INTRODUCTION AND REVIEW OF LITERATURE

CHAPTER 1

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INTRODUCTION

Stress is defined as the total response of an organism to environmental demands or pressure. It is expressed as an external response that can be measured by changes in glandular secretions, skin reactions and other physical functions or it is an internal interpretation of a reaction or reaction to a stressor or it is both. During the stress response, somewhere in the neighborhood, several biochemical reactions can occur in the body. Neurotransmitters are activated, hormones are released, and nutrients are metabolized. Some body systems (e.g., the cardiovascular system) accelerate their functions and others (e.g., the gastrointestinal system) slow down their operations in response to stress. This is commonly referred to as the fight or flight response. The body is being prepared to expend physical energy, which in prehistoric times was necessary for survival. In modern times most human stress is psychosocial in nature, so the need to respond physically in most cases is unnecessary. Unfortunately the byproducts of the stress response continue to circulate in the body and have the potential to create physical illness (e.g., cortisol secretion's impact on the immune system). Regular exercise is useful in removing the byproducts of the stress response by providing the opportunity to simulate the fighting or running away dictated by the fight or flight phenomenon. As such, regular exercise allows the body to return to homeostasis faster and reduce the physical impact of psychosocial stress. Some key factors about the brain are that adrenaline aids in awareness and memory in the short term. Prolonged exposure of the brain to corticosteroid from continued stress can be very damaging, both decreasing hippocampus function and decreasing cell proliferation rates.

Generally, the body acclimatizes in a variety of ways depending upon the environmental stresses to which it is exposed. Passive stresses are those, which are persistent and relatively invariant (e.g., altitude, climate) and active stresses are those, which are occasionally variable (e.g., exercise, emotions, diet). Reactions to excessive stresses are
modified by the individual attributes of each person. Stress in humans results from interaction between persons and their environment that are perceived as straining or exceeding their adaptive capabilities and threatening their well-being. All systems: the heart and blood vessels, the immune system, the lungs, the digestive system, the sensory organs and brain are modified to meet the perceived danger. The causes of stress can include any event or occurrence that a person considers a threat to his or her coping strategies or resources.

Researchers generally agree that a certain degree of stress is a normal part of living organism's response to the inevitable changes in its physical or social environment, and that positive, as well as negative events can generate stress as well as negative occurrences. The symptoms of stress can be either physical or psychological. Stress related physical illness, such as irritable bowel syndrome, heart attack, and chronic headache result from long term over stimulation of a part of the nervous system that regulates the heart rate, blood pressure and digestive system.

External and internal stressors

People can experience either external or internal stressors. External stressors include adverse physical conditions (such as pain or hot or cold temperatures) or stressful psychological environments (such as poor working conditions or abuse relationships). Humans, like animals can sometimes experience external stressors. Internal stressors can also be physical (infection, inflammation) or psychological. An example of an internal psychological stressor is intense worry about a harmful event that may or may not occur.

Acute and chronic stress

Stressors can be short term (acute) or long term (chronic). Acute stress is the reaction to an immediate threat, commonly known as the fight or flight response.
Common acute stressors include

- Noise
- Crowding
- Isolation
- Hunger
- Infection

Imagining a threat or remembering a dangerous event.

Modern life poses on-going stressful situations that are not short lived and the urge to act (to fight or to flee) must be suppressed.

Common chronic stressors include

- Ongoing highly pressured work
- Long-term relationship problems
- Loneliness
- Persistent financial worries

**Effect of acute and chronic stress**

The part of the hypothalamic-pituitary-adrenal (HPA) system is activated by stress. HPA system triggers the production and release of steroid hormones (glucocorticoids: cortisol). Cortisol is very important in marshaling systems throughout the body (including the heart, lungs, circulation, metabolism, immune systems and skin).

Hypothalamic pituitary-adrenal (HPA) system also releases certain neurotransmitters such as dopamine, norepinephrine and epinephrine. Neurotransmitters signal the hippocampus to store emotionally loaded experience in long-term memory. During the stressful event catecholamines also suppress activity in the frontal areas of brain concerned with short-term memory, concentration, inhibition and rational thought. Heart rate and blood pressure increase immediately. Breathing becomes rapid and lungs take in more oxygen.
Blood flow may actually increase 300% to 400%, priming the muscles, lungs and brain for added demands. Spleen discharges red and white blood cells, allowing the blood to transport more oxygen. The immune boosting troops are sent to the body’s front lines where injury or infection is most likely, such as the skin, the bone marrow, and the lymph node. Stress can cause the spasms of the throat muscles, making it difficult to swallow. Stress shuts down digestive activity. Some evidence suggests that repeated release of stress hormone produces hyperactivity in the hypothalamus-pituitary-adrenal axis and disrupts normal level of serotonin. Stress diminishes the quality of life by reducing feelings of pleasure and accomplishment and relationships are often threatened. Mental stress is a trigger for angina as physical stress. Incidents of acute stress have been associated with a higher risk of cardiac events, such as heart rhythm abnormalities and heart attacks, and death from such events in people with heart disease. Stress activates the sympathetic nervous system and affects heart.

1. Sudden stress increases the pumping action and rate of heart beat and causes the arteries to constrict thereby posing a risk for blocking blood flow to the heart.

2. Emotional effects of stress alter the heart rhythms and pose a risk for various arrhythmias in people with existing heart rhythm disturbances.

3. Stress can increase the likelihood of an artery-clogging blood clot.

4. Stress may signal the body to release fat into the bloodstream, raising cholesterol levels in blood, at least temporarily.

5. In woman, chronic stress may reduce estrogen levels, which are important for cardiac health.

6. Stressful events may cause men and women who have relatively low levels of serotonin to produce more of certain immune system proteins - cytokines, which in high amounts cause inflammation and damage to cells including heart cells. People who regularly experience sudden increases in blood pressure caused by mental stress, develop injuries in
The stress implies any condition that harms the body, damages or causes the death of a few or many cells. The body immediately tries to repair the damaged cells, but it can do so only if the diet is adequate, providing a generous supply of all the essential nutrients. If, however, rebuilding of cells is not able to keep pace with their destruction, the condition will result in disease. The most common diseases associated with stress are heart disease, diabetes, head-ache, peptic ulcer, ulcerative colitis, chronic dyspepsia, asthma, psoriasis and sexual disorders. Stress induced homeostatic changes and immune reduction tends to affect the balance between oxidants and antioxidants in the body. Any alteration in this balance in favor of antioxidants may result in pathological responses causing functional disorders and diseases such as cancer and Alzheimers disease. It can also accelerate aging process.

Stress and health are closely linked. It is well known that stress; either quick or constant can induce risky body and mind disorders. Immediate disorders such as dizzy spells, anxiety, tension, sleeplessness and muscle cramps can all result in chronic health problems and it may also affect our immune, cardiovascular and nervous system.

