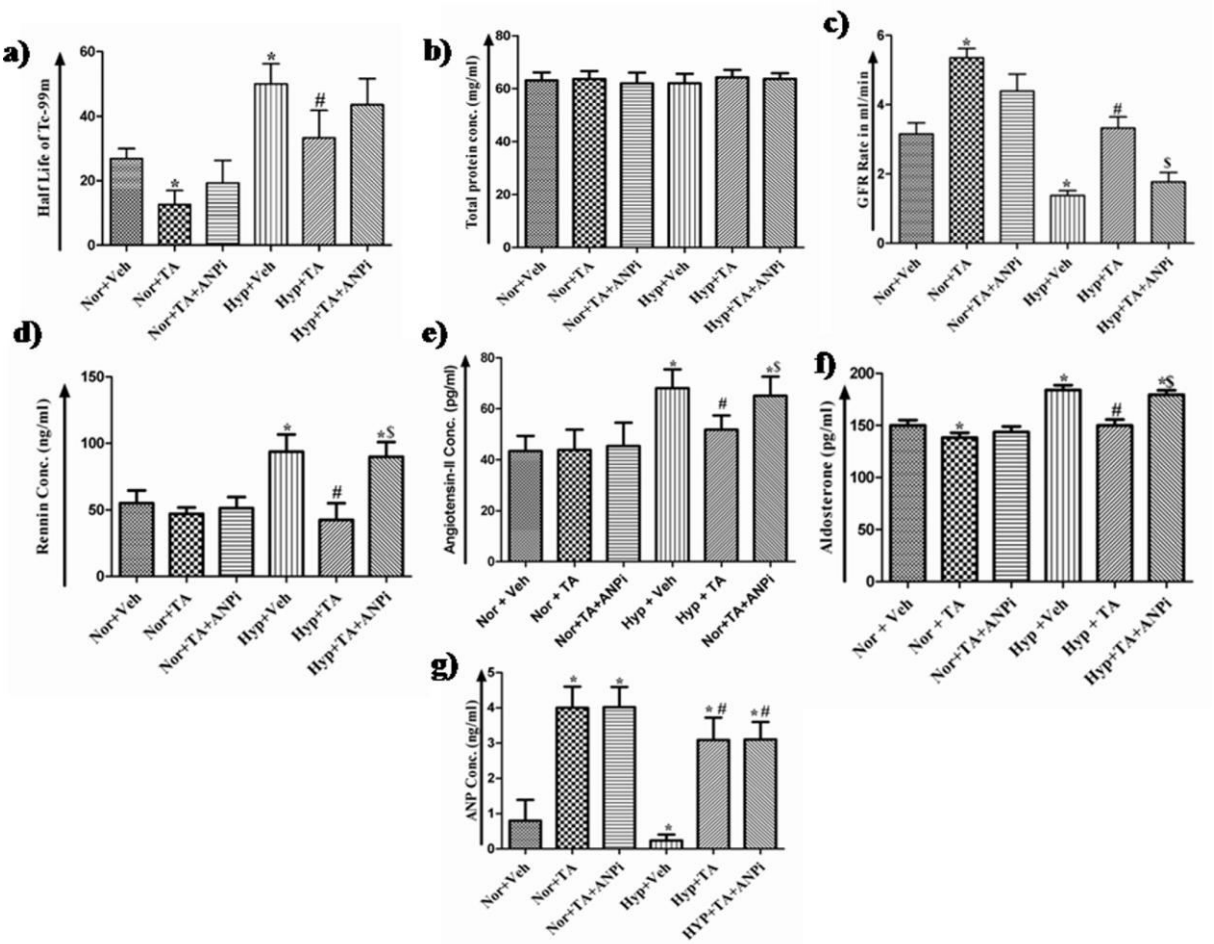


Figure 5.7: Graphs denoting mean ±SD of a)) plasma half-life of Tc-99m, b) total protein concentration, c) glomerular filtration rate, d) serum renin concentration e) serum angiotension-II concentration and f) serum aldosterone concentration g) atrial natriuretic peptide concentration in rats following administration of hydro-alcoholic extract of T. arjuna (150 mg/kg) to hypoxic animals. *denotes $p<0.05$ vs. when compared to Nor + Veh, # denotes $p<0.05$ when compared to Hyp +Veh and $ denotes $p<0.05$ when compared to Hyp +TA. GFR: Glomerular Filtration Rate, ANP: Atrial Natriuretic Peptide, Nor: Normoxia, Hyp: Hypoxia, TA: *Terminalia arjuna*, ANPi: ANP Inhibitor (Anantin)

5.2.2. To study the effect of *Santalum album* heartwood bark extract on acute hypobric hypoxia-induced CVL and neuro-inflammation in SD rats

a). Effect of acute and sub-acute administration of SAE on rats

Acute and sub-acute toxicity studies showed no sign of toxicity and mortality at the doses 300 and 2000 mg/kg as compared to normal control animals. Histological staining with hematoxylin and eosin of vital organs viz., brain, lungs, kidneys, spleen, liver, and heart has shown no sign of toxicity to animals treated with SAE as compared to normal control animals (Figure 5.8).

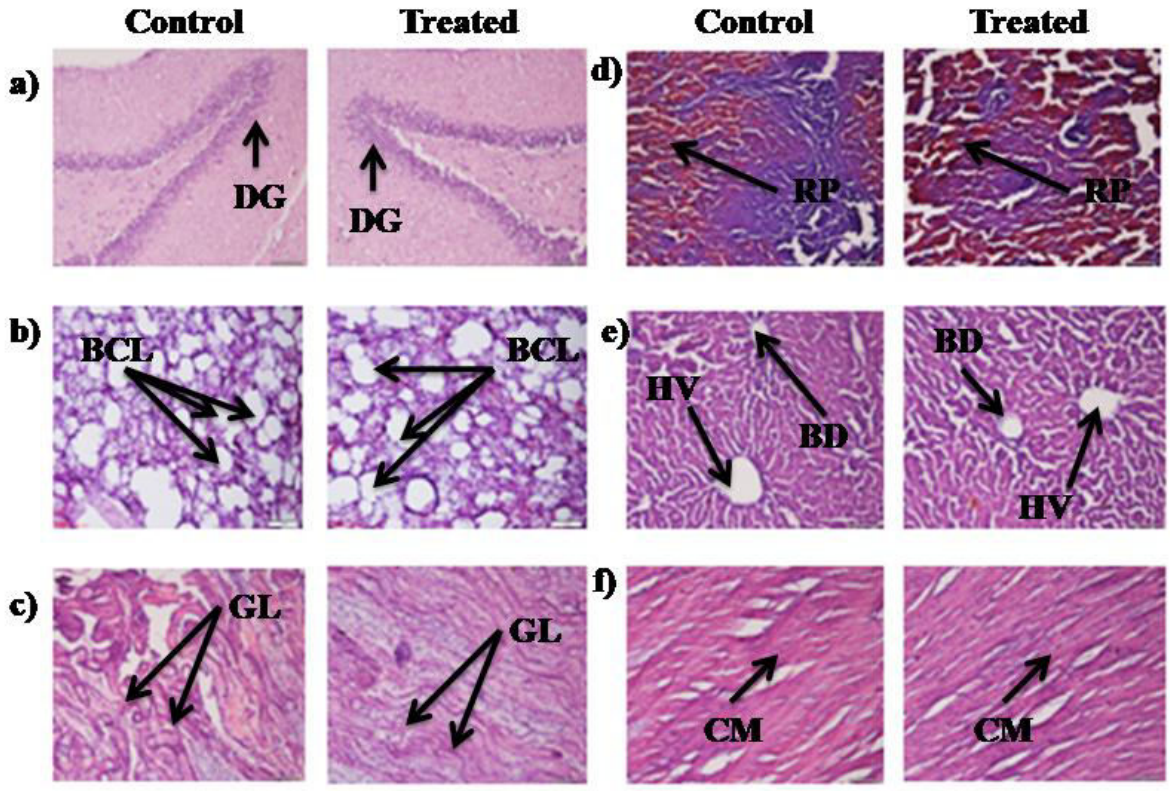


Figure 5.8: Histopathological studies for sub-acute toxicity of SAE at dose of 2000mg/kg *b.w.* Panels showing representative histological sections of different organs of rats viz., a) Brain. Arrow denotes DG neurons. b) Lungs. Arrows denote bronchioles (BCL) c) Kidney. Arrows denote glomerulus (GL) d) Spleen. Arrows denote red pulp (RP) e) Liver. Arrows denote hepatic vein (HV) and bile duct (BD) f) Heart. Arrow denotes cardiac muscles (CM). Scale bar-100 µm. SAE: *S. album* extract.

b). Dose optimization of SAE and effect of S100B protein in acute hypobaric hypoxia-induced neuro-inflammation and CVL in rats

The optimal dose for SAE was determined to be 150 mg/kg *b.w.*, based on hippocampal IL-6 levels and CVL during hypoxia and this dose was used for further experimentation (Figure 5.9).

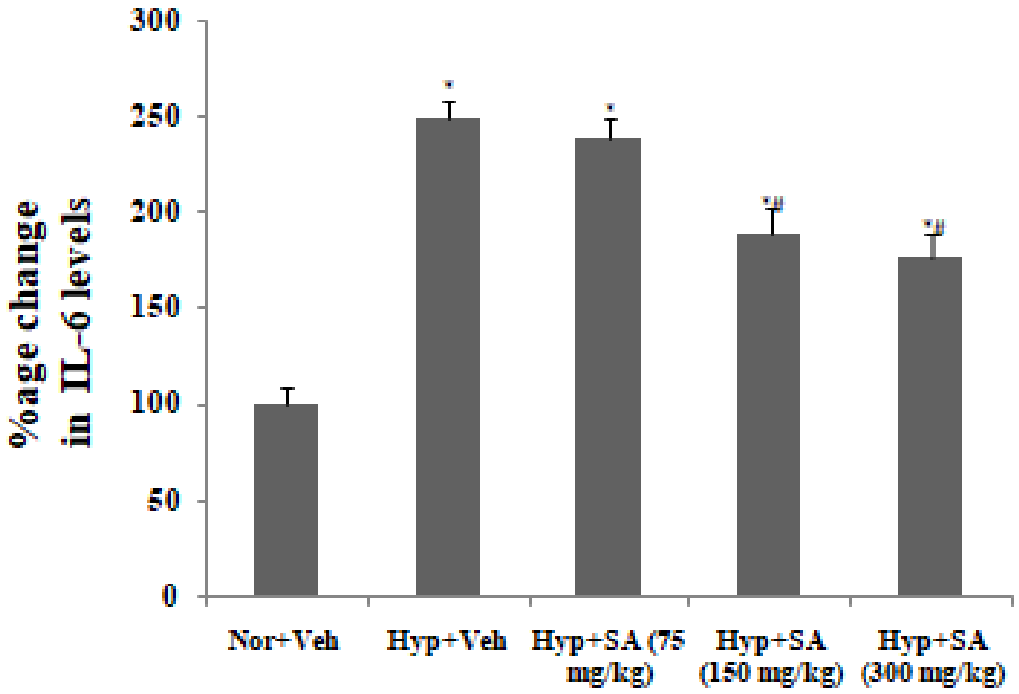


Figure 5.9: Graph denoting mean ±SD of percentage change in IL-6 levels of acute hypoxic animals treated with 75, 150 and 300 mg/kg of FSAE. * denotes $p<0.05$ when compared to Nor + Veh (1167 ft.) and # denotes $p<0.05$ when compared to Hyp + Veh. Nor: Normoxia, Hyp: Hypoxia, Veh: Vehicle; SA: Fractionated S. album extract

In the present study, acute exposure to hypobaric hypoxia had been found in increase expression of the S100B protein in the hippocampus as compared to normal control animals. However, treatment of hypoxic animals with NF-κB blocker and RAGE blocker was found not to alter the expression of S100B protein expression in rat brain hippocampus. In addition

to this, administration of SAE to hypoxic animals was not found to alter the expression of the S100B protein in rat brain hippocampus (Figure 5.10).

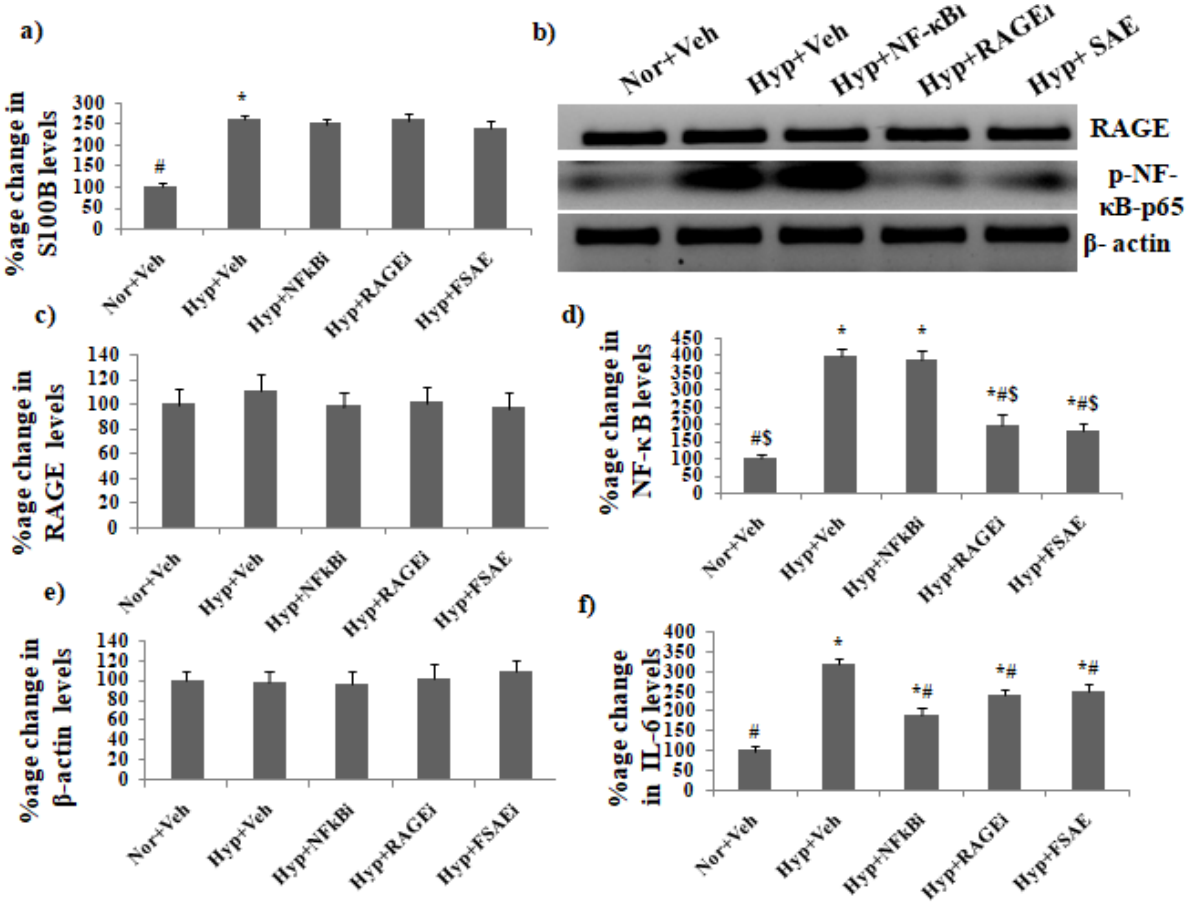


Figure 5.10: Graph denoting mean ±SD of a) %age change in hippocampus S100B levels, b) Representative western blots of RAGE, p-NFkB-p65 and β-actin in total hippocampal lysates., c) %age change in expression of RAGE recpetor, d) %age change in expression of p-NF-κB-p65, e) %age change in expression of β-actin and f) %age change in IL-6 levels in total hippocampal lysate. * denotes $p<0.05$ when compared to Nor + Veh (1167 ft.) , # denotes $p<0.05$ when compared to Hyp + Veh. and $ denotes $p<0.05$ when compared to Hyp+NFkBi. Hyp + Veh. Nor: Normoxia, Hyp: Hypoxia, Veh: Vehicle; SA: Fractionated S. album extract

In the present study, none of the experimental groups showed the significant change in the expression of RAGE receptor in rat brain hippocampus. Phosphorylated NF-κB expression was found to significantly increase in hypoxic animals as compared to normal control animals. However, treatment of animals with RAGE blocker and FSAE treatment significantly reduces the expression of phosphorylated NF-κB as compared to hypoxic

animals. No significant difference in expression of phosphorylated NF-κB was observed in animals treated with the inhibitor of NF-κB as compared to hypoxic animals. Acute exposure to hypobaric hypoxia was found to increase the levels of pro-inflammatory cytokine IL-6 in rat brain hippocampus as compared to normal control animals. However, treatment of hypoxic animals with inhibitors RAGE and NF-κB significantly attenuates acute hypobaric hypoxia-induced increased pro-inflammatory cytokines expressions in rat brain hippocampus as compared to hypoxic control animals (Figure 5.10).

c). In-silico interaction studies of α-santalol present predominantly in FSAE binds with RAGE receptor

Molecular docking studies of α-santalol and evaluation of inter-molecular interactions in terms of docking scores were done with respect to reference ligand named

Table 5.4: Describes 5 best docking pose of α-santalol with RAGE receptors on the basis of D-score (Docking score).

