Introduction
For the development of civilization one of the key factors is the enhancement in physical and mental working efficiency of human and domesticated animals under various environments. The effort has been to increase the work performance and efficiency of man in adverse environments, which otherwise has a limited capacity to work. In addition, the capacity to work deteriorates as the man performs in time; he ages, under mental distress and physiologically adverse working environment. The latter may be due to the presence of physical, chemical, biological or emotional environmental stressors. An example of complex stressful condition is high altitude environment consisting of various stressors viz. hypoxia, cold, humidity, ultraviolet radiation and ionizing radiation, and isolation. Oxidative stress, which is defined as excessive generation of free radicals is also an important factor for development of fatigue and stress induced diseases during exposure to such stressful situations. This needs prevention and if possible restoration of lost strength to carry out the work more efficiently.

The studies on enhancement in work performance required a technique for the quantitative measurement of performance parameter; otherwise it is difficult to define the utility of a tool or a process used as performance enhancer. This depends entirely on the aim of the task being performed. For example a passive Cold-Hypoxia-Restraint (C-H-R) animal model has been used to evaluate performance enhancement efficacy of adaptogenic substances and the parameter for measuring performance studied was rectal temperature, an indicative of hypothalamic and metabolic functions of the organism (Ramachandran et al., 1990). However, there could be several parameters, even more than one in a task, required to be measured for evaluation of the work performance enhancement.
For achieving higher physical work capacity, it is essential to enhance metabolic capacity of the organism to burn high amount of energy fuels to get sufficient energy released. At the same time the tissue homeostasis or dynamic metabolic balance in the body tissues especially in the skeletal muscles, has to be maintained. Availability of sufficient energy reserves in the body is an important factor affecting endurance work being performed. Carbohydrates act as primary source of energy in all mental and physical activity. If taken in adequate amounts before or during the physical activity they provide a ready source of energy and if in excess get stored in skeletal muscles and liver as glycogen. However, a person who can burn fat more efficiently, a compact source of energy, can sustain and work for longer time in adverse situations. Some of the other effective dietary micro-molecules as performance enhancers are peripheral and central neural stimulants such as caffeine, branched chain amino acids (tryptophan, tyrosine, phenyl alanine and glutamic acid), camitine and choline. Arginine is another amino acid, constituents of several well-known herbal adaptogens such as Ginseng, which has been found to be an effective as a performance enhancer (Gupta et al., 2005).

There are several techniques available for increasing physical endurance in physiologically adverse stressful situations (Srivastava and Sen Gupta, 1987). The most common and popular is exposing the organism to increasing amount of effort to do better than earlier. This is often termed as training and practice. Better performance in sports and almost in every situation where there is increased demand of resources on the body, increment in performance can be achieved through this technique.

The technique of Cross adaptation includes the adaptation of an organism to one type of environment and simultaneously comes adaptation to several other types of environments. The enhanced cardiovascular strength achieved in hot environment brings about simultaneously enhanced exercise tolerance especially of the endurance type.

Yoga and Meditation is among the more recently acknowledged techniques, specially the Breathing techniques or Pranayama, for enhancing the physical and mental
performance. Yoga and Meditation brings about strengthening of cardio respiratory system with mental tranquility.

Ayurveda, the science and art of living healthy long life, has proper nutrition and life style at its core. Nutraceuticals, though a recently coined word, had its beginning in Ayurveda some 3500 years ago. Russians, some 50 years ago, gave another word, Adaptogens, to nutrients and biological derived from plants and animals which were useful in enhancing work performance in adverse stressful environments, in sports, among soldiers, miners, students, workers and aged individuals. These substances affected immune system, improved cardio respiratory system, mental vasculature, physical strength and mental alertness. The modern scientific instrumentation and techniques have provided tools for the evaluation of such substances that affect work performance. The utility of these substances for the welfare of mankind is enormous. All of this now forms a part of the medicine called the "Adaptive Medicine" (Brekhman, 1980).

Herbal formulations have been in use for many years not only in Asian countries but also globally for human well-being (Brekhman, 1980; Fulder, 1980). Adaptogens of herbal origin have been claimed to enhance physical endurance, mental functions and non-specific resistance of the body (Brekhman, 1980). During the stressful situations supplementation of various nutrients and single and poly-herbal preparations have been shown to increase stress tolerance (Bhargava and Singh, 1981; Grover et al., 1995; Kumar et al., 1996, 1999, 2000, 2002). It has been hypothesized that plants growing in adverse climatic conditions of high altitude acquire bio-molecules, which help them to sustain in such adverse environment, and supplementation of these plant products to the animals increased their performance during exposure to stressful cold and hypoxic environment (Divekar et al., 1996).

Rhodiola, a high altitude plant, is widely distributed throughout Europe and Asia. The plant belongs to the family Crassulaceae, sub family sedoideae, and genus Rhodiola. There are about 200 species of the plant and its root contains a range of biologically active substance including phenylpropanoids (rosavins, rosin); phenylethanol derivatives (salidroside, tyrosol), organic acids, flavonoids, tannins and phenolic glycosides
(Khanum et al., 2005). Rhodiola root has been used extensively since time immemorial for its medicinal properties in traditional folk medicine in China, Tibet, Mongolia and former Soviet Republics to increase physical endurance, work productivity, longevity and to treat fatigue, asthma, hemorrhage, impotence and gastrointestinal ailments (Kelly, 2001). The extracts of the Rhodiola roots have also been scientifically studied and reported to possess cytoprotective and oxygen scavenging (Kanupriya et al., 2005; Ohsugi et al., 1999), adaptogenic (Rege et al., 1999; Spasov et al., 2000), anti-hypoxic (Kumukov et al., 1986), anti-fatigue (Darbinyan et al., 2000), anti-cancer (Udintsev and Schakhov, 1991), neuroprotective (Mook-Jung et al., 2002) and hepatoprotective (Nan et al., 2003) bio-activities. The available literature of Rhodiola species is supportive of its adaptogenic properties. But most of the available adaptogenic and anti-stress studies are on the species *Rhodiola rosea*. Another species *Rhodiola imbricata* Edgew, (Syn: *Sedum roseum*; *S. imbricata*; *S. rhodiola*) a perennial herb of family Crassulaceae, commonly known as golden or arctic root, grows on rocky slopes of Western Himalaya at an altitude of 4000-5000 m. The roots of *Rhodiola imbricata* was found to possess radio-protective (Arora et al., 2005; Goel et al., 2006), wound healing (Gupta et al., 2007) and immunomodulatory (Mishra et al., 2006) activities. However a detailed dose dependent study of *Rhodiola imbricata* root for its anti-stress and adaptogenic activity, safety studies and biochemical effects remained to be carried out.

