CHAPTER 2

REVIEW OF LITERATURE

2.1 Definition

During 1936, Hans Selye defined, stress as the “non-specific response of the body to any demand for change” [1]. This definition of selye on stress is widely accepted, and still this concept is the basis of evaluation of stress and antistress agents. Different researchers tried to include some other components in stress definition as follows: It may be based on the definition of well-being. “Well-being is a dynamic state of mind characterized by reasonable harmony between a person’s abilities, needs and expectations with environmental demands and opportunities [14]. During 1966, Lazarus defined, “Stress arises when the person perceives that he or she cannot adequately cope with the demands being made on them or with threats to their well-being” [15]. Stress can also be defined as intense and unpleasant condition, on long term it may disturb health, performance and productivity. Stress is the imbalance between environment’s demand and individual’s capabilities [16]. The definition of stress should not be restricted to the semantics of words, but it should include agreement between broad terms and its nature. Stress is indefinable and immeasurable.

2.2 History of Stress concept

Stress is the disruption of homeostasis of the body, but the basis of homeostasis can be traced back in history. During nineteenth century, the French physiologist Claude Bernard stated that organisms maintain constancy with surroundings, even though changes occur in environment by stabilizing inside the body [17].

During 1911, the American physiologist Walter Cannon developed the stress concept, and he coined the word homeostasis. As per his concept, when stress crosses critical point there will be disruption of homeostasis leading to physical damage of organism [17]. In 1930s, Dr. Hans’s selye a Canadian endocrinologist and leading researcher in stress field, expanded stress concept involving homeostasis. During 1930, Selye was working with some experiment on rats, and he was surprised with set of similar responses like ulcers, increased size of adrenal glands and reduced size of immune tissues. He described these changes as nonspecific response. During 1936, Selye conducted experiment on rats with different stressful conditions like cold,
surgery, exercise and toxic drugs. He observed similar sum of body changes or syndrome of responses, and he proposed this syndrome as General adaptation syndrome [1, 17].

Based on this General adaptation syndrome theory, researchers focused on adrenal glands as the central part of stress concept. Later on various experimental investigations supported Sely’s theory of stress, based on link between hypothalamus, pituitary gland and adrenal gland. These biological findings helped to conceptualized that stress involves neuroendocrine system of the body, and during stress this neuroendocrine system produces physiological and biochemical changes to maintain normal homeostasis of the organism [17].

2.3 Selye’s theory of stress (General Adaptation Syndrome)

In 1936, Selye’s proposed general adaptation syndrome as theory of stress, which described body’s response to stressful situation. Stress cannot be manifested by single response, and it must be characterized by sum of responses known as syndrome. This syndrome consists of all nonspecific responses of the body, develop due to stress. According to Selye’s general adaptation syndrome consists of three stages: Initial alarm phase, followed by a phase of resistance and finally the terminal phase of exhaustion [18].

Alarm phase (Fight or Flight Response)

In this phase, the stressor is recognized by the brain, and the body starts to respond either by fight or flight. Alarm phase involves emergency response of the body to cope up with the noxious stimuli or stressful situation. When hypothalamus senses that additional energy is needed to counter disturb homeostasis of the body caused by stress, it sends impulses to activate sympathetic nervous system. This leads to mobilization of large amount of organism’s resources such as glucose and oxygen to the main active organs, such as, Brain, skeletal muscle and heart, which becomes overactive to combat danger during stress. Brain becomes alert, heart pumps enough blood and skeletal muscles works for fight for flight responses. The consequences of these emergency responses are: increase tone of muscle rise of blood sugar level, rise of blood pressure due to vasoconstriction and tachycardia. During alarm phase organism suffer from muscle tension, dryness of mouth, perspiration and increase in respiration. This phase is the beginning of the adaptation of body, and it lasts for a short period [18].
Resistance Phase

As the organisms continue to adapt stress, the syndrome enters into the next stage, called as resistance phase. During this phase the body resists to the stressor, and get adapted to the challenge for a long period. The duration of this phase depends on organism adapting energy reserves and severity of stressor [18].

The resistance phase is initiated by the hypothalamic secretion of corticotrophin realizing hormone (CRH), thyrotrophic releasing hormone (TRH) and growth hormone releasing hormone (GHRH). Among these hormonal responses, corticotrophin releasing hormone plays important role in generating stress response through Adrenocorticotropic hormone (ACTH) and cortisol, which is popularly known as hypothalamic pituitary-adrenal axis [18]. In this pathway, stressor activates nerve cells and stimulates hypothalamus to secret CRH, and then CRH reaches to the pituitary gland through hypothalamic-hypophysial portal system. Anterior pituitary gland secretes ACTH under the regulation of CRH [19]. ACTH is carried by the blood to the adrenal gland, where it stimulates adrenal cortex to secrete corticoid hormones such as cortisol or corticosterone [19, 20].

Corticoids have many actions on the body, which helps organism to deal adaptively with stressors for longer duration. Corticoids promote gluconeogenesis, lipolysis and protein catabolism resulting in the formation of glucose, liberation of free fatty acids and amino acid respectively. These metabolic products such as glucose, amino acid and fatty acids are utilized as fuel in the form of ATP needed for nerve and muscle activity or to repair the damaged cells [18].

The additional energy resources of the body are mobilized by two more hypothalamic releasing hormones like, GHRH and TRH. Growth hormone releasing hormone (GHRH) stimulates anterior pituitary gland to secret growth hormone. Growth hormone increases catabolism of glycogen to glucose through glycogenolysis pathway, and also increases lipolysis [18]. Thyroid stimulating hormone (TSH) is secreted by the anterior pituitary gland by the stimulation from TRH. TSH stimulates thyroid gland to secret thyroid hormone such as thyroid and triiodothyronine. This thyroid hormone stimulates the production of ATP (energy) from glucose [18]. The overall actions of growth hormone and thyroid stimulating hormone are to provide additional resources of energy in the form of ATP required for metabolically hyperactive cells of the brain, heart and muscle [18].
Generally resistance phase helps the organism to cope with stress, restoration of homeostasis, regeneration of cells and bring back to normal conditions. If the stress is prolonged or severe, the acquired adaptation will be insufficient and resistance phase fails to counter stressful stimuli resulting in the exhaustion of the organism [18].

**Exhaustion Phase**

This is the terminal stage of syndrome, during which body’s resources have used up and depleted resulting in exhaustion. The normal functions of the organism are affected and it starts from simple symptoms of sympathetic nervous system (like sweating, increase heart rate etc.), then progress to complicated diseases and disorders (such as ischemia, cell necrosis and suppression of immunity) [18].

**2.4 Selye’s diseases of adaptation (stress and diseases)**

Selye stated that repeated or prolonged physiological responses leads to wear and tear of the body resulting in diseases of adaptation. Chronic activation of HPA axis and high level of cortisol, results in change of physiology of organism in the following ways [21, 22]:

- Wasting of muscle mass leads to fatigue and myopathy.
- Failure of pancreatic beta cell leads to hyperglycemia and even diabetes mellitus.
- Prolonged vasoconstriction may leads to hypertension.
- Lack of supply of glucose and oxygen may lead to ischemia and myocardial infarction.
- Suppression of immunity may cause organism susceptible to host of infection.
- Pathological changes may cause increased susceptibility to cancer.
- Stress-related gastro intestinal tract disorders like gastritis, ulcerative colitis, irritable bowel syndrome.
- Central nervous system disorders like headache, migraine, anxiety and depression.
- Other chronic diseases like asthma, rheumatoid arthritis, and finally premature death may occur.

Even though the exact role of stress in diseases is unknown, they will occur, when body’s homeostasis mechanisms are impaired. As per Selye’s theory of stress, the main reason of stress diseases is due to either excess or deficiency of corticoid produced during stressful stimuli.
FIGURE 2.1: Stress response or General adaptation syndrome proposed by Dr. Hans Selye. Adapted from [18].
2.5 stressors

Selye created a new word stressor to include a wide variety of stimuli, which disturbs homeostasis of the organism. It may be change in environment natural or created artificially, as an experimental stimuli study stress concept. In 1936 Selye used different noxious agents as stressor, during his demonstration of stress, such as cold condition, injury of surgery, shock produced by transcision of spinal cord, increased muscular activity, toxicity of drugs like adrenaline, atropine, formaldehyde, morphine etc. [1]. Stressors can be from internal or external origin. Internal origins of stressor are as follows: Physical source such as infection etc. Psychological sources such as improper perception or worries of life etc. External origins of stressor are as follows: Physical source such as extreme climatic condition like hot or cold. Psychological source such as unfavorable working condition, relationship conflicts etc.

2.6 PHYSIOLOGY OF STRESS

The stress response of organism is composed of neural and endocrinal components. Both the components are important to regulate homeostasis of the organism [17].

2.6.1 Neuronal components of Stress: It involves central and sympathetic neuronal components.

Central nervous system mediated stress response: The brain of CNS plays a crucial role in the body’s perception of stress and initiating response to the perception [25]. Stressful conditions produce deviation of homeostatic state, which results in sensory stimulus, emotions or energy deficiency. These inputs will be interpreted as the initial step across the regions of the brain, from cortical sensory areas to basal structures: that is Amygdala and Hippocampus, where processing of emotions and memory occurs respectively. During stress the blood brain barrier also becomes more permeable for corticosteroids, resulting in the activation of glucocorticoid receptors of Amygdala, Hippocampus and prefrontal cortex. Neurons of these area becomes stimulated, leads to triggering fight or flight response to deal with life-threatening conditions. Glucocorticoids acts on CNS through two types of receptor: that is type I and type II glucocorticoid receptors. Type I glucocorticoid receptors are present mainly in limbic neurons, whereas type II glucocorticoid receptors are found in the hypothalamus, hippocampus, amygdala, lateral septum and nucleus tractus solitarius. During stress, both type I and II glucocorticoid receptors plays role in processing
response to environmental and emotional stimuli, with consequent changes in HPA axis activity [17].

**Sympathetic nervous system stress response:** During mental or physical stress, hypothalamus is stimulated, resulting in release of norepinephrine from locus coeruleus of the brain stem. Signals are passed through reticular formation and spinal cord, to massive discharge of sympathetic nervous system activity. This is known as sympathetic alarm response of stress, which plays a major role during fight or flight response of stress. Sympathetic discharge during fight or flight response improve the ability of the organism to perform vigorous physical activity [26]. Various sympathetic responses are as follows:

- Cell metabolism is promoted in the organism
- Blood glucose level is increased by conversion of glycogen into glucose in liver. Glycolysis is promoted in muscle and liver.
- Blood vessels dilated and blood flow increases in active organs of body (brain, heart and skeletal muscle) to perform more during fight or flight response.

The sum of these responses is to combat the stressful stimuli [26].

**2.6.2 The endocrine components of stress:** It involves activation of hypothalamic pituitary adrenal axis (HPA-axis), and its integration and co-ordination is important to successfully, combat stress. Stressful stimuli trigger hypothalamus to release corticotrophin releasing factor (hormone) from the hypothalamic median eminence into the primary capillary plexus of the hypophysial portal system [26]. Corticotrophin releasing hormone or factor is a peptide consist of 41 amino acids [26], secreted by the neuronal cell bodies of para ventricular nucleus of the hypothalamus [17]. This para ventricular nucleus is linked to limbic system and brain stem [26]. The hypothalamic hypophysial portal vessels is a direct minute blood vessel pathway, linking hypothalamus to anterior pituitary gland, and it helps in transporting CRH to anterior pituitary corticotropic secretory cells. The binding of CRH to the CRH-R1 receptor on corticotropic secretory cells leads to stimulation of Phosphokinase A (PKA) through G protein. This result in phosphorylation of calcium channel and increased calcium influx, leading to exocytosis of Adrenocortico tropic hormone (ACTH) also called as corticotrophin or adrenocorticotropin during stress, ACTH level increases up to ten fold by release from corticotropic secretory cells of anterior pituitary gland into the blood circulation. ACTH binds to its receptor in adrenal
cortex, mainly in zona fasciculata and reticularis, and to produce and secrete glucocorticoids, mineralocorticoids and weak adrenal androgen [17].

**Actions of ACTH**: When ACTH binds to its receptor, which is present on the cell membrane of adrenocortical cells of adrenal gland, leads to stimulation of Gs-protein. Adenylyl cyclase in the cell membrane gets activated; and ATP is converted to cyclic AMP in the cell cytoplasm. Cyclic AMP activates intracellular enzymes like protein kinase A and other enzymes [26].

**Effects of ACTH**: ACTH increases uptake of cholesterol by increasing the number of low-density lipoprotein (LDL) receptors. Cholesterol is the precursor for the biosynthesis of adrenocortical hormones. ACTH activates enzyme desmolase, which converts cholesterol to pregnenolone, and it is the rate limiting step in adrenocortical hormone production [26]. The CYP11A1 gene is important for encoding enzyme involved in cholesterol side-chain cleavage. Pregnenolone is converted to 17-OH-pregnenolone, which then reaches to endoplasmic reticulum to form 11-deoxycortisol; and 11-deoxycortisol undergoes hydroxylation to form cortisol. Adrenal cortex does not store cortisol, but it is secreted when ACTH acts on adrenal gland [17]. When ACTH stimulates adrenal cortex for prolonged period, it leads to hypertrophy and proliferation of the adrenocortical cells in Zona fasciculata and reticularis, from which cortisol and androgens are secreted. ACTH and cortisol are secreted within minutes by any type of physical or mental stress [26]. Different types of stress responsible for cortisol release are: infection, extreme condition of temperature (like hot or cold), Trauma, Surgical operations, administration of sympathomimetic drugs, injecting necrotizing material and immobilizing animals [26].