It is observed that the deleterious effects of stress in the body are brought about by alteration taking place in the concentration of various body parameters. Some of the ways by which stress can be induced experimentally include: extreme temperatures, constriction, handling (of the rats), physical exertion (forced swimming), vibration, and isolation. The changes taking place in the tissue parameters can best be studied in animal models. It is considered important to study the alteration in the metabolism of lipids and also of free radicals in variously stressed animals.
1.0. REVIEW OF LITERATURE

1.1. STRESS

Stress is an inherent part of daily living. Stress in the modern world has become a part of our life style. Prolonged stress can overwork many organ systems, especially the heart, blood vessels, adrenals and immune system. When stress is over-whelming, the response may be general depression, low blood pressure and low heart rate, increased cortisol or decreased sex hormone secretions. Failure to cope with stress can lead to stress related disorders such as head ache, hypertension, heart disease, stroke and ulcers. Stress is a threat or challenge to the integrity and survival of the organism (Herbert Weiner et al, 1991). Stress is an adaptive response that prepares the organism for a threatening situation. It induces strain upon both emotional and physical endurances, which has been considered a basic factor in the aetiology of number of diseases --- cardiovascular diseases, cancer, diabetes mellitus, etc (Halliwell et al 1984). Stressful condition leads to the formation of excessive free radicals which is a major internal threat to cellular homeostasis of aerobic organisms (Yu, 1994). Free radicals are formed in the human body both in physiological and pathological conditions in cytosol, mitochondria, lysosomes, peroxisomes and plasma membranes (Hemnani et al 1988). Stress causes biochemical changes in the body that can raise blood glucose levels. A hormone released from the adrenals causes the liver to convert amino acids into glucose for the raised energy levels needed to deal with the demands of stress. “Stress response” describes the condition caused by a person's reaction to physical, chemical, emotional or environmental factors. Stress can refer to physical effort and mental tension. It's hard to define a high level of emotional or psychological stress to measure in a precise way. All people feel stress, but they feel it in different amounts and react to it in different ways.
More and more evidence suggests a relationship between the risk of cardiovascular disease and environmental and psychosocial factors. These factors include job strain, social isolation and personality traits. But, more research is needed to understand how stress contributes to heart disease risk. Clinical observations continue to suggest that hypertension may be an apparent cause for heart failure in both men and women (Kannel et al, 1996). Acute and chronic stress may affect other risk factors and behaviors, such as high blood pressure and cholesterol levels, smoking, physical inactivity and overeating. The detrimental effects of stress on the cardiovascular system have been documented through research in animal models and humans. Primarily two systems mediate the stress response, by exerting an acute influence on cardiovascular function: the hypothalamic-pituitary-adrenocortical (HPA) axis and the sympat-ho-adrenomedullary system (SAS). Individuals with confirmed cardiovascular disease or its risk factors respond differently to these two systems. Although humans are physiologically equipped to respond to acute stressors, chronic stress disrupts the HPA axis and the SAS, resulting in harmful effects on human health. An exercise-induced increase in myocardial heat shock proteins is a potential mechanism to explain the cardioprotection associated with exercise. A recent study indicates that exercise training in a cold environment provides cardioprotection during I-R (ischemia reperfusion) injury but does not elevate myocardial levels of heat shock proteins (Hamilton et al., 2001).

Briefly, in response to a stress, the hypothalamus releases corticotropin-releasing hormone (CRH). In turn, CRH acts on the pituitary gland triggering the release of another hormone, adrenocorticotropin (ACTH) into the bloodstream. Next, ACTH signals the adrenal glands to release a number of hormonal compounds. These compounds include epinephrine, norepinephrine and cortisol. All three hormones enable the body to respond to a threat. Epinephrine increases blood pressure and heart rate, diverts blood to the
muscles, and speeds reaction time. Cortisol, also known as glucocorticoid, releases sugar (in the form of glucose) from the body reserves so that this essential fuel can be used to power the muscles and the brain. Normally, cortisol also exerts a feedback effect to shut down the stress response after the threat has passed, acting upon the hypothalamus and causing it to stop producing CRH. This stress circuit affects systems throughout the body. The hormones of the HPA axis exert their effect on the autonomic nervous system, which controls such vital functions as heart rate, blood pressure, and digestion.

1.2. **Hormonal changes during stress**

1.2.1. **Release of Steroid Hormones.**

The HPA systems trigger the production and release of steroid hormones (glucocorticoids), including the primary stress hormone cortisol. Cortisol is very important in marshaling systems throughout the body (including the heart, lungs, circulation, metabolism, immune systems, and skin).

1.2.2. **Release of Catecholamines.**

The HPA system also releases the neurotransmitters catecholamines, particularly dopamine, norepinephrine, and epinephrine Catecholamines activate the amygdala, which apparently triggers an emotional response to a stressful event. Neurotransmitters then signal the hippocampus to store the emotionally loaded experience in long-term memory. In primitive times, this combination of responses would have been essential for survival, when long-lasting memories of dangerous stimuli would be critical for avoiding such threats in the future. In post-traumatic stress disorder (PTSD), which is triggered by uncontrollable stress, medial prefrontal cortex activity is reduced. Proposing an analogous mechanism, Maier (Maier et al, 2005) speculated that loss of inhibition from the medial prefrontal cortex might explain increased activity of the amygdala in PTSD.
1.3. Effects of stress on reproductive system

Stress suppresses the reproductive system at various levels. For example, stress hormones inhibit the testes and ovaries directly, hindering production of the male and female sex hormones - testosterone, estrogen, and progesterone. Psychosocial stress influences fertility in females (Sanders, 1987). First, CRH prevents the release of gonadotropin releasing hormone (GnRH), the hormone that signals a cascade of hormones that direct reproduction and sexual behavior. Similarly, cortisol and related glucocorticoid hormones not only inhibit the release of GnRH, but also the release of luteinizing hormone, which promotes ovulation and sperm release.

1.4. The gastro-intestinal tract and stress

Stress can result in digestive problems (Mark G. Swain, 2000). Stress hormones directly hinder the release of stomach acid and emptying of the stomach and can directly stimulate the colon, speeding up the emptying of its contents. Stress is an ever-present part of modern life. The "stress response" constitutes an organism's mechanism for coping with a given stress and is mediated via the release of glucocorticoids and catecholamines. The influence of stress on the clinical course of a number of intestinal diseases is increasingly being recognized (Johan et al, 1998). This article focuses on recent findings related to the effects of stress on mucosal barrier function in the small intestine and colon. Experiments using animal models demonstrate that various types of psychological and physical stress induce dysfunction of the intestinal barrier, resulting in enhanced uptake of potentially noxious material (e.g., antigens, toxins, and other proinflammatory molecules) from the gut lumen. Prolonged stress can disrupt the digestive system, irritating the large intestine and causing diarrhoea, constipation, cramping, and bloating. Excessive
production of digestive acids in the stomach may cause a painful burning. Stress has become an etiological factor in the development of gastric ulcers (Das et al, 1997)

1.4.1. Peptic Ulcers.

Stress-related mucosal disease is common in critically ill patients and can result in significant morbidity (Duerksen et al, 2003). Studies still suggest that stress may predispose someone to ulcers or sustain existing ulcers (Brodie et al, 1960).