The present study was undertaken to evaluate dose dependent anti-stress and adaptogenic activity of *Rhodiola imbricata* root extracts in rats using a passive Cold (5±1°C)-Hypoxia (428 mmHg)-Restraint (C-H-R) animal model (Ramachandran et al., 1990). Further, safety studies of evaluated *Rhodiola imbricata* dried root aqueous extract were also carried out in rats for its toxicity (acute and sub-acute), if any, and effect of biochemical and hematological parameters in the serum and blood and some of the vital organ tissues of animals were determined. To examine anti-stress mechanism of action of *Rhodiola imbricata* root extracts on its oral supplementation, some of the important metabolic regulatory enzymes and other biochemical parameters were estimated in blood and vital tissues viz. liver and skeletal muscle of rats.
Aims and Objectives
AIMS & OBJECTIVES

- To evaluate Rhodiola root herbal extracts for their efficacy in enhancing physical performance in animals during exposure to adverse climatic conditions.

- The identification of a potential herbal extract or bioactive substances with such properties derived from Rhodiola would help in facilitating human adaptation to adverse stressful conditions viz. high altitude.
Chapter-I

Review of Literature
ENVIRONMENTAL STRESS AND ADAPTOGENS

1.1 Environmental stress

Stress is the "wear and tear" our bodies experience as we adjust to continually changing environment. It has physical and emotional effects on us and can create positive or negative feelings. As a positive influence, stress can compel us to act and achieve desired goals in life. As a negative influence, it can result in feelings of disruption, rejection, anger, and depression. So, it is a condition or circumstance that can disturb the normal physical and mental health of an individual. Adverse physiological environments such as heat, cold, noise, vibrations, pollutants, high altitude and work setting beyond a certain threshold limit of the organism result into decreased physical and mental work performance. This is on account of stress induced increased demands posed on body resources including energy, resulting into physiological adjustments affecting the health and performance. The human health care needs and disease pattern have changed significantly with the change in lifestyle and competitive and challenging working conditions. Today, with the rapid diversification of human activity, we come face to face with numerous causes of stress and the symptoms of stress and depression.

Stress can be explained as the sum total of all the reactions of the body, which disturb the normal physiological condition and result in a state of threatened homeostasis. Stress represents a reaction of the body to a stimulus that tends to alter its normal physiological equilibrium or homeostasis and has been defined as a non-specific response of the body to any demand imposed on it. Stress continuously hammers at
the harmony and homeostasis of life. The processes, which keep harmony and homeostasis maintained in spite of the hammering by stress producing factors, are the ones, which constitute adaptation in the living being. The stress comprises a set of reactions in the organism to help its body to overcome the effect of changing conditions. Such reactions mobilize energy and gear up the body to withstand ‘demands’ for successful survival (Cannon and De La-Paz, 1911; Selye, 1946). Thus stress has been responsible for achieving the impossible and performance beyond the ‘capability’ of man. Normally such stress induced changes are compensatory, self limiting and adaptive. Therefore adaptation is required to successfully cope up combat stress and stressful situations and rapidly reassume homeostasis after the stressor is withdrawn. However, in higher animals when stress events of any nature (physical, chemical, biological and emotional) over ride certain ‘threshold’ limits, the changes become rather irreversible. It leads to altered homeostasis and exhaustion, manifesting itself in the pathological form of stress-induced diseases and maladjustment viz. hypertension, diabetes, gastric ulcers etc. (Avtsyn, 1974).

1.2 Stress as a concern for Army personnel

India, together with Nepal, has the world’s highest mountains. The effect of environmental stress due to low oxygen pressure and low temperature is a subject of interest not only to the armed forces but also to those staying at high altitudes for long and short periods. The defence of the country’s borders has necessitated posting of armed forces at high altitude locations, the altitudes ranging from 3500m to 7000m and Siachin glacier with an altitude of 4800m to 7000m where the armed forces of two neighboring countries are facing each other. Soldiers facing the vagaries of war at certain times succumb to extremes of environmental, physiological and emotional stresses. This may lead to decline in the performance of some of them and create behavioural and psychological problems. Success in battlefield depends on high performing troops, who can under any condition, optimally use the equipment to achieve the objectives set for them. The stress experienced by an army personnel in combat situation arises from two diverse milieu: first, the external factors, caused by noise, heat, cold, vibrations; and second, the internal factors, caused by physiological
fatigue, lack of sleep, food and water, psychological fear, anxiety, and apprehension of the consequences of the encounter, i.e., being killed, captured, loss of limbs, separation with near and dears etc. An inability to overcome or cope with emotional stress will result in disorganized and irrational behaviour that will reduce the effectiveness of the individual combatant. The problem of maintaining mission performance under stress has been consistently identified as a priority area for military studies. The concern with countering stress decrements in military performance is shared by many countries, as indicated by studies carried by American (Burke, 1980), Israeli (Friedland and Ketnan, 1986), and Soviet (Solovyeva, 1981) researchers.

In a conflict, the only measure of combat effectiveness is the performance of the army unit in actual combat. Thus, methods and technology for increasing human endurance assumes great significance both in active and passive combat situations. Therefore, an effective psychological and pharmacological therapy is highly desirable to keep the soldiers physically and mentally fighting fit. Several formulations of natural products derived from plants are reported to have anti-stress and physical- and mental-endurance-promoting properties (Brekhman and Dardymov, 1969). The efficacy of a composite Indian herbal preparation (CIHP) in sustaining the mental performance of soldiers engaged in prolonged low-intensity-conflict operations was evaluated using psychological tests (Gopinathan et al., 1999) in a double blind study. The results indicated comparatively better cognitive performance immediately after the mission in the CIHP administered army personnel. CIHP was found effective to sustain mental abilities of soldiers in a low-intensity-conflict environment.