Poses	D-score	No. of residues	Hydrophobic	%age similarity
External Ligand	——	10	LYS42N, ASP65N, TYR155N, GLU153N, TYR62N, ASP14N, LYS15N, GLU11N, GLU44N, ARG66N	—
LP7	-38.431	4	LYS42N, ASP41N, TYR155N, GLU153N	40
LP3	-38.218	4	LYS42N, ASP41N	40
LP8	-37.425	4	TYR155N, GLU153N, LYS42N, ASP41N TYR155N, GLU153N	40
LP10	-36.475	3	LYS42N, ASP41N, GLU153N	30
LP4	-36.436	4	LYS42N, ASP41N, TYR155N, GLU153N	40

as Maltotriose using Vlife MDS 4.4 Biopredicta module Residues present in RAGE viz., Lys42N, Asp41N, Tyr155N and Glu153N are responsible for hydrophobic interactions (Table 5.4). Studies reveal that, LP7 pose of the predominant molecule binds deeply and shows 40% of similarity with external ligand with RAGE receptor. The residues responsible for hydrophobic interactions are Lys42N, Asp41N, Tyr155N and Glu153N as depicted in the 2D plot (Figure 5.11).

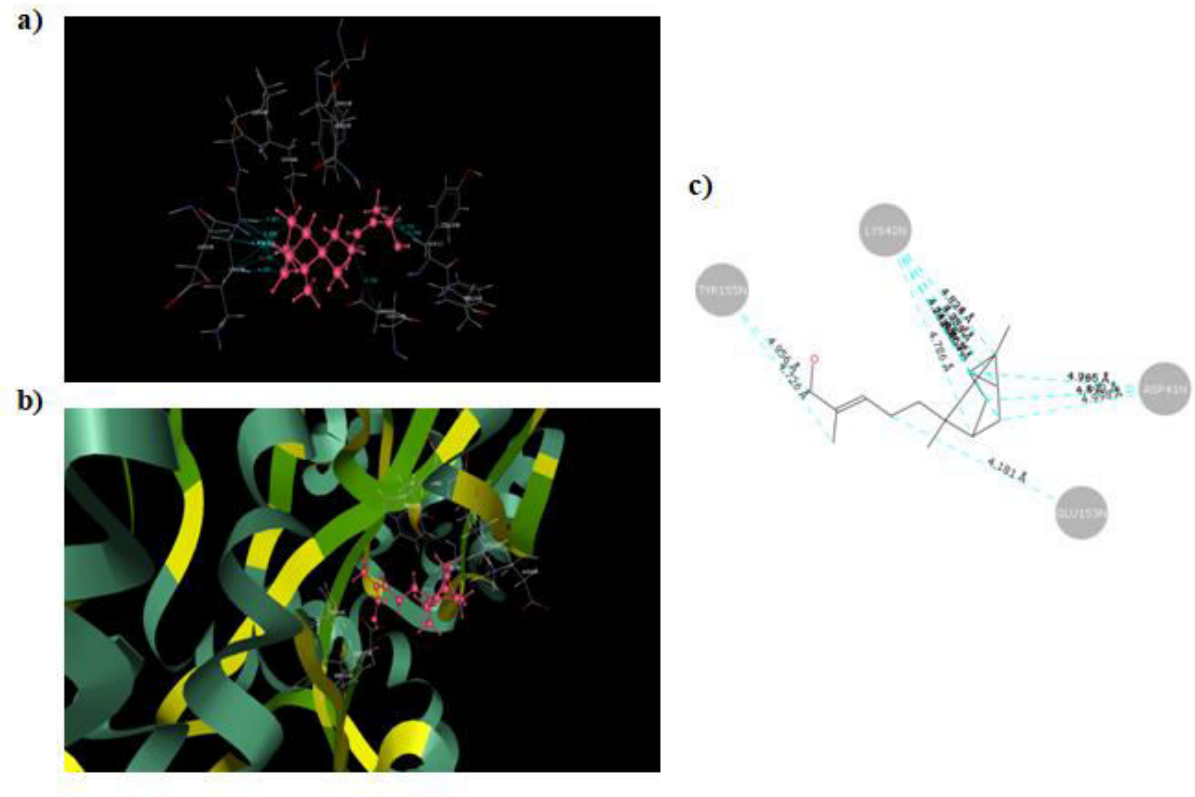


Figure 5.11: *In-silico* interaction study of α-santalol with RAGE receptors. a) In 3D model blue dotted line showing hydrophobic interaction of α-santalol with RAGE receptors protein in respective amino acid sequence LYS42N, ASP41N, TYR155N and GLU153N. b) and c) Ribbon and 2-D model showing the interaction of α-santalol with RAGE receptors.

d). Effect of FSAE on expression of pNF-κB-p65 dependent levels of brain pro-inflammatory cytokines and CVL during acute hypobaric hypoxia

Acute exposure to hypobaric hypoxia was found to significantly increase the levels of phosphorylated NF-κB levels in rat brain hippocampus lysate as compared to normal control animals. While administration of SAE to hypoxic animals decrease phosphorylated NF-κB

expression as compared to hypoxic control animals. However, no significant difference in any of experimental groups in RAGE expression was observed (Figure 5.12).

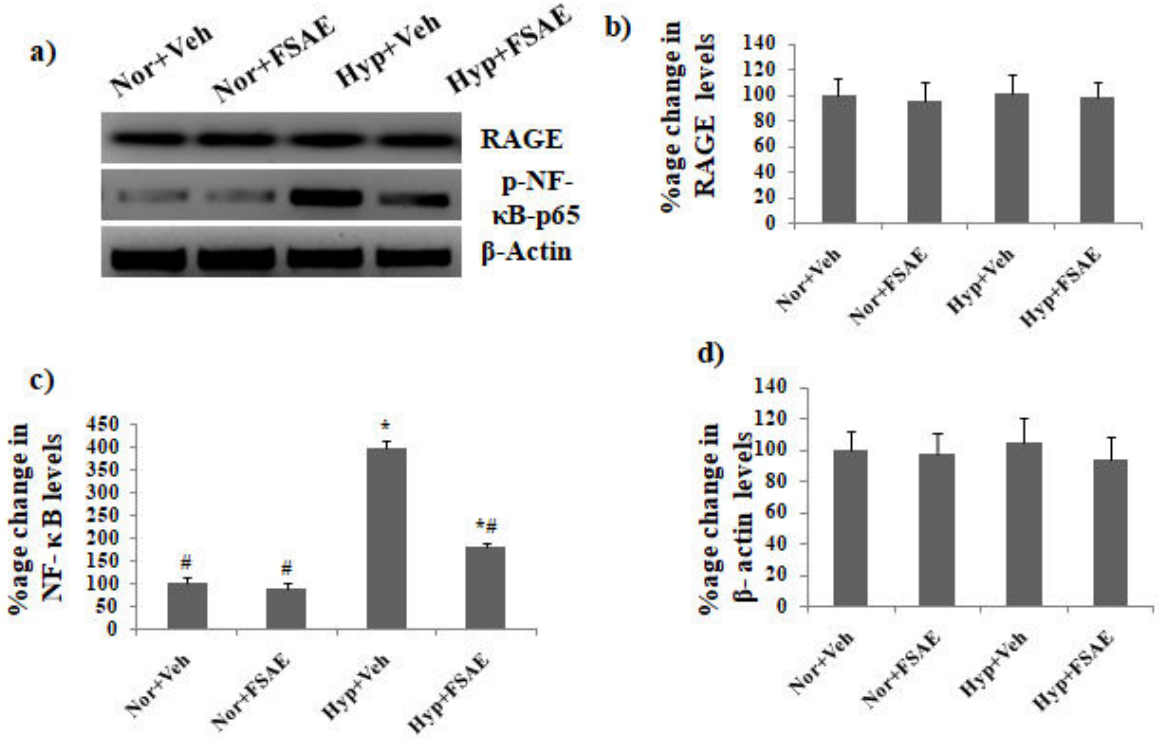


Figure 5.12: a) Representative western blots of RAGE, p-NF-κB-p65 and β-actin in total hippocampal lysates, Graph denoting mean ±SD of b), %age change in expression of RAGE recpetor c) %age change in expression of p-NFkB-p65 and d) %age change in expression of β-actin in rat following administration of FSAE (150 mg/kg) to hypoxic animals. * denotes $p<0.05$ when compared to Nor + Veh (1167 ft.), # denotes $p<0.05$ when compared to Hyp + Veh. Hyp + Veh. Nor: Normoxia, Hyp: Hypoxia, Veh: Vehicle; SA: Fractionated S. album extract.

While, administration of SAE to hypoxic animals, it significantly attenuated acute hypoxia-induced increased level of pro-inflammatory cytokines viz., IL-6, IL-1β, TNFα levels, CVL and the S100B levels in rat serum as compared to hypoxic control animals (Figure 5.13).

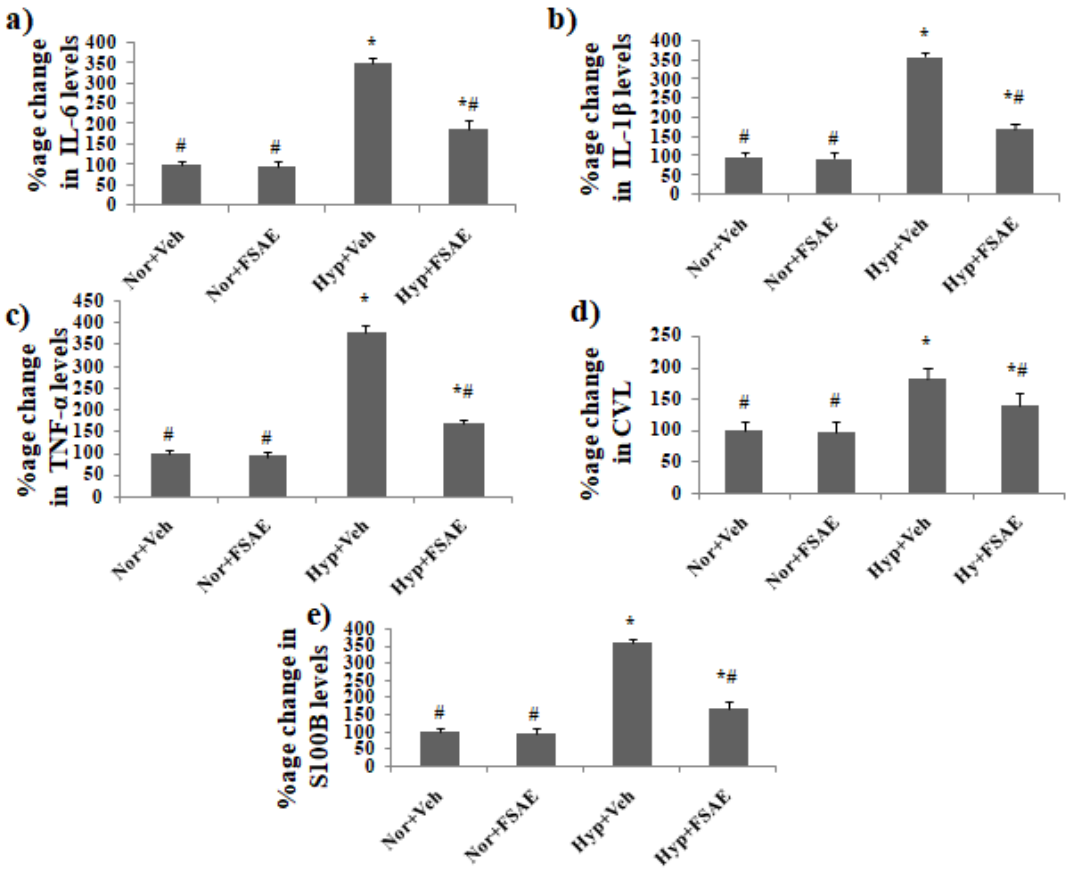


Figure 5.13: Graph denoting mean ± SD of a) %age change in IL-6 levels, b) %age change in IL-1 β levels c) %age change in TNF-α levels d) %age change in CVL and d) %age change in S100B levels in rat following administration of FSAE (150 mg/kg) to hypoxic animals. *denotes $p<0.05$ vs. when compared to Nor + Veh, # denotes $p<0.05$ when compared to Hyp +Veh. Nor: Normoxia; Hyp: Hypoxia; Veh: Vehicle; SA: Fractionated S. album extract; IL: Interleukin; TNF: Tumor nacrosis factor

5.2.3. To study the effect of *Nymphaea rubra* on dehydration induced increased RBC fragility in SD rats

a). Phytochemical fingerprinting of alcoholic N. rubra rhizome extract

RP-HPLC analysis of extract reveals the presence of phenolic compounds viz., rutin, caffic acid, and quercetin as inferred from comparison of the retention time of standard compounds with the extract (Figure 5.14).

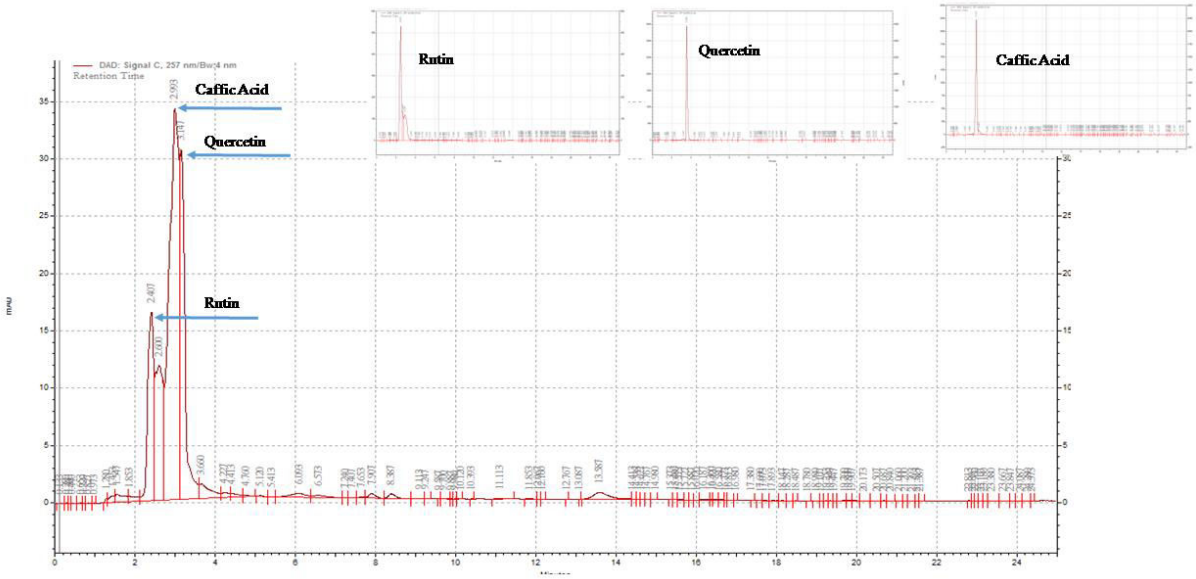


Figure 5.14: Representative chromatogram of methanolic *N. rubra* rhizome bark extract indicating presence of rutin, caffic acid and quercetin with other un-identified compounds.

b). Effect of acute and sub-acute administration of N. rubra rhizome extract

Acute and sub-acute toxicity studies showed no sign of toxicity and mortality at the doses 300 and 2000 mg/kg as compared to normal control animals. Histological staining with hematoxylin and eosin of vital organs viz., brain, lungs, kidneys, spleen, liver, and heart has shown no sign of toxicity to animals treated with *N. rubra* as compared to normal control animals (Figure 5.15).

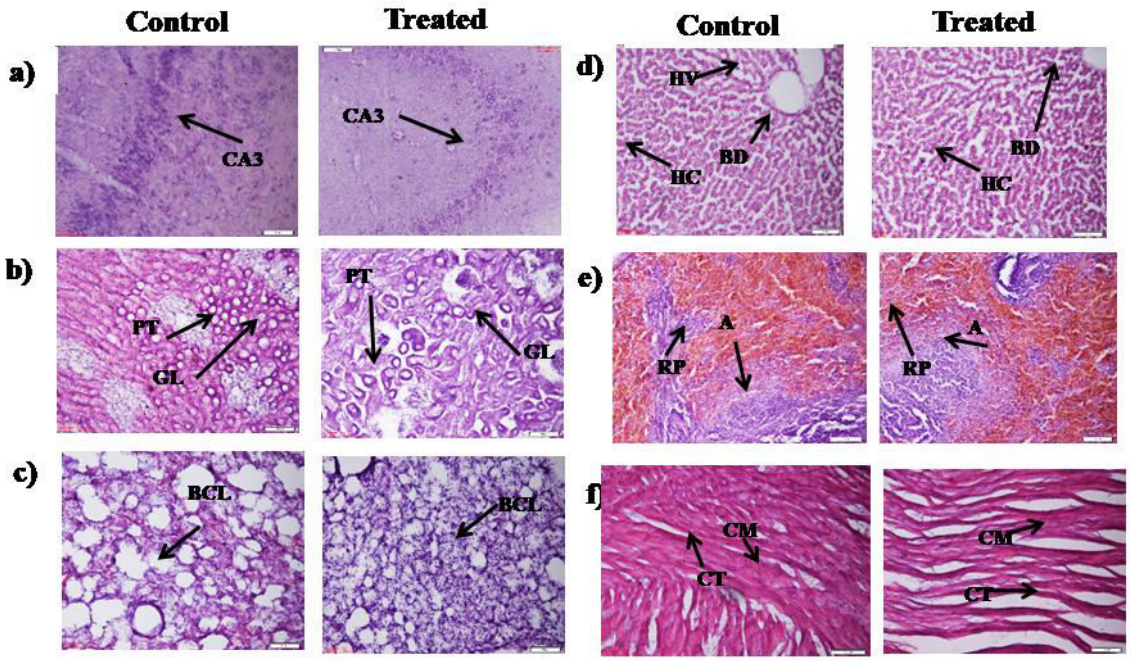


Figure 5.15: Histological studies of sub-acute regulatory toxicity studies of *N. rubra* extract at the dose of 2000 mg/kg *b.w.* Panels representing histological sections of different organs of rats viz., a) Brain, arrow denote CA3 region of hippocampus, b) Kidney, arrows denote proximal tubules (PT) and glomerulus (GL), c) Lungs, arrows denote bronchioles (BCL), d) Liver, arrows denote hepatocytes (HT), hepatic vein (HV) and bile duct (BD), e) Spleen, arrows denote red pulp (RP) and arteries (A), and f) Heart, arrows denote cardiac muscle (CM) and connective tissues (CT). Scale bar- 100 µm.

c). Dose optimization and effect of N. rubra rhizome extract on dehydration induced alterations in hematological parameters and RBC fragility in rats

Exposure to dehydration results in significant increase in RBC fragility in rats. When animals were treated with extract, we observed significant decrease in RBC fragility during dehydration stress. But no significant difference in RBC fragility was observed when animals treated at the dose of 200 and 400 mg/kg of extract. Hence, the optimized dose for preventing dehydration induced increased RBC fragility was found to be 200 mg/kg of *N. rubra* rhizome extract (Figure 5.16).