1.4.2. Inflammatory Bowel Disease.

Chronic stress seems to impair the immune system's capacity to respond to glucocorticoid hormones that normally are responsible for terminating an inflammatory response following infection or injury (Gregory E. Miller, 2002)

1.5. The Immune System's Response to Acute Stress

The steroid hormones dampen parts of the immune system, so that infection fighters (including important white blood cells) or other immune molecules can be redistributed. These immune-boosting troops are sent to the body's frontlines where injury or infection is most likely, such as the skin, the bone marrow, and the lymph nodes. Stress interacts with the immune system (Gregory E Miller, 2002), making more vulnerable to colds and flu, fatigue and infections. In addition, the high cortisol levels resulting from prolonged stress could serve to make the body more susceptible to disease by switching off disease-fighting white blood cells. Conversely, too little corticosteroid, can lead to a hyperactive immune system and increased risk of developing autoimmune diseases. Breast cancer patients who feel high levels of stress concerning their diagnosis and treatment show evidence of a weakened immune system compared to patients experiencing less stress. A preliminary study (Felicia et al, 1995) found that the highly stressed women had lower levels of natural killer cells than women who reported less stress. However, the immune system is a highly specialized network whose activity is
affected not only by stress but also by a number of other factors. It has not been shown that stress-induced changes in the immune system directly cause cancer. Chronic stress not only makes people more vulnerable to catching illnesses but can also impair their immune system's ability to respond to its own anti-inflammatory signals that are triggered by certain hormones, say researchers, possibly altering the course of an inflammatory disease (*Health Psychology*, published by the American Psychological Association - APA, 1998). It's no surprise that being diagnosed with cancer is stressful. A more interesting problem for many researchers (Beth Azar, 1998) is whether heightened stress can increase a person's susceptibility to cancer or worsen the prognosis of a person with cancer.

1.6. The Brain's Response to Acute Stress

The stress system orchestrates body and brain responses to the environment. Corticosteroid hormones secreted by the adrenal cortex are implicated in both modes through their high affinity type 1 (mineralocorticoid receptors - MR) and lower affinity type 2 (glucocorticoid receptors - GR) receptors that are co-localised in limbic neural circuitry (De Kloet. et al 2003). MR controls in specific afferents the threshold or sensitivity of the fast CRH-1 driven stress system mode and thus prevents disturbance of homeostasis, while GR facilitates its recovery by restraining in these very same circuits stress responses and by mobilizing energy resources. In preparation for future events GR facilitates behavioural adaptation and promotes storage of energy. The balance in the two stress system modes is thought to be essential for cell homeostasis, mental performance and health. Imbalance induced by genetic modification or chronic stressors changes specific neural signalling pathways underlying psychic domains of cognition and emotion, anxiety and aggression. This Yin-Yang stress concept is fundamental for
genomic strategies to understand the mechanistic underpinning of cortisol-induced stress-related disorders such as severe forms of depression and co-morbid diseases.

Rats exposed to uncontrollable stress develop learned helplessness, a syndrome similar to depression and post traumatic stress disorder (PTSD) (Amat et al, 2005). They lose the ability to learn how to escape stressors. Activation of a brain stem area (dorsal raphe nucleus) has been implicated in such reactions. But this area is too small and lacks the proper sensory inputs to judge whether a stressor is controllable. Many of its inputs come conspicuously from the mid-prefrontal cortex area (medial prefrontal cortex), seat of higher order functions, such as problem solving and learning from experience. These signals are sent via the chemical messenger, serotonin, which is involved in mood regulation and in mediating the effects of the most widely prescribed antidepressants. In response to seeing the stress, a part of the brain hypothalamic-pituitary-adrenal (HPA) system is activated. An increased activity of serotonergic neurons in the brain is an established consequence of stress. An increase in brain tryptophan levels on the order of that produced by eating a carbohydrate-rich/protein-poor meal causes parallel increases in the amounts of serotonin released into synapses (Takeda et al, 2004). Eating is suppressed during stress, due to anorectic effects of corticotrophin releasing hormone, and considered to be increased during recovery from stress, due to appetite stimulating effects of residual cortisol.

1.7. Response by the Heart, Lungs, and Circulation to Acute Stress

Breathing becomes rapid and the lungs take in more oxygen and blood flow may actually increase 300% to 400%, priming the muscles, lungs, and brain for added demands. The spleen discharges red and white blood cells, allowing the blood to transport more oxygen.
1.8. The Acute Response in the Mouth and Throat

As the threat increases, fluids are diverted from nonessential locations, including the mouth. This causes dryness and difficulty in talking. In addition, stress can cause spasms of the throat muscles, making it difficult to swallow.

1.9. The Skin's Response to Acute Stress

The stress effect diverts blood flow away from the skin to support the heart and muscle tissues. The physical effect is a cool, clammy, sweaty skin. The scalp also tightens so that the hair seems to stand up.

1.10. Metabolic Response to Acute Stress

Stress shuts down digestive activity, a nonessential body function during short-term periods of physical exertion or crisis.

1.11. The Relaxation Response: the Resolution of Acute Stress

Once the stress has passed and the effect has not been harmful the stress hormones return to normal. This is known as the relaxation response. In turn, the body's systems also normalize.

Stress-related conditions that are most likely to produce negative physical effects include: an accumulation of persistent stressful situations, particularly those that a person cannot easily control (for example, high-pressured work plus an unhappy relationship), persistent stress following a severe acute response to a traumatic event (such as an automobile accident), an inefficient or insufficient relaxation response, acute stress in people with serious illness, such as heart disease.

1.12. Stress and cardiovascular system

The detrimental effects of stress on the cardiovascular system have been documented through research in animal models and humans. Primarily two systems
mediate the stress response, by exerting an acute influence on cardiovascular function: the hypothalamic-pituitary-adrenocortical (HPA) axis and the sympatho-adrenomedullary system (SAS). Individuals with confirmed cardiovascular disease or its risk factors respond differently to these two systems. Although humans are physiologically equipped to respond to acute stressors, chronic (longtime) stress disrupts the HPA axis and the SAS, resulting in harmful effects on human health. Moreover, cross-sectional (and, to a lesser extent, longitudinal) epidemiological data show that chronic job stress and cardiovascular reactivity in response to stress are associated with hypertension, coronary heart disease, and stroke (Kenneth et al, 1976). However, the specific physiological and behavioral mechanisms, as well as the degree of cardiovascular risk attributable to chronic stress, remain poorly understood. Besides the mobilizing of our body for flight, the chronic stress increased flow of adrenaline produces a number of other less helpful and more serious consequences.