1.3 Oxidative Stress: Biological adaptation in adverse environment

Oxygen is the primary oxidant in metabolic reactions to derive energy from the oxidation of a variety of organic molecules. It plays a vital role as the terminal electron acceptor during respiration. More than 90% of the $O_2$ taken up by the human body is used by mitochondrial cytochrome oxidase, which adds four electrons onto each $O_2$ molecule to form water ($O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$) (Halliwell, 1992). Four-
electron reduction of oxygen in the respiratory chain is accompanied with a partial one- to three- electron reduction; intermediates produced in this process are reactive oxygen species (ROS) that are highly reactive. Excessive production of ROS disturbs the equilibrium status of pro-oxidant/anti-oxidant systems in intact cells. Oxidative stress occurs when the pro-oxidant system outbalance the anti-oxidant, causing oxidative damage to lipids, proteins, carbohydrates and nucleic acids, ultimately leading to cell death (Ames et al., 1993; Halliwell and Gutteridge, 1993; Jenkins and Goldfarb, 1993; Kehrer and Smith; 1994). Increased energy demand during stressful adverse environmental situations or physical exercise, especially of the aerobic type, necessitates a multifold increase in oxygen supply to active tissues to maximize energy yield. But organisms have to pay a price for this increased aerobic metabolism due to generation of oxygen free radical (OFR) and reactive oxygen species (ROS) (Halliwell and Gutteridge, 1989). The "ROS" and "OFR" are not synonymous terms; unlike the latter, ROS represent a broader spectrum of species including non-radical derivatives (e.g., H$_2$O$_2$, peroxides, hydro peroxides etc.) that are capable of inciting and propagating oxidative tissue damage. The formation of ROS also occurs as a result of endogenous and exogenous conditions. Some of these exogenous sources are ultraviolet light, anoxia, pollutants, inflammation, smoking, and radiation (Haramaki and Packer, 1994; Jenkins and Goldfarb, 1993). Endogenous sources include direct reduction of molecular oxygen by oxidases in the electron transport chain, phagocytic cells, D-amino acid oxidases, xanthine oxidase, epinephrine, coenzyme Q10, vitamin K and the cytochrome P$_{450}$ system (Ames et al., 1993; Halliwell and Gutteridge, 1993; Kehrer and Smith, 1994).

A free radical is a molecule that contains an unpaired electron in its outer orbit and that can exist independently. Molecular oxygen is a bi-radical, containing 2 unpaired electrons with parallel spin configurations. Because electrons must have opposite spin to occupy the same orbit, electrons added to molecular oxygen must be transferred one at a time during its reduction (Sen, 1995), resulting in several highly reactive intermediates (Yu, 1994). A univalent reduction of oxygen refers to the reduction of molecular oxygen by one electron at a time. The complete reduction of oxygen to H$_2$O requires 4 steps and the generation of several free radicals and H$_2$O$_2$. H$_2$O$_2$ is not
a free radical in itself because it contains no unpaired electrons but considered a reactive oxygen species (ROS) due to its ability to generate highly reactive hydroxyl free radicals through interactions with reactive transition metals (Aruoma and Halliwell, 1987). The complete reduction of oxygen is summarized in the following equations:

- \( \text{O}_2 + e^- \rightarrow \text{O}_2^- \) superoxide radical (1)
- \( \text{O}_2^- + \text{H}_2\text{O} \rightarrow \text{HO}_2^- + \text{OH}^- \) hydroperoxyl radical (2)
- \( \text{HO}_2^- + e^- + \text{H} \rightarrow \text{H}_2\text{O}_2 \) hydrogen peroxide (3)
- \( \text{H}_2\text{O}_2 + e^- \rightarrow \cdot\text{OH} + \text{OH}^- \) hydroxyl radical (4)

In biological systems, bi-radical oxygen is reduced to produce energy and water. Due to its bi-radical nature, oxygen, and its metabolites are potentially cytotoxic (Fridovich, 1978). The oxygen-derived intermediates are considered highly reactive because their unstable electron configurations allow them for the attraction of electrons from other molecules, resulting in another free radical that is capable of reacting with yet another molecule. This chain reaction is thought to contribute to lipid per-oxidation (Hochstein and Emster, 1963), DNA damage (Kasai et al., 1986), and protein degradation (Griffith et al., 1988) during oxidative stressful events. Although all the intermediates are potentially reactive, the intermediates vary in their biological importance. The superoxide radical (\( \text{O}_2^- \)) is the most well known oxygen-derived free radical (Yu, 1994) and, unlike the other oxygen-derived intermediates, can lead to the formation of additional reactive species (Harris, 1992). Although \( \text{H}_2\text{O}_2 \) is relatively un-reactive, it is more lipophilic than the superoxide anion radical and can traverse biological membranes to act on intracellular phospholipids, carbohydrates, metalloproteins and DNA via a site-specific Fenton reaction (Marx and Chevion, 1985). The formed \( \cdot\text{OH} \) radicals are highly reactive capable of initiating deleterious reactions such as lipid per-oxidation and DNA damage. These active oxygen intermediates are generated in living cells physiologically, but produced in excessive amounts in pathologic situations and also during exposure to stressful environmental insults.
In a biological system, oxidative stress may arise either from deficiencies of antioxidants compounds (such as glutathione, uric acid, metal chelating agents [transferring, lactoferrin, ceruloplasmin], β-carotene, ascorbate, or α-tocopherol); decreased activity of antioxidant enzymes (superoxide dismutase [SOD], catalase, glutathione peroxidase) and/or from increased formation of ROS. These highly ROS can cause glutathione depletion, lipid per-oxidation, membrane damage, DNA strand breaks, protein destruction, and activation of proteases.