Exposure to dehydration results in increased RBC, PCV, and Hb in rats. Administration of *N. rubra* rhizome extract during dehydration did not decrease the dehydration-induced increased PCV and Hb. No significant difference in MCH and MCHC was observed between control drug-treated and dehydrated animals (Figure 5.17 a). Exposure to dehydration results in a significant increase in osmotic fragility of RBC in rats when compared with normal control animals. Administration of *N. rubra* rhizome extract significantly attenuates the dehydration-induced increase in RBC fragility in rats (Figure 5.17 b and Figure 5.18).

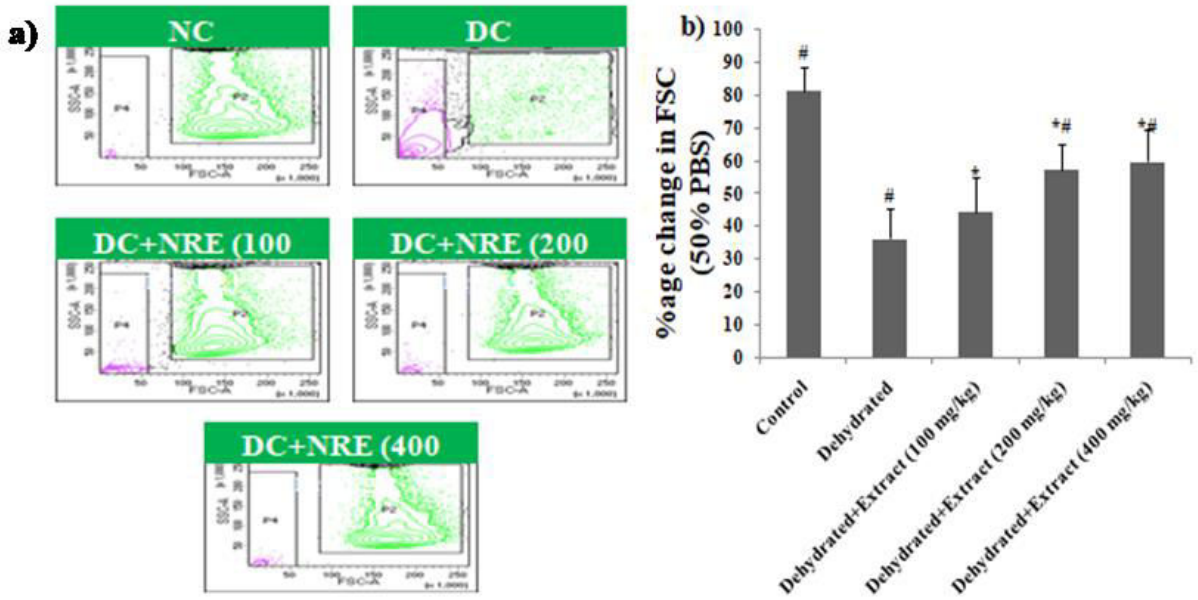


Figure 5.16: Figure representing a) dot blot histogram of RBC fragility and b) figure denoting mean ±SD of FSC following administration of *N. rubra* rhizome extract at the doses of 100, 200 and 400 mg/kg. Where NC represents normal control animals, NC+NRE represents normal control animals treated with N. rubra rhizome extract, DC represents dehydration control animals, DC+NRE represents dehydration control animals treated with extract and FSC represents forward scattering.

a)

	NC	NC+NRE	DC	DC+NRE
RBC ($\times 10^6$/ µl)	8.02± 0.58	8.25± 0.33	8.95±0.08*	8.97±0.11*
PCV (% age)	43.12± 2.64	43.30± 2.68	48.97± 2.33*	49.45±3.72*
Hb (g/dl)	14.47± 1.04	14.23± 0.55	17.23± 0.9*	17.73±1.4*
MCH (pg)	18.73± 0.86	18.70± 0.53	18.87±0.7	19.90±0.53
MCHC (g/dl)	36.73±1.62	36.57± 1.04	37.70±1.04	37.50±1.08

b)

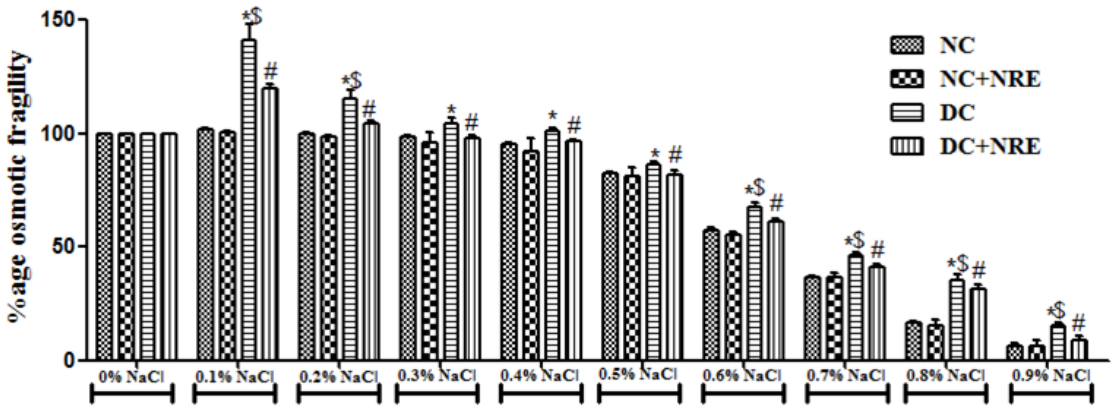


Figure 5.17: Tables denoting mean ±SD of a) effect of N. rubra extract treatment on hematology in rats and b) Figure representing effect of N. rubra treatment on RBC fragility in rats. Where NC represents normal control animals, NC+NRE represents normal control animals treated with N. rubra rhizome extract, DC represents dehydration control animals and DC+NRE represents dehydration control animals treated with extract. Note: * vs. NC; # vs DC; $ vs NC+NRE. Where NC represents normal control animals, NC+NRE represents normal control animals treated with N. rubra rhizome extract, DC represents dehydration control animals, DC+NRE represents dehydration control animals treated with extract, FSC represents forward scattering and SSC representing side scattering.

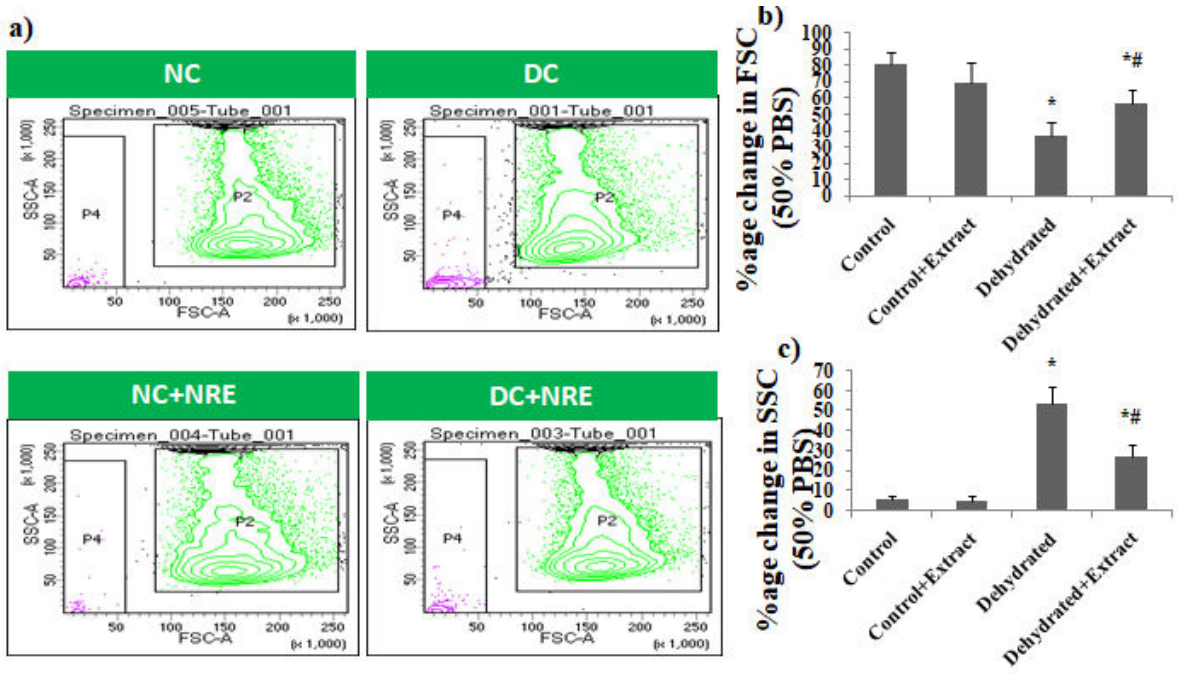


Figure 5.18. Figure representing a) dot blot histogram of RBC fragility and b) figure denoting mean ±SD of FSC and c) SSC following administration of *N. rubra* rhizome extract at the doses of 100, 200 and 400 mg/kg. Where NC represents normal control animals, NC+NRE represents normal control animals treated with *N. rubra* rhizome extract, DC represents dehydration control animals, DC+NRE represents dehydration control animals treated with extract, FSC represents forward scattering and SSC representing side scattering.

5.3. To study the prophylactic efficacy of poly-herbal formulation comprising of *Terminalia Arjuna*, *Santalum album,* and *Nymphaea rubra* during CHD stress

5.3.1. Preparation of poly-herbal formulation

For the composition optimization, poly-herbal formulation, it was mixed in eight different proportions in order to optimize the composition of the poly-herbal formulation. When the animals were treated with 1:1:1 proportion of *T. arjuna, S. album and N. rubra*, there was significant increase in GFR and the significant increase in RBC fragility and brain hippocampal IL-6 levels was observed in poly-herbal formulation treated animals as compared with CHD control animals. However, when we reduced the proportion of *N. rubra* in poly-herbal formulation to 0.5, the increased RBC fragility was found to be attenuated in the same manner when the composition of *N. rubra* in poly-herbal formulation was 1. Same results were observed with respect to attenuation of increased RBC fragility when the composition of *N. rubra* was reduced to 0.25. So, the proportion of *N. rubra* in poly-herbal formulation was fixed to 0.25.

When we increased the proportions of *T. arjuna* to 2, the diuresis was found to increase but the effect of poly-herbal formulation on CHD stress-induced increased IL-6 levels were compromised, same effect on GFR was observed when the composition of *T. arjuna* was increased to 2. So, the final optimized composition was found to be 1:1:0.25 for *T. arjuna, S. album and N. rubra* (Table 5.5).

Table 5.5: Table denoting composition optimization of *T. arjuna, N. rubra and S. album*

GFR: Glomerular Filtration Rate; RBC: Red Blood Cells; IL-6: Interleukin-6; CHD: Combined hypobaria and dehydration model; N: Normal Control Animals; T: Treated with various composition of poly-herbal formulation

Composition (*T. arjuna: N. rubra: S. album*)	**%age change in GFR rate**				**%age change in RBC fragility**				**%age change in hippocampaI IL-6 levels**			
	N	**CHD**	**N+T**	**CHD+T**	**N**	**CHD**	**N+T**	**CHD+T**	**N**	**CHD**	**N+T**	**CHD+T**
1:1:1	**100 ±12**	**50 ±09**	**251 ±10**	**115 ±12**	**100 ±08**	**313 ±13**	**98± 06**	**186 ±11**	**100 ±06**	**284 ±16**	**98 ±11**	**182 ±11**
1:0.5:1	**100 ±12**	**50 ±09**	**246 ±12**	**118 ±10**	**100 ±08**	**313 ±13**	**96± 08**	**182 ±14**	**100 ±06**	**284 ±16**	**96 ±12**	**184 ±10**
1:0.25:1	**100 ±12**	**50 ±09**	**254 ±13**	**116 ±11**	**100 ±08**	**313 ±13**	**98± 09**	**180 ±12**	**100 ±06**	**284 ±16**	**98 ±08**	**186 ±13**
2:1:1	**100 ±12**	**50 ±09**	**300 ±12**	**180 ±09**	**100 ±08**	**313 ±13**	**102 ±09**	**181 ±13**	**100 ±06**	**284 ±16**	**96 ±14**	**180 ±11**
1:1:2	**100 ±12**	**50 ±09**	**246 ±14**	**110 ±08**	**100 ±08**	**313 ±13**	**96± 10**	**182 ±14**	**100 ±06**	**284 ±16**	**94 ±07**	**160 ±09**
1:2:2	**100 ±12**	**50 ±09**	**240 ±12**	**109 ±08**	**100 ±08**	**313 ±13**	**92± 09**	**178 ±11**	**100 ±06**	**284 ±16**	**92 ±09**	**158 ±12**
2:2:1	**100 ±12**	**50 ±09**	**312 ±09**	**176 ±13**	**100 ±08**	**313 ±13**	**90± 12**	**176 ±12**	**100 ±06**	**284 ±16**	**98 ±10**	**192 ±14**
2:1:2	**100 ±12**	**50± 09**	**310 ±10**	**178±**	**100 ±08**	**313 ±13**	**93± 09**	**182 ±08**	**100 ±06**	**284 ±16**	**90 ±12**	**162 ±09**

5.3.2. Dose optimization and efficacy evaluation of poly-herbal formulation in CHD model

CHD animals treated with the normal saline solution, there was the significant increase in CVL was observed as compared to normal control animals. When the CHD animals were

treated with 150 and 300 mg/kg of poly-herbal formulation, there was no significant difference in CVL was observed as compared to CHD control animals. However, treatment with 600 mg/kg of the poly-herbal formulation to CHD animals, there was the significant decrease in CVL levels were observed as compared to CHD control animals. At the doses of 600 and 900 mg/kg, there was no significant difference in CVL levels was observed, but these animals were significant to CHD control animals in terms of changes in CVL levels. So, the optimized dose of the poly-herbal formulation was found to be 600 mg/kg of body weight (Figure 5.19).

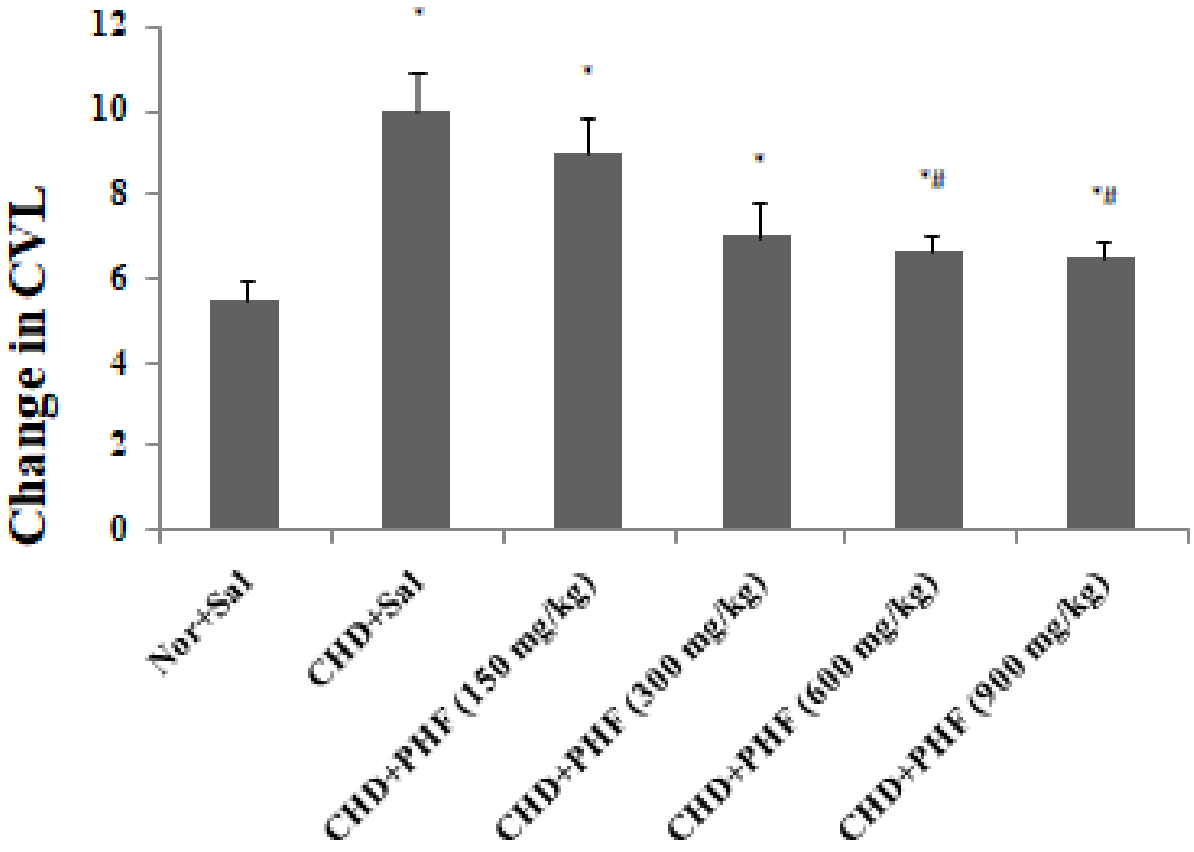


Figure 5.19: Graphs denoting mean ±SD of change in CVL during treatment with PHF at the dose of 150, 300, 600, 900 and 1200 mg/kg, *p.o.* * denotes $p<0.05$ when compared to Nor + Sal and # denotes $p<0.05$ when compared to CHD+PHF (300 mg/kg). PHF: Poly-herbal formulation; Sal: Saline; Nor: Normal; CVL: CVL; CHD: Combined hypoxia and dehydration.