1.12.1. Biochemical changes:

- An increase in the production of blood cholesterol
- A narrowing of the capillaries and other blood vessels that can shut down the blood supply to the heart muscle.
- A decrease in the body's ability to remove cholesterol
- An increase in the blood's tendency to clot
- An increase in the depositing of plaque on the walls of the arteries

1.12.2. Stress related cardiovascular system disorders

1. High blood pressure
2. Atherosclerosis
3. Heart attacks
4. Strokes
5. Angina pectoris
6. Myocardial infarction

Acute or chronic psychological stress could induce an acute phase response and subsequently a chronic inflammatory process such as atherosclerosis (Black, 2003). Stress can induce an acute phase response and inflammation, has been extended to include a chronic inflammatory process, characterized by the presence of certain cytokines and acute phase reactants (APR), which is associated with certain metabolic diseases. The loci of origin of these cytokines, particularly interleukin 6 (IL-6), and their induction, have been considered. Evidence is presented that the liver, the endothelium, and fat cell depots are the primary sources of cytokines, particularly IL-6, and that IL-6 and the acute phase protein, C-reactive protein (CRP), are strongly associated with, and likely play a dominant role in the development of this inflammatory process. This process leads to insulin resistance, non-insulin dependent diabetes mellitus type II, and metabolic syndrome X. The possible role of psychological stress and the major stress-related hormones as etiologic factors in the pathogenesis of these metabolic diseases, as well as atherosclerosis, is discussed. The fact that stress can activate an acute phase response, which is part of the innate immune inflammatory response, is evidence that the inflammatory response is contained within the stress response or that stress can induce an inflammatory response. The evidence that the stress, inflammatory, and immune systems all evolved from a single cell, the phagocyte, is further evidence for their intimate relationship which almost certainly was maintained throughout evolution. Mental stress is as major a trigger for angina as physical stress. Incidents of acute stress have been
associated with a higher risk for serious cardiac events, such as heart rhythm abnormalities and heart attacks, and even death from such events in people with heart disease.

Stress activates the sympathetic nervous system (the autonomic part of the nervous system that affects many organs, including the heart). Such actions and others may negatively affect the heart in several ways. Sudden stress increases the pumping action and rate of the heart and causes the arteries to constrict, thereby posing a risk for blocking blood flow to the heart. Emotional effects of stress alter the heart rhythms and pose a risk for serious arrhythmias in people with existing heart rhythm disturbances. Stress causes blood to become stickier (possibly in preparation of potential injury), increasing the likelihood of an artery-clogging blood clot. Stress may signal the body to release fat into the bloodstream, raising blood-cholesterol levels, at least temporarily. In women, chronic stress may reduce estrogen levels, which are important for cardiac health.

Stressful events may cause men and women who have relatively low levels of the neurotransmitter serotonin (and therefore a higher risk for depression or anger) to produce more of certain immune system proteins (called cytokines), which in high amounts cause inflammation and damage to cells, including possibly heart cells (Rabin, 1999).

Recent evidence confirms the association between stress and hypertension. People who regularly experience sudden increases in blood pressure caused by mental stress may, over time, develop injuries in the inner lining of their blood vessels.

1.3. Responses to Stressful Life Events

A person is not only in constant interaction with the world around him, but also in continuous contact with his inner reality. Human responses to life stress of diverse nature
can, therefore, be divided into four groups: psychological, physiological, social, and spiritual.

**TABLE I- Physiological response- Adaptive and maladaptive responses**

**Release of hormones:**
- ACTH, Endorphins, Norepinephrine...

- **Cardiovascular: Changes**
  - Adaptive — in pulse, respiration, blood pressure...
  - Central nervous system and other systemic changes

- **Physiological Response**
  - Bronchial
  - Hypertension
  - Maladaptive — Peptic ulcer
    - Angina, other coronary heart diseases
    - Migraine

Stress can be considered as a state of disharmony or threatened homeostasis. Stress condition varies and can range from physical to psychological, from mild to severe and from acute to chronic (Vogel, 1993 and Selye, 1950). An animal or organism is said to be in a state of stress, if it is required to make some abnormal or extreme adjustments in its
physiology or behaviour in order to cope with adverse aspects of its environment as quoted (Frazer et al, 1990). Adrenocorticotropic hormone (ACTH) is released by anterior pituitary during stress bringing about the release of corticosteroids as cortisol and cortisone resulting in a relative decrease in carbohydrate metabolism, an increase in protein metabolism and mobilization of fat deposits. Stress research in the laboratory animals has assumed an important role in the biological and psychological sciences over the past decade due to the view that stressful stimulus may influence the onset and progression of a number of diseases in human beings leading to hypertension, stroke, depression, etc. Cardiovascular response during and after psychological stressful situation has been frequently investigated with an objective to correlate behavioral and physiological aspects of stress (Koolhas, 1991). Stress, age and behavioral characteristics are considered to be risk factors for disturbances of the cardiovascular system in animal and man. It has been suggested that chronic stress can contribute to the development or exacerbation of cardiovascular dysfunction. Previous studies have revealed significant interaction between individual responsiveness to changing environment and susceptibility for high blood pressure leading to hypertension in chronically stressed rats [Henry et al, 1981 and De Quattro et al, 1985, and Muldoon et al, 1995]. Psychological stress affected cardiovascular and adrenal physiology in five different types of rat strains (Henry et al, 1993). Stress is known to induce more secretion of epinephrine and norepinephrine from adrenal medulla (Axelrod et al, 1984). The body and mind react to any stress factor. A large number of physical changes take place at the time of stress, stress induced nervous system become intensively active, the pupils of the eye dilate, digestion slows down muscles become tense, the heart start pumping blood harder and faster, hormones such as adrenaline are released into the system along with glucose from the liver and sweating starts. Stress implies an ability to withstand a defined amount of strain. Dr. Hands Selye
(Hands Selye, 1956) described stress as a state manifested by specific syndrome, which consists of all the non-specifically induced changes within a biological system. The term implies any condition that harms the body and damages or causes the death of a few or many cells. The body immediately tries to repair the damaged cells but it can do so only if the diet is adequate, providing a generous supply of all the essential nutrients. If however, rebuilding of cells is not able to keep pace with destruction, the condition will result in diseases, such as diabetics, headache, peptic ulcer and ulcerative colitis, chronic dyspepsia, asthma, psoriasis and sexual disorders. Reaction to stress is manifold. No one situation is stressful to all the people all the time. Stress is a pervasive factor in everyday life that critically affects development and functioning. Severe and prolonged stress exposure impairs homeostatic mechanisms, particularly associated with the onset of depressive illness. An increased activity of serotonergic neurons in the brain is an established consequence of stress. An increase in brain tryptophan levels on the order of that produced by eating a carbohydrate-rich/protein-poor meal causes parallel increases in the amounts of serotonin released into synapses (Takeda, 2004). Eating is thought to be suppressed during stress, due to anorectic effects of corticotrophin releasing hormone, and increased during recovery from stress, due to appetite stimulating effects of residual cortisol. A short single experience of stress can have long-term consequences for the animal’s stress responsivness and behaviour (Sutantowet et al, 1994). One predominant feature of chronic stress is the finding that repeated stress leads to adaptation or habituation. Chronic or repeated stress can cause a wide range of physiological and neuroendocrine changes (Naleson et al, 1988). Swimming in small laboratory animals has been widely used for studying the physiological changes and capacity of the organism in response to stress (Tan et al, 1988 and Greenen et. al., 1988). The amount of work done during swimming exercise is far greater than that during excercise of identical time
duration. Forced swimming is not always a simple exercise stress, because emotional factors are difficult to be eliminated (Kramer et al., 1993). The forced swimming stress developed by Porsolt (Porsolt et al., 1977) has now become a widely accepted model for studying the physical stress in animals. Water temperature is another factor in the forced swimming test. By varying the temperature Richter (Richter et al., 1957) found that rats could survive as long as 80 hours in lukewarm water (36°C). Increase or decreasing the temperature above/ below this point influence the overall behaviour of the animal and changes the involvement of glucocorticoids (Abel et al., 1991). Enzyme activities after swimming were studied (Di Simplicio et al, 1997) and the enzyme activities found to be modified in a complex way. A decrease in the enzyme activity observed in the adductor muscle seem to confirm the sensitivity of this organ to overproduction of reactive oxygen species, oxidized glutathione (GSSG) decrease observed in blood was a new and unexpected findings, one that indicates a very prompt adaptation of red cells to increased oxidant stress. Effect of immobilization stress was also studied (Hemlata et al, 2004). The forced immobilization stress enhances lipid profiles (Sighal et al,1997). Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameter in broilers were studied (Altan et al, 2003). Catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities and MDA concentration were increased. Cows exposed to moderate heat stress (39.52°C) due to summer temperature, show high erythrocyte SOD, GSH, GPx and TABRS, indicating a condition of oxidative stress in summer transition cows (Bernabuci et al 2002). Ronchi and Trout (Ronchi et al1999 and Trout et al, 1999 ) reported no effects of heat stress on plasma concentration of vitamin E and β-carotene or on muscle content of thiobarbituric acid reactive substances. Calamari [Calamari et al, (1999)] observed weak negative effects of heat stress on some plasma markers of oxidative status in midlactating cows.
The body and mind react to stress factor. A large number of physical changes take place at the time of stress-induced arousal. The brain and nervous system become intensively active, the pupils of the eye dilate, the digestion slows down, muscle become tense the heart starts pumping harder and faster, hormones such as adrenaline are released into the system along with glucose from the liver and sweating starts. All theses changes take place in a split second under the direction of nervous system. If the stress factors are immediately removed no harm occurs and all the changes are reversed. Stress in earlier stage leads to poor sleep, bad temper, continual grumbling, longer hours of work with lesser achievement, domestic conflict with spouse and children, repeated minor sickness, absenteeism and prolonged absence of each spell of sickness, accident proneness, feeling of frustration and persecution by colleagues and complaints of lack of cooperation and increase in alcohol intake. Harmon (Harmon et al, 1997) reported a reduction of antioxidant activity of plasma in midlactating heat-stressed Holstein cows.