**Actions of Glucocorticoids**: Adrenal cortex synthesizes and release glucocorticoid into the blood, which then reaches and act on target cells. Glucocorticoids easily penetrate cell membrane as it is lipid soluble, and interact with cytosolic glucocorticoid receptors. When glucocorticoid binds with receptor, the heat shock protein 90 and HSP 70 are dissociated from the receptor, due to its conformational changes. It helps in removing inhibitory influence of heat shock protein on the receptor, and subsequently the hormone translocate to the nucleus for dimerization. The hormone receptor complex interacts with specific DNA sequence of genes (glucocorticoid responsive element) on the chromatin. The expression of these genes as gene transcription is either inhibited or promoted. Due to transcription, ribosome synthesizes protein, which modifies cell function [27].
FIGURE 2.2: Activation of Hypothalamic-Pituitary-Adrenal axis during stress. Adapted from [17].
FIGURE 2.3: Synthesis of Cortisol. Adapted from [17].

Effects of Glucocorticoids: The corticoids help the organism to combat all kinds of noxious stimuli and stress, as a result of activation of hypothalamic-pituitary-adrenal axis.
a) **Carbohydrate metabolism:** Corticoids increases metabolism in such a way that excess of glucose should be available and avoid starvation of glucose dependent organs (Brain, heart and muscles).

![Diagram of glucocorticoid actions](image)

**FIGURE 2.4: Actions of Glucocorticoid. Adapted from [17].**

During stress, more energy is required by the active organs of the body. Glucocorticoid promotes glucose deposition as glycogen in liver by promoting gluconeogenesis and then inducing hepatic glycogen synthetase. They also stimulate rate of gluconeogenesis by six to tenfold as a result of two different effects of cortisol. First, enzymes are synthesized, when glucocorticoids activate DNA transcription in the nuclei of liver cells with formation of protein for enzymes. This enzyme converts amino acid into glucose by gluconeogenesis process, thereby promoting glucose formation. Then glucocorticoid breaks peripheral muscle tissue into amino acid, and mobilizes amino acid through blood into the liver for gluconeogenesis process. This promotes the formation of glucose [26].

The side effects of glucocorticoid’s muscle breakdown are: muscle wasting leading to decreased muscle mass, lympholysis leading to atrophy of lymphoid tissue,
thinning of skin and loss of osteoid from bone. Cortisol also diminishes peripheral cell utilization of glucose, resulting in increased blood glucose levels; and the cause of this action is not fully recognized. It may be alteration between the point of entry of glucose and its final metabolism in the cell. It may be due to either inhibition of translocation of glucose transporter from cell membrane to deeper sites, so that glucose uptake and its peripheral utilization is decreased or by decreasing oxidized form of Nicotinamide-adenine dinucleotide (NADH), which is important for rapid glycolysis [26].

(b) **Protein metabolism:** Cortisol inhibits mRNA in the extra hepatic tissue, so that enzyme required for protein synthesis are decreased, resulting in less protein synthesis. Whereas cortisol stimulate mRNA in the liver, so that enzyme required for protein synthesis are increased resulting in more protein synthesis. Cortisol also increases catabolism of already existing cell protein [26].

(c) **Fat metabolism:** Cortisol promotes mobilization of fatty acids from adipocytes into the blood, and during starvation or stress, this fatty acid is utilized for energy generation. Chronic effect of cortisol in the body is abdominal obesity with emaciated limbs, moon face, fish mouth and buffalo hump characters. Because in peripheral adipose tissues, corticoids enhances lipolytic actions of growth hormone and adrenaline, resulting in loss of fat from subcutaneous tissues of peripheral extremities; and this produces emaciated limbs. The fat from peripheral extremities is also redistributed and deposited over face, neck and shoulder, resulting in moon face, fish mouth and buffalo hump in a person. The adipose tissue of trunk region responds preferentially to elevated insulin levels under the influence of corticoids, resulting in fat deposition in the trunk and abdominal obesity [26-27].

(d) **Glucocorticoid and immunity:** Glucocorticoid decreases lymphocytes and eosinophils in circulation, leading to lymphocytopenia and eosinopenia. Cortisol in large amount produces atrophy of lymphoid tissue, leading to decrease output of both T cells and antibodies from the lymphoid tissue. Due to this immunity is suppressed for foreign invaders; and occasionally it may lead to infections. Cortisol increases the number of red blood cells by stimulating spleen. Large amount of glucocorticoid suppress immunity, and its inhibition at many level of immune system. Glucocorticoids have negative regulation of gene for cytokines in macrophages, endothelial cells and lymphocytes: it results in decrease production of interleukin-1, interleukin-2, interleukin-3, interleukin-6, Tumor necrosis factor α, granulocyte
macrophage colony stimulating factor and γ interferon. This leads to suppression of fibroblast proliferation and T-lymphocyte function [27].

(e) **Glucocorticoid and inflammation:** Glucocorticoid prevents or control inflammation due to stress in the following ways [26]:

- Cortisol stabilizes the lysosomal membrane, so that it prevents lysosome rupture and release of inflammatory proteolysis’ enzymes.
- Cortisol prevents loss of plasma into the tissues by decreasing the capillary permeability.
- Cortisol prevents migration of white blood cell and also it prevents phagocytosis of the injured cells.
- Cortisol prevents promotion of inflammatory process by suppressing immune system, and especially T lymphocytes are suppressed.
- Cortisol reduces release of interleukin-1 from white blood cell, which leads to decrease in body temperature, and it results in vasodilatation [26].

(f) **Other actions of Glucocorticoid are as follows:**

- Glucocorticoid increases secretion of gastric acid, which may aggravate peptic ulcer [27].
- Glucocorticoid produces loss of calcium from bone, which effects spongy bones like vertebrae, ribs etc [27].
- Glucocorticoid may play a permissive role in the rise of blood pressure by influencing adrenaline and angiotensin [27].
- Glucocorticoid is required for the normal muscular activity. Weakness of muscle develops, if its optimum level is not maintained. Sometimes due to excess of cortisol, person cannot move from squatting position because of muscle weakness [26].
- Immunity is also suppressed due to malfunctioning of lymphoid tissue, as a result of atrophy of lymphoid tissue by cortisol [26].

2.7 **Stress and living system**

As stress produces non-specific response in the body, it affects normal physiology of living system through different mechanisms.

2.7.1 **Stress and immunity:** Stress shows complex immune response of both acquired and innate immunity [21]. Stress induced immunomodulation involves neuroendocrine system of the organism: where both central and sympathetic nervous
system are involved, and hypothalamic-pituitary-adrenal axis will integrate and coordinate during stress [13, 17]. This concept is called as **immune-hypothalamic-pituitary-adrenal axis**, a well-known and widely studied concept. The coordination between stress neuron, HPA axis and immunity occurs through Interleukin-I. As shown in the figure 2.5, stressful stimuli (like infection, tissue damage or toxic agents), activates immunocompetent cells (T-cell and B-cells), which leads to release of cytokines (IL-1 and IL-6) [13, 17]. Both cytokines [IL-1 and IL-6] acts on HPA axis, and induce the release of CRH from hypothalamus [13]. CRH stimulates pituitary gland to secrete ACTH, and ACTH stimulates adrenal cortex to release corticosteroid. Glucocorticoid produces resistance to stress, anti-inflammatory effect and suppression of immunity [13, 17-18].

**Glucocorticoid weakens or suppresses immunity by the following mechanism** [17]:

- Inhibition of recruitment of leukocyte at antigen contacting place.
- Interference of cell mediated immunity, where T cells are affected.
- Suppression of further production of cytokines by inhibiting cytokine gene transcription (as with IL-1, IL-2, IL-3, IL-8).
- By decreasing IL-I production and secretion from macrophage.
- Glucocorticoid inhibits IL-2 synthesis by making T-cells unresponsiveness to interleukin-1. The Glucocorticoid mediated immunosuppression; protect cells and whole organism from excessive immune response, during acute stress.

Acute stress induced level of glucocorticoid, benefit the organism from overreacting to stressor [13, 17]. Chronic stress also produces dysfunction of immunity by shifting TH₁ response (cellular immunity) to a TH₂ response (humeral immunity). Due to this switch from cellular immunity to humeral immunity, certain infections and autoimmune disorders are favored [21]. During chronic stress, infections (Common cold, Mycobacterium tuberculosis, Helicobacter pylori) persist due to immune shift from TH₁ (cellular immunity) to TH₂ (humeral immunity). Some allergic conditions and even autoimmune diseases (such as Grave’s disease and systemic Lupus erythematosus) are the results of switch of TH₁ to TH₂ response [21].
It was found that increase in score of stress index, correlated with greater severity of cold symptoms of persons infected with the respiratory virus [28]. During stress,
HIV progression and development of AIDS is rapid, in HIV infected patient [88]. People under stressful events, such as distressed marriage have shown weaker lymphoproliferative response [29]. Modulation of immune response is required to avoid diseases [30].

### 2.7.2 Stress on growth and development:
Chronic stress impairs growth and development by inhibiting pituitary gland secretion of growth hormone, and also the sensitivity of target tissue to Somatomedin-C is reduced. The physical and mental development of a person is affected by stressful psychosocial life [17]. Emotional stress may cause abuse dwarfism syndrome consisting of delayed physical development, retarded intellectual development and under developed social maturation [17]. As shown in figure 2.6, chronic stress affects growth hormone through the prolonged activation of HPA axis.

![Image](image.png)

**FIGURE 2.6: Effect of Stress on reproduction, growth and development through Hypothalamic-Pituitary-Adrenal axis. Adapted from [32].**

CRH released during HPA-axis activation, increases secretion of Somatostatin and this inhibit growth hormone secretion. Also glucocorticoid inhibits growth hormone secretion and inhibits Somatomedin C effect on target tissue [31].

### 2.7.3 Stress and Reproductive system:
Stress disturbs reproductive functions of both male and female. In males, stress suppresses libido, testosterone secretion and spermatogenesis. Whereas in female, stress have shown abnormality of sexual and related functions such as delayed puberty, inhibition of sexual receptivity, inhibition
of ovum formation, failure of implant of embryo, abortion or death of infant [17]. As shown in the figure 2.6, during stress, reproduction is suppressed by the hormones of HPA-axis in the following ways:

- CRH inhibit the release of gonadotropin-releasing hormone from hypothalamus, directly as well as indirectly via β-endorphin [17].
- Glucocorticoid inhibits gonadotropin-releasing hormone secretion from hypothalamus.
- Glucocorticoid inhibits pituitary gonadotroph responsiveness to gonadotropin-releasing hormone, resulting in decrease secretion of luteinizing hormone and follicular stimulating hormone [17].
- Glucocorticoid inhibits sex steroidogenesis in ovaries and testis, resulting in decrease sex steroid output. Glucocorticoid reduces testicular LH receptor, resulting in decrease sensitivity of testicular leydig cell to luteinizing hormone [17].

Stress decreases luteinizing hormone and sex steroid, resulting in sexual dysfunction, decrease sexual behaviors and inactivity of ovaries and testis. Stress affects the ability of female for conception and fecundity. Stress has produced smaller litter size in rodents. Studies have shown that stress and corticosteroid in sheep have increased embryo loss [17].

2.7.4 Stress and memory: During chronic stress, hippocampus of the brain is susceptible to be damaged [33]. It has been found that, long term potentiating of memory in the hippocampus is blocked by the corticosteroid. Dose-dependent relationship occurs between corticosteroid and long term potentiating. Corticosteroid shows biphasic effect on long term potentiating (LTP) of memory. At low level, corticosteroid shows positive correlation with LTP, and at high level corticosteroid shows negative correlation with LTP [24]. Animal studies have shown that, corticosterone increases the extinction rate of shock avoidance response]. In another study, it was found that corticosterone in low amount facilitated extinction of avoidance response [17]. Corticosterone occupy type 1 receptors in hippocampus at the basal level and enhances primed burst potentiating (low threshold type of LTP), and at the stress level or high level, corticosteroid occupy type II receptor in hippocampus leading to suppression of LTP. Glucocorticoid indirectly increases cytosolic calcium in neuron, it results in calcium dependent degeneration of neuron in
the hippocampus; and which may affect memory. Stress affects memory probably by inhibition of glucose transport to the hippocampus via glucocorticoid. Glucocorticoid translocate glucose transporter from cell membrane to the intracellular site and also inhibit mRNA for glucose transporters [17].