5.3.3. Toxicological evaluation of poly-herbal formulation

Acute and sub-acute toxicity studies were conducted according to OECD guidelines. No mortality was observed at dose of 2,000 mg/kg of *b.w.* Anatomical and hislogical examination revealed no adverse effect on vital organs viz., brain, lungs, kidneys, spleen, liver and heart (Figure 5.20).

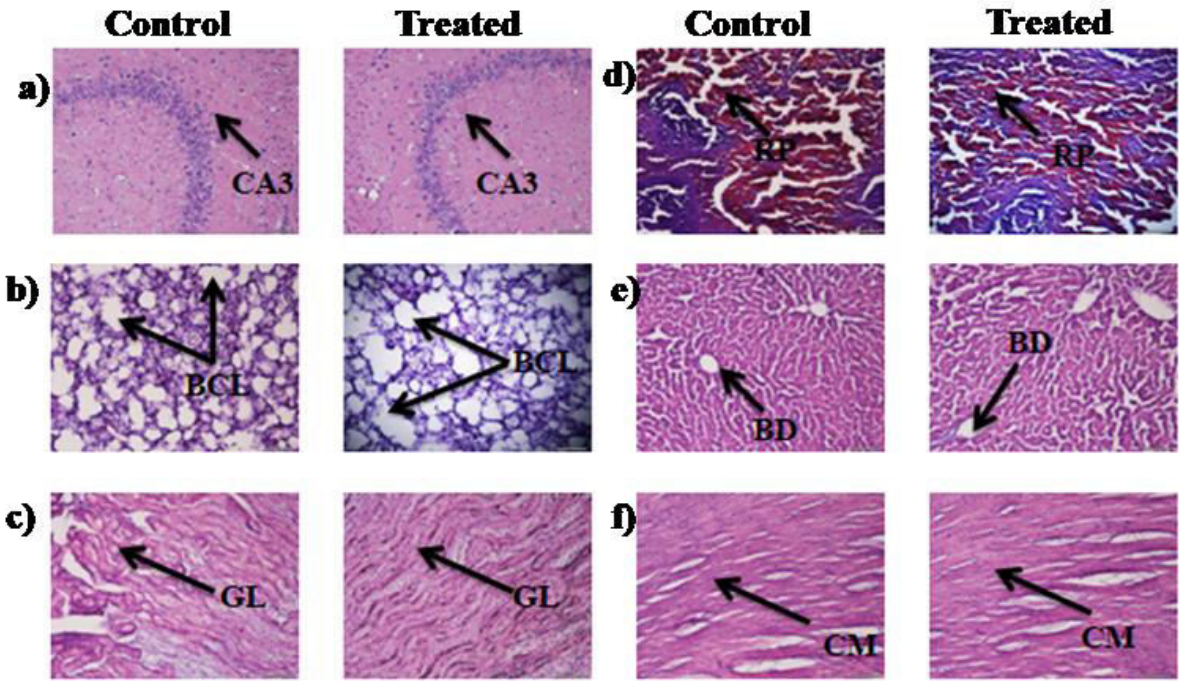


Figure 5.20: Histological studies of sub-acute regulatory toxicity studies of poly-herbal formulation at the dose of 2000 mg/kg *b.w.* Panels representing histological sections of different organs of rats viz., a) Brain, arrow denote CA3 region of hippocampus, b) Lungs, arrows denote bronchioles (BCL), c) Kidney, arrows denote glomerulus (GL), d) spleen, arrows denote red pulp e) Liver, arrows denote bile duct (BD) and f)) Heart, arrows denote cardiac muscle (CM). Scale bar- 100 µm.

5.3.4. Minimal optimal dose determination and efficacy evaluation in humans

The human equivalent dose as described in material and methods sectioned was found to be 97 mg/kg of body weight for humans.

DISCUSSION

6. DISCUSSION

6.1. Development of animal model to study the effect of acute hypobaric hypoxia on GFR, fluid and electrolyte imbalance, neuro-inflammation, CVL and RBC fragility in rats

The world's toughest, coldest, highest and bravest battle is being fought on Siachen Glacier for over the last 25 years. This is the most glaciated area outside the polar region comprising 22 glaciers. Troop deployment on the forward locations is from 18,000 ft. to 21,000 ft. Acute mountain sickness (AMS) have been seen widely reported in the troops at these locations and these symptoms may be fatal if not treated timely. However, due to absence of animal model for AMS, pre-clinical research on therapeutic and prophylactic potential of pharmaceutical and bio-active compounds against AMS is sparse. The patho-physiological markers associated with AMS also remain to be conclusively identified. We hypothesized on a combined role of acute hypobaric hypoxia and dehydration on glomerular filtration rate (GFR), fluid and electrolyte balance, neuro-inflammation, cerebral vascular leakage (CVL) and red blood cells (RBC) fragility in rats which could result in symptoms of AMS. In the present study, three experimental approaches were used. In the first experimental approach, animals simulated at different altitudes and the effect of acute hypobaric hypoxia on GFR, fluid and electrolyte balance, neuro-inflammation and CVL was studied. In the second set of experiment, the animals were exposed to dehydration and the effect of dehydration on hematological parameters, GFR, and RBC fragility was studied. In the last set of experiments, the combined effect of dehydration and acute hypobaric hypoxia on GFR, fluid and electrolyte balance, neuro-inflammation, CVL and RBC fragility was studied.

Exposure to hypoxia has been previously found to cause an alteration in fluid and electrolyte balance [3, 127]. Interestingly with the onset of AMS, the renal activity switches from

net loss to gain of fluid [4]. The exact molecular mechanism pertaining to altered renal responses in AMS positive patients during hypobaric hypoxia is not clear. This could be due to the burst release of hormones responsible for anti-diuresis which may be triggered by nervous system during hypoxia [4]. Previous reports suggest that, acute exposure to hypobaric hypoxia increases sympathetic drive in AMS patients resulting in up-regulation of circulating rennin-angiotensin-aldosterone system (RAAS) [1, 2].

Over-expressed circulatory RAAS has been implicated in various cardiovascular and renal diseases by collective effect on kidneys, heart and blood vessels [3]. RAAS augmented flow of flow determined the GFR and electrolytic balance. Under, both, physiological and pathological conditions, RAAS directly reported to control GFR [128-129]. Under patho-logical conditions, over-expressed RAAS play a major role in controlling GFR in negative manners [5, 130-131]. Over-expressed RAAS was suggested to alter balance of renal medullary oxygen demand and supply during chronic exposure to hypoxia in some subjects [132]. Altered balance between demand and supply of oxygen to renal medulla has been found to cause altered renal blood flow [133]. As earlier suggested my many published studies [134-135], blocking any of the component associated with RAAS can be use as a therapeutic strategies for treating disorders associated with acute as well as chronic renal diseases. During our studies, we have observed circulatory over-expressed RAAS augmented decrease GFR and half-life of ^{99m}Tc in rats exposed to 27,000 ft. of high altitude (Figure 5.1 and 5.2). The predicted half-life of ^{99m}Tc has been clinically used to diagnostics of renal disease [136-138]. Atrial natriuretic peptide (ANP), a flight and fight peptide hormone, synthesized in atria has gained its attention for its properties in controlling renal flow and electrolytic balance possibly by blocking the over-expressed circulatory RAAS [139]. Interestingly, clinically, in sojourns travels to high mountains, ANP

level was found to low in AMS positive subjects and ANP levels was found to be high in AMS negative sojourns [141]. We have done studies and found interesting results about the correlation of GFR with ANP and circulatory RAAS. Acute simulated exposure to animals at the altitude of 27,000, the animals with low level of ANP and with over-expressed circulatory RAAS were associated with decreased GFR and altered electrolytic balance (Figure 5.1 and Table 5.1). In addition to over-expressed circulatory RAAS, decrease circulatory ANP could be additively working to alter GFR and electrolytic balance.

High metabolic rate and restricted glucose storage capacity of brain demands accurately auto-regulated blood flow to the brain in order to maintain constant supply of nutrients and oxygen [92]. In has been reported that, altered oxygen supply to brain, changes in partial pressure of carbon dioxide, mean arterial pressure and autonomic nervous system majorly regulate cerebral blood flow [92-93]. Acute exposure to hypobaric hypoxia results in decreased blood supply to the brain and hyperventilation-induced excessive accumulation of carbon dioxide could be working synergistically in controlling cerebral blood flow at high altitude [9, 93]. In order to compensate for decreased cerebral blood flow, vascular resistance has been found to significantly reduce in small arteries and arterioles to facilitate gaseous exchange [10]. Increase cerebral blood flow and vasodilatation in arteries and arterioles causes augmented brain volume and intracranial pressure in brain [94]. Augmented cerebral blood flow and vasodilatation may overcome capillary vasoconstriction, boost blood-brain barrier (BBB) permeability that finally resulting in CVL [12]. Finally results in augmented CVL referred to as ‘tight fit hypotheses of symptoms associated with AMS [95-96]. BBB, formed by the brain endothelial cells, dominantly maintain the electrolytic balance of brain by regulating the flux of fluid and substances between the systemic circulation and brain microenvironment. At the same time BBB protect the brain from

harmful xenobiotics that may cause damage to neuronal and non-neuronal cells present in brain microenvironment [12-13]. Acute exposure to hypobaric hypoxia has been well reported to cause disruption of BBB permeability and is associated with increased CVL [15].

Hypobaric hypoxia-mediated increased neuro-inflammation has been reported to play a key role in acute hypobaric hypoxia-induced altered BBB permeability [12]. Under physiological conditions, homeostasis between pro- and anti-inflammatory cytokines play a pivotal role in maintaining the immune responses in the body [16]. During physiological conditions, there was a balance maintained between inflammatory cytokines that control the neuronal immunological responses [16, 17]. However, under patho-physiological conditions, dys-regulation of homeostasis between cytokines leads to neuro-inflammation and disruption of the BBB [17]. 21 different types of S100 class of proteins has been reported in the literature and considered as damage-associated molecular patterns (DAMPs) [19]. The major function of S100 class of proteins is Ca^{2+} sensing and once activated they may interact with other proteins resulting in regulation of target proteins activity. S100B one of the firstly identified S100 class of protein, has been reported to be secreted by astrocytes [20]. Secreted S100B protein may exert regulatory activities through intra- and extracellular signals and has been considered as specific marker of brain injury [21-22]. More specifically elevated serum S100B has been considered as a marker of neuro-inflammation associated with acute and chronic injury [23]. High serum S100B protein levels have been found in subjects exposed to acute hypobaric hypoxia with symptoms of AMS [24]. In the same line, in the present study, we have found altitude dependent increase in serum S100B levels could be an indicator of hypobaric hypoxia-induced neuro-inflammation and resulting in BBB disruption and CVL (Figure 5.2). Extra- and intra-cellular fluid balance was found to being regulated by electrolytes movement across the cellular membrane. Majorly the

movement of two pivotal ions Na^+ and K^+ decide the fate of cellular fluid movement [142]. Even re-absorption of above mentioned electrolytes has also been principally implicated in deciding the fate of body fluid [3]. As discussed earlier over-expressed circulatory RAAS could be the player behind altered electrolytic balance and fluid balance. Principally, aldosterone, a component of RAAS has an impact on controlling re-absorption of Na^+, K^+ and water [143-145]. In the present study during simulated altitude (27,000 ft.) resulted in increased circulatory aldosterone levels in rat serum that finally causing excessive Na^+ re-absorption and K^+ excretion (Figure 5.6). However, increased BBB permeability-mediated increased CVL and Na^+ deposition not directly correlated. But, circulatory Na^+ deposition mediated excessive deposition of fluid could be associated with increased intra-cranial pressure. Increased intra-cranial could finally lead to BBB disruption and CVL.

Exposure to high altitude has been found to decrease plasma volume and has been positively correlated with the symptoms of AMS [97]. The mechanism behind high altitude induced dehydration and hypovolemia is not clear. It could be due to the cold environment at high altitude that leads to excessive diuresis and poor availability of water [97-98]. Low humidity at high altitude could be another contributing factor for dehydration [89]. Water is an essential nutrient and water deprivation for only 2% can result in impaired physical and performance [27]. Dehydration referred to as state of deficiency in body water, which may be due to the excessive loss of body water and/or inadequate intake of water resulting in hypo-hydration in organs and tissues [28]. The decrease in the plasma volume, increased pack cell volume, decreased body weight and GFR is a clear indicator of dehydration [123]. In the present study, decreased plasma volume and increased pack cell volume, GFR, and increased RBC fragility observed in

dehydrated animals as compared with the normal control animals providing a clear indication of symptoms of dehydration in animals exposed to water restrictions for three days (Table 5.2).

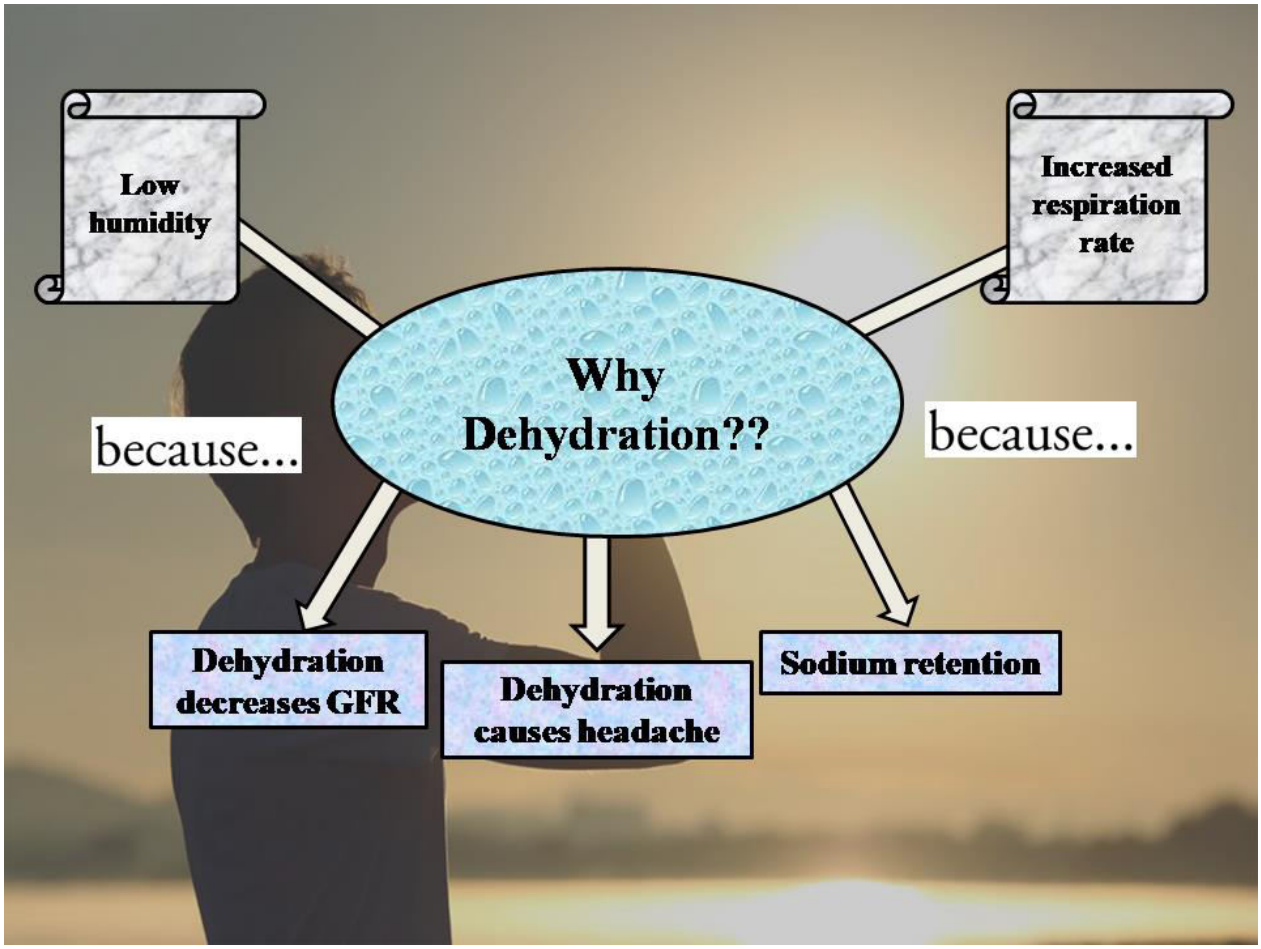


Figure 6.1: Can dehydration along with hypobaric hypoxia is an additive factor for the symptoms associated with AMS??

It has been well reported that dehydration is a contributing factor at high altitude in the symptoms associated with AMS along with hypobaric hypoxia [97]. Recently, Castellani et al. provided a clear indication of the importance of hypo-hydration in additively increasing severity of symptoms associated with AMS [89]. However, there is no study reported in the literature that specifically correlated dehydration at high altitude with AMS by taking specific pathological

markers associated with AMS. Though, acute exposure to hypobaric hypoxia and dehydration has been separately reported to decrease the GFR in rats, the combined effect of both remains to be studied [3, 123].