1.4. **Stress response**

Stress may be caused by a variety of factors both outside the body and within. External factors include loud noises, blinding lights, extreme heat or cold, X rays and other forms of radiation, drugs, chemicals, bacteria and other toxic substances, and pain and inadequate nutrition. Stress is known to worsen many immune related medical conditions, including diabetes. Cortisol produced during stress situations may suppress the body’s immune response and increasing susceptibility to infectious diseases.

Stress has become an etiological factor in the development of gastric ulcer during experimental procedures (Piere et al, 1998, Brodie et al, 1960) found rats highly susceptible to restraint stress and noted ulceration of gastric mucosa. Bharihole, (Bharihole et al, 2000) subjected their experimental animals to stress of immobilization and cold in a plexi glass container for 2 hours for five days. Sanchez (Sanchez et al,1996)
studied the restrained stress in rats and noted a rise in cortisol level. Francis (Francis et al, 1979) established the serum cholesterol level as an index of stress. Use of temporary immobilization in small plastic devices as stress induced in rats has been commonly employed in their study (Coscum et al, 1995 and Brodie et al, 1960 and Brennan et al, 1996 and Bharihoke et al, 2000). This stress was sufficient as evidenced by production of gastric ulcer (Brodie et al, 1960). Varley (Varley et al, 1992) reported higher values of serum cholesterol in men as compared to that of women. Vyas (Vyas et al, 1992) noted a rise of 20% cholesterol level in pregnant women as compared to that of nonpregnant women. Various theories have been propounded to explain this rise of serum cholesterol. Patterson (Patterson et al, 1993) are of the view that increased cholesterol synthesis in immobilization stress is due to hypovolemia, while Koob (Koob, 1985) associated stress response with hyperinsulinemia and increased cholesterol synthesis, (Alveraz et al, 1989 and Mayer et al, 1988). The forced immobilization stress enhances significant increase in heart weight was observed after the rats were exposed to stress (Horie et al, 1991 and Gelsema et al, 1992). Stress is known to induce more secretion of epinephrine and norepinephrine from adrenal gland (Axelord et al, 1984). These hormones acting on heart exert positive influence on the force of contraction (inotropism) which gradually might have caused the cardiac hypertrophy. [During stress there is uniform arousal of both the fight-flight sympatho-adrenal and pituitary-adrenal cortical systems. These two systems acting together participate in the stress response of an organism. Increased heart rate and force of cardiac contraction is considered as the immediate response of the organism to stress (Herd et al, 1991). Several workers reported tachycardia as a response to both acute and chronic stress (Gomez et al, 1989 and Vike et al, 1968). Vasopressin (VP) stimulates pituitary ACTH secretion through interaction with receptors of the V1b subtype (V1bR, V3R), located in the plasma membrane of the pituitary corticotroph, mainly by
potentiating the stimulatory effects of corticotropin releasing hormone (CRH). Chronic stress paradigms associated with corticotroph hyper-responsiveness lead to preferential expression of hypothalamic VP over CRH and upregulation of pituitary V1bR, suggesting an important role for VP during adaptation of the hypothalamic-pituitary-adrenal (HPA) axis to stress. (Volpi et al, 2004)

"Stress response" describes the condition caused by a person's reaction to physical, chemical, emotional or environmental factors. Stress can refer to physical effort and mental tension. It's hard to define a high level of emotional or psychological stress to measure in a precise way. All people feel stress, but they feel it in different amounts and react to it in different ways. More and more evidence suggests a relationship between the risk of cardiovascular disease and environmental and psychosocial factors. These factors include job strain, social isolation and personality traits. But more research is needed on how stress contributes to heart disease risk. It is not known whether stress acts as an "independent" risk factor for cardiovascular disease. Acute and chronic stress may affect other risk factors and behaviors, such as high blood pressure and cholesterol levels, smoking, physical inactivity and overeating. The experience of stress affects cellular immunity, an important aspect of many medical problems, including controlling/curing cancer and the immunobiology of autism. Treating disease with immunological components means also treating and managing psychological stress. Free radicals play an important role as mediators of skeletal muscle damage and inflammation after strenuous exercise. Simmons (Simmon et al, 1990) reported a decrease in glutathione levels in mice on exposure to cold.