2.7.5 Stress and Thyroid functions: As shown in the figure 2.7, stress inhibits thyroid function through HPA axis with resultant decrease production of TSH; and reduced amount of T3 and T4 hormones. CRH stimulate production of somatostatin, and somatostatin inhibits TSH secretion. During inflammatory stress, cytokines act on HPA axis to produce somatostatin, leading to decrease secretion of TSH [34].

2.7.6 Stress and Gastrointestinal tract: As shown in the figure 2.8, stress decreases gastric motility of stomach via vagus nerve, and colonic motility of large bowel increases by stress via sacral parasympathetic system [21]. Acute stress stimulates CRH, and CRH inhibit gastric motility with stimulation of colon motility through autonomic nervous system. When dorsal vagal complex is inhibited, it results in reduced gastric motility.

![Figure 2.7](image-url)

**FIGURE 2.7:** Effect of Stress on thyroid function through Hypothalamic-Pituitary-Adrenal axis. Adapted from [34-35].

When CRH projections are stimulated, there will be activation of sacral parasympathetic nervous system resulting in increased motility of colon. CRH-R₁ receptor mediates the central action of CRH on colonic motility, whereas CRH-R₂
receptors mediate central medullar action on gastric emptying. So CRH is involved in stress induced diarrhea of irritable bowel syndrome [21].

FIGURE 2.8: Effect of Stress on gastric colonic motility. Adapted from [35].

2.7.7 Stress and cardiovascular system: Stress exerts its action on heart and blood vessel by stimulating hypothalamus to release norepinephrine from locus ceruleus of the brain stem.

FIGURE 2.9: Effect of Stress on cardiovascular system. Adapted from [35].
Signals acts on spinal cord to discharge norepinephrine from sympathetic nervous system. Acute stress increases heart rate and arterial blood pressure through sympathetic nervous system [21].

2.7.8 Stress and Appetite-Satiety regulators: Stress affects appetite and satiety of organism by influencing hypothalamic CRH, neuropeptide Y and melanocyte-stimulating hormone.

![Diagram showing effect of stress on appetite-satiety centers through hypothalamic-pituitary-adrenal axis. Adapted from [35].](image)

Food intake is regulated by the arcuate nucleus and paraventricular nucleus of the hypothalamus. CRH causes loss of appetite, whereas neuropeptide Y stimulates food intake. Neuropeptide Y also stimulates CRH secretion by acting on Y1 receptors. NPY inhibit LC/NE-sympathetic system. Neurons of arcuate nucleus and paraventricular nucleus of hypothalamus releases neuropeptide Y, which stimulates food intake. This occurs when leptin level in the body is low. Leptin is the satiety stimulating hormone secreted by adipocyte in proportion to stored triglycerides of adipocytes. When leptin level is high, it acts on hypothalamus to inhibit the secretion of neuropeptide Y. Also leptin stimulates POMC neurons between arcuate and paraventricular nuclei, release melanocortin, and melanocortin acts on melanocortin’s receptor to inhibit food intake.

2.7.9 Stress and Mesocorticolimbic dopaminergic system: During stress, both mesocortical and mesolimbic components of dopaminergic system are activated by
catecholamine through LC/NE-Sympathetic system as well as by CRH/glucocorticoid through PVN neurons. Stress may be suppressed by the activated neurons of mesocortical system. The mesocortical dopaminergic system links the ventral tegmentum to the prefrontal cortex, which is implicated in cognitive function such as planning, attention, problem solving etc. The mesolimbic dopaminergic system links the ventral tegmentum to the nucleus accumbens, which plays important role in motivational reward phenomena [21].

**Amygdala:** Stress activates the central nucleus of the amygdale in acute and temporary way. The CRH system of Amygdala is involved in the generation of fear and/or anger. Glucocorticoid activates CRH neurons of amygdala leading to stress response and anxiety [21]. Activation of CRH neurons in amygdala is crucial for CRH induced neuroendocrine, autonomic and behavioral effects. Amygdala activation is important for modulating stress response during fear related behavior [37].

**Hippocampus:** Stress activates the hippocampus in an acute manner. Circulating glucocorticoid exert negative input on hippocampus through paraventricular nucleus of hypothalamus. It exerts inhibitory input upon the stress system [21]. During stress, prior memories through Hippocampus enhance or suppress stress response. It is susceptible to damage during chronic stress [33].

2.8 **Stress, diseases and disorders:**

Severe or repeated stress may develop diseases or disorders like allergy, endocrinal, mental, metabolic, developmental, and cardiovascular disorders [21]. Organisms are more vulnerable to stress, especially during prenatal stage, infancy, childhood and adolescence life.

I. **Acute stress related conditions:**
- Allergic disorders like asthma, urticaria, or eczema.
- Angiokinetic phenomena like migraine, hypertensive and hypotensive attacks.
- Gastrointestinal disorders like indigestion, pain, diarrhea, constipation.

II. **Chronic stress related conditions:**
- Cardiovascular disorders like hypertension, Atherosclerosis.
- Metabolic disorders like obesity, Diabetes mellitus.
- Mental disorders like anxiety, depression, and insomnia.
- Degenerative disorders like osteoporosis.
Acute stress related disorders are due to the release of mediator like corticotrophin releasing hormone and norepinephrine [21]. Asthma and eczema develops due to immune-CRH induced degranulation of mast cells in lungs and skin respectively. Migraine headache is due to the local vasodilatation of meningeal blood vessel by the degranulated mast cells, as a result of activated immune and CRH. When CRH is released in central amygdala, it results in fear or panic attack.

FIGURE 2.11: Chronic stress and development of metabolic syndrome. Adapted from [21].

During stress, hypertension or hypotension develops due to excessive sympathetic or parasympathetic nervous system stimulation respectively. Chronic stress disorders are
due to release of mediators; and their influence on multiple homeostatic systems activities [21]. Anxiety disorder is due to activation of fear system by the stress mediators like CRH, NE, Cortisol. Insomnia is due to suppression of sleep system by the stress mediators.

Following disorders are due to chronic stress and its somatic consequences [21].

- Growth and development of child may be suppressed due to decrease functioning of growth hormonal axis.
- Loss of libido and/or hypo fertility in adult is due to stress induced hypogonadism.
- Hypertension is due to excess stimulation of sympathetic system.
- Chronic stress mediator action leads to visceral fat accumulation due to hypercortisolism, hypersecretion of insulin, decrease secretion of growth hormone and hypogonadism.
- Polycystic ovary syndrome may develop in susceptible women during chronic stress.
- Stress related IL-6 release with hypercytokinemia and hypercortisolism leads to hypercoagulation of blood.
- Atherosclerosis may develop due to endothelial dysfunction as a result of dyslipidemia, hypercoagulation, hypercytokinemia, hypertension, insulin resistance.
- Chronic stress contributes to persistence of infection with *Helicobacter pylori*, *Mycobacterium tuberculosis* and common cold virus. This is due to immune shift from T_H1 to T_H2 by the stress.
- Chronic stress may develop autoimmune diseases (Grave’s disease, Systemic lupus erythematosus) and some allergic disorders. This is also due to immune shift from T_H1 to T_H2 by the stress.

2.9 **Adaptogens or antistress agents:**

Adaptogens or antistress agents are the plant base medicine which induces a state of nonspecific resistance of the organism in order to better resist stressful stimuli [13]. Dr. Nikolai Vasilievich lazarev (1895-1974), a Russian pharmacologist and toxicologist, coined the concept of adaptogen. In 1947, lazarev found the unexpected response of dibazol (2-benzyl benzimidazol). Dibazol was developed in France as an arterial dilator, but it produced a state of Non-specific resistance in experimental research. So lazarev described dibazol as adaptogen [13].
Dr. Israel Itskovitch Brekhman (1921-1994), a soviet scientist in pharmacology and Toxicology, defined adaptogen as follows [8, 13]:

- The adaptogen must show non-specific resistance to all types of stresses, such as physical, chemical or biological nature.
- Adaptogen must normalize and prevents disturbance of stressors.
- Adaptogen must be innocuous or harmless to the organism and should not disturb normal body functions.

According to this concept, adaptogen must produce nonspecific resistance, in such a way that organism with stand or adapt to the stressor stimuli, leading to normalizing overall physiological response.

The concept of adaptogen or antistress agent correlates Selye’s theory of general adaptation syndrome in the following ways:

As shown in the figure 2.12, adaptogen may reduce the alarm phase or prolong the phase of resistance (stimulatory effect); and prevent or delay the stage of exhaustion, to provide protection against stress of long period [39].

![Figure 2.12: Effect of Adaptogens on stress response or General adaptation syndrome. Adapted from [39].](image)

Plants like *Eleutherococcus Senticosus*, *Rhodiola rosea* and *Schisandra chinensis* were incorporated into USSR medical practice as antistress, on the basis of their ability to increase nonspecific resistance to stress. Number of clinical trials demonstrated that plants like *Eleutherococcus senticosus*, *Rhodiola rosea* and *Schisandra chinensis* have increased mental and physical working capacity in situations of fatigue and stress [39]. Soviet scientists found that some medicinal plants
induced state of nonspecific increased resistance in experimental animals and human being [8].

Table No. 2.1: List of well-established adaptogen [4, 41].

<table>
<thead>
<tr>
<th>Sl. NO.</th>
<th>PLANT NAME</th>
<th>BOTANICAL NAME</th>
<th>FAMILY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Eleuther root</td>
<td><em>Eleutherococcus senticosus</em></td>
<td>Araliaceae</td>
</tr>
<tr>
<td>2</td>
<td>Rhodiola root</td>
<td><em>Rhodiola rosea</em></td>
<td>Crassulaceae</td>
</tr>
<tr>
<td>3</td>
<td>Bryonia root</td>
<td><em>Bryonia alba</em></td>
<td>cucurbitaceae</td>
</tr>
<tr>
<td>4</td>
<td>Wu Wei Zi seed</td>
<td><em>Schisandra chinensis</em></td>
<td>Magnoliaceae</td>
</tr>
<tr>
<td>5</td>
<td>Ginseng root</td>
<td><em>Panax ginseng</em></td>
<td>Araliaceae</td>
</tr>
<tr>
<td>6</td>
<td>Shatavari roots</td>
<td><em>Asparagus racemosus</em></td>
<td>Liliaceae</td>
</tr>
<tr>
<td>7</td>
<td>Ashwagandha</td>
<td><em>Withania somnifera</em></td>
<td>Solanaceae</td>
</tr>
<tr>
<td>8</td>
<td>Holy Basil herb</td>
<td><em>Ocimum sanctum</em></td>
<td>Lamiaceae</td>
</tr>
<tr>
<td>9</td>
<td>Guduchi stem</td>
<td><em>Tinospora cordifolia</em></td>
<td>Menispermacae</td>
</tr>
<tr>
<td>10</td>
<td>Amla fruit</td>
<td><em>Emblica officinalis</em></td>
<td>Euphorbiaceae</td>
</tr>
</tbody>
</table>

2.9.1. Active constituents of plant adaptogen: Panossian and other researchers suggested that, active constituents of plant responsible for adaptogenic activity, falls into three groups of compounds in the following way [4]:

**Group I:** Plant constituents include those compounds containing phenolic, phenylethane derivative and lignans [40]:

a. Phenolic compounds, such as phenyl propanoids.

b. Phenyl ethane derivatives, such as salidroside (hodioloside), rosavin, syringin, triandrin, tyrosol [39].

c. Lignans, such as eleuthoroside E, Schisandrin B [39].

R. rosea, S. chinensis and E. senticosus are the adaptogenic plant containing relatively high amount of phenolic compounds, particularly phenyl propanoids or phenyl ethane derivatives. These groups of active constituent are synthesized from tyrosine, and it is similar to the biosynthesis of catecholamine of sympathetic nervous
system. Due to their structural resemblance to catecholamine, they may act on the early stage of stress response, through sympathetic and central nervous system [39].

**Table No. 2.2: List of polyherbal antistress formulation [4].**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>POLYHERBAL PRODUCT</th>
<th>HERBAL INGREDIENTS</th>
</tr>
</thead>
</table>
| 1       | GERIFORTE           | *Asparagus Racemosus, Withania somnifera*  
*Piper longum, Glycyrrhiza glabra, Shilajit,*  
*Centella asiatica, Myristica fragrans*  
*Terminalia chebula, Cichorium intybus* |
| 2       | AVM                 | *Withania somnifera, Emblica officinalis,*  
*Asparagus Racemosus, Ocimum sanctum,*  
*Tribulus terrestris, Piper longum & Dioscorea* |
| 3       | ADAPT-232           | Fixed combination of *Schisandra chinensis,*  
*Rhodiola rosea & Eleutherococcus senticosus* |
| 4       | Zeeters             | *Withania somnifera, Ocimum sanctum* and  
*Emblica officinalis.* |
| 5       | AP-3000             | *Panax ginseng, Withania somnifera,*  
*Myristica fragrans and Piper longum.* |
| 6       | TRIPHALA MEGA EXT   | *Terminalia chebula, T.bellerica,* and  
*Emblica officinalis.* |
| 7       | TRIKATU MEGA EXT    | *Piper longum, Piper nigrum* and  
*Zingiber officinale.* |
| 8       | VEDIC CALM          | Bacopa monnieri, Centella asiatica |

**Group-II:** Plant constituents include those compounds containing tetracyclic triterpenes, such as cucurbitacin R diglucoside, ginsenoside, Phytosterol-glycoside (e.g. SG, eleutheroside A, sitoindosidin, daucosterol) [39]. Phytosterols and
phytoecdysteroids of triterpenes have adaptogenic property in human being and mammals [4]. Panax ginseng, Eleutherococcus senticosus, Withania somnifera, Aralia mandshurica and B.alba are the adaptogenic plants; and they contain triterpenoid saponins. Triterpenes structurally resembles to corticosterone; and corticosterone is the stress hormone, as well as involved in inhibition of overreaction to stressors by auto feedback inhibition. Triterpenes involve with HPA-axis mediated regulation of immune and neuroendocrine system [4]. Eleutheroside E may be responsible for antistress activity of Eleutherococcus senticosus [42].