The well-described consequence of the generation of free radicals and ROS is lipid per-oxidation (Hochstein and Ernster, 1963). Polyunsaturated fatty acids (PUFA), the main components of membrane lipids are susceptible to per-oxidation. As a consequence of per-oxidation of membrane phospholipids, the membrane becomes rigid, loses channel function, and finally fails to preserve membrane integrity (Patel and Block, 1988). ROS have also been suggested to cause per-oxidation of membrane phospholipids by activating phospholipase A₂, which can result in increased membrane fluidity and permeability and loss of membrane integrity (Freeman and Crapo, 1982). The disruption of membrane permeability or fragmentation of the cell membrane leads to Ca²⁺ influx and irreversible cell damage. The proteins modified by oxidative damage are shown to activate proteolytic enzymes and become susceptible to proteolysis (Davies, 1991). Ribo- and deoxyribo nucleic acids are also targets of ROS attack resulting in strand breaks (single/double), sister chromatin exchange, DNA-DNA and DNA-protein cross links and base modifications (Teebor et al., 1988).

1.3.1 Sources for the Generation of Free Radicals

Some of the important sources for the generation of free radicals are as follows (Nakazawa et al., 1996):

1. Mitochondria: Partially reduced oxygen is known to escape as superoxide radical in electron transport chain (Turrens et al., 1980; 1985). The superoxide rapidly dismutates to form H₂O₂.

2. Auto-oxidation of reduced flavin, thiols, and small molecules such as hydroquinones and catecholamines.
3. Arachidonic acid pathway.
4. Endoplasmic reticulum and nuclear membrane electron transport systems.
5. Peroxisomes: potent source of cellular $H_2O_2$.
6. Respiratory burst: various types of stimulants such as opsonized bacteria, viruses, immunoglobulins, activated polymorphonuclear (PMN) leukocytes and macrophages, which take up large amount of oxygen and convert it into superoxide anion.
7. Transition metals, iron and copper, promote the generation of the most highly reactive class of active oxygen known as the hydroxyl radical.
8. Hypoxanthine and xanthine oxidase: activation of xanthine oxidase from xanthine dehydrogenase. Hypoxanthine [formed as a stepwise breakdown product of adenosine triphosphate (ATP)] is converted to xanthine and uric acid by xanthine oxidase when re-oxygenation occurs; it gives rise to formation of superoxide.

1.3.2 Detection of ROS

ROS can be detected either by using direct method or indirect method:

1. Direct method: To date, use of spin trapping agents and detection by electron spin resonance spectroscopy is the only available direct method (Zweir et al., 1987).

2. Indirect method: Thiobarbituric acid reaction - A semi quantitative test to detect the presence of lipid peroxides by reaction with thiobarbituric acid in acid medium. A colored product malondialdehyde (MDA), a lipid per-oxidation by-product, forms during this reaction and is the most common parameter that has been studied with respect to oxidative injury. In most cases, the thiobarbituric acid reactivity approach has been used for malondialdehyde (MDA) assay. In addition, hydrocarbon by-products viz. ethane and pentane produced by lipid per-oxidation may be collected in expired air and could be measured using gas chromatograph and hydrogen flame detectors.
1.3.3 Antioxidant System

To protect the organism from deleterious effects of free radicals generated in an oxygen rich environment, a biochemical antioxidant defense system has evolved whose net effect is to lower the cellular concentration of free radicals and thereby inhibit excessive damage to cellular components. An antioxidant may be defined as any substance that, when present in low concentrations compared with those of an oxidizable substrate viz. proteins, carbohydrates, lipids and DNA, significantly prevents generation of free radicals during oxidation of these substrates (Halliwell and Gutteridge, 1989). The antioxidant defense is essentially a two-component system consisting of 1) low molecular weight compounds that quench free radicals and 2) enzymes that metabolizes free radicals to less reactive species.

I. Low-Molecular-Weight Compounds

The principal low molecular weight compounds that function as endogenous free-radical scavengers are vitamins E, C, A and glutathione.

Vitamin E is a lipid-soluble and therefore can insert into cellular membranes. Thus it protects against lipid per-oxidation most efficiently through its chain-breaking antioxidant action. Vitamin E reacts with free radicals and donates its labile hydrogen to them. Thus, the chain reaction of per-oxidation terminates since these chain-propagation radical are scavenged. During these reactions, vitamin E is consumed and converted to the radical form (Vit-E'). Vitamin-E' is reduced back to vitamin E by ascorbic acid (vitamin C) (McCay, 1985). Carotenoid is a pro-vitamin A and is found in membrane as a protein complex form. It is shown to scavenge singlet oxygen (Foote and Denny, 1968). Ubiquinol present in the mitochondrial inner membrane acts as a chain-breaking antioxidant by donating hydrogen to free radicals (Frei et al., 1990).

Vitamin C is a water-soluble antioxidant in the cytosol and extracellular fluid. Its chemical properties allow it to interact directly with $O_2^-$ and •OH in the aqueous phase such as plasma thus preventing damage to erythrocyte membrane (Beyer, 1994). Vit C also reduces Vit E radical and the oxidized semidehydroascorbate is reduced by a GSH and or dihydrolipoic acid redox cycle (Halliwell and Gutteridge,
1989). Ascorbic acid has an ability to scavenge a wide range of ROS viz. superoxide anion, singlet oxygen and hydrogen peroxide, and acts as a chain-breaking antioxidant (Beyer, 1994).

Reduced glutathione (GSH) is considered to be one of the most important components of the antioxidant defense of living cells. GSH is a potent cellular reductant with a broad redox potential. It acts as a scavenger of peroxides and serves as a storage and transport form of reduced sulphur. GSH is a tripeptide (L-γ-glutamyl-L-cysteinyl-glycine) containing a thiol (sulfhydryl) group. Due to redox active thiol group GSH act as a defence compound against oxidative stress. GSH protects cells against free radicals and oxidants. GSH is regarded as the most important intracellular low molecular mass antioxidant, and its concentration is indicative of the cellular redox state during any stressful event (Jones et al., 1981). GSH is a water soluble antioxidant found in the cytosol and mitochondria (Halliwell and Gutteridge, 1989). Mammalian erythrocytes are rich in GSH and account for most of the blood GSH. The –SH pool contributed by reduced GSH plays a central role in regenerating vitamin C and E from their radical forms. Free GSH scavenges O$_2^{-}$ and •OH by forming the disulfide form, GSSG. Glutathione homeostasis is essential for the pro-oxidant-antioxidant balance and circumvention of cellular oxidative stress during any stressful situation.