. Human immune function is mediated by the release of cytokines and nonantibody messenger molecules from a variety of cells of the immune system and from other cells such as endothelial cells. There are Th1 and Th2 cytokines. Autoimmune and
allergic diseases involve a shift in the balance of cytokines toward Th2. The autoimmune aspect of autism has been related to excessive Th2 cytokines resulting, in part, from vaccination. Gulf War syndrome and asthma have been similarly linked to excess immunization in the presence of increased environmental toxins and pollutants (high antigenic load). Cytokines stimulate cellular release of specific compounds involved in the inflammatory response. Stress-induced activation of the sympathetic nervous system and the sympathetic-adrenal medullary and hypothalamic-pituitary adrenal axes lead to the release of cytokines (Rabin et al, 1999). Blocking the response of the sympathetic nervous system by pre-treating subjects in stressful experiments with adrenergic antagonists can reduce this release of cytokines and decrease the resulting inflammatory response (Bachen et al, 1994 and Benschop et al, 1994). Discrete areas of the brain (for example, the hypothalamus and the locus coeruleus) regulate the sympathetic nervous system and therefore the levels of circulating adrenergic stress hormones, thereby influencing the activity of the immune system (Wetmore et al, 1991 and Rassnick et al, 1994). Adrenergic stress hormones alter the synthesis and release of cytokines by white blood cells. The effects of stress on immunity has been experimentally studied in animals. The stress of crowding prior to and following tuberculosis infection affects the outcome of the infection in mice (Tobach et al, 1956). Social disruption in mice causes reactivation of latent herpes simplex virus (Padgett et al, 1998). Stress enhances the reactivation of latent herpes viruses including the Epstein-Barr virus in humans (Glaser et al, 994). Psychological stress inhibits many aspects of the immune response including innate immunity (eg, natural killer cell lysis), T-cell responses, and antibody production (Rabin et al, 1999). Outside of proven clinical interventions, there is reason to think that certain changes in lifestyle might increase host resistance to infectious diseases.
1.5. Oxidative stress and free radicals

Oxidative stress resulting from increased production of free radicals and reactive oxygen species, and a decrease in antioxidant defense, leads to damage of biological macromolecules and disruption of normal metabolism and physiology (Trivisan et al, 2001). When reactive forms of oxygen are produced faster than they can be safely neutralized by antioxidant mechanisms, oxidative stress results (Sies, 1991).

Free radical or reactive oxygen species (ROS) were formed by various biochemical reactions. It was towards the end of the 18th century that oxygen emerged as the paragon among the elements that sustained life, promoted physical health and stimulated mental vigor. But too much of even the best is bad and it is known that oxygen in high concentration can damage the brain, lungs and other organs. The phenomenon of oxygen toxicity in early days referred as the toxic effects of oxygen at high pressure. Free radicals are an unstable and extremely reactive chemical species, which have an unpaired electron in their structure. The most important free radicals are the by products of energy generation and are formed during oxidation, such as that occurs in the electron transport chain.

The term reactive oxygen species (ROS) collectively describes free radicals such as superoxide radicals (O$_2^-$), hydroxyl radical (OH$^-$), and hypochlorous acid (HOCl). These reactive oxygen intermediates form in reactions, which give rise to free radicals species. Unstable free radical species attack cellular components, causing damage to lipids, proteins and DNA, which can initiate a chain of events which results in the onset of diseases. Oxidative stress results when the balance between the reactive oxygen species overrides the antioxidant capability of the target cells. ROS may interact with and modify cellular proteins, lipids and DNA, which results in altered target cell function.

Free radicals are extremely reactive. Their half-life is only a few milliseconds. When a
radical reacts with a new compound more free radicals are generated. This chain reaction leads to thousands of events. Peroxidation of polyunsaturated fatty acids (PUFA) in the plasma membrane results in the inhibition and loss of membrane functions such as absorption, secretion, inhibition of protein and enzyme synthesis and indirectly cause cell death.

1.6. Free radicals can originate in various ways

Biochemical redox reactions involving oxygen, which occur as a part of normal metabolism. Eg. $O_2$, NO, $H_2O_2$. 

By phagocytosis as a controlled inflammatory reaction. Eg. $HOCl$. Occasionally in response to exposure to ionizing radiations, UV light, environmental pollution, cigarette smoke, stress, hyperoxia, excessive exercise and ischemia eg $O_2'$, $OH'$, and $ROO'$

Many free radicals and ROS have been implicated in disease development. OH is a highly reactive radical, which can travel in the blood and attack a number of biological targets. $O_2$ can also act as a vasodilator and may have a role in intracellular signaling and growth regulation. NO act on smooth muscle cells in vessel wall causing relaxation. $H_2O_2$ crosses cellular membrane easily and may cause alteration in the expression of virus gene in infected cells. Eg HIV. This ROS has only cellular targets but can result in the production of hydroxyl radicals. It is now widely believed that stress play important role in the development of free radicals and diseases.

1.7. Biologically relevant ROS

Superoxide radical
Hypochlorous acid
Hydrogen peroxide
Hydroxy radical
Though all classes of biomolecules may be attacked by free radicals, lipids are the most susceptible. Human cells are rich in polyunsaturated fatty acids (PUFA) and hence are readily attacked by oxidizing radicals by a process known as lipid peroxidation, which is a highly damaging self-perpetuating chain reaction.

1.7. The antioxidant system

Body possesses a number of mechanisms both to control the production of ROS and to limit or repair the damaged tissues. The integrated antioxidant system consists of (i) preventive antioxidants, which prevent the formation of new ROS [ceruloplasmin (Cu), transferrin (Fe), ferritin (Fe) and myoglobin (Fe)]. (ii) Scavenging antioxidants which remove ROS once formed, thus prevent free radical chain reaction [e.g., superoxide dismutase (SOD), catalase (CAT) glutathione peroxidase (GPx) and glutathione reductase]. (iii) Repair enzymes, which repair or remove ROS-damaged biomolecules (e.g., DNA repair enzymes and methionine sulfoxide reductase). The first defense against ROS is mainly by the antioxidant enzymes. By the combined action of these enzymes, the free radicals are removed very effectively.

1.7.1. Superoxide dismutase

Superoxide dismutase (SOD) is a metalloenzyme that convert superoxide radical to hydrogen peroxide.

\[ \text{O}_2^+ + \text{O}_2^- + 2\text{H} \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]
SOD is most important enzyme because it is found virtually in all aerobic organisms. SOD is found in four different isoforms: copper dependent (Cu-SOD), copper-zinc dependent (Cu-Zn-SOD), Manganese dependent (Mn-SOD) and iron dependent (Fe-SOD). Human SOD is the Cu-Zn-SOD. The transition metals of the enzyme react with oxygen radical by abstracting its electron (Oberely and Oberley, 1984). The only known substrate for SOD is superoxide radical, which is converted to hydrogen peroxide by the action of the enzyme.