**Group-III**: Plant constituents include those compounds containing oxylipins. They are unsaturated trihydroxy or epoxy fatty acids, resembling to leukotrienes and lipoxines [40]. Bryonia alba plant contains polyhydroxylated oxylipins, which are responsible for adaptogenic activity. These three groups of plant constituents originate from dissimilar biosynthetic pathways with different chemical structure.
FIGURE 2.13: Active constituents of plant adaptogen. Adapted from [39].

2.9.2. Antistress mechanism of adaptogens: Adaptogen shows stress protective effect by restoring normal homeostasis of a disordered or highly stressed system. As shown in the figure 2.14, the mechanism of antistress action involves effect on the neuroendocrine-immunologic axis that constitutes the stress system [40].
FIGURE 2.14: Effect of Adaptogens on neuroendocrine-immunologic axis and symptoms of stress. Adapted from [4].

HPA axis and sympatho adrenal system are the primary site of action of adaptogens, whereas components of immune system are their secondary site of action [4, 40].

Adaptogen: Regulation of Homeostasis at cellular level: A cell exists in one of the following stage:
- Cells under homeostatic balance.
- Cells under threatened homeostatic imbalance (Stress).
- Cells under adaptation to stress.
- Cells under apoptosis.

Cell under threatened homeostatic imbalance (stress): cells homeostatic balance is threatened under different stressful conditions like cold, heat, infection, radiation, physical load, emotional stress. Stress signal activates JNK (a stress activated protein kinase), which transduce stress stimuli into intracellular stress responses as shown in the figure 2.15. The stress responses of JNK are as follows:
- JNK indirectly diminishes energy required for normal functioning of cells. ATP production is reduced with inhibition of glycolysis by modification of SH group of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [17]. Due to diminished ATP, several cell functions are suppressed. Aggressive radicals and nitric oxide production is increased by JNK. Stress induced nitric oxide, strongly inhibit the ATP generation in the cell [4].
JNK indirectly increases Cortisol level. Feedback inhibition of Cortisol is required to protect organism from overreaction to stress. But due to JNK, this protection is threatened. Glucocorticoid receptors for cortisol are suppressed by JNK, which leads to cease of feedback inhibition of Cortisol. [39]. But JNK also help in stress resistance by translocating FOX O transcription factor to the nucleus, resulting in synthesis of proteins. This leads to stress resistance, survival and increase life span [39].
FIGURE 2.16: Molecular mechanism behind antistress effect of adaptogen. Adapted from [39].

Cells under adaptation to stress or effect of adaptogen such as ADAPT-232 on cell during stress: ADAPT-232 is a fixed combination of three adaptogenic plants extracts, namely *Schisandra chinensis*, *Eleutherococcus senticosus* and *Rhodiola rosea*. ADAPT-232 has shown vaccine like activity against stress by activating stress induced self-defense mechanism, so that cell and organism adapt with counteracting stress induced harmful effects. As shown in the figure 2.16, this is achieved by decreasing nitric oxide, cortisol and JNK. ADAPT-32 also stimulates the expression of HSP 70, FOX O under stress [39].

The key mechanism of the adaptogenic property through molecular chaperons (HSP 72, Heat shock protein of 72 K Da) is as follows:

- When ADAPT act, a protein (HSF-1) bind to gene and initiate transcription or production of HSP. Heat shock Protein assist in repair of damaged or misfolded proteins by promoting correct three dimensional folding and by preventing their aggregation [39].
- HSP 70 inhibits stress induced expression of nitric oxide synthase II gene resulting in reduced level of nitric oxide. So ATP level will be normal in adapted cell. Due to less nitric oxide, mitochondrial respiration is not interfered; and normal glycolysis pathway leads to efficient ATP production. Thus performance and endurance are improved by adaptogen [17].
- HSP inhibit JNK, and consequently stress responses of JNK are suppressed. Because glucocorticoid receptor are not suppressed by inhibited JNK. Glucocorticoid receptor will be normal, so that feedback inhibition of Cortisol work to protect organism from over reaction of stress [39].
- HSP 72 acts as a chaperone by binding antigenic peptide, then deliver to antigen presenting cells (APC). HSP 72 also functions as cytokine by stimulating pro-inflammatory cytokine release. So HSP 72 Posses chaperokine activity due to their dual function as chaperone and cytokine [39].

Adaptogens phosphorylate FOX O and its translocation to the nucleus resulting in synthesis of proteins; and this leads to stress resistance and increase life span [39].

**Adaptogen regulate following key mediators of the stress response:**
- HPA axis mediator, such as cortisol: cortisol inactivates overreacting stress response [39].
- Nitric oxide mediator: Nitric oxide is intracellular signaling molecule involved in modulation [39].
- Stress activated protein kinase mediator, such as C-Jun N-terminal protein kinase 1 (JNK1). JNK1 is involved in signaling system through which cells transduction of extra cellular stimuli into intracellular response occurs [39].
- Molecular chaperons, such as Heat shock protein HSP 70, HSP 16: They are involved in stress induced cytoprotection and adaptation to repeated stress [39]. During stress, Carnosic acid has shown neuroprotective property by the activation of HSP 70 expression [43]. Even *Cichorium intybus* extracts was found to increase HSP 70 expression [43].
- Energy providing molecule such as ATP: The biosynthesis of ATP, Provides energy required during stress [39].
- Transcription factor such as Fork head BOX O (FOX O) transcription factor DAF-16. They control synthesis of protein involved in stress resistance [39].
- Beta-endorphin [39].
Adaptogen may work on balance between “switch on” and “switch off” system or reactivity: According to this concept, stress activates sympathoadrenal system and HPA axis; and with the formation of various endogenous regulators of cells and organs function, such as catecholamine, cytokines, nitric oxide etc. Due to this switch on system or reaction, activates energetic and metabolic resources of the organism [4, 13, and 40]. Switch off system is the counteracting mechanism, which protects cells and the whole organism from damaging overreaction to stress. This switch off system includes corticosteroids, CRF, PGE₂, HSP, interleukins, antioxidant enzymes, such as catalase glutathione peroxidase, superoxide dismutase [40]. They prevent defense mechanisms from overreacting. Plant adaptogen could be the agents that reduce the reactivity of the host defense system to stressor, and restore homeostasis [40].

Summary of mechanism of Adaptogen: Stress system involve immune, sympathoadrenal and HPA axis; and they share several mediators with effects in common [17]. So antistress or Adaptogen may act at any target.

- HPA axis is one of the main targets for antistress drug [40]. Adaptogen on subchronic pretreatment, normalize levels of stress hormone. Antistress drug re-establish the functioning of HPA-axis, improve the sensitivity of receptors involved in negative feedback mechanism and protect organism from over reaction to stress. This is achieved through mediators like Heat shock protein 70 [39].

- Another target for Adaptogen is the stress hormone neuropeptide Y: it has been found that stimulation and release of NPY or HSP 72, is a defense response of mild stressors; and it increases adaptation to strong stress [44]. Thus Adaptogen may work like vaccine by inducing mild activation of the stress response in order to adopt severe stress [39].

- Another mechanism for antistress effect is that, prevention of stress induced increases in nitric oxide. Consequently rise of energy causes increase in performance and endurance [17, 45].

- Antioxidant property of certain plants may contribute to antistress effect [17]. Because stress induced radicals impair defense mechanism [4] with disturbed homeostasis.
2.10 Literature review of medicinal Plants: It includes *Vitis vinifera* and *Cichorium intybus* plants.

2.10.1 Literature review of *Vitis vinifera* Plants:

**Introduction**: *Vitis vinifera* is a large deciduous climber plant, climbing by means of tendrils. Fruit of *Vitis vinifera* (Grape) is considered one of the world largest fruit crops; it comes to us, out of the abyss of antiquity [46]. The worldwide annual production approximates 58 million metric tons. Recently, *Vitis vinifera* polyphenols attracts attention of investigator, due to their strong beneficial health effects; and it has been directly attributed to the so called “French paradox”. The tradition of regular consumption of red wine by the French population, have contributed to their reduced mortality from coronary heart diseases; and it has happened, inspite of the presence of a local diet rich in saturated fats [47]. Proanthocyanidins present in red wine are responsible for this phenomenon [48]. The unique combination of bioactive components of *Vitis vinifera*, flavonoids glycosylation, type of sugar residues and subsequent acyl esterification are responsible for their wide spectrum of pharmacological actions and traditional uses [49].

**Synonym**: wine grape, European grape, common grape.

**Vernacular names (common names)** [50, 51]:

English: Grapes.
French: Vigne, Cultive.
Hindi: Angur, Dakh, Prakh.
Telugu: Draksha-pandu.
Kannada: Drakshi.
Sanskrit: Dakha, mridirka, draksha.
Tamil: kotumuntiri, Tiratcai, Aravavam.
Gujarathi: Mudrake, Draksh [50, 51].

**Plant Taxonomy**

Kingdom: Plantae.
Subkingdom: Viridae Plantae.
InfraKingdom: Streptophyta.
Division: Tracheophyta.
Subdivision: Spermatophyta.
Infradivision: Angiospermae.
Class: Magnoliopsida.
Superorder: Rosanae.
Order: Vitale.
Family: Vitaceae.
Genus: Vitis.
Species: *Vitis vinifera*.

**Distribution and habitat:** *Vitis vinifera* is cultivated throughout India. Its cultivation is large in Afghanistan, Baluchistan, Kashmir, Punjab and north-Western India [50, 51]. It is cultivated on every continent in the temperate climatic regions with sufficient rain, mild winter and dry summer [49]. It is one of the most economically important plant species, which are grown worldwide for their edible fruits, raisins, and beverages such as wines, currents, sultanas [52].

**Historical aspects of *Vitis vinifera***: Since Neolithic period or new Stone Age (10,200 BC), humans have interacted with the *Vitis vinifera* plant. Ancient jars of olden days (5400-5000 BC) were found in well preserved condition; and these jars were used for wine production in the Middle East. The historical importance of wines and grapes in ancient societies were demonstrated by their images on coins, temples, ritual potteries and mosaic sculptures. Grape and wine were considered divine in eastern Mediterranean and Balkans. They dedicated grape and wine to various deities: “Dionysus” by Greeks, “Zagreus” by Thracians and “Bacchus” by the Romans [49].

**Description of the plant:** *Vitis vinifera* is a large deciduous climber, climbing by means of tendrils to 35m tall with flaky bark. Leaf is opposed and often bifid. Leaves are simple and alternate with more or less deeply 3-5 lobed. Leaves are orbicular-cordate, irregularly toothed, 5-20 cm long and board. Leaves are thin membranous, glabrescent above and grey tomentose beneath. Flowers are small green, paniched cymes and hermaphrodite. They are pollinated by insects. Fruit is a bluish black berry, ovoid to globose upto 3cm long. It can be green, red or purple. Seeds are 2-4 with a discoidal tubercle on the back [51].

**Phytochemistry:** Reviewing the current literature of *Vitis vinifera*, it was found that, the characteristic compounds of this plant are: simple phenolic and poly phenolic compounds. Other constituents include sugar, sterols, amino acid, minerals, vitamin E, protein and lipids. *Vitis vinifera* includes unique combination of bioactive phytochemicals, such as simple phenolic compounds, flavonoids, proanthocyanidins, anthocyanins, stilbenes and vitamin E [49].
A: *Vitis vinifera* plants

B: *Vitis vinifera* fruits

C: *Vitis vinifera* seeds

FIGURE 2.17: Photograph of *Vitis vinifera*
Simple phenolic compounds are derivatives of hydroxycinnamic acid and hydroxybenzoic acid [49]. Like P-coumaric acid, caffeic acid, sinapic acid and ferulic acid, gallic acid, gentisic acid, protocatechuic acid, P-hydroxybenzoic acid [49].

Polyphenolic compounds or Flavonoids: The most common flavonoids of Vitis vinifera are anthocyanins, flavonols, flavanols and proanthocyanidins [49].

I. **Anthocyanins** are either as monoglucosides and diglucosides or derivatives as their acetyl, P-coumaroyl- and/or caffeoyl-esters.