Selenium is an important antioxidant substance; it functions as a cofactor of glutathione peroxidase (Burk, 1983). Uric acid and bilirubin are present in plasma as an end product of purine metabolism and hemoglobin, respectively. Both can scavenge O$_2^{-}$ and •OH at their physiological concentrations (Ames et al., 1981).

2. **Enzymes and Proteins**

Superoxide dismutase (SOD) is the first enzyme involved in the antioxidant defense, which accelerates the dismutation reaction of O$_2^{-}$ to H$_2$O$_2$. The enzyme is present in all aerobic organisms and in all sub-cellular compartments susceptible to oxidative stress. Three types of SOD have been identified: Cu-Zn-SOD, Mn-SOD (McCord, 1979) and extra-cellular SOD (EC-SOD) (Marklund, 1984). Cu- Zn-SOD is found
abundantly in cytosol and nucleus. Mn-SOD is located in the mitochondrial matrix and EC-SOD is present in plasma, bound to heparin sulphate on the surface of endothelial cells. The presence of SOD in various compartments of our body enables it to dismutate $O_2^-$ immediately at the site where they are formed.

Catalase is another important enzyme, which executes the next step in the detoxification of ROS. It is a hemoprotein and catalyses the decomposition of $H_2O_2$ to water and oxygen and thus protects the cell from oxidative damage by $H_2O_2$ and $•OH$ (Deisseroth and Dounce, 1970). Catalase is also found in all the major organs, but especially concentrated in liver and erythrocytes (Schonbaum and Chance, 1976). However its activity is low in the brain, heart, lung and skeletal muscle (Marklund et al., 1982). Catalase has four subunits, each of which contains a heme group as an active site and NADPH helps to stabilize the subunit (Kirkman and Gaetani, 1984).

Glutathione peroxidase catalyses the oxidation of the reduced form of glutathione (GSH) to the oxidized form (GSSG) at the expense of $H_2O_2$ or lipid peroxides. GSSG produced intracellularly may be reduced back to GSH in the presence of glutathione reductase, which requires NADPH as a cofactor for its activity. It is a selenoenzyme; two-third of which (in liver) is present in the cytosol and one-third in the mitochondria (Freeman and Crapo, 1982).

Glutathione-S-transferase (GST, EC 2.5.1.18) catalyze the conjugation of GSH via the -SH and potential alkylating agents, thereby neutralizing their electrophilic sites and rendering them more water-soluble. This activity is useful in the detoxification of endogenous compounds such as peroxidized lipids as well as the metabolism of xenobiotics. Vani et al. (1990) reported a significant increase in hepatic GST activity subsequent to swimming training.

Some of the proteins also act as antioxidants (Nakazawa, 1996). Transferrin present in interstitial space and lactoferrin in both interstitial space and cytosol bound to iron, minimize free ionic iron and prevent generation of $•OH$. Iron storage protein ferritin present in cytosol protect against Fenton reaction-related damage in cytosol. Ceruloplasmin, a glycoprotein, inhibits copper-dependent lipid per-oxidation.
Albumin present in plasma is able to bind copper tightly and iron weakly (Halliwell and Gutteridge, 1989).

3. *Natural Molecules of Plant Products as Antioxidants*

Plant-derived compounds, also called phytochemicals, have demonstrated antioxidant capacities. The polyphenols present in plant products possess ideal structural chemistry for free radical scavenging activity, and they have been shown to be more effective antioxidants than tocopherols and ascorbate. Anti-oxidative properties of polyphenols arise from their high reactivity as hydrogen donor or electron donors, ability to stabilize and delocalize the unpaired electron (chain-breaking function), and capacity to chelate transition metal ions (Rice-Evans et al., 1997). Another mechanism underlying the anti-oxidative properties of phenolics is the ability of flavonoids to alter per-oxidation kinetics by modification of the lipid packing order and to decrease fluidity of the membranes (Arora et al., 2000). The plant derived phenolic antioxidant viz. apocyanin has been shown to induce GSH synthesis in human alveolar epithelial cells (Lapperre et al., 1999).

1.3.4 Relationship of Oxidative Injury and Decreased Performance during Stress

Oxidative stress may progress to oxidative damage involving cellular proteins (contractile, structural, and enzymatic), lipids, DNA, and other molecules in ways that might lead to abnormal cellular function. Excessive oxidative damage results in the onset of poor health and a variety of stress induced diseases (Dhalla et al., 2000). Though excessive ROS production is always problematic but low levels always appears necessary for important physiological functions such as cell signaling, immune response, and apoptosis (Volaard et al., 2005). The production of ROS in the biological systems increases during exposure to both environmental and physical stressors (Halliwell and Cross, 1994). It is reported that intermittent exposure to hypoxia (Radak et al., 1994), cold (Bhaumik et al., 1995) and immobilization (Hisao et al., 1993) cause increased production of ROS. Free radicals stimulate rate of autocatalytic process of lipid per-oxidation including damage in the muscular structure (Benzi, 1993). Free radicals induced muscular damage result in muscular
atrophy (Kondo and Itokawa, 1994) and muscle fatigue (Barclay & Hansel, 1991), resulting in decreased performance of an individual.

1.4 Available Techniques and Management of Stress

With change in life style and day-to-day increase in stress producing factors beyond the threshold limits, a large number of populations suffer from stress-induced diseases. The management of unusual oxidative and psycho-physiological stress has acquired enormous significance in day-to-day life. The optimum performance of a task in any work setting requires the confluence of physical and mental processes. The reduced endurance of man both physical and mental during a long and strenuous task results primarily due to decreased availability of energy to the system.