1.7.2. Catalase

Catalase also serves as a free radical scavenging enzyme. It is present in almost all the cells especially in erythrocytes. Catalase catalyses the decomposition of hydrogen peroxide to water and oxygen.

\[ 2H_2O_2 \rightarrow 2H_2O + O_2 \]

The toxic hydrogen peroxide formed by the action of SOD on superoxide radical is converted to water by catalase. Catalase is a haeme containing protein and is found to act 104 times faster than peroxidases. It is localized mainly in mitochondria and in subcellular respiratory organelle (Pryor et al, 1986). Catalase is also present in peroxisomes and cytosol.

1.7.3. Glutathione peroxidase

Glutathione peroxidase (GPx) is another well known enzyme defense against oxidative stress, which in turn requires glutathione as cofactor. Among the many functions of glutathione it is involved in the generation of nucleotide precursors of DNA via the reduction of ribonucleotides to deoxyribonucleotides (Meister, 1994). Gpx catalyses the oxidation of GSH to GSSG at the expense of hydrogen peroxides.

\[ ROOH + 2GSH \rightarrow ROH + H_2O + GSSG. \]
The reverse reaction is catalysed by the glutathione reductase (GR) to retain the reduced glutathione.

\[
\text{NADPH} + \text{H}^+ + \text{GSSG} \rightarrow \text{NADP}^+ + 2\text{GSH}
\]

The combined action of these enzymes the oxidative stress induced by free radicals has been eliminated. Other antioxidant molecule such as glutathione, albumin, bilurubin and uric acid also found to defense against the oxidative stress induced by free radicals or ROS.

**1.7.4. Glutathione-S-transferase**

Glutathione-S-transferase (GST) utilizes glutathione in reaction contributing to the transformation of a wide range of compounds and products of oxidative stress. GST act by catalyzing the reaction of glutathione with an acceptor molecule to form an S-substituted glutathione. R is an electrophilic xenobiotic.

\[
\text{R} + \text{GSH} \rightarrow \text{R-S-G}
\]

**1.7.5. Reduced glutathione**

Glutathione a cofactor for the enzyme GPx and GST. GSH is known as a cofactor in both conjugation and reduction reactions, catalysed by glutathione-S-transferase enzymes in cytosol, microsomes and mitochondria. GSH antioxidant system participate directly in the destruction of reactive oxygen compounds. Glutathione destroys free radicals involved in detoxification. The reaction is catalysed by glutathione peroxidase.

\[
\text{H}_2\text{O}_2 + \text{GSH} \rightarrow 2\text{H}_2\text{O} + \text{GSSG}
\]

The ratio of GSSG/ GSH in the cell is an important marker of oxidative stress. Glutathione reduces the toxic substances before they can damage other molecule or important part of cell.
1.8. Alcohol

Alcohol abuse is the commonest cause of liver damage (Doll et al, 1994). Light or moderate ingestion of alcohol may reduce the risk of coronary artery disease (Fuchs, 1995). Intake of more than 30gm alcohol per day has been associated with cirrhosis in both sexes (Becker et al, 1996). The mechanisms involved in alcohol induced liver disease are poorly understood (Friedman, 1993). Only 8 to 30% of long term alcohol abusers develop alcoholic cirrhosis and a minority of individuals will not progress beyond stage of fatty liver despite persistent drinking. The risk of severe liver damage is dose dependent (Bellantani, 1997). The technique of feeding ethanol as part of a totally liquid diet was invented two decades ago. This technique results in much higher ethanol intake than the conventional procedures. As a consequence, various complications observed in alcoholics were reproduced in animal models. The amount of ethanol metabolized by the individual differs by sex (Frezza et al, 1990) and that chronic ethanol consumption induces the microsomal ethanol oxidizing system (Liber et al, 1967). Chronic liver damage caused by alcohol consumption diminishes livers capacity to synthesize and export lipids (Baraona et al). The involvement of free radicals in the pathogenesis of liver injury has been investigated for many years in a few well defined system (Poly, 1993). There is now good evidence that moderate consumption of alcohol is associated with a lower risk of coronary artery disease than either teetotalism or heavy drinking (Steinberg et al, 1991). Increased alcohol consumption is associated with an increased risk of cardiovascular and other diseases due to hypertension and haemorrhagic stroke or sudden arrhythmic death (Kauhnen et al, 1997)

1.8.1. Ethanol metabolism

Alcohol reaching the liver in the portal blood is oxidized by alcohol dehydrogenase (ADH) in the hepatocyte cytosol to acetaldehyde, with simultaneous
reduction of the cofactor NAD to NADH. Acetaldehyde is highly reactive substance whose toxicity greatly exceeds that of ethanol, and is metabolized to acetate by the mitochondrial isoenzyme of aldehyde dehydrogenase (ALDH). The cytochrome P450 system present in endoplasmic reticulam provides an alternative pathway for alcohol metabolism that may account for up to 10% of ethanol oxidation in chronic abusers due to enzyme induction. Factors that increase ethanol oxidation or reduce acetaldehyde clearance will result in increased acetaldehyde levels in the liver and greater injury.

1.8.2. Ethanol administration and alteration in cell biochemistry

During ethanol oxidation, acetaldehyde forms both stable and unstable adducts with proteins, glycoproteins and membrane phospholipids (Sorrel et al, 1985). Evidence from rat model suggests that this process is dependent upon ADH activity (Lin et al, 1990). Free-radical formation results in increased lipid peroxidation, which exacerbates membrane injury (Lauterburg et al, 1988). Many enzyme systems are effected by the redox shift that occurs due to increased NADH production relative to NAD (Lieber et al, 1998). In addition profound changes occur in lipid, carbohydrate and protein metabolism, leading to ketosis, the accumulation of triglycerides and lactate and very occasionally hypoglycemia occurs in acute alcoholic states. Chronic ethanol ingestion induces oxidative stress in the liver as result of the generation of superoxide radicals by ethanol metabolism via cytochrome P450 system. In alcoholic hepatitis infiltration of neutrophils may contribute to this oxidative stress (Williams et al, 1987). Free radical mediated damage is aggravated by a reduction in cytoprotective enzymes and other antioxidants. Glutathione synthesis is reduced due to the interaction of acetaldehyde with precursor essential amino acids. In addition, nutritional deficiencies and malabsorption contribute to the reduced levels of natural antioxidants (Bjorneboe et al, 1993).
The interaction of ethanol with lipid metabolism is complex. When ethanol is present, it becomes a preferred fuel for the liver and displaces fat as a source of energy. This favours fat accumulation. In addition, the altered redox state secondary to the oxidation of ethanol promotes lipogenesis, through enhanced formation of acylglycerols. The high-density lipoprotein (HDL) have been suggested to be responsible for the lower incidence of coronary complications of moderate drinkers compared to teetotalers (Lieber, 1984). Schlorff (Schlorff et al, 1999) found that ethanol ingestion perturbs the plasma antioxidant system in a dose and time dependent manner. The significant changes in the ratios of catalase/superoxide dismutase, glutathione peroxidase/superoxide dismutase, glutathione reductase/glutathione peroxidase and reduced glutathione/oxidised glutathione in the plasma may be used as an index of alcohol induced oxidative stress. Krikun (Krikun et al, 1986) reported that microsomes isolated from chronic ethanol fed rats displayed elevated rates of malondialdehyde production when compared to pair fed controls. Husain (Husain et al, 1998) studied the effect of exercise training and chronic ethanol ingestion and combination selectively inhibited hypothalamic cholinesterase activity and the inhibition was correlated with increased lipid peroxidation which may perturb hypothalamic function. Leptin, the hormone that regulates appetite discovered in 1994, has implications beyond its originally designated role In rats, one month of alcohol use stimulates leptin (Nicolas et al. 2001), presenting another potential and intriguing mechanism for alcohol-induced hypogonadism.