II. **Flavonols** or Flavon-3-ols are as following:

   A. Flavonols as 3-0-glycosides (in Grape skin): Quercetin 3-0-glycoside, Kaempferol 3-0-glycoside, myricetin 3-0-glycoside, Laricitrin 3-0-glycoside, isorhamnetin 3-0-glycoside, Syringetin 3-0-glycoside.
   
   B. Flavonols as aglycones: They found mainly in wine and juice.

III. **Flavanols** or Flavan-3-ols are as following:

   A. Monomeric flavan-3-ols: (+)-catechin, (-)-epicatechin and (-)-Epicatechin-3-0-gallate.
   
   B. Oligomeric flavan-3-ols: procyanidin or proanthocyanidin.

FIGURE 2.18: Structures of anthocyanins (flavonoids) of Vitis vinifera. Adapted from [49]
Phytochemical investigation of different parts of *Vitis vinifera* plant, revealed the presence of following phytochemical constituents. Investigators have utilized different technique for the extraction, separation and identification of phenolic constituents from *Vitis vinifera* plant [53-68]. It is summarized as follows:
Fruits: *Vitis vinifera* fruits are the richest source of polyphenols. The most abundant bioactive polyphenol of this fruit are flavonoids, which are found in the outer epidermal cells of skin, flavanols found in skin of the berries as flavonoids [49].

The second most common abundant flavonoids in grapes skin are flavonols in the form of 3-0-glycosides of quercetin, kaempferol, isorhamnetin and myricetin derivatives [49]. Both red and white varieties of *Vitis vinifera* consist of derivative of Quercetin, Kaemferol and isorhamnetin, whereas myricetin derivatives are found only in red variety [49]. Quercetin-3-0-glucoside and Quercetin-3-0-glucuronide is the predominant flavonols compound present in *Vitis vinifera* [49].

Monagas M. et. al. have identified following anthocyanin in the skin of *Vitis vinifera* fruit [69]. Delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, delphinidin-3-(6-acetyl)-glucoside, cyanidin-3-(6-acetyl)-glucoside, petunidin-3-(6acetyl)-glucoside, peonidin-3-(6-acetyl)-glucoside, malvidin-3-(6-P-coumaroyl)-glucoside, peonidin-3-(6-caffeoyl)-glucoside, malvidin-3-(6-caffeoyl)-glucoside, delphinidin-3-(6-p-coumaroyl)-glucoside, petunidin-3-(6-p-coumaroyl)-glucoside and malvidin-3-(6-P-coumaroyl)-glucoside [69].

Fruit contain other phytochemical constituents, such as glucose, tannin, tartaric acid, malic acid, citric acid, racemic acid, gum, potassium chloride, sodium chloride, potassium sulphate, tartrate of lime, magnesia, aluminum, iron. Ash content is 0.05%. Tartaric acid is the characteristic acid of *Vitis vinifera* [50].

Seeds: It contains lipid, protein, carbohydrate and polyphenol [70]. 60-70% of total polyphenols are accumulated in seeds [49]. Seeds are rich source of procyanidin type of proanthocyanidin [71]. Seeds have a higher content of proanthocyanidin than rest of the grape [72].

Polyphenols in grape seeds are mainly flavonoids such as monomeric flavan-3-ols (catechin, epicatechin, gallocatechin, epigallo catechin and epicatechin 3-0 gallate), procyanidin (dimers-trimer and more highly polymerized procyanidin bigger than pentamer), gallic acid [70]. Monagas et. al. analyzed *Vitis vinifera* seed extract and identified phytoconstituent like Gallic acid, monomeric flavans-3-ols [(+ )- catechin, (-)-epicatechin, epicatechin-3-0-gallate] and procyanidin [B1, B2, B3, B4, B3´-3-0-gallate and trimers] [69]. One more study have reported presence of monomers [(+) catechin, (-)-epicatechin, (-)-epicatechin-3-0-gallate], 14 dimeric, 11trimeric procyanidin, one tetrameric procyanidin and highly polymerized procyanidin in the
seeds of *Vitis vinifera* [73]. In another study it has been reported that, seeds have seventeen phytoconstituent, such as 11% of (+)-catechin, (-)-epicatechin (10%), 6% of epicatechin-(4β→8)-epicatechin, 7% of epicatechin-3-0-gallate-(4β→8)-catechin, 9% of (-)-Epicatechin-3-0-gallate [73]. Anthocyanin or flavonol does not present in the seed [71].

**Leaves:** Monagas M.et. al. have identified following phytoconstituent in the leaves of *Vitis vinifera* [69]: Delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, cyanidin-3-(6-acetyl)-glucoside, peonidin-3-(6-acetyl)-glucoside, delphinidin-3-(6-p-coumaroyl)-glucoside, petunidin-3-(6-p-coumaroyl)-glucoside and malvidin-3-(6-P-coumaroyl)-glucoside [69].

**Traditional uses of *Vitis vinifera***: Different parts of *Vitis vinifera* have been used traditionally for the treatment of diseases.

**Fruit:** Traditionally the fruits (Grape) have been used as a laxative, diuretic, stomachic and demulcent. They are also recommended in some cases of bilious dyspepsia, dysuria, and hemorrhages. Fruits show some beneficial effect in chronic bronchitis, Bright’s disease and heart disease. During jaundice, *Vitis vinifera* fruits were used as diet. In Bruises and Sprains conditions, juices of sour fruit have been used [50]. Fruits of *Vitis vinifera* (grapes) have been used as cardiotonic, haematinic, aphrodisiac, nerve tonic, febrifuge, de purative, antispasmodic, stomachic, expectorant and tonic [51].

**Leaves:** The leaves of *Vitis vinifera* plant are found to be useful in scabies, syphilis, hemorrhoids, splenomegaly, stomatorrhagia and skin diseases [51].

**Stem:** Ash of the stem has been used for arthralgia, hemorrhoids, vesical calculi and orchitis [51].

**Flowers:** The flowers have been used as expectorant, haematinic and emmenagogue. They are recommended for treatment of bronchitis, hepatopathy, anaemia, amenorrhoea and dysmenorrhea [51].

**Pharmacological actions.**

Polyphenols as phytoconstituent from plant sources were found to possess different biological activity [74]. Polyphenols have been proved as adaptogen scientifically [75]. Phenolic contents of fruit seeds exhibited antioxidant activity [76]. Polyphenols as flavonoids have shown antioxidant activity [77–78], and protection against myocardial ischemia reperfusion injury [79].
**Action on brain function:** Brain function and CNS performance can be improved by consuming flavonoids of *Vitis vinifera* [49]. Anthocyanin flavonoids of grape, can avoid neuro-degeneration by inhibiting oxidative stress and neuro-inflammation. In a clinical investigation, fruit juice has improved memory function in cognitive impaired patient [49]. *Vitis vinifera* seed derived polyphenol have shown recently significant ultra-structural alteration of native paired helical filaments in Alzheimer’s patient [49]. Feng y et. al., [80] found that grape seed extract exhibited neuroprotective effect in the neonatal rat of hypoxia-ischemic brain injury model. The authors attributed the observed effect to the antioxidant and anti-lipid peroxidation activity of extracts constituents [80]. Proteomics study of the actions of grape seed extract in rat brain demonstrated that extract might in fact mitigate or accentuate the actions of some psychoactive drug by maintaining an overall viability of the nervous system [81]. Grape seed extract exhibited improvement in memory and brain long term potentiation deficits due to ischemia in rats [82].

**Hepatoprotective action:** Polyphenols of *Vitis vinifera* have the capability to protect liver from diseases due to environmental factors such as viral infection, alcohol, pollutants; and this hepatoprotective action can be attributed to the anti-inflammatory and antioxidant activity [49]. Recent research showed that polyphenol-rich grape skin extract has been found to protect against diet-induced adiposity and hepatic steatosis; these effects were probably due to the inhibition of lipogenic enzymes in liver and adipose tissue, and also by regulation of mRNA expression enzymes, which are involved in regulation of lipogenesis and fatty acid oxidation. Researchers have shown that ethanol grape seed extracts possesses hepatoprotective activity against ethanol-induced cytotoxicity in the liver [49]. Ethanolic extract of *Vitis vinifera* seeds exhibited liver protection activity during diabetic condition [83].

**Action on cardiovascular diseases and disorders:** Review of different literature revealed that, consumption of fruits of *Vitis vinifera* have beneficial effect on cardiovascular system, and it may be due to decreasing of LDL oxidation, improvement of vascular function, enhancing of endothelial function, alteration of blood lipids or modulation of inflammatory process [49]. A Clinical study demonstrated that flavonoid rich *Vitis vinifera* fruit juice consumption has inhibited thrombosis; and this effect was probably due to the suppression of platelet-dependent inflammation [49].
Karthikeyan et. al., [84] found that grape seed proanthocyanidin exhibited cardioprotective and hypolipidemic properties in isoproterenol induced myocardial injury in rats. Sano T et. al., [85] investigated that the antithrombotic effect of grape seeds proanthocyanidin, on laser induced thrombus in carotid artery of mice. Authors found that antithrombotic effect of proanthocyanidin was due to direct inhibitory effect on platelets; and they also reported that proanthocyanidin could be useful in thrombotic diseases in human being.

**Action on obesity, diabetes and metabolic syndrome:** Recently it was found that polyphenols of *Vitis vinifera* have reduced metabolic syndrome and prevented development of obesity and Diabetes; and it could be due to their antioxidant and anti-inflammatory effects [49].

Terra et. al., [86] found that daily consumption of grape seed procyanidins have prevented both systemic and local low grade inflammation in muscle, liver and adipose tissue; and it could improve obesity-induced insulin in these tissue.

Abir et. al., [87] demonstrated that grape seed proanthocyanidins have protective effect against diabetes, induced by alloxan in rats. The authors reported that such protection was mediated via prevention and restoration of pancreatic antioxidant defense system [87].

Sivaprakasha Pillai et. al., [88] conducted a clinical study to determine effect of grape seed extract on blood pressure of adults with metabolic syndrome. The authors found that antioxidant properties of phenolic compounds, reduced the concentration of oxidized low-density lipoprotein; and this effect may be responsible for decreasing blood pressure [88]. *Vitis vinifera* seed extract exhibited protective effect against streptozotocin induced hyperglycaemia in rats [89].

**Anticancer activity:** Review of scientific literature revealed that, flavonoids of *Vitis vinifera* possesses anticancer activity [49]. In a research, it was found that grape skin polyphenol exhibited antitumor and antimetastatic activities against breast cancer in model system [49]. In another study, researchers have demonstrated that extracts of *Vitis vinifera* have cancer preventive efficacy on colon cancer cells; and the authors attributed the observed effect to the antioxidant and anti-inflammatory effects of extract’s constituents [49]. In a study, proanthocyanidin of *Vitis vinifera* seed, significantly reduced cell viability and induced apoptosis in human pancreatic cancer cells; and they observed inhibition of migration of human pancreatic cancer cell is by inactivation of the inflammatory transcription factor NF-κB [49].
**Antioxidant action:** Review of scientific literature revealed that flavonoids of *Vitis vinifera* exhibited suppressive effect on oxidative stress and prevented oxidative damage [49].

Pari et. al., [90] have demonstrated that grape leaf extract have protective effect against alcohol induced oxidative stress toxicity in rats; and the authors attributed the observed effect to antioxidant and anti-lipid peroxidation activities of extract’s constituents.

Balu M. et. al., [91-93] have found that grape seed extract enhanced the antioxidant status in the CNS of aged rats, by reducing the free radical induced lipid peroxidation.

Jayaprakash et. al., [73] have reported antioxidant activity of *Vitis vinifera* seed extract on peroxidation with invitro model; and also they reported that grape seed extract might be useful for health supplements and nutraceuticals.

Baydar et. al., [94] have found strong antioxidant activity in grape seed extract than bagasse extract in a study; and they attributed the observed effect for the high phenolic content in seed extract.

Polyphenols of red wines possess antioxidant activity [95]. Polyphenolic compounds of grape skin extracts have exhibited antioxidant and antimicrobial activities [96]. Black grape extract have exhibited antioxidant property against cyclosporine A induced oxidant stress and peroxidation in rat ovary tissue [97].

**Anti-inflammatory action:** Proanthocyanidins of grape seeds have shown anti-inflammatory action, because they target multiple pathways, such as scavenging free radicals, prevention of lipid peroxidation, and inhibition of formation of pro-inflammatory cytokines [49]. In a study proanthocyanidins of *Vitis vinifera* have shown immune-modulatory action in inflammatory condition [49]. Extracts of grape pomaces have shown suppression of chronic inflammation, induced by lipopolysaccharide and galactosamine, and it could be due to suppression of activating inflammatory transcription factor NF-κB [49].

**Antimicrobial and antiviral activities:** Review of literature revealed that, antimicrobial activities of *Vitis vinifera* have been widely studied. It has been found that *Vitis vinifera* seed extract exhibited stronger antibacterial activity against Gram-positive bacteria compound to the Gram-negative bacteria. The authors found that, gram-positive bacteria like *Bacillus cereus*, *Bacillus coagulants*, *Bacillus subtilis* and *Staphylococcus aureus* were inhibited completely at 850-1000 ppm, whereas gram-
negative bacteria like E. coli and Pseudomonas aureginosa were inhibited at 1250-1500 ppm concentration [98].