The available techniques (Srivastava and Sen Gupta, 1987) for increasing endurance performance can be classified as following:-

1. Physical Training for Endurance Work
2. Yogic and Meditation Practices
3. Supplementation With Food Components or 'Neutraceuticals'
4. Intervention by 'Adaptogens'

**Physical Training for Endurance Work**

To increase physical endurance, tolerance and resistance to a wide variety of stressors, the most widely utilized method is exposure to gradually increasing load of such a stressor. The organism over a prolonged period varying from few hours to several months depending upon the nature of stressors, acquire a better capacity to overcome the insults and develops tolerance or resistance to face an increasing load of strenuous work. Endurance training also serves to enhance lipolytic response to catecholamines (Izagawa, 1991), lipid oxidation (Mole et al., 1971) and increased enzyme levels viz. Citrate synthase and Hexokinase (Berthon et al., 1995).
**Yogic and Meditation Practices**

Yoga comprises a rich treasure of physical and mental techniques which can be effectively used to create physical and mental well being. It is an ancient Indian science and way of life. It has been claimed that yogic practices can lead to remarkable resistance and tolerance to cold and hypoxic conditions (Anand et al., 1961; Selvamurthy et al., 1988). Yogic practices are claimed to increase the pulmonary vital capacity, strengthen heart muscle and improve mental tranquility. Three months yogic training resulted in decreased lipid profile, cardiovascular risk factor and blood pressure in human subjects especially in those with elevated levels of these parameters (Schmidt et al., 1997). Pranayamas have been shown to modify oxygen consumption and heart rate (Telles and Desiraju, 1992). Yogic practices might be useful for amelioration of various stress-induced deteriorations.

**Supplementation of Food Components or ‘Neutraceuticals’**

During the state of increased energy requirements or exposure to various stressful situations, one major limiting factor for decreased physical and mental endurance is known to be the depletion of body energy reserves. Additional quantities of macro and micronutrients are required to cater for the caloric needs of working man in alien hostile environments (Askew, 1995) and maintain his endurance capability.

Various dietary substances have been studied for their efficacy during stressful situations. Caffeine has been shown to be effective at maintaining alertness and to function as a potent reinforcer (Griffiths & Woodson, 1988). The precursor for serotonin is tryptophan while for dopamine and norepinephrine is tyrosine. Stress elevates the brain level of tryptophan, an effect that has not been observed for tyrosine (Curzon et al., 1972). Under stressful conditions concentration of tyrosine is limited by its supply. Tyrosine supplementation in human (100 mg/kg) was found to ameliorate many of the adverse consequences of environmental stress viz. headache, nausea, mood, performance, vigilance and anxiety (Banderet & Lieberman, 1989). In another study by Vij and Satija, (1998), administration of tyrosine during combat stress (hypoxia) modulates the neurobehavioral changes in rats. Choline an essential
component of the human diet is important for a wide variety of metabolic processes in
the body. Best-known function of choline is as acetylcholine, an important
neurotransmitter. Choline supplementation, in the form of lecithin, showed anti-stress
and adaptogenic activity during cold-hypoxia and restraint stress (Kumar et al., 2002).
Carnitine facilitates transport of long chain fatty acids transport into matrix of
mitochondria, site of β oxidation. Carnitine supplementation prior to exercise
increased work output (Vecchiet et al., 1990).

**Intervention by Adaptogens**

Adaptogens were discovered in 1947 by the Russian scientist Dr. Nicolai Lazarev,
who in fact coined the name "adaptogen", for the substances which enhanced
performance and produced a “state of non-specific resistance”. Much of the early
research in the field of adaptogens was done by Dr. I.I. Brekhman who, in the late
1950's, studied *Panax ginseng*. The word and concept of an “adaptogen” is a
relatively new way of describing a type of remedy commonly found in traditional
Chinese (Qi tonic), African (Manyasi), Tibetan and Ayurvedic oriental systems of
medicine.

According to modern science, adaptogens are natural plant products that increase non-
specific resistance of the body and ability to cope up with internal and external stress
factors. They help to maintain the stable internal environment inside the organism
known as homeostasis. An important characteristic is that they are safe, possessing
minimal side effects. Adaptogens, increase the ability of the organism to adapt in
physiologically adverse situations. Chemical interventions for increasing endurance
performance are largely concerned with increasing energy supply and influencing
biosynthesis of proteins and nucleic acids. Adaptogens have been found to possess
such activity without leading to addiction. These substances have wide range of
therapeutic activity having minimal alterations of body functions; manifest action
under conditions of challenge to the system and their actions are non-specific.

The preparations of plant origin are gaining popularity and being investigated for
remedies of a number of disorders and anti-stress adaptogenic activity. There are
several substances known to have ‘adaptogenic’ properties, used in countries other
than India viz. Panax ginseng, Ginkgo biloba, Eleutherococcus senticosus etc.
(Brekhman and Dardymov, 1969). The endurance for running and doing hard work
and working in adverse environmental conditions significantly increased in
experimental animals administered Panax ginseng (Avakian et al., 1984). Using a
preparation of Panax ginseng, Revital, it was again confirmed in animals that it
increased performance during exposure to Cold (5±1°C)-Hypoxia (428 mmHg) and
Restraint (C-H-R) stress (Kumar et al., 1996). In human subjects Panax ginseng was
also found to restrict extreme high altitude induced deteriorations (Srivastava et al.,
1994). Extract of E. senticosus improved the mental and physical power of Soviet
athletes, cosmonauts and workers (Fulder, 1980; Wagner et al., 1994).

The Indian traditional medicines, specially, Ayurveda has been using food
supplements, dietary elements, herbs and minerals for increasing physical endurance
and mental performance. In Ayurveda these substances, Rasayanas, have been
described to possess properties similar to Adaptogens (Nadkami, 1954). The initial
studies on anti-stress activity of some of the Indian medicinal plants such as Ocimum
sanctum, Asparagus racemosus and Withania somnifera opened a vast area of
adaptogen research to improve performance during stressful conditions (Bhargava and
Singh, 1981; Singh et al., 1982; Singh and Dhawan, 1982). Withania somnifera roots
were investigated for its adaptogenic activity against a rat model of chronic stress and
the chronic stress induced perturbations were attenuated by Withania somnifera (25
and 50 mg/kg po), administered 1h before foot shock for 21 days. The results
indicated that Withania somnifera had significant anti-stress adaptogenic activity
(Bhattacharya and Muruganandam, 2003).