1.9. Cigarette smoke

Tobacco smoking leads to increased leukocytosis and elevation of acute phase reactants. Cigarette smoke is produced by incomplete combustion of tobacco. It is a heterogeneous aerosol containing more than 4000 substances (Anthony et al, 1998). Cigarette smoke contains various cytotoxic, mutagenic and carcinogenic agents like
biphenyl and polycyclic aromatic hydrocarbons and oxidants like oxygen, nitrous oxide and free radicals. Free radicals are capable of independent existence and can cause oxidative tissue damage (Ansari, 1997). Free radicals are capable of initiating and promoting oxidative damage (Chow et al, 1993 and Church et al, 1985). Smoking can significantly increase CAD mortality and morbidity related to the amount of tobacco smoked daily and the duration of smoking (Manson et al, 1992 and Wilhelmsen, 1998). Nicotine stimulates release of adrenaline leading to increased serum concentrations of fatty acids (Shepherd et al, 1978). Free fatty acid is a stimulant of hepatic secretion of LDL and triglycerides (TG). The free fatty acids can also stimulate hepatic synthesis and release of cholesterol (Banonome et al, 1992). In addition to this, cigarette smoking can alter coagulation system, produce various free radicals, all of which may contribute to atherosclerosis. The benefit of smoking cessation is seen regardless of how long and how much the person previously smoked (Kawachi, 1993). Tobacco is the most commonly known carcinogen for human society. Two most important etiological factors, implicated in the development of oral cancer are tobacco smoking and alcohol consumption.

1.9.1. Toxicity of cigarette smoke

Cigarette smoke predisposes to emphysema, lung and several other cancers, atherosclerosis and many other diseases.

1.9.2. Chemistry of cigarette smoke

Cigarette smoke is a complex mixture of toxic agents, some of which are free radicals themselves, others are redox cycling agents, cytotoxic aldehydes and carcinogens such nitrosamines and benzpyrene. Other constituents of cigarette smoke are ammonia, carbon monoxide, hydrogen cyanide, ethanol, formaldehyde, benzene vapour, vinyl chloride, tar, nicotine, phenols, lead, iron and carcinogenic hydrocarbons etc. The tar in smoke contains about \(10^{17}\) radicals per gram; most of them are highly stable, persisting
for hours. The tar contains 3000 aromatic compounds and at least four different radical species. Aqueous extracts of cigarette tar generates $O_2$ free radicals and hydrogen peroxide and have been shown to damage isolated DNA. It has been estimated that more than one microgram of iron is inhaled per pack of cigarettes. Lung macrophages and respiratory tract lining fluids in smokers have elevated iron contents. Fresh cigarette smoke contains high concentrations of NO and NO$_2$.

1.9.3. Mechanisms of damage by cigarette smoke

i. $RO_2$ and oxides of nitrogen can cause direct damage, stimulating lipid peroxidation and oxidizing DNA bases. Plasma and urine from cigarette smokers show elevated levels of isoprostanes. Cigarette smokers exhale more pentane immediately after smoking.

ii. The aldehyde present, especially acrolein, other unsaturated acetaldehyde and formaldehyde, can cause GSH depletion and modify protein - SH groups and amino groups.

iii. The hydroquinones/quinines in the tar phase may leach out into lung lining fluids, diffuse across the cell membranes and undergo both extracellular and intracellular redox cycling to generate semiquinones.

iv. The carcinogens are partially absorbed and usually after the metabolism can initiate and promote carcinogenesis.

v. Cigarette smoke acts as an irritant to lung macrophages and may activate them to produce hydrogen peroxide. It also promotes recruitment and retention of neutrophils in the lung.

vi. Both surfactant and alpha-1-antiproteinase can be inactivated by species within cigarette smoke or generated by activated phagocytes. Smoking predisposes to the development of emphysema and bronchitis. The term chronic obstructive
pulmonary diseases (COPD) is often used to encompass both chronic bronchitis
and emphysema, since the two conditions often co-exist to some extent in
smokers. Cigarette smoking is the primary risk factor for COPD.

vii. Cigarette smoke not only contains but also be capable of releasing iron from
ferritin in lining fluids, possibly leading to hydroxyl formation from hydrogen

1.10. Aim of the present study

From the above review it is evident that stress both physical, and psychological as
well as oxidative stress have a major role to play in the pathogenesis of number of
diseases. Our present study is aimed to study the effect of different types of stress on lipid
metabolism and antioxidant status.

1.11. Relevance of the present study

The assay of lipid profiles- total cholesterol, triglycerides, HDL- cholesterol and
LDL-cholesterol and scavenger enzymes of reactive oxygen species- superoxide
dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione
content are important to understand the role of stress in the incidence of various diseases.

Hence the objectives are

1) To study the effect of various types of stress (Fresh water swimming, cold
water swimming, isolation, overcrowding, alcohol administration and
cigarette smoke exposure) on the lipid parameters ( serum and tissue
cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides ) and
lipogenic enzymes (glucose-6- phosphate dehydrogenase, malic enzyme
and HMG Co A reductase) in the liver tissue of experimental animals.

2) To study the effect of above mentioned stress conditions on antioxidant
enzymes (Superoxide dismutase, catalase, glutathione peroxidase and
glutathione-S-transferase) and reduced glutathione content in tissue of
experimental animals.

3) The study also envisages to evaluate the combined effect of alcohol and cigarette smoke on lipid parameters and antioxidant enzymes in serum and tissues of experimental animals.

4) The assay of the above parameters (lipids and antioxidant enzymes) are done with a view to understand the effects of various stress conditions on the incidence of coronary heart disease.

5. The study is also extended to understand the effect of S-allyl cysteine sulphoxide and diallyl disulphide (SACS and DADS) on animals treated with alcohol.