In another study, grape extracts exhibited strong inhibitory effect on the growth of Listeria monocytogenes [49]. In a study, grape skin extracts have shown antiviral activity against influenza virus and this study revealed that antiviral activity could be due to extract’s constituents such as catechin, epicatechin and rutin [49]. Wine phenolic have shown antifungal activity on Candida albicans [49].

Baydar et. al., [46] have demonstrated grape seed extract may be useful as antibacterial agents to avoid stored food deterioration.

**Other activities:** Polyphenols as procyanidin in grape seed possess bioactivity in glucose and lipid metabolism with macrophage functionality [99]. It has been found that Vitis vinifera seed extract exhibited antilisterial activity [100] and antiulcer activity [101]. Vitis vinifera exhibited potential antiallergic, antianaphylactic and mast cell stabilizing activity in the management of asthma [102]. Vitis vinifera have beneficial effect in the psychosomatic disorders [103].

Satyanarayana et. al., [104] demonstrated the antioxidant and nootropic activities of Vitis vinifera seed extract. They also demonstrated adaptogenic activity by measuring urinary excretion of vanillylmandelic acid and ascorbic acid [104]. Vitis vinifera extract exhibited neuroprotective effect on prediabetic mice [105]. Grape seeds proanthocyanidins have shown protective effect against lipid peroxidation and DNA fragmentation [106]. Proanthocyanidins of grape seeds have shown protective effect on stress induced oxidative gastrointestinal injury in rats [107].

**2.10.2. Literature review of Cichorium intybus plant.**

**Introduction:** Cichorium intybus belonging to family Asteraceae is an erect fairly woody perennial medicinal herb. Its height is around 1 meter with large basal leaves, bright blue flowers (rarely white or pink) and fleshy taproot of length upto 75cm [108]. Six species of the genus Cichorium are distributed mainly in Europe and Asia.

**Synonyms:** Chicory, blue sailor’s succory and coffee weeds are the popular common names. Other are Wild chicory, Kasani, Italian dandelion, Garden chicory, Witloof chicory, Cikorie, Chicon, Blue dandelion, Blue wegwarte, Brunswick, Curly endive.

**Vernacular names (common names)** [50, 109]:

English: Chicory, Wild endive.
Greek: Kichora, Kikori.
Hindi: Kasni.
Gujrathi: Kasani.
Tamil: Kashini Virai, Tsikorie.
Telugu: Kasini vittulu.
Kannada: Kacani, Kachani.

**Plant Taxonomy:**

Kingdom: Plantae.
Sub kingdom: Viridaeplantae.
Infra kingdom: Streptophyta.
Division: Tracheophyta.
Sub-division: Spermatophytina.
Infradivision: Angiospermae.
Class: Magnoliopsida.
Superorder: Asteranae.
Order: Asterales.
Family: Asteraceae.
Genus: Cichorium L.
Species: *Cichorium intybus* L.

**Distribution and Habitat:** *Cichorium intybus* is native to the temperate area. It can be found along the roadsides, field edges, railways and hedge-rows. It grows wildly almost in all types of soil and in areas upto 1,800 m elevation. The plant is cultivated throughout India, such as in north western area, Punjab, Kashmir, Maharashtra, Andhra Pradesh, and Karnataka. Countries which cultivate *Cichorium intybus* are France, Germany, Netherland, Switzerland, Belgium, United Kingdom, West Asia, South Africa, Waziristan, Persia and Baluchistan [109].

**Description of plant:** *Cichorium intybus* is a hardy, erect, glandular perennial plant. All plant parts exudates milky latex on breaking it. It can withstand extreme temperature during vegetative and growth condition [108]. Leaves are oblong lanceolate crowded at the base and arranged spirally on the stem. Leaf is simple with 3-10 lobes per side, leading edge of the lobe often toothed. Flowers are bright blue to pale blue, blooming occurs in a month of May-October. Flowering heads are open once in the morning and wilt in the afternoon in the month of May-October. 10-15mm long, 5 heads open per day per plant. Floral branches are ascending, 1-120cm long, ridged and green. Fruit are smooth angled achene, with 1 seeded, 2-3mm long, 1-1.5
mm wide, 1mm thick, 4 or 5 ridges near the base and dark brown, crowned with a ring of pappus scales.

A: *Cichorium intybus* plants

B: *Cichorium intybus* roots

C: *Cichorium intybus* roots (Dried)

FIGURE 2.22: Photograph of *Cichorium intybus*
Pappi of 28-45, imbricate scales are 0.3mm long. Stem is erect, tough, branched, rigid, spreading, hollow, 1-3 long angled and grooved. It is very hairy near the reddish stiff base. Roots are fleshy, 10-75cm long, 1cm wide, tapering and somewhat branched. When fresh, inside and outside color is white. It is covered by dense rootlets. The root tastes sweetish and then very bitter.

**Microscopy:** A typical dicotyledenous structure with secondary growth is present in the cross section of *Cichorium intybus* root. Central core of a tri or pent arch xylem and the phloem lying between two xylem arms are present in the lateral root. Then a zone of cortex, which is externally lined by a single layered epidermis, is covered. It possesses few tracheids. Phloem shows sieve tubes, few fibers and parenchymatous medullary rays traverse radially from xylem to phloem. Cork cells and lactiferous vessels are also found [109].

**Phytochemistry:** Reviewing the current literature of *Cichorium intybus*, it was found that the characteristic compounds of this plants are Inulin, sesquiterpene lactones, phytosterols, triterpenoids, flavonoids, coumarins (including cichorin), caffeic acid derivatives, vitamins, tannins, pectins and fats [110-112]. The major compound in methanolic extract of chicory was found to be chicoric acid [108]. The main constituents of plant are aliphatic compound and their derivatives; whereas minor constituents are terpenoids. *Cichorium intybus* contains mainly Sesquiterpene lactones such as lactucin, 8-deoxylactucin and lactucopicrin [113].

Phytochemical investigation of different parts of *Cichorium intybus* plant, revealed the presence of following phytochemical constituents, and it is summarized as follows: Flowers contain glucoside, cichorrin, lactucin, intybin. It contains flavonoids, saccharides, methoxy-coumarin, cichorine and essential oils. The blue color of the perianth is due to anthocyanin [108]. Other compounds of flowers identified are as follows [113, 114]: Delphinidin-3,5-di-O-(6-O-malonyl-beta-D-glucoside), Delphinidin-3-O-(6-O-malonyl-beta-D-glucoside)-5-O-beta-D-glucoside, Delphinidin-3-O-beta-D-glucoside-5-O-(6-O-malonyl-beta-D-glucoside), Cynadin 3-malonylgucoside, Delphinidin 3,5-di-O-beta-D-glucoside and 3-O-p-coumaroyl quinic acid [113].
FIGURE 2.23: Structure of Sesquiterpene lactones of *Cichorium intybus*. Adapted from [113].

Seeds contain oil, with 21.7% saturated fatty acids and 78.3% unsaturated fatty acid. Stems contain coumarin as umbelliferon, esculetin (6, 7-dihyrocumarin) scopoletin, esculetin and cichorin (esculetin 7-o-β-D-glucoside) [113].

Leaves contain chicoric acid, caffeic acid, flavonoids as isorhamnetine, apigenin, apigenin-7-O-L-arabinoside, Luteolin-7-O-glucuronide, quercetin-3-o-glucoronide, campheroil-3-O-glucoside, C-glycosiflavone, selenium compounds [113]. In another study, researchers investigated on free sterols and polyamine present in *Cichorium intybus* leaves and they found sitosterol, stigmasterol, campesterol, putrescine and spermidine [115].

Roots contain inulin, mucilage, nitrates and sulphate of potash [113]. Researchers have established structural characteristics of inulin present in *Cichorium intybus* roots, particularly degree of polymerization [116]. Milk juice of root contains sesquiterpene, lactone of guajanolid type, lactucin and lactucopicrin (8-p-Hydroxy phenylacetillactucin). Fresh root contains moisture, fats, cellulose, inulin, fiber, glucose, bitter extractives and ash-0.8%. Sesquiterpenes lactone are *Sonchusides A*
and C, Cytokinin, Crepdiase B, Cichoriolide A, Cichoriosids B and C, ribosylzeatin a nucleotide sugar, lactucopicrin, lactucin, caffeic and chicoric acid [109]. The root produce latex, inulin, bitter compound of lactucin, lactucopicrin, intybin, cichorin, tannins, pectin, fructose, fixed oils and alkaloids [109]. Some of the reported phytoconstituents of *Cichorium intybus* are as follows: Bischoff et. al., [117] isolated and reported the presence of lactucin and lactucopicrin sequiterpene lactones from *Cichorium intybus*. Shah et. al., [118] isolated and reported the presence of sequiterpene lactones in *Cichorium intybus* like 1, 5-hydroxytaraxacin, 6, 8, 11-epi-desacetyl-matricarine, desacetylmaticarine, 11-13-dihydrolactucin and 11β-13-dihydrolactucopicrin.

Other investigators have identified various compounds of *Cichorium intybus*, such as Lactucin, Jacquilenin, Dihydrolactucin, Dihydrolactucopicrin, Dihydro-15-dihydrolactucopicrin, Magnolialedge, Ixerisoside D, Loliolide, cichorioside B, Sonchuside A, Artesin, Cichoriolide, Cichorioside, Sonchuside C and Cichopumilide [108]. In another research, phenolic acids and flavonoids were characterized in *Cichorium intybus* by using HPLC with mass spectrometry [119]. In another study, volatile composition of roots aerial parts of *Cichorium intybus* has been investigated and reported [120]. In a study, chemical composition and biological properties of different parts of *Cichorium intybus* has been determined. Root inulin with phenolic was characterized in extracts and they were found to be responsible for physiological activity [121].

Shad et. al., [122] have determined biochemical, phytochemical and antioxidant properties of different parts of *Cichorium intybus*. They found tannins, saponins, flavonoids, terpenoids, cardiac glycosides and anthocyanins in each part of the plant. They also reported antioxidant property of *Cichorium intybus* leaves [122].

In another study, various phenolic constituents of *Cichorium intybus* were identified as follows [108]: Lactucopicrin; Lactucin; Loliolide; Jacquilenin; 8-Deoxylactucin; 11,13 Dihydrolactucin; Artesin; 3,4-Dihydro-15-dehydrolactucopicrin; Cyanin 3-O-p-(6-O-malonyl)-D-glucopyranoside; 11,13-Dihydrolactucopicrin; Ixerisoside D; Cichorioside B; Dimethoxycinnamoyl shikimic acid; Kaempferol-3-O-glucosyl-7-O-(6-Omalonyl)-glucoside; Cichorioside B; Cichoriolide; Putrescine; Cichorioside; Stigmasterol; Chlorogenic acid; Spermidine; Sonchuside C; Sitosterol; Sonchuside A; Cichopumilide; Campesterol; Cyanidin; Caffeic acid; Crepidiaside A; Cichoralexin; 3-Caffeoylquinic acid; Malic acid; 4,5-
Dicafeoylquinic acid; cis-Caftaric acid; 5-Caffeoylquinic acid; 3,5-Dicafeoylquinic acid; trans-Caftaric acid; 4-Caffeoylquinic acid; 5-p-Coumaroylquinic acid; 5-O-Feruloylquinic acid; cis-5-Caffeoylquinic acid; 5-Caffeoylshikimic acid; Quercetin-3-O-glucuronide-7-O-(6-Omalonyl)-Glucoside; Cyanidin-3-O-galactoside; sorhamnetin-7-O-(6-O-acetyl)-glucoside; Kaempferol-7-O-glucosyl-3-O-(6-malonyl)-glucoside; Quercetin-7-O-glucoside; Quercetin-7-O-glucuronide; Kaempferide glucuronide; Malvidin-3-O-glucoside; Kaempferol-7-O-glucoside; Quercetin-3-O-(6-O-malonyl)-glucoside; Quercetin-7-O-(6-O-acetyl)-glucoside; Kaempferol-3-O-sophoroside; Cyanidin-3-O-glucoside; Quercetin-7-O-galactoside; Cyanidin-3-O-(6-O-acetyl)-glucoside; 3,4-Dicafeoylquinic acid; Pelargonidin-3-O-monoglucuronide; Kaempferol-7-O-glucuronide; Succinic acid; Quercetin 3-O-β-D-glucoside; Oxalic acid; Shikimic acid; Quinic acid; Cyanidin 3-O-β-(6-O-malonyl)-glucopyranoside; Kaempferide-3-O-(6-O-malonyl)-Glucoside; Kaempferol-3-O-(6-O-malonyl)-glucoside; Dicafeoyl tartaric acid (chicoric acid); Kaempferol-3-O-glucuronide; Kaempferol-3-O-glucoside; Delphinidin-3-O-(6-O-malonyl)-glucoside-5-O-glucoside; Chrysoeriol-3-O-gluco side; Tricin-3-O-glucoside; 1,3-Dicafeoylquinic acid; 1,4-Dicafeoylquinic acid; Kaempferol-7-O-rutinoside; Quercetin-7-O-p-coumaroylglycoside; Magnolialide; Isorhamnetin-7-O-neohesperidoside; Kaempferol-7-O-(6-O-malonyl)-glucoside; Magnolialide; Kaempferol-3-O-glucuronide-7-O-glucoside; Myricetin-7-O-(6-O-malonyl)-glucoside; Kaempferol-7-O-(6-O-acetyl)-glucoside; Kaempferol-3-O-(6-O-acetyl)-glucoside; Isorhamnetin-7-O-glucoside; Isorhamnetin-7-O-glucuronide; Delphinidin3,5-di-O-(6-O-malonyl-β-D-glucoside); Delphinidin 3-O-(6-O-malonyl-β-D-glucoside)-5-O-β-D-glucoside; Delphinidin 3-O-β-D-glucoside-5-O-(6-O-malonyl-β-D-glucoside); Delphinidin 3,5-di-O-β-D-glucoside; 3-O-p-Coumaroyl quinic acid; (7S, 8R)-3-Demethyldehydrodiconiferyl alcohol-3-O-β-glucopyranoside; Cyanidin -3-O-(6-O-malonyl)-glucoside; Petunidine-3-O-(6-O-malonyl)-glucoside; 4-O-Feruloylquinic acid; and Apigenin-7-O-glucoside [108].
TABLE NO. 2.3: Traditional uses of different preparations of *Cichorium intybus*.
Adapted from [108].