A poly-herbal formulation Siotone (ST), comprising of Withania somnifera, Ocimum
sanctum, Asparagus racemosus, Tribulus terrestris and shilajit, was found to possess
significant adaptogenic activity, qualitatively comparable to Panax ginseng, against a
variety of behavioural, biochemical and physiological perturbations induced by stress
(Bhattacharya et al., 2000). A Composite Indian Herbal Preparation-I (CIHP-I) was
tested for its adaptogenic activity using a passive Cold-Hypoxia-Restraint (C-H-R)
animal model (Ramachandran, et al. 1990) and found to have a strong cumulative endurance promoting activity (Srivastava et al., 1996). CIHP-I intake also alleviated the combat stress induced deteriorations in physical and mental performance of Border Security Force personnel engaged in anti-insurgency operations (Srivastava et al., 1996). The CIHP-I intake acted as an anti-stress and increased performance of animals in adverse environmental situation by increasing glucose turnover rate and fat utilization (Kumar et al., 1999). Another Composite Indian Herbal Preparation-II (CIHP-II) was also tested and found to possess anti-stress and endurance promoting activity (Singh et al., 1978; Srivastava et al., 1995). The mechanism of action of CIHP-II was also investigated and found that its intake conserved glucose utilization, facilitated oxygen delivery system and improved cell membrane permeability (Grover et al., 1995). Further, it was observed that CIHP-II intake helped in generating energy by increased glucose turnover and increased adipose fat mobilization and its myocardial oxidation. It had a carbohydrate sparing effect during stressful situations (Kumar et al., 2000). In a double blind placebo controlled trial, CIHP-II intake for 3 months in army personnel, who stayed and worked at extreme high altitude (4800-6000m) helped in accelerating the acclimatization process and restoring the normal functions in high altitudes (Srivastava et al., 1995). It also improved the mental performance of soldiers engaged in prolonged low-intensity-conflict operations (Gopinathan et al., 1999). Another Composite Indian Herbal Preparation III (CIHP-III) intake, containing herbs that are reputed for having beneficial effect on mental performance, was found to increase memory of animals when tested for avoidance learning of stressful stimuli of electrical shock (Bhardwaj and Srivastava, 1995).

A number of plants growing at high altitude were screened for their hypoxia and cold resistance inducing activity and hypothesized that plants growing in adverse environmental conditions acquires bio-molecules, which help them to sustain in such type of harsh climate. Such bio-molecules, derived from plants, might be of immense importance in improving the human acclimatization in similar conditions (Divekar et al., 1996).
Chapter 1

1.5 Rhodiola – A High Altitude Plant

Rhodiola is a perennial herbaceous plant of the family Crassulaceae and the roots of the plant are used for the medicinal applications. The outer peel of the root has a light golden color, so that the herb is sometimes referred to as golden root, the inside of the root is pink. The yellow flowered taxon of the rose root is mainly found on rocky slopes at 3500-5000m (11000-16000 feet). Rose root has an approximate plant height of 75cm, with the characteristic flower scent of roses, which impart its name. Some taxonomists separated different species of Rhodiola into an independent genus, belonging to the subfamily Sedoidae (Hegi, 1963). Afterwards Rhodiola was reclassified as a subgenus of the larger genus Sedum (Ohba, 1975). The traditional Tibetan use of both sedum and Rhodiola is for lung disease. The pharmacological and medicinal properties of Rhodiola are species dependent phenomena. (Saratikov and Krasnov, 1987).

Rhodiola, also known as “Roseroot,” has a long history of use as an adaptogen and sexual tonic in the traditional medicine. In more recent times, Rhodiola preparations have been listed in the national pharmacopoeias of France, Sweden, Denmark, and the former USSR, as an adaptogen and “brain tonic”. Four decades of animal studies and controlled clinical trials in humans clearly demonstrated that Rhodiola extract is a true adaptogenic botanical.

Rhodiola as a Genus originated in the mountainous regions of Southwest China and the Himalayas, but varied species of genus Rhodiola are distributed in Asia (China, Kazakhstan, Russia Federation), Europe and North America (Canada, United states). There are over 200 different species of Rhodiola that grow throughout Europe, Asia, and Alaska (Kelly, 2001), with over 70 of these in China. At least 20 of these species have been used in Asian traditional medical systems, including Rhodiola alterna (R. alterna), R. brevipetiolata, R. crenulata, R. kirilowii, R. quadrifida, R. sachalinensis, R. sacra, and R. rosea (Kelly, 2001). Of all the Rhodiola species, R. rosea has been the predominant subject of phytochemical, animal, and human studies. (Darbinyan et al., 2000; Saratikov and Krasnov, 1987). Approximately 51 percent of all animal studies and 94 percent of all human studies conducted on Rhodiola are on the species
Chapter I

*R. rosea.* In Chinese medicine, it is used to enhance resistance against fatigue and extend life (Tolonen et al., 2003). This species has been studied intensively in Russia and Scandinavia for over 35 years, and literature from these areas indicate that it has antidepressant, anticancer, cardio-protective, and central nervous system enhancing properties (Kelly, 2001). The phytochemistry of *R. rosea* root has revealed the presence of about 28 compounds classified into 6 distinct groups (Kurkin and Zapesochnaya, 1985) viz. Phenylpropanoids [rosavin, rosin, rosarin (rosavins is the general term for all 3)]; Phenylethanol derivatives (salidroside, tyrosol); Flavanoids (rodolin, rodionin, rodiosin, acetylrodalgin, tricin); Monoterpenes (rosiridol, rosarin); Triterpenes (daucosterol, beta-sitosterol); Phenolic acids (chlorogenic and hydroxycinnamic, gallic acids). Extracts of *R. rosea* root have been found to contain powerful adaptogenic activity and it protected animals and humans against mental and physical stress, toxins, and cold (Saratikov and Krasnov, 1987). A single oral administration of hydro-alcoholic extract of *R. rosea* (containing 3% rosavin and 1% salidroside) significantly induced antidepressant, adaptogenic, anxiolytic and stimulating effects in mice (Perfumi and Mattioti, 2007). Root extract of *R. rosea* increased physical work capacity of normal individuals exposed to maximal work on a bicycle ergometer and also in Olympic-level cross country skiers and athletes (De Bock et al., 2004). *R. rosea* is found to be rich in phenolic compounds and known to have strong antioxidant properties (Bolshakova et al., 1997). In female rats behavioral and physiological changes induced by chronic exposure to mild stress for 6 weeks were strongly inhibited by chronic administration of hydro-alcoholic extract of *R. rosea* containing 3% rosavin and 1% salidroside (Mattioli et al., 2009).