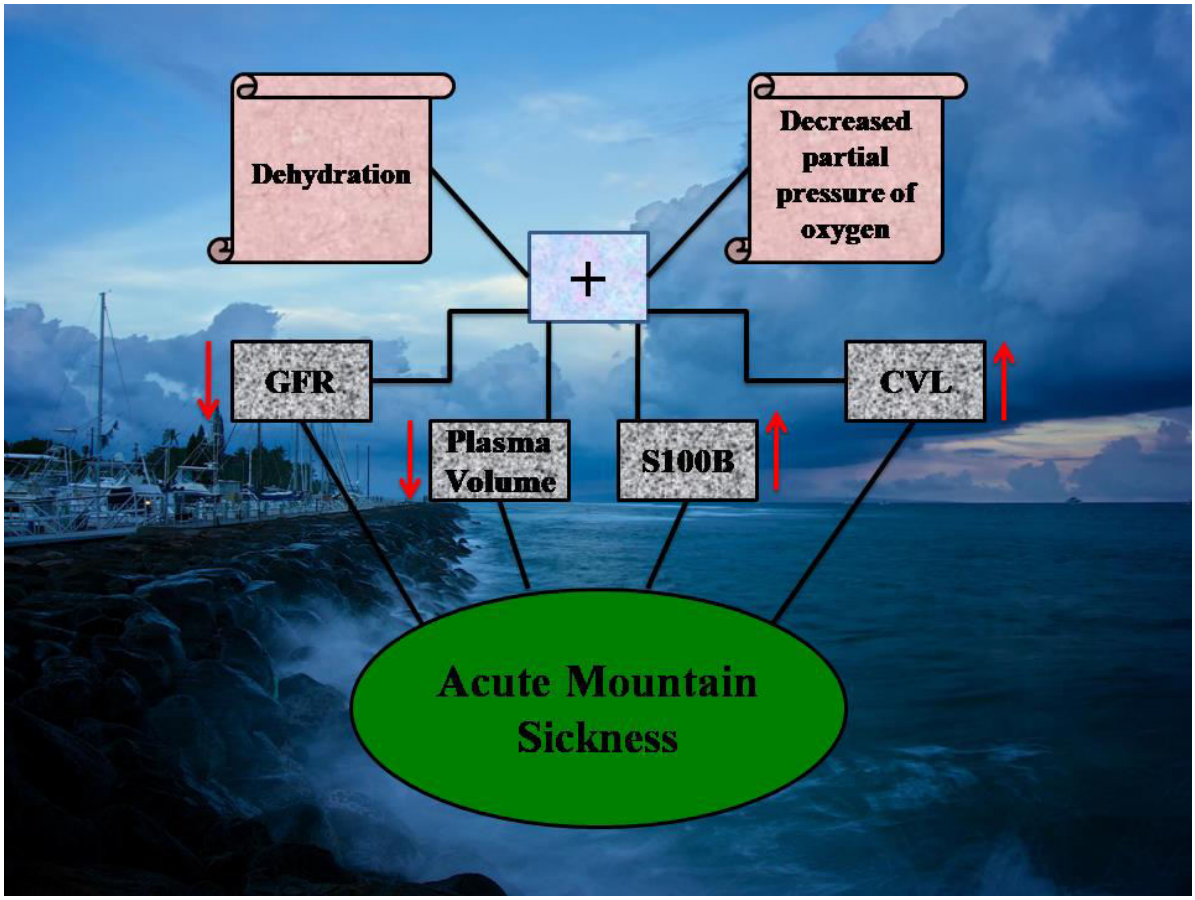


Figure 6.2: Dehydration as a contributing factor along with decreased partial pressure of oxygen in symptoms associated with AMS

So, this was the first study to see the combined effect of dehydration and acute hypobaric hypoxia on AMS symptoms by taking specific pathological markers viz., GFR, neuro-inflammation, plasma volume and RBC fragility in rats.

During combined hypoxia dehydration (CHD) exposure, in the present study, dehydration has been found to additively affect acute hypobaric hypoxia-induced decreased GFR in rats.

Apart from decreased GFR in rats during CHD stress, we also observed increase in S100B levels, clearly indicating additive neuro-inflammatory changes (Table 5.3).

In summary, dehydration and hypobaric hypoxia have an additive effect of high altitude and result in increased neuro-inflammation, RBC fragility and decreased in GFR in rats.

During the present study, exposure to hypoxia was found to negatively regulate GFR and cause electrolytic imbalance at the altitude of 25,000 ft. and 27,000 ft. and this was found to be augmented by altered balance between RAAS and ANP, as discussed earlier (Figure 5.1, Table 5.1). In addition to this, serum S100B protein levels were found to increase with the increase in altitude along with increase in percentage prevalence of CVL (Figure 5.2). Increased serum S100B levels which is an indicator of neuro-inflammation and further leads to BBB permeability and CVL, support the role of vasogenic edema theory of AMS. We have also studied the effect of dehydration on various serological parameters in order to find out the correlation between dehydration and the symptoms associated with acute mountain sickness. Increased pack cell volume has been reported to be a cardinal sign of dehydration. During CHD, increased pack cell volume along with decreased GFR and increased serum S100B protein levels were observed (Table 5.3). This indicates dehydration to be an additive factor for the symptoms associated with AMS. Thus, dehydration, along with the hypobaric hypoxia could be an additive factor in the progression of symptoms associated with AMS during initial exposure to high altitude in un-acclimatized sojourns, which travel to high altitude areas.

6.2. Phyto-prophylactics for the prevention of Acute Mountain Sickness

6.2.1. To study the effect of *T. arjuna* bark extract on acute hypobaric hypoxia-induced decrease GFR in rats

From the ancient times, *T. arjuna* was famous for its medicinal properties in treating cardiac and renal disorders viz., congestive heart failure, hypertension and renal failure [42-43]. Growing body of evidences suggested potential of arjuna tree bark as a modulator of over-expressed circulatory RAAS, thus showed therapeutic efficacy in renal disorders [43, 146-148]. In addition to this, red colored bark extract was previously found to shown efficacy in dehydration-induced alteration in acute renal failure [43]. In the present study, we studied the efficacy of hydro-alcoholic *T. arjuna* bark extract during acute hypobaric hypoxia mediated altered electrolytic balance and GFR in SD rats.

As discussed earlier, modulation of over-expressed circulatory RAAS by controlling release of ANP could be beneficial in hypoxia mediated altered electrolytic balance and GFR. This study deciphering the efficacy of hydro-alcoholic *T. arjuna* extract during acute hypoxia by significantly attenuating the activity of ANP and circulatory over-expressed RAAS. Administration of red-colored bark extract to hypoxia animals attenuated hypoxia-mediated negatively regulated GFR (Figure 5.7). Decreased half-life of ^{99m}Tc was recovered significantly by orally administration of arjuna bark extract (Figure 5.7). We have found that, the arjuna bark extract administration showed recovery in half-life of ^{99m}Tc. The recovery could be due the efficacy of bark extract in modulation ANP and RAAS. Diuretics effect of many formulations was studied by the prediction of GFR as well as quantifying the amount of urine production in animals [116, 149]. It has been found that GFR and urine volume has positive correlation [150].

Animals administered with *T. arjuna* during 27,000 ft. of exposure, the urine production significantly improved, supporting the efficacy of red-colored bark extract (Figure 5.6).

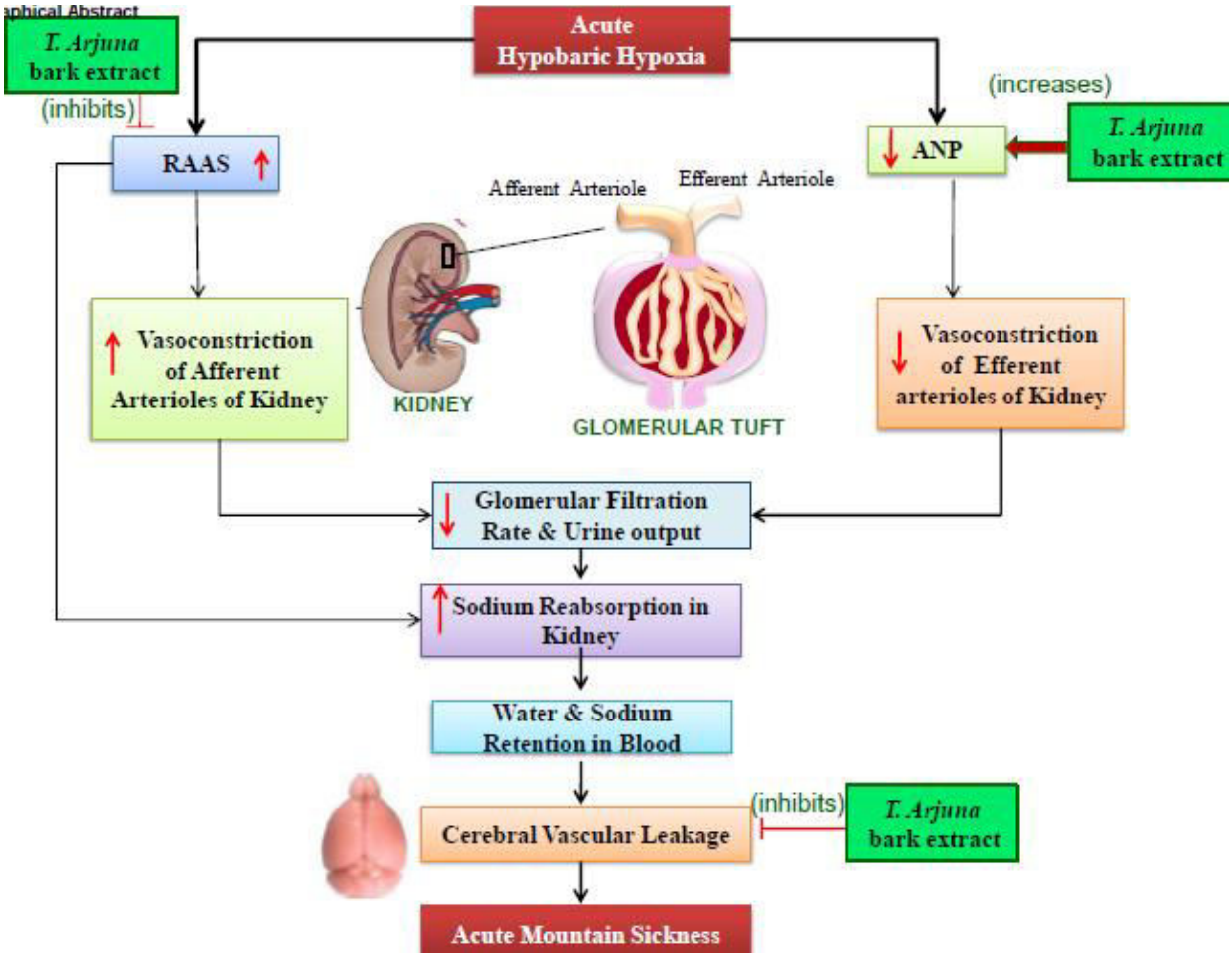


Figure 6.3: Prophylactic efficacy of *T. arjuna* bark extract in acute hypobaric hypoxia induced decreased GFR in rats [3].

Acute hypoxic exposure mediated negatively altered ANP level was found to be recovered by the treatment of *T. arjuna* to hypoxic animals by modulation circulatory over-expressed RAAS (Figure 5.7 and 6.3). However, the effect by ANP on RAAS modulation was found to be completely blocked by anantin (Figure 5.7). Even arjuna bark administration mediated increased GFR was also blocked (Figure 5.7).

Conclusively, red colored *T. arjuna* bark extract could a therapeutic strategies in maintaining acute hypobaric hypoxia-mediated altered electrolytic balance and GFR possibly by modulating the activity of over-expressed circulatory RAAS by ANP (Figure 6.3).

6.2.2. To study the effect of *Santalum album* heartwood extract on acute hypobaric hypoxia-induced CVL and neuroinflammation in rats

The present study demonstrates the anti-inflammatory potential of SAE (*Santalum album* extract) during acute hypobaric hypoxia though "receptor for advanced glycation end products/ nuclear factor kappa-light-chain-enhancer of activated B cells" (RAGE/NF-κB) dependent mechanisms. Growing body of evidence suggests the role of S100B protein up-regulation in neuro-inflammation during various stress disorders. S100B has been considered as a marker of astrocytic damage in recent studies [151-152]. In addition to this, increased S100B levels have been found in the serum of AMS patients [24]. Over the last decades, elevated S100B expression has been considered as a marker of BBB permeability [22]. Alterations in BBB have been well documented to increase CVL during acute hypoxic stress [3, 153]. Altered BBB dependent increased CVL leakage could be associated with potential symptoms of AMS [154]. In the present study we observed altitude dependent increase in serum S100B levels which was correlated to increase in CVL [155] (Figure 5.2). S100B could be a released either from adipocytes or from astrocytes during stress. We, therefore, estimated the levels of S100B in rat brain hippocampal lysate as well in rat serum during acute hypobaric hypoxia (Figure 5.10). We observed increased levels of S100B protein in serum as well as in rat brain hippocampal lysate. Hence, increased S100B levels during acute hypoxic stress released from brain astrocytes and could be responsible for acute hypobaric hypoxia-induced increased BBB permeability and resulting neuro-inflammation and CVL in rats (Figure 5.2 and 5.10).

Growing body of evidence supported the concept of acute hypobaric hypoxia-induced neuro-inflammation and CVL as triggers for the symptoms of AMS. However, the molecular mechanism pertaining to increased S100B protein-dependent increased neuro-inflammation and CVL during acute hypobaric hypoxia was not still clear. RAGE, a multi-ligand receptor protein has been reported to interacts with S100B protein and regulate various cellular functions of neuronal cells [156]. It has been well reported in the literature that, activation of RAGE/NF-κB signaling mediates production of pro-inflammatory cytokines [17, 157]. Interestingly, specifically blocking RAGE receptor with FPS-ZM1, a RAGE receptor blocker, has been reported to reduce BBB disruption during intra-cerebral hemorrhage in rats [158]. Increased production of pro-inflammatory cytokines could finally lead to neuro-inflammation resulting in BBB disruption and increased CVL. In the present study, we have observed that acute exposure to hypoxia in animals significantly increases the expression of S100B, NF-κB and pro-inflammatory cytokines viz., interleukin-6 (IL-6). However, no significant difference in the expression of RAGE receptor was observed in the present study (Figure 5.10).

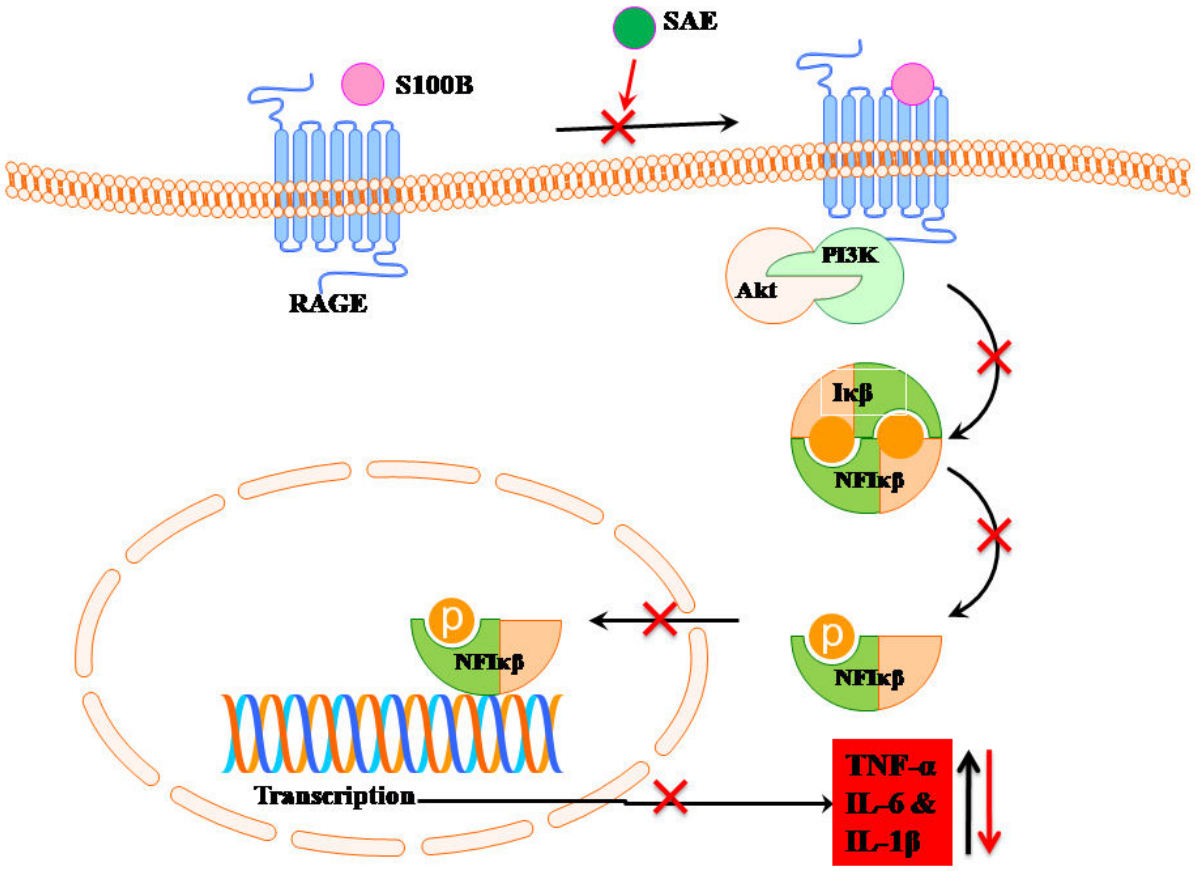


Figure 6.4: Prophylactic efficacy of *Santalum album* heartwood extract in acute hypobaric hypoxia-induced increased neuro-inflammation in rats

As discussed earlier, could be responsible for acute hypobaric hypoxia-induced neuro-inflammation and CVL in rats by activating RAGE/NF-κB signaling. Administration of SAE to hypoxia animal's attenuated acute hypobaric hypoxia-induced RAGE/NF-κB signaling dependent increased pro-inflammatory cytokines viz., IL-6, interleukin-1 β (IL-1β) and tumor necrosis factor- α (TNF-α) level in rat brain hippocampus (Figure 5.13). Integration of experimental strategies with computational strategies could be of high value in prediction of the exact molecular mechanism of predominant molecule present in formulations [159]. Our *in-silico*

approach revealed the interaction of α-santalol with RAGE receptor hydrophobically (Table 5.4 and Figure 5.11). So, we hypothesized that, α-santalol present in SAE could have attenuated acute hypobaric hypoxia-induced increase in neuro-inflammation and CVL by inhibiting RAGE/ NF-κB mediated signaling mechanisms.