<table>
<thead>
<tr>
<th>COUNTRY</th>
<th>TRADITIONAL USES</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>Liver disorders, Diabetes, Jaundice, gout, Liver enlargement, Rheumatism and Cough.</td>
</tr>
<tr>
<td>Italy</td>
<td>High blood pressure, Blood purification, arteriosclerosis and antiarthritis.</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>Cholagogue, For gastric secretion and hypoglycemic</td>
</tr>
<tr>
<td>South Africa</td>
<td>Jaundice, tonic</td>
</tr>
<tr>
<td>Jordan</td>
<td>Internal hemorrhage and sedative in typhoid</td>
</tr>
<tr>
<td>Morocco</td>
<td>Renal disease and Kidney disorders,</td>
</tr>
<tr>
<td>Poland</td>
<td>Digestive complaints</td>
</tr>
<tr>
<td>Afghanistan</td>
<td>Malaria</td>
</tr>
<tr>
<td>Turkey</td>
<td>Cancer, kidney stones, Wound healing, Hemorrhoids and urinary disorders</td>
</tr>
<tr>
<td>Pakistan</td>
<td>Diabetes</td>
</tr>
<tr>
<td>Serbia</td>
<td>Diarrhea, Diuretic, laxative, Anti-inflammatory, Liver complaints and hypoglycemic.</td>
</tr>
</tbody>
</table>

**Traditional uses of *Cichorium intybus***: Traditionally decoction of the *Cichorium intybus* seeds have been used in obstructed or disordered menstruation, whereas infusion of powdered seeds have been used in liver obstruction and spleen enlargement. Roots have found to be useful as substitute for coffee, and also in the treatment of dyspepsia, fever etc. Juice of flowers has been used in liver disorder [50]. Reported various uses of *Cichorium intybus* are as follows; such as anti-inflammatory, liver tonic, cardio tonic, diuretic, digestive, cholagogue, appetizer, stomachic, febrifuge, emmenagogue and depurative [51].

In South Africa, tea preparation of roots, leaves have been used for jaundice treatment, whereas chicory syrup as a tonic and purifying medicine for infants [108]. Folkloric
reports of Afghanistan revealed the use of root extract of *Cichorium intybus* for the treatment of malaria. Later on this has been confirmed by the identification of sesquiterpene lactones such as lactucin and lactucopicrin as antimalarial compound [117]. Traditional use of *Cichorium intybus* roots, mentioned in European monograph include the treatment of digestive disorders, such as feeling of abdominal fullness, flatulence, slow digestion, temporary loss of appetite [108].

**Pharmacological actions of Cichorium intybus**

*Cichorium intybus* possesses various pharmacological and therapeutic actions. Some of these actions have been proved scientifically, by different investigations, and they are reported as follows:

**Gastro protective activity:** In a study, antiulcer property of *Cichorium intybus* roots has been demonstrated in rats, and it inhibited 95% of ulcerogenesis induced by ethanol [108, 123].

**Anti-inflammatory activity:** Researchers demonstrated that, chicory root extract have inhibited expression and activity of cyclooxygenase in the human colon carcinoma HT29 cell line. They also found that production of prostaglandin E$_2$ was inhibited by ethyl acetate extract in a dose-dependent manner; and authors also reported that chicory extract suppressed TNF-α mediated induction of cyclooxygenase-2 expression. Rizvi et. al., [124] have investigated and reported anti-inflammatory activity of roots of *Cichorium intybus*. They also reported that anti-inflammatory activity was due to its inhibitory effect on various cytokines and antioxidant activity.

**Anti-oxidant Activity:** Researchers studied antioxidant activity of chicory in chemical and biological system. They established antioxidant activity of *Cichorium intybus* in coupled model of linoleic acid and β-carotene [125]. Whereas in biological system, prevention of lipid peroxidation on microsomal membrane of rat hepatocytes due to carbon tetrachloride; and it was established as antioxidant property. The authors reported that, *Cichorium intybus* showed strong antiradical activity in both systems [64, 126].

Researchers evaluated antioxidant activity of red chicory by using the synthetic 2, 2-diphenyl-1-(2, 4, 6-trinitro-phenyl) hydrazyl radical and enzyme catalyzed reaction (xanthine oxidase, myeloperoxidase, diaphorase); and they reported that significant antioxidant activity of chicory in both the models [108]. In another study, extract of aerial parts of *Cichorium intybus* was found to be inhibiting xanthine oxidase enzyme
in a dose dependent manner [108]. In another study, *Cichorium intybus* exhibited inhibition of hydrogen peroxide, chelation of ferrous ion and DPPH radical scavenging activity [108]. Ozgen et. al., [127] have demonstrated antioxidant activity of *Cichorium intybus* along with other species of Asteraceae family. Red chicory was found to be a potential source of antioxidant anthocyanin [128].

**Anti-diabetic activity:** Pushparaj et. al., [129] investigated hypoglycemic and hypolipidemic activities of *Cichorium intybus*; and the effect of ethanol extract of whole plant on Streptozotocin induced diabetes in rats was observed. The authors found that *Cichorium intybus* at a dose of 125mg/kg body weight, attenuated serum glucose level in the oral glucose tolerance test; and a marked decrease in the serum triglyceride and cholesterol was also observed. The authors reported that, reduction in the hepatic glucose-6-phosphatase activity could have decreased hepatic glucose production, and this has resulted in lower concentration of blood glucose in Cichorium treated diabetic rats [129].

Researchers investigated effect of *Cichorium intybus* on glucose tolerance test with metabolic profile, during early and late stage of diabetic condition in rats. The researchers induced early-stage and late-stage of diabetes in rats by using Streptozotocin- niacinamide and Streptozotocin alone respectively. They found that chicory treatment prevented weight loss in both stages, resisted excessive increase in fasting blood sugar, and normalized blood parameters, such as Alanine aminotransferase, Triacylglycerol, Total cholesterol, Glycosylated hemoglobin. They also reported that chicory treatment during early-stage of diabetes have increased insulin level, showing insulin sensitization [108].

**Hepatoprotective activity:** A clinical study was conducted for the efficacy of Hepatoprotective effect of tonic liv-52, containing *Cichorium intybus*, on liver cirrhotic patients. In this randomized double blind study, liv-52 medication reduced the serum levels of Alanine aminotransferase and Aspartate amino transferase [108].

Researchers evaluated Hepatoprotective action of Jigrine, a polyherbal formulation containing leaves of *Cichorium intybus*. Galactosamine was used to induce hepatopathy in rats. They found that, pretreated rats with Jigrine significantly reduced the levels of Alanine transaminase, Aspartate transaminase and urea; and they also found to increased level of glutathione in blood and tissue. Jigrine pretreatment have decreased inflammation of hepatic cells caused by galactosamine [108].
Researchers have evaluated hepatoprotective activity of *Cichorium intybus* seed extract on carbon tetrachloride and acetaminophen induced liver damage in mice. They found that *Cichorium intybus* pretreatment have decreased both the death rate and the serum level of glutamyl oxaloacetate transaminase, alkaline phosphatase and glutamyl pyruvate transaminase [108].

Zafar and Mujahid, [130] studied antihepatotoxic effects of root and root callus extracts of *Cichorium intybus* in rats against carbon tetrachloride induced hepatic damage. They found that pretreatment of root and root callus extracts prevented elevation of bilirubin and serum enzyme levels such as aspartate transaminase, alanine transaminase; and decrease in albumin and protein levels in ccl4 treated rats. They also reported that *Cichorium intybus* root callus extract could afford a better antihepatotoxic activity against carbon tetrachloride induced hepatocellular damage, compared to the root extract [130]. Yasser et. Al., [131] have investigated and reported that, hepatoprotective activity of *Cichorium intybus* root extract was due to the prevention of lipid peroxidation, sustaining of endogenous antioxidant molecules and overexpression of genes encoding antioxidant enzymes. Sultana et. al., [132] found that hepatoprotective effect of *Cichorium intybus* extract was due to its ability to suppress the oxidative degradation of DNA. Aktay et. al., [133] have demonstrated hepatoprotective activity of *Cichorium intybus* in carbon tetra chloride induced hepatotoxicity model in rats. Neha et. al., [134] have demonstrated antioxidant and hepatoprotective activity of *Cichorium intybus*. They also reported that, flavonoids are responsible for the antioxidant activity [134].

**Anti-malarial activity:** Bischoff et. al., [117] identified and extracted light sensitive Sesquiterpenes lactones from roots of *Cichorium intybus*. They demonstrated the *Cichorium intybus* lactones inhibitory effect on HB 3 clone of the *Honduras-1* strain of *Plasmodium falciparum*. They reported that Sesquiterpenes lactones, such as lactucin and luctucopicrin, showed anti-malarial activity at a concentration of 10µg/ml and 50µg/ml respectively [117].

**Anti-microbial activity:** Nandagopal and Ranjitha kumari, [135] have demonstrated antibacterial activity of *Cichorium intybus*; and they found that root extracts have potential antibacterial effect on *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella typhi* than *Escherichia coli* and *Micrococcus luteus* [135]. Petrovic et. al., [111] have investigated antibacterial activity of *Cichorium intybus*, and they reported that, it has antibacterial action against *P.aeruginosa, Pseudomonas fluorescens,*
_Erwinia carotovera_ and _Agrobacterium radiobacter_ [111]. In another study, leaf extract of _Cichorium intybus_ have shown anti-microbial activity against multidrug resistant salmonella typhi [108]. Guaianolides-rich root extract of cichorium intybus have shown antifungal action against anthropophilic fungi, such as _Trichophyton tonsurans_, _T. violaceum_ and _T. rubrum_ [108].

**Tumor-inhibitory activity:** Hazra et. al., [136] studied tumor inhibitory activity of chicory root extract, and they reported that its crude ethanol extract exhibited a significant inhibition of Ehrlich ascites tumor carcinoma in mice. They also reported that, treatment with 500mg/kg/day intra peritoneal dose of chicory root extract has increased 70% in the life span with respect to the control [136].

Researchers investigated effect of magnolialide constituent of _Cichorium intybus_ on human leukemia cells; and they found that magnolialide (1β-hydroxyeudesmanolide isolated from the roots of _Cichorium intybus_) inhibited several tumor cell lines and also induced the differentiation of human leukemia cells (HL-60 and U-937) to monocyte or macrophage-like cells [108].

**Anti-allergic activity:** Researchers investigated and reported that _Cichorium intybus_ have inhibited the mast cell mediated immediate type of allergic reaction in mice. They found that, _Cichorium intybus_ extract restrained the systemic anaphylactic reaction in mice, and it was in dose dependent manner. They also found that, _Cichorium intybus_ inhibited passive cutaneous anaphylactic reaction caused by anti-dinitrophenyl IgE in rats. _Cichorium intybus_ treatment resulted in increased cAMP level, decreased plasma histamine level and decreased histamine released from rat peritoneal mast cells [108].

**Immunomodulatory activity:** Amirghofran et. al., [137] have demonstrated immunoactive potential of _Cichorium intybus_ root extract by a mitogen proliferation assay and mixed lymphocyte reaction (MLR). They found inhibitory effect on lymphocyte proliferation in the presence of phytohemagglutinin and a stimulatory effect on MLR.

Researchers have investigated effect of _Cichorium intybus_ on the immunotoxicity by ethanol in mice. They found restoration in the markers of immunity, namely, hemagglutination titer, secondary IgG antibody production, plaque forming cells of spleen, phagocytic activity, number of circulating leucocytes, NK cell activity, delayed type of hypersensitivity reaction (in response to subcutaneous administration
of sheep red-blood cells to paw), cell proliferation and production of interferon-\(\gamma\), was registered [108].