Some of the other Rhodiola species were also studied. *Rhodiola quadrifida,* is traditionally used in Asia as a tonic, adaptogen, antidepressant and anti-inflammatory drug. A study by Zhang et al. (1989) showed that administration of *Rhodiola kirilowii* (regel.) Maxim prevented the human cardiopulmonary function at high altitude (2500 - 4475m). *Rhodiola sacra* radix has been used in Tibetan medicine for promoting circulation and preventing hypertension and cardiovascular diseases. Intravenous injection of water-soluble fraction of *Rhodiola sacra* exhibited dose-dependent
hypotension and increase in heart rate and cardiac contractility in anesthetized Sprague-Dawley rats. (Shih et al., 2008).

In India, high altitude region of Himalayas are well known for their rich sources of quality medicinal plants. The extremely harsh high altitude climatic conditions of hypoxia and cold provide favorable environment for high altitude plants to acquire potential medicinal property. Ladakh has a unique traditional medicinal system called Amch system of medicine and is able to provide cure and relief to more than 60 percent of tribal population using high altitude plant products. The high medicinal and nutritional value of these plants makes the products highly beneficial for local residents as well as for troops deployed at high altitude (Ballabh et al., 2007).

In India the Rhodiola species found in the Western Himalayan regions is *Rhodiola imbricata* Edgew. This plant also belongs to family Crassulacease and subfamily Sedoidae. It is known as *Sedum roseum*. It is also known by names Golden Arctic root, Himalayan Rose root and Shrolo. It is a perennial soft cushion herb with thick rootstocks and small yellow flowers in heads. The plant is found growing on high alpine open moist slopes at 13500-17500 ft of Changthang valley and Indus valley, near Khardumla region of Leh, Jammu and Kashmir. It is an erect, perennial herb ranging from 10-25 cm tall. The leaves are 1.3-3 cm long, lanceolate to elliptic, imbricate, nearly entire, glabrous, acute, and rounded at the base, sessile. Flowers are pale yellow in color with green or often reddish sepals, in a very-dense compact terminal cluster surrounded by involucres of leaves. Rootstock is massive, 2-2.5cm across, without suckers and seeds are reddish, brown, ellipsoids. Flowering and fruiting period of the plant is from June to August. The roots are useful in stress and fatigue, and also restore memory. The Indian Rhodiola species *Rhodiola imbricata* has not been extensively studied. In India the studies on this available plant species were started recently and some of the studied biological properties of *Rhodiola imbricata* are as given below:
Chapter I

Anti-oxidant

Oxidative stress is known to be one of the major causes of different pathological states such as diabetes, aging, arthritis, atherosclerosis, ischemia and reperfusion injuries (Ames et al., 1993). Therefore use of anti-oxidants can significantly reduce the severity and progression of these diseases. Kanupriya et al. (2005) observed that aqueous and alcoholic extracts of *Rhodiola imbricata* roots at a concentration of 250 \( \mu g/ml \) inhibited tert-BHP induced free radical production, apoptosis and also restored the anti-oxidant levels to that of the control cells in U-937 human macrophages. The extracts of *Rhodiola imbricata* possessed potent cytoprotective and antioxidant activities.

Anti-radiation

*Rhodiola imbricata* root has been tested for its radio-protective efficacy. The administration of optimal doses (350mg/kg body weight) of aqueous (RD-I) and aqua-alcoholic (RD-II) extracts of *Rhodiola imbricata* roots 30 min before lethal doses of irradiation (10Gy) significantly increased colony forming units per spleen (17.3 +/- 0.67 and 15.6 +/- 0.61, respectively vs. 1.91 +/- 0.15) (Goel et al., 2006). In another study by Arora et al. (2005) radio-protective efficacy of *Rhodiola imbricata* (REC-7004) (maximally effective dose: 400 mg/kg body weight, intraperitoneal administration) 30min prior to lethal (10 Gy) total body gamma-irradiation in mice rendered 83.3% survival. It was proposed that *Rhodiola imbricata* root extracts renders *in vitro* and *in vivo* radioprotection via multifarious mechanisms and also exhibited significant anti-hemolytic capacity by preventing radiation-induced membrane degeneration of human erythrocytes.

Wound Healing

Wound healing efficacy study of *Rhodiola imbricata* root ethanol extract, at different doses, was carried out and found to augment the healing process (Gupta et al., 2007) by improving rate of wound contraction and decreased time taken for epithelialization. The extract treatment increased cellular proliferation and collagen synthesis at the wound site, as evidenced by the increase in DNA, protein,
hydroxyproline and hexosamine contents in comparison to a positive control treated with povidone-iodine ointment. The results suggested that aqueous root extract of *Rhodiola imbricata* promoted wound healing, due to increased antioxidant levels in the granulation tissue.

**Immune System**

Immunostimulatory potential of aqueous extract of *Rhodiola imbricata* root in human peripheral blood mononuclear cells (PBMCs) and mouse macrophage cell line RAW 264.7 was studied and the extract was found to up regulate immune response in patients with inadequate functioning of the immune system (Mishra et al., 2006), it also showed potent anti-cancer activity, which might be useful in leukemia cancer treatment (Mishra et al., 2008). Aqueous extract of *Rhodiola imbricata* root also stimulated the innate immune pathway in mouse splenocytes (Mishra et al., 2009).

But the studies so far conducted lacked the evaluation of *Rhodiola imbricata* species of Rhodiola for its anti-stress adaptogenic properties. Hence, the proposed study was undertaken to determine the dose dependent adaptogenic bioactivity of *Rhodiola imbricata* root extracts and also to determine the toxicity studies as well as its mechanism of action.