This is the first report demonstrating the anti-inflammatory role of *S. album* extract in rat brain. S100B protein has been found to play a dominant role in acute hypobaric hypoxia induced neuro-inflammation, altered BBB permeability and CVL in rats. Secreted S100B protein could lead to neuro-inflammation through RAGE/NF-κB pathways. Preventive administration of SAE to hypoxic animals significantly reduced the activation of NF-κB-p65 signal cascade protein in the hippocampus, supporting the role of SAE in reducing neuro-inflammation (Figure 5.12). *In-silico* studies revealed α-santalol present in *S. album* binds to RAGE receptor and could block RAGE receptor, thus preventing neuro-inflammation during acute hypobaric hypoxia.

6.3. To study the effect of *Nymphaea rubra* rhizome extract on dehydration-induced increased RBC fragility in rats

Nymphaea x rubra Roxb. ex Andrews (*N. rubra)*, has been reported in the literature for its anthelmintic, immunomodulation, insulin resistance, anti-hyperglycemic, anti-dyslipidemic, anti-inflammatory, anti-pyretic, hepatoprotective and free radical scavenging activity [49, 52-54]. However, literature is sparse on regulatory toxicological evaluation and the potential of plant for use as phytomedicine in hematological disorders has been less studied.

Though several medicinal plants have found widespread application in folklore and traditional medicine, regulatory toxicological parameters of these plants still remain to be studied. Considering previous reports on potential adverse effects of plant extracts that could lead to degenerative changes or mortality even on single dose administration, toxicological evaluation of

plants with phyto medicinal properties is of paramount importance [160-161]. Though several phyto compounds may not cause immediate adverse physiological effects on administration, bioaccumulation due to repeated dosing or damage to subcellular components may be manifested as delayed effects. Histo-pathological examination of tissue has therefore been considered as a gold standard to examine the effects phyto-components on the vital organs [162]. Our findings on microscopic examination of tissue samples showed no adverse effect of *N. rubra* rhizome extract on heart, lungs, brain, kidney, spleen, liver, testis and ovary. The no observed adverse effects levels (NOAEL) of *N. rubra* rhizome extract was determined to be > 2000 mg/kg *b.w.* (Figure 5.15).

Previous reports on use of *N. rubra* extract on blood-related disorders formed our basis for investigating hematological parameters [50]. Since dehydration stress has been previously reported to alter hematological parameters like packed cell volume (PCV), hemoglobin (Hb) and plasma osmolality, it was the obvious choice for our experimental model [29, 163]. Dehydration has been well reported to increase plasma osmolality through decrease in plasma volume [164]. Increased plasma osmolality, in turn, has been reported to decrease RBC deformability and increased RBC fragility [165-167]. Consistent with previous reports, the results of the present study show increased plasma osmolality and RBC fragility in dehydrated animals (Figure 5.17 and 5.18). Previous reports show high mineral content in rhizome of *N. rubra* [168] which in turn could influence serum electrolyte concentration in dehydration. Since maintenance of electrolyte balance could contribute towards decreased RBC fragility in dehydrated rats administered with *N. rubra* extract, we investigated the plasma electrolytes during the present study. Administration of *N. rubra* rhizome during dehydration, on the other hand, decreased RBC fragility by increasing serum magnesium concentration through

mechanisms similar to that suggested by Franceschi et al., (1997) [37]. Detailed studies are however required to determine the molecular signaling mechanisms contributing toward the efficacy of *N. rubra* extract in dehydration.

6.3. To study the prophylactic efficacy of poly-herbal formulation comprising of *T. arjuna*, *Santalum album* and *Nymphaea rubra* during CHD stress

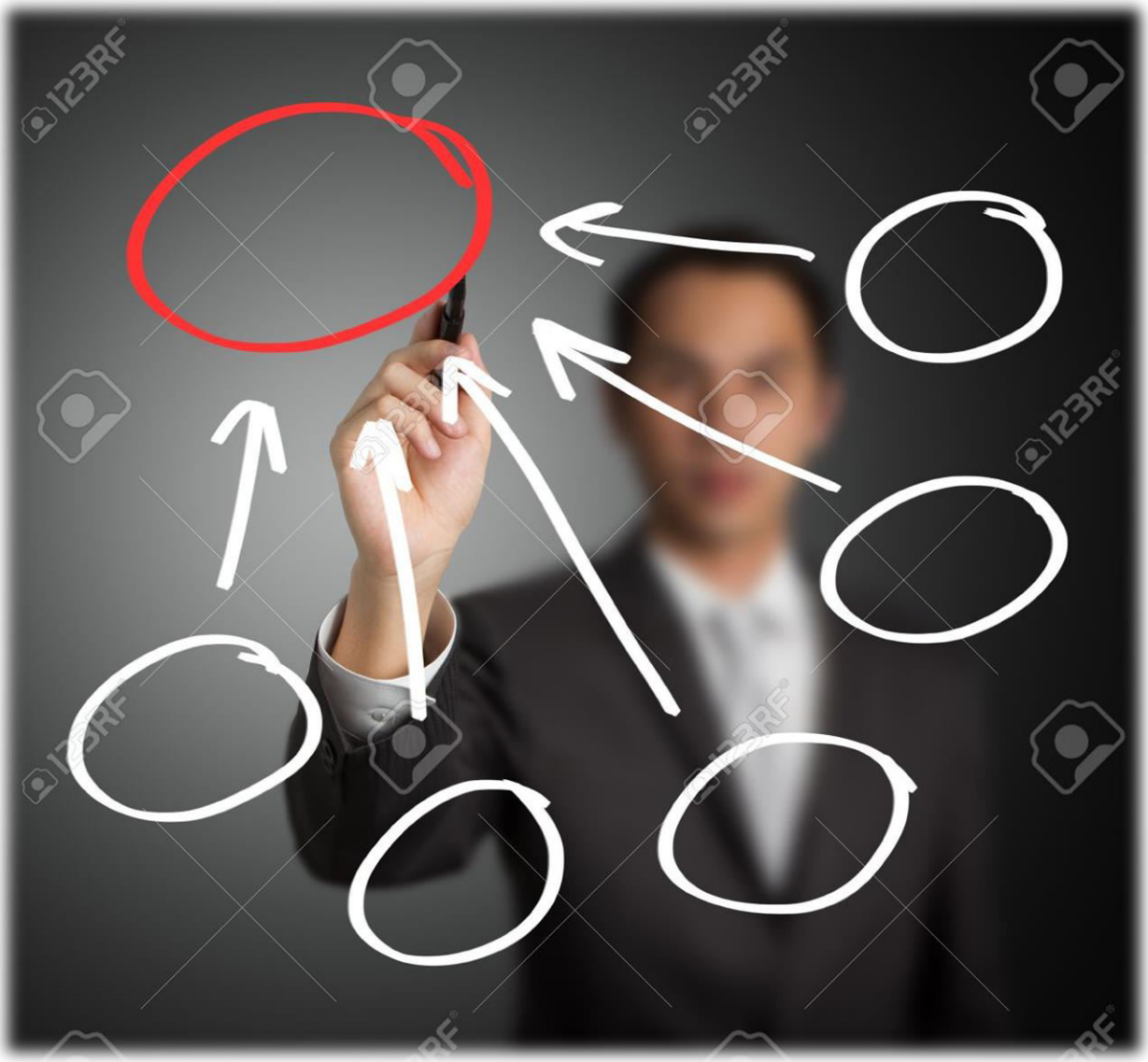
Usage of medicinal plants for the treatment of acute and chronic maladies has been quoted as one of the best oldest known methods in various traditional medicinal systems viz., Ayurveda, Chinese, Unani and African system of traditional medicines [169]. In Ayurveda, the formulations consist of either, one herbal plant, known as mono herbal formulation or the formulation consist of more than one plant, known as poly-herbal formulation [170]. However, the less availability of scientific advancement in terms of techniques to optimize the composition of poly-herbal formulations, made them questionable in recent ages [171]. So, the composition optimization of poly-herbal formulations in relation to specific pharmacological targets of specific herbal formulation constituents could over ride the question of composition optimization of poly-herbal formulation [172-173]. We also have first optimized the composition of poly-herbal formulation in relation to *T. arjuna*, *N. rubra* and *S. album*. The dose was optimize by taking specific targets associated with specific herbal formulation and optimize composition of poly-herbal formulation was found to be 1:0.25:1, for *T. arjuna*, *N. rubra* and *S. album* respectively (Table 5.5).

Despite wide usage of Ayurvedic herbal formulations, the scientific data on their toxicological evaluation is less or not available [174]. It has been found that, many of the poly-herbal formulations have serious toxic effects, could be correlated with, intrinsic toxicological properties, over-dosing, drug-drug interactions and contamination of herbal drug formulations [175]. So, before doing clinical studies on poly-herbal formulations, it is essential to evaluate the safely profile of herbal formulation [3]. So, we have evaluated the poly-herbal formulation for both acute and sub-acute toxicity studies according to the organization for economic corporation and development (OECD) guidelines. Based on the previously published studies, single or

multiple dose administration of herbal formulations could lead to degenerative changes in vital organs viz., brain, heart, lungs, liver, kidneys, and spleen [123]. However, in this study, we have not found any degenerative changes on organs studies during acute and sub-acute toxicity studies on poly-herbal formulation (Figure 5.20). The NOAEL of poly-herbal formulation was found to be greater than 2000 mg/kg of body weight. Thus, in conclusion, we could say that, poly-herbal formulation is safe for usage either pre-clinically and clinically. The human equivalent dose as described in material and methods sectioned was found to be 97 mg/kg of body weight for humans.

The herbal prophylactic formulation for acute mountain sickness comprised of Arjun Tree (*T. arjuna*) bark powder, Water Lily (*Nymphaea rubra*) rhizome powder and Sandalwood (*Santalum album)* heartwood powder mixed in the ratio 1:1:0.25. Pre-clinically, poly-herbal formulation was found to be free from toxicity during acute and sub-acute toxicity studies. This poly-herbal formulation was found to be having high efficacy during pre-clinical studies. The optimized dose of poly-herbal formulation was 600 mg/kg and human equivalent dose was 97 mg/kg of *b.w.* for human subjects.

SUMMARY AND CONCLUSION



7. SUMMARY AND CONCLUSION

Acute mountain sickness (AMS) is the highest prevalent illness occurring on the rapid ascent to high altitude. AMS have been seen widely reported in the troops and these symptoms may be fatal if not treated timely. However, due to no availability of animal model for AMS, pre-clinical research on therapeutic and prophylactic potential of pharmaceutical and bioactive compounds against AMS is sparse. So, the present study was aimed at developing animal model and phyto-prophylactics for AMS.

1. Acute hypobaric hypoxia was found to decrease glomerular filtration rate (GFR), fluid and electrolyte balance and cause neuro-inflammation in rats. Exposure to dehydration was found to decrease plasma volume and GFR in rats. During combined hypoxia dehydration stress, dehydration augments the hypobaric hypoxia in progression of patho-physiological markers associated with AMS such as GFR, fluid and electrolyte balance, neuro-inflammation and RBC fragility.

2. To summarize, this is the first report demonstrating the adaptogenic and prophylactic potential of *Terminalia arjuna (T. arjuna)* bark extract for high altitude maladies. *T. arjuna* bark extract ameliorates hypobaric hypoxia-induced decrease in GFR and sodium ion accumulation in the serum through arterial natriuretic peptide-induced modulation of renin-angiotensin-aldosterone-system. *T. arjuna* could be beneficial in acute hypobaric hypoxia induced decreased GFR and altered fluid and electrolyte balance.

3. This is also the first report demonstrating the anti-inflammatory role of *Santalum album (S. album)* extract (SAE). S100B protein has been found to play a dominant role in acute hypobaric

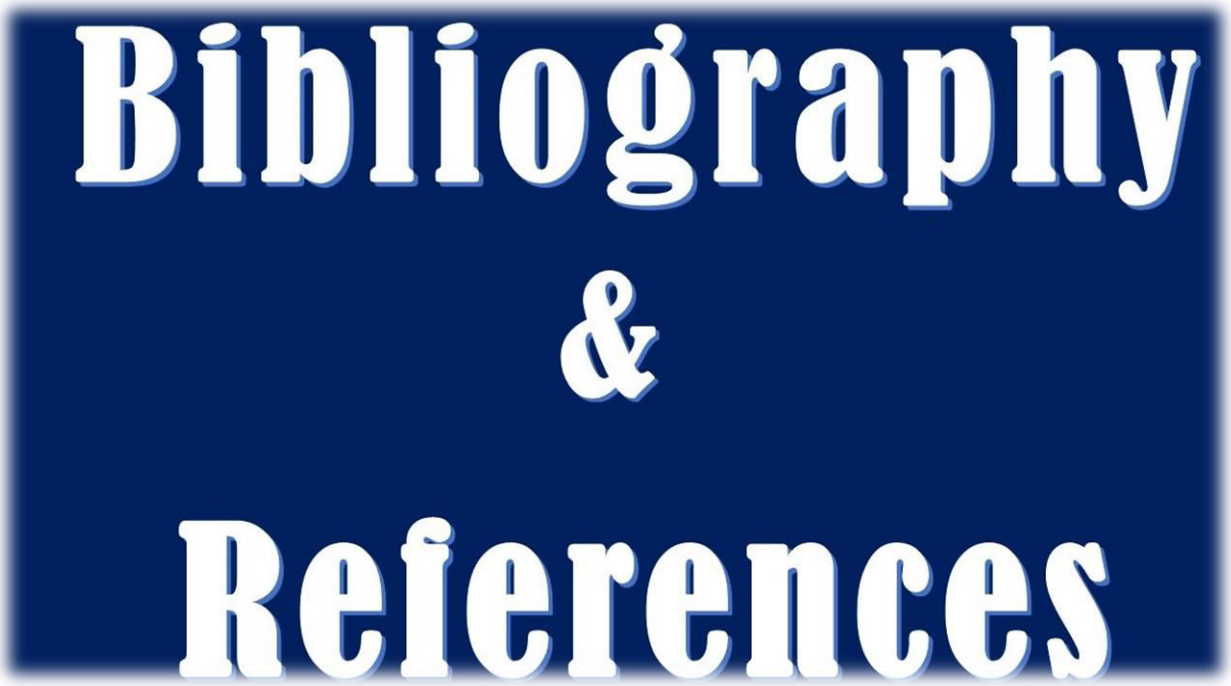
hypoxia induced neuro-inflammation, altered blood brain barrier permeability and cerebral vascular leakage in rats. Secreted S100B protein could lead to neuro-inflammation through RAGE/NF-κB pathways. Preventive administration of SAE to hypoxic animals significantly reduces the activation of NF-κB-p65 signal cascade protein in the hippocampus, supporting the role of SAE in reducing neuro-inflammation. *In-silico* studies reveals, α-santalol present in *S. album* binds to RAGE receptor and could block RAGE receptor, thus preventing neuro-inflammation during acute hypobaric hypoxia.

4. Administration of *Nymphaea rubra* rhizome extract during dehydration was found to decrease RBC fragility in rats. However, the exact molecular mechanism associated with *Nymphaea rubra* extract mediated protection of dehydration induced increased RBC fragility was still not clear. This could be due to the high concentration of magnesium present in the extract and magnesium has been well reported to prevent RBC fragility during many stress disorders.

5. The optimized composition of poly herbal formulation was found to be 1:1:0.25 for *T. arjuna, S. album* and *N. rubra* respectively. Study concludes that, poly herbal formulation is free from any toxicity and could be safe for clinical usage.

In the milieu of the above findings, it can be concluded that, dehydration along with the acute hypobaric hypoxia could be equally responsible for symptoms associated with AMS. Herbal prophylactics or formulation made from *T. arjuna, S. album and N. rubra* may be useful in ameliorating symptoms associated with AMS. However, further studies on human subjects need to be conducted to evaluate the efficacy of poly-herbal formulation made from *T. arjuna, S. album and N. rubra.*

REFERENCES



REFERENCES

1. Swenson ER. *High altitude diuresis: Fact or fancy. In: Houston CS, Coates G, editors. Hypoxia: Women at altitude. Burlington, VT:* Queen City Printers. 1997;272-283.

2. Loeppky JA, Icenogle MV, Maes D, Riboni K, Scotto P, Roach RC. *Body temperature, autonomic responses, and acute mountain sickness.* High Alt Med Biol. 2003;4(3): 367-373.

3. Kumar K, Sharma S, Vashishtha V, Bhardwaj P, Kumar A, Barhwal K, Hota SK, Malairaman U Singh B. *Terminalia arjuna bark extract improves diuresis and attenuates acute hypobaric hypoxia induced cerebral vascular leakage.* J Ethnopharmacol. 2016;180:43-53.

5. Vos PF, Koomans HA, Boer P, Dorhout Mees EJ. *Effects of angiotensin II on renal sodium handling and diluting capacity in man pretreated with high-salt diet and enalapril.* Nephrol Dial Transplant. 1992;7(10):991-996.

6. Baxter JD, Lewicki JA, Gardner DG. *Atrial Natriuretic Peptide*. Nature Biotechnology. 1988;6:529 – 546.

7. Laragh JH. *Atrial natriuretic hormone, the renin-aldosterone axis, and blood pressure-electrolyte homeostasis.* N. Engl. J. Med. 1985;313:1330-1340.