**Other activities**

Keshri et. al., [138] have demonstrated postcoital contraceptive activity of Cichorium *intybus*. Christinaa et. al., [139] have reported antilithiatic activity of Cichorium *intybus* against glycolic acid induced stone formation. Jindal et. al., [140] have investigated and reported various actions of *Cichorium intybus* root extracts, like analgesia, anti-inflammatory, potentiation of drug induced hypnosis and anti convulsant. *Cichorium intybus* have shown protective effect against experimental cerulean induced acute pancreatitis in albino mice [141]. *Cichorium intybus* leaf extract exhibited antibacterial and antifungal activity [142].

**Toxicological studies:** Schmidt et. al., [143] have evaluated safety of the root extract of *Cichorium intybus*. An Ames test and a 28 day sub-chronic toxicity study were conducted using male and female rats. They evaluated mutagenic property of Sesquiterpenes rich extract using *Salmonella typhi murium* strains TA97a, TA98, TA100, TA1535 and *Escherichia coli* strain WP2 uvrA. It was found that chicory extract did not have mutagenic activity, although it was cytotoxic to certain strains of Salmonella at higher doses. Subchronic toxicity study has not shown mortality up to the dose of 1000mg/kg/day.

In another study, toxicity of *Cichorium intybus* was evaluated by using *vibrio fischeri* bioluminescence inhibition test. Decrease in light emission from the marine luminescent bacteria *V. fischeri* was measured, when exposed to organic extracts. It was found that extracts showed less than 20% inhibition of bioluminescence. Hence it was reported that *Cichorium intybus* to be safe for human use [108].

In recent research, acute toxicity of *Cichorium intybus* in albino mice was investigated, and reported that death were not observed in mice due to *Cichorium intybus* after oral administration at 625mg/kg body weight and also after intra peritoneal administration of 125mg/kg body weight. They also reported that, LD\(_{50}\) of *Cichorium intybus* was found to be 8900 mg/kg body weight [108].

**2.11. Experimental models for evaluation of antistress agent**

Literature survey revealed that, various animal models have been employed to induce non-specific stress; and to evaluate antistress activity of a drug. Stress method should be able to determine physical stress aspect associated with neuroendocrine changes, and psychological stress aspect associated with behavioral changes [19].
Each model have been developed and used to determine specific aspects of the stress response. Various stress models are as follows:

2.11.1. **Swimming endurance of mice**: This test is widely used to evaluate antistress activity of drugs, since swim endurance reflects physical endurance [144]. It has been reported that, antistress agent have improved swimming endurance and overall performance of the animals [144]. In this model, animals like rodents are subjected to swim in a water vessel. Initially an animal shows vigorous activity, trying to escape from the restricted space, but after some period, they become immobile representing behavioral despair or mental depression [145]. During stress, behavioral depression is a common response. The depression was postulated to be the result of depletion of central nor adrenaline and serotonin, when animals are subjected to inescapable and severe stress [145].

2.11.2. **Effect of adaptogenic plant extracts on drug induced narcosis test in mice**: Antistress agent (Adaptogen) produce different effects on organism, to protect from unfavorable stressful stimuli. The mechanism of antistress activity could be capacity of Adaptogen or antistress agent to depress the CNS. It can be demonstrated by observing the ability of the antistress agent to synergize with CNS depressant such as barbiturate [146].

In this model the effect of antistress agent on righting reflex of mice is observed as a parameter to determine CNS depression. Even it is postulated that antistress agent may involve in the stimulation of inhibitory synapse of the brain [147].

2.11.3 **Effect of adaptogenic plant extract on cold and restraint stress induced Brain lipid peroxidation**: In this model, rats are immobilized on wooden plank, and they are placed at 4-6 °C to accomplish cold and restraint stress [19, 38]. Synergism between two stressful stimuli (such as cold, restraint) can be achieved in this model. Cold environment activates temperature regulatory center in the hypothalamus; and it results in HPA axis activation, leading to release of adrenocortical hormones responsible for stressful response [19]. Whereas restraint of animal produces physical as well as mental stress, and animal does not exhibit adaptation [19]. Physiological and neuroendocrine changes have been found in chronic stress model. HPA axis activation results in elevated level of ACTH and Cortisol [148].

In this model, chronic exposure of rats to cold and restraint stressful situation leads to oxidative damage and this result into lipid peroxidation [149]. Brain is particularly sensitive to lipid peroxidation, because it contains high concentration of easily
peroxidizable poly unsaturated fatty acids, which is susceptible to oxidative stress induced damage by oxygen free radical [149-150]. Oxygen free radicals are generated in various stressful conditions such as cold and restraint stress [151]. During restraint of animal, they suffer from both emotional as well as physical components of stress leading to robust increase in basal oxidative stress [152]. This oxidative stress is capable of inducing lipid peroxidation [150]. Lipid peroxidation involves three stages. It includes in initiation, progression and termination. During initiation hydrogen is abstracted from poly unsaturated fatty acid (LH) or oxygen radical is added. During this initiation stage PUFA undergoes oxidative breakdown resulting in lipid alkyl radical (L$^\ast$). During propagation stage, this lipid alkyl radical reacts with oxygen yielding lipid peroxy radical (LOO$^\ast$). This lipid peroxy radical can abstract H form another PUFA resulting in another lipid alkyl radical (L$^\ast$) and lipid hydro peroxides (LOOH). In this way of propagation of lipid peroxidation, numerous PUFA$\_8$ are converted into lipid hydro peroxides and subsequently can produce alkyl, peroxy and alkoxy radical. Finally during termination stage, the alkyl radical may be stabilized into conjugated diene (fatty acid dimer) through rearrangement. Termination of lipid peroxidation occurs with the formation of peroxy bridged fatty acid dimer [153-154].

Alternatively due to instability of lipid peroxy radical, it can form endoperoxide which decomposes to malondialdehyde (MDA), hexanal and 4-hydroxy- 2-nonenal (HNE) etc [153-154].These lipid peroxidation products can inactivate DNA, RNA, protein and other biomolecules by cross linking and altering structure [155].

![Figure 2.24: Stages of Lipid peroxidation. Adapted from [153].](image-url)
This tissue damage due to lipid per oxidation can be determined by measuring malondialdehyde formed as an end product of lipid per oxidation. MDA can be measured by a well-accepted thiobarbituric acid assay. Here malondialdehyde reacts with thiobarbituric acid to give characteristic chromogenic dithiobarbituric acid adduct MDA (TBA)_2, which absorbs strongly at 532 nm [153].

2.11.4 Effect of adaptogenic plant extracts on swimming stress induced gastric ulceration in rats: The fact that stress is one of the pathogenic factor of gastric ulcers in human, was published by a clinical endocrinologist and experimental biologist, Dr. Hans Selye during 1930’s; and he used restraint as stress for the first time in animal studies [1]. Stress can be given to animal for a single short period (acute stress) or repeatedly for a long period on daily basis (chronic stress). Physiological and neuroendocrine changes have been found in chronic stress model, and this activate HPA axis resulting in elevated level of ACTH and Cortisol [148]. Animal shows stress response, when they are unable to escape noxious stimuli, and this principle was utilized while developing model, such as forced swimming stress induced gastric ulceration in albino rats [19]. In this model, rats are forced to swim 15 m daily for 10 consecutive days in the tub, containing water to the depth of 40 cm [19].

2.11.5 Effect of Adaptogen plant extract on adrenocortical activity in stress induced rats: Any type of stress, regardless of the nature (whether physical or psychological), will stimulate Hypothalamic-Pituitary-Adrenal (HPA) axis of the organism [20]. In this pathway, stressors are able to activate nerve cells and hypothalamus, so that CRH is secreted from the paraventricular nucleus. Then CRH reaches the pituitary gland through hypothalamicohypophysial portal system. Anterior pituitary gland releases adrenocorticotropic hormone (ACTH), under the regulation of CRH [12]. ACTH is carried by the blood to the adrenal gland; there it stimulates adrenal cortex to secrete corticoid hormones such as Cortisol or corticosterone into the blood [20]. Several studies have reported that stress increases plasma corticosterone level [156] and altered adrenal gland weight [156]. So estimation of plasma corticosterone with assessment of adrenal gland weight is the index of hypothalamo-hypophyseal-adrenocortical axis activation, and important markers to evaluate stress [145].

Cold swimming stress has been used frequently to induce acute stress. Even it is well documented that cold swimming stress elicits an increase in the plasma corticosterone level [157]. In this model, rodents are subjected to swim in a tank of
cold water, at a temperature of 10°C till exhaustion [157]. Due to cold swimming stress, temperature regulatory center in the hypothalamus is activated, and this results in activation of HPA axis, which is responsible for stressful response [19].

2.11.6. Effect of Adaptogen plant extract on liver glycogen of albino rats during weight loaded forced swimming stress: To determine effect of plant extracts on liver glycogen due to stress, an adapted version of the Porsolt’s forced swimming test is used with little modification. In this model rats are forced to swim in the transparent tub containing water to the depth of 40 cm. But rats tail are loaded with a steel weight (4% of their body weight), which maintain continuous rapid leg movements [158].

Physical stress, such as swimming induced stress, involves physiological changes inside organism: to meet energetic demand as well as to maintain homeostasis [159]. During stress more energy is required by the active organs of the body like heart, brain and muscle. When hypothalamus senses that additional energy is needed to counter stress, it sends impulses to activate sympathetic nervous system and adrenal medulla, so as to release epinephrine and norepinephrine. This leads to mobilization of large amount of organisms resources (such as glucose, oxygen) to the main organs: like brain to become alert, skeletal muscle to fight or flee, heart to pump enough blood. Even corticosteroid increases metabolism in such a way that excess of glucose should be available, and avoids starvation of glucose dependent organs.

Swimming of rodents in water is now commonly accepted as an experimentally induced physical exercise in animals [157]. To study physical stress in animals, Porsolt et al., have developed forced swimming stress test, that has been widely accepted and used model [145]. It has been reported that, physical exercise as a stress have produced complex hemodynamic and biochemical changes [160]. During physical exercise, energy is provided from breakdown of stored glycogen in liver and muscles [161]. It has been reported that, severe depletion of liver glycogen was observed in guinea pigs, when subjected for swimming [158]. Depletion of glycogen in liver and skeletal muscle during stressful condition, such as prolonged exercise has been well established [158]. Even it has been well established that, immobility during prolonged exercise is due to development of fatigue, and it is related by depletion of glycogen in liver [158].

Depression occurs if stress is continued for prolonged period of time [145,152]. This method is based on observation of passiveness and immobility of rats after a period of vigorous activity, when they are subjected for forced swimming [145, 162].
This model is widely used to induce depression in animal by the stress. Behavioral despair represents an experimental model of depression in animal [145]. Stress results in increased expression of nitric oxide, which in turn inhibits production of cellular energy (ATP) [45]. Antistress agent prevents stress induced expression of nitric oxide and the associated decline in ATP production. Ultimately rise of energy (ATP), results in increased endurance capacity and overall performance of the organism [45].

2.11.7 Studies on the Immunomodulatory activity of adaptogenic plants:

Influence of stress on immunity is a well-known phenomenon, and it shows complex immune response [145]. Physical or psychological stress influences function and efficiency of the immune system, resulting in immunosuppression [163]. Immune response can be measured by two basic ways as follows [164]:

1) Quantitative measurement of immune response, which involves measuring number of cells in a given volume of blood or a percentage of each type of cell.

2) Whereas in functional measurement, non-specific antigen (mitogen) are administered to observe lymphocyte function.

In animal model immune response to stress is evaluated by measuring number of cells and percentage of each type of cells in blood. Stress induces significant alteration in absolute numbers of leukocytes and its relative proportions in the blood [165]. Earlier, blood leukocyte measurement was the method to determine stress, when hormone measurement methods were not developed [165].

Numerous studies have been reported on stress induced changes in blood leukocyte numbers in mice, rats, rabbits, humans, non-human, primates, horses, hamsters and fish [165]. It has been found that stress in rodents have induced decrease of leucocytes number in blood with decrease in lymphocyte and monocyte percentage, whereas increase in neutrophils percentage [165]. Even it has been reported that, leukocyte numbers are rapidly reversed back to normal on the withdrawal of stress [165]. Decrease in lymphocyte and monocyte are due to glucocorticoid hormone released during stress [165].

During initial period of stress, norepinephrine of activated sympathetic nervous system induces distribution of leukocytes from spleen to blood, resulting in high level of blood leukocyte. During later stages of stress, activation of HPA axis releases glucocorticoid, and this induces redistribution of leukocytes from blood to immune challenge areas such as skin, lung, gastrointestinal tract, urinary-genital tract, mucosal
surfaces, and lymph nodes [165]. During later stages of stress, blood leukocyte number decreases due to redistribution, while preparing for immune challenges of stressor. It has been reported that blood leukocyte distribution as an adaptive response during stress [165]. During chronic stress, corticosterone level remains high, resulting in reduction of white blood cell numbers in circulating blood [166]. Certain medicinal plants are able to modulate pathophysiological processes, so they are called as Immunomodulatory agents [163].