8. Yasue H, Obata K, Okumura K, Kurose M, Ogawa H, Matsuyama K, Imura H. *Increased secretion of atrial natriuretic polypeptide from the left ventricle in patients with dilated cardiomyopathy.* Journal of Clinical Investigation. 1989;83(1):46–51.

9. Xu F, Liu P, Pascual JM, Xiao G, Lu H. *Effect of hypoxia and hyperoxia on cerebral blood flow, blood oxygenation, and oxidative metabolism.* Journal of Cerebral Blood Flow & Metabolism. 2012;32(10):1909-1918.

10. Otis SM, Rossman ME, Schneider PA, Rush MP & Ringelstein EB. *Relationship of cerebral blood flow regulation to acute mountain sickness.* J Ultrasound Med. 1989;8:143–148.

11. Cipolla MJ. *The adaptation of the cerebral circulation to pregnancy: mechanisms and consequences.* Journal of Cerebral Blood Flow & Metabolism. 2013;33(4):465-478.

12. Julian CG, Subudhi AW, Wilson MJ, Dimmen AC, Pecha T, Roach RC. *Acute mountain sickness, inflammation, and permeability: new insights from a blood biomarker study.* Journal of Applied Physiology.1985;111(2):392-399.

13. Abbott NJ. *Astrocyte-endothelial interactions and blood-brain barrier permeability*. J Anat. 2002;200(6):629-638.

14. Wilson MH, Milledge J. *Direct measurement of intracranial pressure at high altitude and correlation of ventricular size with acute mountain sickness:* Brian Cummins' results from the 1985 Kishtwar expedition. Neurosurgery. 2008;63(5):970-975.

15. Song TT, Bi YH, Gao YQ, et al. *Systemic pro-inflammatory response facilitates the development of cerebral edema during short hypoxia.* Journal of Neuroinflammation. 2016;13:63.

16. Jeon SW, Kim YK. *Neuroinflammation and cytokine abnormality in major depression: Cause or consequence in that illness?* World Journal of Psychiatry. 2016;6(3):283-293.

17. Wang W-Y, Tan M-S, Yu J-T, Tan L. *Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease.* Annals of Translational Medicine. 2015;3(10):136.

18. Marenholz I, Heizmann CW, Fritz G. *S100 proteins in mouse and man: from evolution to function and pathology (including an update of the nomenclature)".* Biochemical and Biophysical Research Communications. 2004;322(4):1111–1122.

19. Memari B, Bouttier M, Dimitrov V, Ouellette M, Behr MA, Fritz JH, White JH. *Engagement of the Aryl Hydrocarbon Receptor in Mycobacterium tuberculosis-Infected Macrophages Has Pleiotropic Effects on Innate Immune Signaling.* J Immunol. 2015;195(9):4479-1491.

20. Sorci G, Bianchi R, Riuzzi F, et al. S100B Protein, *A Damage-Associated Molecular Pattern Protein in the Brain and Heart, and Beyond.* Cardiovascular Psychiatry and Neurology. 2010;2010:656481.

21. James NT, Forough M, Thomas GP. Intracellular and Extracellular *Effects of S100B in the Cardiovascular Response to Disease.* Cardiovascular Psychiatry and Neurology. 2010. doi:10.1155/2010/206073.

22. Sun B, Liu H, Nie S. *S100B protein in serum is elevated after global cerebral ischemic injury*. World J Emerg Med. 2013; 4(3):165-168.

23. Michetti F, Corvino V, Geloso MC, Lattanzi W, Bernardini C, Serpero L, Gazzolo D. *The S100B protein in biological fluids: more than a lifelong biomarker of brain distress.* J Neurochem. 2012;120(5):644-659.

24. Winter CD, Whyte TR, Cardinal J, Rose SE, O'Rourke PK, Kenny RG. *Elevated plasma S100B levels in high altitude hypobaric hypoxia do not correlate with acute mountain sickness.* Neurol Res. 2014;36(9):779-785.

25. Ramasamy R, Yan SF, Schmidt AM. Receptor *for AGE (RAGE): signaling mechanisms in the pathogenesis of diabetes and its complications.* Annals of the New York Academy of Sciences. 2011;1243:88-102.

26. Aggarwal BB, Prasad S, Reuter S, Kannappan R, Yadev VR, Park B, Kim JH, Gupta SC, Phromnoi K, Sundaram C, Prasad S, Chaturvedi MM, Sung B. *Identification of novel anti-inflammatory agents from Ayurvedic medicine for prevention of chronic diseases: "reverse pharmacology" and "bedside to bench" approach.* Curr Drug Targets. 2011;12(11):1595-1653.

27. Kleiner SM. *Water: an essential but overlooked nutrient.* J Am Diet Assoc. 1999;99(2):200-206.

28. Steiner MJ, DeWalt DA, Byerley JS. *Is this child dehydrated?* JAMA. 2004;291(22):2746-2754.

29. Netti VA, Vatrella MC, Chamorro MF, Rosón MI, Zotta E, Fellet AL, Balaszczuk AM. *Comparison of cardiovascular aquaporin-1 changes during water restriction between 25- and 50-day-old rats.* Eur J Nutr. 2014;53(1):287-295.

30. Wu SG, Jeng FR, Wei SY, Su CZ, Chung TC, Chang WJ, Chang HW. *Red blood cell osmotic fragility in chronically hemodialyzed patients.* Nephron. 1998;78(1):28-32.

31. Swaminathan R. *Magnesium Metabolism and its Disorders.* The Clinical Biochemist Reviews. 2003;24(2):47–66.

32. Keen CL, Lowney P, Gershwin ME, Hurley LS, Stern JS. *Dietary magnesium intake influences exercise capacity and hematologic parameters in rats.* Metabolism. 1987;36(8):788-793.

33. Cox IM, Campbell MJ, Dowson D. *Red blood cell magnesium and chronic fatigue syndrome.* Lancet. 1991;337(8744):757-760.

34. Buchman AL, Keen C, Commisso J, Killip D, Ou CN, Rognerud CL, Dennis K, Dunn JK. *The effect of a marathon run on plasma and urine mineral and metal concentrations.* J Am Coll Nutr. 1998;17(2):124-127.

35. Lukaski HC, Bolonchuk WW, Siders WA, Milne DB. *Chromium supplementation and resistance training: effects on body composition, strength, and trace element status of men.* Am J Clin Nutr. 1996;63:954–965.

36. Casoni I, Guglielmini C, Graziano L, Reali MG, Mazzotta D, Abbasciano V. Changes of magnesium concentrations in endurance athletes. Int J Sports Med. 1990;11:234–237.

37. De Franceschi L, Bachir D, Galacteros F, et al. *Oral magnesium supplements reduce erythrocyte dehydration in patients with sickle cell disease.* Journal of Clinical Investigation. 1997;100(7):1847-1852.

38. Leaf DE, Goldfarb DS. *Mechanisms of action of acetazolamide in the prophylaxis and treatment of acute mountain sickness.* J Appl Physiol. 1985;102(4):1313-1322.

39. Saito H, Ogasawara K, Suzuki T, Kuroda H, Kobayashi M, Yoshida K, Kubo Y, Ogawa A. *Adverse effects of intravenous acetazolamide administration for evaluation of cerebrovascular reactivity using brain perfusion single-photon emission computed tomography in patients with major cerebral artery steno-occlusive diseases.* Neurol Med Chir (Tokyo). 2011;51(7):479-483.

40. Dwivedi S. *Terminalia arjuna Wight &Arn.-a useful drug for cardiovascular disorders*. J Ethnopharmacol. 2007;114(2):114-129.

41. Maulik SK, Katiyar CK. *Terminalia arjuna in cardiovascular diseases: making the transition from traditional to modern medicine in India*. Curr Pharm Biotechnol. 2010;11(8):855-860.

42. Dwivedi S, Jauhari R. *Beneficial effects of Terminalia arjuna in coronary artery disease.* Indian Heart J. 1997;49(5):507-510.

43. Das K, Pratim CP, Ghosh D, Kumar ND. *Protective Effect of Aqueous Extract of Terminalia arjuna against Dehydrating Induced Oxidative Stress and Uremia in Male Rat.* Iranian Journal of Pharmaceutical Research. 2010;9(2):153-161.

44. Guo H, Zhang J, Gao W, Qu Z, Liu C. *Anti-diarrhoeal activity of methanol extract of Santalum album L. in mice and gastrointestinal effect on the contraction of isolated jejunum in rats.* J Ethnopharmacol. 2014;154(3):704-710.

45. Misra BB, Dey S. *Biological activities of East Indian Sandalwood Tree, Santalum album.* PeerJ PrePrints. 2013;1:e96v1 https://doi.org/10.7287/peerj.preprints.96v1.

46. Okugawa H, Ueda R, Matsumoto K, Kawanishi K, Kato A. *Effect of α-santalol and β-santalol from sandalwood on the central nervous system in mice*. Phytomedicine. 1995;2(2):119-126.

47. Kumar R, Anjum N, Tripathi YC. *Phytochemistry and pharmacology of Santalum album l.: a review.* World Journal of Pharmaceutical Research. 2015;4(10):1842-1876.

48. Dkhar J, Kumaria S, Rao SR, Tandon P. *New insights into character evolution, hybridization and diversity of Indian Nymphaea (Nymphaeaceae): evidence from molecular and morphological data.* Systematics and Biodiversity. 2013;11(1):77–86.

49. Behera SK, Mohapatra TK, Dash V. *Anthelmintic Activity of Rhizomes of Nymphaea rubra linn*. Ancient Science of Life. 2010;29(3):33-36.

50. Sonowal R, Barua I. *Ethnomedical Practices among the Tai-Khamyangs of Assam, India.* Ethno Med. 2011;5(1):41-50.

51. Yumnam RS, Dev CO, Abujam SKS. *Study of the ethanomedicinal system of Manipur.* Int J Pharm Biol Arch. 2012;3(3):587-591.

52. Cheng JH, Lee SY, Lien YY, Lee MS, Sheu SC. *Immunomodulating Activity of Nymphaea rubra Roxb. Extracts: Activation of Rat Dendritic Cells and Improvement of the T(H)1 Immune Response.* International Journal of Molecular Science. 2012;13:10722–10735.

53. Rahuja N, Mishra A, Gautam S, Tamrakar AK, Maurya R, Jain SK, Srivastava AK. *Nymphaea rubra Ameliorates TNF-α-Induced Insulin Resistance via Suppression of c-Jun NH2-Terminal Kinase and Nuclear Factor-κB in the Rat Skeletal Muscle Cells.* International Journal of Pharmaceutical Sciences Review and Research. 2013;22:121–133.

54. Gautam S, Rahuja N, Ishrat N, Asthana RK, Mishra DK, Maurya R, Jain SK, Srivastava AK. *Nymphaea rubra Ameliorates TNF-α-Induced Insulin Resistance via Suppression of c-Jun NH2-Terminal Kinase and Nuclear Factor-κB in the Rat Skeletal Muscle Cells.* Appl Biochem Biotechnol. 2014;174:446–2457.

55. Daffodil ED, Mohan VR. *In vitro anti-oxidant activity of Nymphaea rubra L. rhizome.* World Journal of Pharmaceutical Research. 2014;3(4):2178-2189.

56. Barry JW, Pollard AJ. *Altitude illness*. BMJ 2003; 326:915-919.

57. Deweber K, Scorza K. *Return to activity at altitude after high-altitude illness*. Sports Health. 2010 Jul;2(4):291-300.

58. Hultgren H. *High Altitude Medicine*. Hultgren Publications; 1997.

59. Gilbert DL. *The first documented report of mountain sickness: the China or Headach Mountain story*. Respir Physiol. 1983;52(3):315-326.

60. White AP. *High Life: A History of High-Altitude Physiology and Medicine*. The Yale Journal of Biology and Medicine. 2001;74(2):139-141.

61. Moraga FA, Osorio JD, Vargas ME. *Acute mountain sickness in tourists with children at Lake Chungará (4400 m) in northern Chile*. Wilderness Environ Med. 2002;13(1):31-35.

62. Carozzi Albert V., Crettaz Bernard, Ripoll David, Les plis du temps, Mythe, science et H.-B. de Saussure, *Musée d'Ethnographie, Annexe de Conches*, Genève. 1998:368p.

63. Grainge C. *Breath of life: the evolution of oxygen therapy*. Journal of the Royal Society of Medicine. 2004;97(10):489-493.

64. Doherty MJ. *James Glaisher's 1862 account of balloon sickness: altitude, decompression injury, and hypoxemia*. Neurology;60(6):1016-1018.

65. Houston C S. 1980. *Going High*. Burlington, VT: Queen City Printers.

66. Windsor JS, Rodway GW. *Heights and haematology: the story of haemoglobin at altitude*. Postgraduate Medical Journal. 2007;83(977):148-151.

67. West JB. Alexander M. *Kellas and the physiological challenge of Mt. Everest.* J Appl Physiol (1985). 1987;63(1):3-11.

68. West JB. *Joseph Barcroft's studies of high-altitude physiology*. Am J Physiol Lung Cell Mol Physiol. 2013;305(8):L523-9.

69. Hackett PH, Roach RC. *High-altitude illness*. N Engl J Med 2001;345:107–114.

70. Hackett PH, Hornbein T. *Disorder of high altitude. In: Murray JF, Nadel JA (eds) Textbook of respiratory medicine.* Saunders, Philadelphia. 1988:1646–1663.

71. Ravenhill T. *Some experiences of mountain sickness in the Andes.* J. Trop. Med. & Hyg. 1913;20:313-320.

72. Carod-Artal FJ. *High-altitude headache and acute mountain sickness*. Neurologia. 2014;29(9):533-540.

73. Luks AM, McIntosh SE, Grissom CK, Auerbach PS, Rodway GW, Schoene RB, Zafren K, Hackett PH; *Wilderness Medical Society. Wilderness Medical Society practice guidelines for the prevention and treatment of acute altitude illness: 2014 update.* Wilderness Environ Med. 2014 Dec;25(4 Suppl):S4-14.

74. Sakamoto R, Okumiya K, Norboo T, Tsering N, Yamaguchi T, Nose M, Takeda S, Tsukihara T, Ishikawa M, Nakajima S, Wada T, Fujisawa M, Imai H, Ishimoto Y, Kimura Y, Fukutomi E, Chen W, Otsuka K, Matsubayashi K. *Sleep quality among elderly high-altitude dwellers in Ladakh.* Psychiatry Res. 2017;249:51-57.

75. Imray C, Wright A, Subudhi A, Roach R. *Acute mountain sickness: pathophysiology, prevention, and treatment.* Prog Cardiovasc Dis. 2010;52:467-484.

76. Silber E, Sonnenberg P, Collier DJ, Pollar AJ, Murdoch DR, Goadsby PJ. *Clinical feature of headache at altitude: a prospective study*. Neurology. 2003;60:1167-1671.

77. Serrano Duenas ˜ M. *High altitude headache. A prospective study of its clinical characteristics.* Cephalalgia. 2005;25:1110-1116.

78. Sanchez del Rio M, Moskowitz MA. *High altitude headache – lessons from headaches at sea level. In: Roach RC, Wagner PD, Hackett PH, eds. Hypoxia: Into the Next Millenium.* New York, Kluwer Academic/Plenum Publishers, 1999; pp. 145–153.

79. Westerterp-Plantenga MS, Westerterp KR, Rubbens M, Verwegen CR, Richelet JP, Gardette B. *Appetite at "high altitude" [Operation Everest III (Comex-'97)]: a simulated ascent of Mount Everest.* J Appl Physiol (1985). 1999;87(1):391-399.

80. Westerterp KR, Kayser B. *Body mass regulation at altitude*. Eur J Gastroenterol Hepatol. 2006;18:1–3.

81. Palmer BF, Clegg DJ. *Ascent to Altitude as a Weight Loss Method: The Good and Bad of Hypoxia Inducible Factor Activation*. Obesity (Silver Spring, Md). 2014;22(2):311-317. doi:10.1002/oby.20499.

82. Nussbaumer-Ochsner Y, Ursprung J, Siebenmann C, Maggiorini M, Bloch KE. *Effect of short-term acclimatization to high altitude on sleep and nocturnal breathing.* Sleep. 2012;35(3):419-423.

83. Dong JQ, Zhang JH, Qin J, Li QN, Huang W, Gao XB, Yu J, Chen GZ, Tang XG, Huang L. *Anxiety correlates with somatic symptoms and sleep status at high altitudes.* Physiol Behav. 2013;112-113:23-31.

84. Hornbein TF. *Long term effects of high altitude on brain function*. Int J Sports Med. 1992;13(suppl 1):S43-S45.

85. Jeong JH, Kwon JC, Chin J, Yoon SJ, Na DL. *Globus pallidus lesions associated with high mountain climbing.* J Korean Med Sci. 2002;17(6):861-863.

86. Dellasanta P, Gaillard S, Loutan L, Kayser B. *Comparing questionnaires for the assessment of acute mountain sickness.* High Alt Med Biol. 2007;8(3):184-91.

87. Maggiorini M., Buhler B., Walter M., and Oelz O. *Prevalence of acute mountain sickness in the Swiss Alps.* BMJ. 1990;301:853–854.

88. Kayser B, Aliverti A, Pellegrino R, Dellaca R, Quaranta M, Pompilio P, Miserocchi G, Cogo A. *Comparison of a visual analogue scale and Lake Louise symptom scores for acute mountain sickness*. High Alt Med Biol. 2010;11(1):69-72.

89. Castellani JW, Muza SR, Cheuvront SN, Sils IV, Fulco CS, Kenefick RW, Beidleman BA, Sawka MN. *Effect of hypohydration and altitude exposure on aerobic exercise performance and acute mountain sickness*. J Appl Physiol (1985). 2010;109(6):1792-1800.

90. Rehrer NJ. *Fluid and electrolyte balance in ultra-endurance sport.* Sports Med. 2001;31(10):701-715.

91. Hackett P, Mangione MP. *Fluid and Electrolyte Balance*. Basic Clinical Anaesthesia. 2015. 89-100.

92. Willie CK, Tzeng Y-C, Fisher JA, Ainslie PN. *Integrative regulation of human brain blood flow*. The Journal of Physiology. 2014;592(Pt 5):841-859.

93. A, Balaban DY, Machina MA, Han JS, Katznelson R, Minkovich LL, Fedorko L, Murphy PM, Wasowicz M, Naughton F, Meineri M, Fisher JA & Duffin J. *The interaction of carbon dioxide and hypoxia in the control of cerebral blood flow.* Pflugers Arch. 2012;464:345–351.

94. Cipolla MJ. *The Cerebral Circulation,* San Rafael (CA): Morgan & Claypool Life Sciences; 2009. Available from: http://www.ncbi.nlm.nih.gov/books/NBK53081/.

95. Schoch HJ, Fischer S, Marti HH. *Hypoxia-induced vascular endothelial growth factor expression causes vascular leakage in the brain.* Brain. 2002;125:2549–2557.

96. Brown RC, Mark KS, Egleton RD, Huber JD, Burroughs AR, Davis TP. *Protection against hypoxia-induced increase in blood-brain barrier permeability: role of tight junction proteins and NFkappaB*. J Cell Sci. 2003;116:693–700.

97. Nerin MA, Palop J, Montano JA, et al. *Acute mountain sickness: influence of fluid intake.* Wilderness Environ Med 2006 ;17 (4):215 -220.

98. Hansen JE, Evans WO. *A hypothesis regarding the pathophysiology of acute mountain sickness.* Arch Environ Health. 1970;21(5):666-669.

100. Luks AM, McIntosh SE, Grissom CK, Auerbach PS, Rodway GW, Schoene RB, Zafren K, Hackett PH; *Wilderness Medical Society.. Wilderness Medical Society consensus guidelines for the prevention and treatment of acute altitude illness*. Wilderness Environ Med. 2010;21(2):146-155.

101. Montgomery AB, Mills J, Luce JM. *Incidence of acute mountain sickness at intermediate altitude.* JAMA. 1989;261(5):732-734.

102. Pigman EC, Karakla DW. *Acute mountain sickness at intermediate altitude: military mountainous training.* Am J Emerg Med. 1990;8(1):7-10.

103. Lyons TP, Muza SR, Rock PB, Cymerman A. *The effect of altitude pre-acclimatization on acute mountain sickness during reexposure*. Aviat Space Environ Med. 1995;66(10):957-962.

104. Schneider M, Bernasch D, Weymann J, Holle R, Bartsch P. *Acute mountain sickness: influence of susceptibility, preexposure, and ascent rate.* Med Sci Sports Exerc. 2002;34(12):1886-1891.

105. Coote JH. *Pharmacological control of altitude sickness*. Trends in Pharmacological Sciences. 1991;12: p450–455.

106. Rabold MB. *Dexamethasone for prophylaxis and treatment of acute mountain sickness.* 1992;3(1):54-60.

107. Ellsworth AJ, Meyer EF, Larson EB. *Acetazolamide or dexamethasone use versus placebo to prevent acute mountain sickness on Mount Rainier.* West J Med. 1991;154(3):289-293.

108. Pandit A, Karmacharya P, Pathak R, Giri S, Aryal MR. *Efficacy of NSAIDs for the prevention of acute mountain sickness: a systematic review and meta-analysis.* Journal of Community Hospital Internal Medicine Perspectives. 2014;4(4):10.

109. Ellsworth AJ, Larson EB, Strickland D. *A randomized trial of dexamethasone and acetazolamide for acute mountain sickness prophylaxis.* Am J Med. 1987;83(6):1024-1030.

110. Zell SC, Goodman PH. *Acetazolamide and dexamethasone in the prevention of acute mountain sickness.* West J Med. 1988;148(5):541-545.

111. Hota SK, Barhwal K, Singh SB, Sairam M, Ilavazhagan G. *NR1 and GluR2 expression mediates excitotoxicity in chronic hypobaric hypoxia.* J Neurosci Res. 2008;86(5):1142-1152.

112. Hota KB, Hota SK, Chaurasia OP, Singh SB. *Acetyl-L-carnitine-mediated neuroprotection during hypoxia is attributed to ERK1/2-Nrf2-regulated mitochondrial biosynthesis.* Hippocampus. 2012;22(4):723-736.

113. Zhang M, Mao Y, Ramirez SH, Tuma RF, Chabrashvili T. 2010. *Angiotensin II induced cerebral microvascular inflammation and increased blood-brain barrier permeability via oxidative stress.* Neuroscience. 2010;171(3):852-858.

114. Nankivell BJ, Fawdry RM, Harris David CH. *Assessment of glomerular filtration rate in small animals by intraperitoneal 99mTc DTPA.* Kidney International. 1992;41:450–454.

115. Belcher EH, Harriss EB. *Studies of plasma volume, red cell volume and total blood volume in young growing rats*. J. Physiol. 1957;139:64-78.

116. Gasparotto JA, Prando TB, Leme TS, Gasparotto FM, Lourenço EL, Rattmann YD, Da Silva-Santos JE, Kassuya CA, Marques MC. *Mechanisms underlying the diuretic effects of Tropaeolummajus L. extracts and its main component isoquercitrin*. J Ethnopharmacol. 2012;141(1):501-509.

117. Billett HH. *Hemoglobin and Hematocrit. In: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical, and Laboratory Examinations.* 3rd edition. Boston: Butterworths. 1990;Chapter 151.

118. El-Ayoubi R, Menaouar A, Gutkowska J, Mukaddam-Daher S. *Urinary responses to acute moxonidine are inhibited by natriuretic peptide receptor antagonist*. Br J Pharmacol. 2005;145(1):50-56.

119. Raj CD, Shabi MM, Jipnomon J, et al. Terminalia arjuna's antioxidant effect in isolated perfused kidney. Research in Pharmaceutical Sciences. 2012;7(3):181-188.

120. Varghese A, Pandita N, Gaud RS. *In vitro and in vivo Evaluation of CYP1A Interaction Potential of Terminalia Arjuna Bark.* Indian J Pharm Sci. 2014;76(2):138-147.

121. Li J, Qi Y, Liu H., et al. *Acute high-altitude hypoxic brain injury: Identification of ten differential proteins.* Neural Regeneration Research. 2013;8(31):2932-2941.

122. Biswal S, Sharma D, Kumar K, Nag TC, Barhwal K, Hota SK, Kumar B. *Global hypoxia induced impairment in learning and spatial memory is associated with precocious hippocampal aging.* Neurobiol Learn Mem. 2016;133:157-170.

123. Kumar K, Sharma S, Kumar A, Bhardwaj P, Barhwal K, Hota SK. *Acute and sub-acute toxicological evaluation of lyophilized nymphaea x rubra roxb. Ex andrews rhizome extract.* Regul Toxicol Pharmacol. 2017;88:12-21.

124. Bradford MM. *Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding*. Anal. Biochem. 1976;72:248–254.

125. Nelson JM, Duane PG, Rice KL and Niewoehner DE. *Cadmium ion induced alteration of Phospholipid Metabolism in Endothelial Cell.* Am J Respr Biol. 1991;5:328-336.

126. Yamamoto A, Saito N, Yamauchi Y et al. *Flow Cytometric Analysis of Red Blood Cell Osmotic Fragility.* Journal of Laboratory Automation. 2014;19(5):483-487.

127. Loeppky JA, Icenogle MV, Maes D, Riboni K, Hinghofer-Szalkay H, Roach RC. *Early fluid retention and severe acute mountain sickness.* J Appl Physiol (1985). 2005;98(2):591-597.

128. Remuzzi G, Perico N, Macia M, Ruggenenti P. *The role ofrenin-angiotensin-aldosterone system in the progression of chronic kidneydisease.* Kidney Int Suppl. 2005;99:S57-65.

129. Atlas SA. *The renin-angiotensin aldosterone system: pathophysiological roleand pharmacologic inhibition.* J Manag Care Pharm. 2007;13(8B): 9-20.

130. Wilson SK. *The effects of angiotensin II and norepinephrine on afferent arterioles in the rat*. Kidney Int. 1986;30(6):895-905.

131. Zeng GB, Duan SF. *Role of the renin-angiotensin-aldosterone system in the pathogenesis of edema formation in chronic obstructive pulmonary disease*. ZhonghuaNeiKeZaZhi. 1989;28(5):283-285.

132. Al-Hashem FH, Alkhateeb MA, Shatoor AS, Khalil MA, Sakr HF. *Chronic exposure of rats to native high altitude increases in blood pressure via activation of the renin-angiotensin-aldosterone system.* Saudi Med J. 2012;33(11):1169-1176.

133. Skytte Larsson J, Bragadottir G, Redfors B, Ricksten SE. *Renal function and oxygenation are impaired early after liver transplantation despite hyperdynamic systemic circulation*. Crit Care. 2017 Apr 11;21(1):87.

134. Moutzouri E, Daios G, Elisaf M, Milionis HJ. *Pharmaceutical modulation of the Renin Angiotensin aldosterone system for stroke prevention: a review ofexperimental and clinical evidence*. CNS Spectr. 2010;15(11):619-629.

135. Lambers Heerspink, HJ. *Inhibition of the renin-angiotensin-aldosterone systemfor cerebrorenal protection.* Contrib Nephrol. 2013;179:07-14.

136. Hendy-Willson VE, Pressler BM. *An overview of glomerular filtration rate testing in dogs and cats.* Vet J. 2011;188(2):156-165.

137. Holness JL, Fleming JS, Malaroda AL, Warwick JM. *(99m)Tc-DTPA volume of distribution, half-life and glomerular filtration rate in normal adults*. Nucl Med Commun. 2013; 34(10):1005-1014.

138. Vogel K, Opfermann T, Wiegand S, Biermann J, Busch M, Winkens T, Freesmeyer M. *Relationship between estimated glomerular filtration rate and biological half-life of 131I.* Retrospective analysis in patients with differentiated thyroid carcinoma. Nuklearmedizin. 2013;52(5):164-169.

139. Johnston CI, Hodsman GP, Harrison RW, Mendelsohn FA, Tsunoda K. *Regulation of cardiac preload by atrial natriuretic peptide in congestive cardiac failure*. Am J Med. 1988;84(3A):105-111.

140. Woods, D., Hooper, T., Mellor, A., Hodkinson, P., Wakeford, R., Peaston, B., Ball, S., Green, N., 2011. *Brain natriuretic peptide and acute hypobaric hypoxia in humans*. J Physiol Sci. 61(3), 217-220.

141. Milledge JS, Beeley JM, Mc Arthur S, Morice AH. Atrial natriuretic peptide, altitude and acute mountain sickness.ClinSci (Lond). 1989;77(5):509-514.

142. Terry J. *The major electrolytes: sodium, potassium, and chloride.* J Intraven Nurs. 1994;17(5):240-247.

143. Davis JO, Urquhart J, Higgins JT. *The Effects of Alterations of Plasma Sodium and Potassium concentration on Aldosterone Secretion.* Journal of Clinical Investigation. 1963;42(5):597–609.

144. Miller WL, Borgeson DD, Grantham JA, Luchner A, Redfield MM, Burnett JC. *Dietary sodium modulation of aldosterone activation and renal function during the progression of experimental heart failure.* Eur J Heart Fail. 2015;17(2):144-150.

145. Kotchen TA. *Effects of potassium on renin and aldosterone*. Arch Mal Coeur Vaiss. 1984;77:87-91.

146. Rao NK, Nammi S. *Antidiabetic and renoprotective effects of the chloroform extract of Terminalia chebula Retz. seeds in streptozotocin-induced diabetic rats*. BMC Complement Altern Med. 2006;7:0 6:17.

147. Kumar S, Enjamoori R, Jaiswal A, Ray R, Seth S, Maulik SK. *Catecholamine-induced myocardial fibrosis and oxidative stress is attenuated byTerminalia arjuna (Roxb.).* J Pharm Pharmacol. 2009;61(11):1529-1536.

148. Sherif IO. *Amelioration of cisplatin-induced nephrotoxicity in rats by triterpenoid saponin of Terminalia arjuna.* Clin Exp Nephrol. 2014; (4):591–597.

149. Kau ST, Keddie JR, Andrews D. *A method for screening diuretic agents in the rat*. J Pharmacol Methods. 1984;11(1): 67-75.

150. Legrand M, Payen D. *Understanding urine output in critically ill patients*. Ann Intensive Care.2014; 24:1(1):13.

151. Chaves ML, Camozzato AL, Ferraira et al. *Serum levels of S100B and NSE proteins in Alzheimer's disease patients.* Journal of Neuroinflammation 2010;7:6.

152. Donato R, Cannon BR, Sorci G et al. *Functions of S100 Proteins*. Curr Mol Med. 2013;13(1):24–57.

153. Purushothaman J, Suryakumar G, Shukla D, Malhotra AS, Kasiganesan H, Kumar R, Sawhney RS, Chamic A. *Modulatory effects of seabuckthorn (Hippophaerhamnoides L.) in hypobaric hypoxia induced cerebral vascular injury.* Brain Research Bulletin. 2008;77:246–252.

154. Bailey DM, Roukens R, Knauth M, Kallenberg K, Christ S, Mohr A, Genius J, Storch-Hagenlocher B, Meisel F, McEneny J, Young IS, Steiner T, Hess K, Bärtsch P. *Free radical-mediated damage to barrier function is not associated with altered brain morphology in high-altitude headache.* J Cereb Blood Flow Metab. 2006;26(1):99-111.

155. Gonçalves CA, Leite MC, Guerra MC. *Adipocytes as an Important Source of Serum S100B and Possible Roles of This Protein in Adipose Tissue*. Cardiovascular Psychiatry and Neurology. 2010;2010:790431. doi:10.1155/2010/790431.

156. E, Fritz G, Vetter SW, Heizmann CW. *Binding of S100 proteins to RAGE: an update*. Biochim Biophys Acta. 2009;1793(6):993-1007.

157. Saha A, Kim S-J, Zhang Z, et al. *RAGE signaling contributes to neuroinflammation in infantile neuronal ceroid lipofuscinosis*. FEBS letters. 2008;582(27):3823-3831.

158. Yang F, Wang Z, Zhang JH et al. *Receptor for Advanced Glycation End-Product Antagonist Reduces Blood–Brain Barrier Damage After Intracerebral Hemorrhage.* Stroke. 2015;46:1328-1336.

159. Ferreira LG, Dos Santos RN, Oliva G, Andricopulo AD. *Molecular docking and structure-based drug design strategies*. Molecules. 2015;20(7):13384-421.

160. Nana HM, Ngane RA, Kuiate JR, Mogtomo LM, Tamokou JD, Ndifor F, Mouokeu RS, Etame RM, Biyiti L, Zollo PH. *Acute and sub-acute toxicity of the methanolic extract of Pteleopsis hylodendron stem bark*. J Ethnopharmacol. 2011;137(1):70-76.

161. Xu K, Lin Y, Zhang R, Lan M, Chen C, Li S, Zuo C, Chen C, Zhang T, Yan Z. *Evaluation of safety of iridoids rich fraction from Valeriana jatamansi Jones: Acute and sub-chronic toxicity study in mice and rats*. J Ethnopharmacol. 2015;172:386-394.

162. Kulkarni YA, Veeranjaneyulu A. Toxicological Evaluation of the Methanol Extract of Gmelina arborea Roxb. Bark in Mice and Rats. Toxicology International. 2012;19(2):125–131.

163. Naylor JR, Bayly WM, Schott HC 2nd, Gollnick PD, Hodgson DR. *Equine plasma and blood volumes decrease with dehydration but subsequently increase with exercise.* J Appl Physiol. 1993;75(2):1002-1008.

164. Nose H, Mack GW, Shi XR, Nadel ER. Role of osmolality and plasma volume during rehydration in humans. J Appl Physiol. 1985;65(1):325-331.

165. Nash GB, Meiselman HJ. Red cell and ghost viscoelasticity. Effects of hemoglobin concentration and in vivo aging. Biophys J. 1983;43(1): 63-73.

166. Mohanty JG, Nagababu E, Rifkind JM. Red blood cell oxidative stress impairs oxygen delivery and induces red blood cell aging. Frontiers in Physiology. 2014;5: 84.

167. Cahalan SM, Lukacs V, Ranade SS, Chien S, Bandell M, Patapoutian A. Piezo1 links mechanical forces to red blood cell volume. eLife. 2015;4: e07370.

168. Mohan VR, Kalidass C. Nutritional and antinutritional evaluation of some unconventional wild edible plants. Tropical and Subtropical Agroecosystems 2011;12:495- 506.

169. WHO (WorldHealth Organization). *WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems*. WHO (World Health Organization), Geneva, Switzerland. 2008.

170. Parasuraman S, Thing GS, Dhanaraj SA. *Polyherbal formulation: Concept of ayurveda.* Pharmacognosy Reviews. 2014;8(16):73-80.

171. Ekor M, Osonuga OA, Odewabi AO, Bakre AG, Oritogun KS. *Toxicity evaluation of Yoyo 'cleanser' bitters and fields Swedish bitters herbal preparations following sub-chronic administration in rats.* Am. J. Pharmacol. Toxicol. 2010;5:159–166

172. WHO. *National Policy on Traditional Medicine and Regulation of Herbal Medicines*.Report of World Health Organization Global Survey. Geneva, Switzerland: WHO. 2005b

173. WHO. *WHO global atlas of traditional, complementary and alternative medicine*. in *Map Volume* eds Ong C. K., Bodeker G., Grundy C., Burford G., Shein K., editors. (Geneva, Switzerland:World Health Organization;). 2005a.

174. Neergheen-Bhujun VS. *Underestimating the toxicological challenges associated with the use of herbal medicinal products in developing countries.* Biomed Res Int. 2013;2013:804086.

175. Ekor M. *The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety*. Frontiers in Pharmacology. 2013;4:177.